

# Poster Presentations

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### Heart, Circulation(I)

#### 1PK-001

##### Regulation of Cardiomyocyte Survival by Neuronal Calcium Sensor-1

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Identifying the molecular targets regulating myocyte survival is essential for therapeutic purposes for heart failure. Neuronal Ca<sup>2+</sup> sensor-1 (NCS-1) is a small EF-hand Ca<sup>2+</sup>-binding protein that is important for neuronal functions. Using NCS-1<sup>-/-</sup> (KO) mice, we recently identified cardiac functions of NCS-1 as a positive regulator of Ca<sup>2+</sup> signaling in immature and diseased hearts (*Circ. Res.* 2011). Since neonatal KO mice had high mortality rate, and we reported that NCS-1 is neuroprotective (*JCB* 2006); we hypothesized that NCS-1 plays beneficial roles in cardiac survival especially under stressed conditions. We found that KO mouse hearts were more susceptible than WT group to several kinds of stressors including serum/glucose depletion in cultured myocytes, and to ischemia-reperfusion injury in whole heart. In KO group, a major survival pathway PI3K/Akt signaling, and mitochondrial function, were both significantly reduced. As molecular mechanisms for these, we identified two NCS-1 targets; 1) phosphatidylinositol 4-kinase (PI4K), located upstream of the PI3K/Akt pathway, and 2) IP<sub>3</sub>R, a Ca<sup>2+</sup>-release channel regulating cellular and mitochondrial Ca<sup>2+</sup> and ATP levels. We ensued that NCS-1 binds to and activates both of these proteins in the heart; and inhibition of these proteins by RNAi resulted in decreased resistance to stress-induced cytotoxicity. These results demonstrate that NCS-1 promotes cardiomyocyte survival via activation of PI4K and IP<sub>3</sub>R, leading to amplification of PI3K/Akt pathway and regulated mitochondrial functions, respectively.

#### 1PK-002

##### Abnormal Modulation of hERG and KCNQ1 Channels by KCNE1 Subunit with a G38S Mutation

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The normal modulation of human ether-a-go-go-related gene (hERG) and KCNQ1 K<sup>+</sup> channels by wild-type KCNE1 [KCNE1 (WT)] auxiliary subunit is important for maintaining the repolarizing kinetics of cardiomyocytes and preventing the arrhythmic activities. A G38S substitution is a common polymorphism of KCNE1 whose association with heart failure is unclear. We examined how this mutation affects the K<sup>+</sup> channels, performing whole-cell voltage-clamp measurements in HEK-293T cells transfected with the KCNE1 and K<sup>+</sup> channel genes. The density of hERG channel current in KCNE1 (G38S)-transfected cells was smaller by ~30% than that in KCNE1 (WT)-transfected cells. This effect of KCNE1 (G38S) was obscured by co-transfection of KCNE1 (WT). The voltage-sensitivity of hERG channel current was not different between KCNE1 (G38S)- and KCNE1 (WT)-transfected cells. In contrast, the I-V plot of KCNQ1 channel current in KCNE1 (G38S)-transfected cells was right-shifted by ~10 mV as compared with that in KCNE1 (WT)-transfected cells. This effect of KCNE1 (G38S) was obscured by co-transfection of KCNE1 (WT). The density of KCNQ1 channel current was not different between KCNE1 (G38S)- and KCNE1 (WT)-transfected cells. These findings suggest that the mutant KCNE1 subunit reduces the activity of the hERG and KCNQ1 channels in different manners.

#### 1PK-003

##### Characterization of a New Mutant KCNQ1 Channel Subunit with a C-Terminal Truncation

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KCNQ1 encodes the alpha subunit of a delayed-rectifier K<sup>+</sup> channel responsible for the repolarization of cardiomyocytes and the prevention of arrhythmic cardiac activities. We identified a new mutation causing a C-terminal truncation at the Y461 of the subunit [KCNQ1 (Y461X)] from a patient with a mild QTc prolongation. We characterized the function of this mutant subunit, performing whole-cell voltage-clamp measurements in HEK-293T cells transfected with the gene encoding KCNE1 auxiliary subunit and either of wild-type KCNQ1 [KCNQ1 (WT)], KCNQ1 (Y461X), or their mixture (WT, Y461X, and Y461X/WT cells, respectively). Y461X cells displayed no delayed-rectifier current, suggesting that KCNQ1 (Y461X) subunit for itself cannot form functional channels. In contrast, Y461X/WT cells displayed delayed-rectifier currents whose density and voltage-dependence were similar to WT cells. In Y461X/WT cells, KCNQ1 (WT) subunit predominated in membrane immunoreactivity, suggesting that homomeric KCNQ1 (WT) channel might carry a large portion of the current seen in the Y461X/WT cells. These findings indicate the importance of the C-terminus for functional channel formation and trafficking. Moreover, the heterozygous inheritance of KCNQ1 (Y461X) may cause minor cardiac symptoms whereas the homozygous inheritance might cause severe symptoms.

## 1PK-004

### Sphingosine-1-phosphate receptor-2 plays a protective role against anaphylactic shock through inhibiting eNOS

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The role of sphingosine-1-phosphate (S1P) receptor S1P<sub>2</sub> in systemic anaphylaxis is still controversial, which contrasts to the established protective role of S1P. We demonstrate that S1P<sub>2</sub> deletion in mouse anaphylaxis models enhances stimulation of eNOS, which generates NO to mediate hypotension, vascular leak, hypothermia and lethality, markedly compromising survival from anaphylaxis after antigen challenge and platelet-activating factor (PAF) injection. In *S1pr2*<sup>-/-</sup> mice, PAF-induced activation of Akt and eNOS in aorta and lung was enhanced compared with wild-type mice. Consistently, PAF-induced increase in the cGMP level in aorta was enhanced in *S1pr2*<sup>-/-</sup> mice. Either pharmacological inhibition or genetic deletion of eNOS rescued *S1pr2*<sup>-/-</sup> mice from exacerbation of anaphylaxis after antigen challenge and PAF injection. Endothelial cells (EC) isolated from *S1pr2*<sup>-/-</sup> mice showed greater stimulation of Akt and eNOS with enhanced NO production in response to either S1P alone, PAF alone, or S1P plus PAF, compared with wild-type EC. In addition, *S1pr2*<sup>-/-</sup> EC showed more severe disassembly of adherens junctions with increased S-nitrosylation of  $\beta$ -catenin in response to PAF, which was restored by pharmacological inhibition of eNOS. These observations demonstrate that S1P<sub>2</sub> plays a protective role against anaphylaxis and is a novel therapeutic target for anaphylaxis.

## 1PK-005

### A novel nucleic acid analogue COA-Cl promotes angiogenesis by way of purinergic receptors and Src family tyrosine kinases

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We have recently developed a novel nucleic acid analogue termed COA-Cl that elicits strong angiogenic activities via ERK1/2 MAP kinases. We explored how COA-Cl modulates cellular signaling machineries to promote angiogenesis using cultured human umbilical vein endothelial cells (HUVEC) as a model. Phospho-western analyses revealed that ERK1/2 activation by COA-Cl was sensitive to suramin, an inhibitor of P2 family purinergic receptors; BAPTA-AM, an intracellular calcium chelator; and U73112, an inhibitor of phospholipase C (PLC). Immunoprecipitation assay showed that COA-Cl induces remarkable phosphorylation of p130Cas, a major substrate protein of Src-family kinase (SFK), which is abrogated by PP2, a SFK inhibitor. Conversely, PP2 significantly attenuated COA-Cl-elicited promotion of both ERK1/2 activation and tube formation. These results are consistent with a model in which COA-Cl exerts its angiogenic activity by way of P2 family purinergic receptors to activate PLC and mobilize intracellular calcium, leading to activation of a protein kinase cascade comprising Src-family tyrosine kinases and MAP kinases, thus identifying a novel endothelial machinery regulating angiogenesis.

## 1PK-006

### Proteomic analysis identified prostanoid E2-induced proteins in tissues from patients with aortic aneurysm

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Aortic aneurysm (AA) is common in the elderly. Degradation of extracellular matrices (ECM) induced by inflammation is known to play a pivotal role in AA progression. In the previous study, we showed that prostaglandinE2 (PGE2) up-regulated IL-6 and MMP-2 in mouse AA models. In this study, we applied proteomic approach to comprehensively search for effects of PGE2 on AA etiology.

Human AA tissues were obtained at a blood vessel prosthesis implantation with written informed consents. The tissues were cultured in transwells in which we separated luminal and adventitial fraction (LF and AF) and were stimulated with PGE2 for 24 h. Trypsinized proteins were labeled with iTRAQ and were analyzed by LC/MS/MS for comparative quantification of the proteins. Identified proteins were classified by GO terms.

Numbers of identified proteins were 396 in LF and 354 in AF. Increased expression (>1.3-fold) by PGE2 was found in 17 putative secretomes in LF, 15 in AF, and 6 in common. Decreased expression (<0.77-fold) was found in 6 secretomes in LF, 5 in AF, and 1 in common. The increased proteins included 2 cytokines, 5 ECM, and 2 proteinase inhibitors in LF, and 2 cytokines, 3 ECM, and 2 proteinase inhibitors in AF.

Using MS spectrometry, proteins potentially related to PGE2 signaling in AA progression were identified.

## 1PK-007

### Effect of capsaicin microinjection into the cardiovascular-related region in the nucleus of the solitary tract of the adult rat

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Capsaicin, as a member of the vanilloids, desensitizes the sensory nerve endings giving a paradoxical antinociceptive effect. Vanilloids are a group of compounds which are related to capsaicin structurally. It is known they demonstrate actions via the vanilloid receptor which is a subgroup of TRP (transient receptor potential) cation channels, very sensitive to temperature. The respiratory response to microinjection of capsaicin into the nucleus tractus solitarius (NTS) reduced respiratory frequency. However, to date there is little information on the role of vanilloid receptors at the NTS in relation to cardiovascular effects. This report deals with the response of capsaicin on blood pressure and heart rate after microinjection into the different sites of NTS of urethane anaesthetized rats. Microinjection reduced blood pressure and heart rate at the NTS 0.5 mm rostral, 0.5mm lateral from calamus scriptorius and 0.5mm below from the brain surface. These results suggest the possibility that capsaicin may play an important role on cardiovascular regulation in the NTS.

## 1PK-008

### Preliminary analysis of differentiation and proliferation program of cardiomyocytes in developmental stages

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Adult cardiomyocytes are terminally-differentiated cells and lose their self-renewal capacity, and thus no more regenerate upon their pathological loss. While in developmental stages, immature myocytes and its progenitors coordinate their proliferation and differentiation program to form a complicated structure of the heart. Although many past studies used proliferative marker like Ki67 or BrdU to state when myocytes exit the cell cycle, the actual division process has not been observed in live cells. Moreover, the critical signals to induce cell cycle exit during pre/post-natal stages remain mostly unknown. To realize this developmental program, preliminary experiments were performed. Immunofluorescent analysis of Ki67 in the heart sections of neonatal C57BL/6 mice aged at post-natal (P) day 1 to 24 indicated that proliferative capacity of myocytes is lost at P10-P15. Next, using isolated myocytes from mice before P10, we successfully captured the cell division processes in live cells including the generation of mitotic spindles, karyokinesis, and cytokinesis. Complete cytokinesis is very rare even before P10 (<1% of total cells), indicating that the expression of proliferative marker alone does not show its actual division activity. To avoid the effect from contaminated fibroblasts, we now successfully obtain >99% purity of myocytes expressing cardiomyocyte-specific  $\alpha$ -actinin. These preliminary data will drive future investigations to figure out the regulation of cardiomyocyte proliferation and differentiation in heart development.

## 1PK-009

### Theoretical analysis of mitochondrial NCX (NCLX) -mediated regulation of cardiac automaticity

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We reported that reduction of mitochondrial Na-Ca exchanger (NCLX) resulted in the prolongation of beating rate in HL-1, a spontaneously beating cardiac cell line derived from mouse atrial myocytes. From experimental as well as theoretical analyses, it was shown that NCLX fine-tunes sarcoplasmic reticulum (SR) Ca dynamics, thereby modulates "Ca clock" mechanism. However, the "Ca clock" mechanism, in which a spontaneous Ca leak from SR determines the automaticity, is still controversial among researchers especially when discussing about the automaticity of intact pacemaker cells (SA node cells), while the "membrane clock" mechanism, in which membrane channels determines the automaticity, is well accepted. In order to clarify the contribution of mitochondrial Ca dynamics on the automaticity of SA node cells, we incorporated mitochondrial Ca dynamics into two different SA node cell models, Maltsev-Lakatta (ML) model and Himeno (HSMN) model, the automaticity of which is driven by "Ca clock" and "membrane clock", respectively. In both models, reduction of mitochondrial Na-Ca exchange resulted in the marked decrease of SR Ca content. However, the beating rate was prolonged in "Ca clock"-driven ML model but shortened in "membrane clock"-driven HSMN model. These results suggest that contribution of mitochondrial Ca fluxes on the pacemaker activity is mechanism dependent.

## 1PK-010

### Vidarabine, an anti-herpesvirus agent, prevents atrial fibrillation in mice

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Atrial fibrillation (AF) is one of the most common tachyarrhythmia in clinical practice. Beta-adrenergic blockers ( $\beta$ -blockers), established agents for treatment of heart failure, are reported to be effective in preventing adrenergically induced AF. Adenylyl cyclase (AC) mediates the beta-adrenergic receptor signal transduction. Recently, it was demonstrated that an anti-herpesvirus agent Vidarabine, a selective inhibitor of cardiac AC, ameliorated the development of heart failure in mice. Here, we assessed the anti-arrhythmic effect of Vidarabine in a mouse model of AF. AF was reproducibly induced by transesophageal atrial burst pacing. Both Metoprolol (a  $\beta$ -blocker) and Vidarabine (44.5 vs. 18.1 sec,  $P < 0.001$ ) significantly reduced the duration of AF. Consistent with the results, sympathetic activation by norepinephrine (NE) (1.5 mg/kg) strikingly increased the duration of AF (38.9 vs. 739 sec,  $P < 0.001$ ). The NE-elongated AF was also shortened by Vidarabine. Moreover, the NE-elongated AF was shorter in mice deficient for type 5 AC, a major cardiac AC isoform, than that in wild-type mice. Further, Vidarabine did not affect the cardiac systolic function. On the other hand, Metoprolol decreased the left ventricular ejection fraction as reported previously. These findings suggest that Vidarabine may prevent AF via inhibition of cardiac AC activity, while causing no adverse effects on heart function.

## 1PK-011

### The roles of EP4 signaling in cardiac fibroblasts

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Prostaglandin E2 (PGE2) is known to be increased in the ischemic heart. Previous studies demonstrated that PGE2 receptor EP4 plays a protective role in the ischemic heart. Although EP4 signaling in cardiac myocytes (CM) is suggested to promote hypertrophy to reduce infarct size, the role of EP4 in cardiac fibroblasts (CF) remains unknown. [Materials and Methods] EP4 hetero (HT) mice and littermates wild type (WT) of 3 months-old male were used. CM and CF were isolated by Langendorff collagenase perfusion. CF were cultured up to 6 days. mRNA expression was measured by qRT-PCR. Catheter and echocardiography were performed. Collagen fiber was visualized by Masson trichrome stain and was quantified using a color extraction method. [Results] In WT mice, expression levels of EP4, collagen I and collagen III mRNAs were much higher in CF than in CM (12-fold, 211-fold, and 123-fold,  $n = 5$ , respectively,  $p < 0.01$ ). Expression level of collagen III mRNA was significantly lower in CF of EP4HT mice than in WT mice (0.5-fold,  $n = 5$ ,  $p < 0.05$ ). There were no difference in mRNAs of collagen I and the collagen cross-linking enzyme lysyl oxidase between in EP4 HT and WT mice. In the basal condition, cardiac systolic and diastolic function, and area of fibrosis were not different between in EP4HT and WT mice. [Conclusions] In the heart, EP4 was predominantly expressed in CF rather than in CM. EP4 in CF may play a protective role in ischemic heart through producing collagen III.

## 1PK-012

### Effect of cibenzoline on Na/Ca exchange current in guinea pig cardiac ventricular myocytes

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The effect of cibenzoline, a class I antiarrhythmic drug, on Na<sup>+</sup>/Ca<sup>2+</sup> exchange current ( $I_{NCX}$ ) was investigated using the patch clamp method. Cibenzoline inhibited the bi-directional  $I_{NCX}$  in a concentration-dependent manner. The  $IC_{50}$  values of cibenzoline for the outward and inward components of  $I_{NCX}$  were 77  $\mu$ M and 84  $\mu$ M, respectively, with Hill coefficients of 1. Intracellular application of trypsin via the pipette solution did not change the inhibitory effect of cibenzoline. The inhibitory effect of cibenzoline on  $I_{NCX}$  at pH 6.5 was smaller than those at pH 7.4 and pH 8.2. We conclude that cibenzoline inhibits  $I_{NCX}$  in supra-therapeutic concentrations.

## 1PK-013

### Positive correlation between $I_{st}$ and $I_{Ca,L}$ in guinea-pig sinoatrial node cells

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The sustained inward Na<sup>+</sup> current ( $I_{st}$ ) has been identified in sinoatrial node (SAN) cells of various mammals and suggested to play a pivotal role in cardiac pacemaker activity. Although the channel molecule mediating  $I_{st}$  remains unidentified, the pharmacological profile of the current predicts that some unknown variants of  $I_{Ca,L}$  channel might be involved in  $I_{st}$ . In the present study, we investigated the expression of  $I_{st}$  in morphologically different types of pacemaker cells dissociated from guinea-pig SAN and its functional relationship to  $I_{Ca,L}$ .  $I_{st}$  was preferentially detected in spontaneously active SAN cells with spindle and spider morphology, but was less expressed in elongated cell type and practically absent in atrial-like nodal cells. By linear regression analysis, we found a significant positive correlation between the densities of  $I_{st}$  and  $I_{Ca,L}$  in spindle and spider cells. However, the slope of regression line was blunted in the elongated cell type and no significant correlation was observed in atrial-like cells. On the other hand, the voltage dependence of  $I_{Ca,L}$  activation also varied in cell types, suggesting the heterogeneous composition of  $Ca_v1.2$  and  $Ca_v1.3$   $I_{Ca,L}$  subunits with distinct voltage sensitivity. The half-maximal voltages for  $I_{Ca,L}$  activation were -31.2, -27.9, -20.1 and -11.4 mV in spindle, spider, elongated and atrial-like cells, respectively, which was strongly correlated with relative expression level of  $I_{st}$ . Taken together, these results suggest that the expression level of  $I_{st}$  is dependent on the abundance of low voltage-activated  $Ca_v1.3$   $I_{Ca,L}$  channel.

## 1PK-014

### Retrograde perfusion for blood and oxygen supply to hindlimb in relation with venous valves in rats

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The vascular surgery for distal vein arterialization, DVA, has been developed to rescue arteriosclerotic hind limbs from amputation in the clinic. However, basic questions remain unstudied. The arterial blood introduced into the vein was suspected to fill up the vein only and to flow through thick collaterals into other veins without oxygen delivery to peripheral tissues. To elucidate this question DVA surgery was conducted in rat hind limbs as a model for DVA surgery. In the first group of rats the venous valves were dissected only in the femoral vein, followed by the DVA surgery. The femoral vein was clearly dilated. A high skin temperature was detected in the groin. But the thigh and foot remained cool. In the second group venous valves were dissected also in the popliteal vein to the ankle. The skin temperature rose in the thigh and foot to peripheral regions as seen on the thermo camera recordings. Valves are present in venules as small as 0.15 mm in diameter. These small valves can not probably resist the high blood pressure and the periphery tissues can be perfused via venular network as suggested by Özbek et al. Thus, it seemed probable that the arterial blood flows through the venular networks by the DVA surgery, arriving at venous anastomoses and finally join to the vein. Since the venular length density is large enough, the Krogh's tissue cylinder around the hypothetical venule can be supplied with oxygen, assuming the oxygen consumption rate at rest. The present study was made with Drs. Sasajima T, Kikuchi S, Ishikawa N, Dept. Vascular Surgery, Asahikawa Medical Univ.

## 1PK-015

### Spatiotemporal chaos during tachycardia-like excitation in the rat atrium revealed by optical mapping studies

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Spatiotemporal patterns of chaotic spread of electrical excitation in isolated rat atrial preparations were mapped by multiple-site optical recording methods using a multi-element photodiode array together with a fast merocyanine-rhodanine voltage-sensitive dye. The excitation spread during the event of the *tachycardia-like excitation* evoked by electrical stimulations were assessed by timing the initiation of optical action potentials. Then maps of the excitation spread patterns were constructed. From these maps, the characteristics of tachycardia-like excitation are summarized as follows: 1) Tachycardia-like excitation is the generation of (or "transition to") a new pattern of excitation spread evoked by perturbing the "normal" pattern. 2) During its generation, transitional unstable "chaotic" patterns of excitation spread, with blocked areas and ectopic foci, are observed. 3) After this phase, a new quasi-stable pattern appears. It is "quasi-stable" because the newly emerged pattern often returns to the original "normal" pattern spontaneously. 4) Among these events, *event-to-event variations*, which seem to result from the physiologically trivial difference (s) in the initial conditions, are always observed. This nature of "complex system" strongly supports the idea that tachycardia-like excitation is an example of the *functional "self organizing systems"*. We consider the tachycardia-like excitation observed here as the "physiology-specific attractor".

## 1PK-016

### Elucidation of the chick ductus arteriosus remodeling during development

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**Introduction :** The ductus arteriosus (DA) is a fetal artery connecting between the pulmonary artery and the descending aorta, and functions as a bypass that arrows the blood from the right atrium to flow into the aorta. The normal DA closes immediately at birth, but the condition that DA remains open after birth is called as patent DA. Our previous studies reveal that PGE<sub>2</sub> promotes the mammal DA closure through the hyaluronic acid (HA) production toward the lumen by the PGE<sub>2</sub>-EP4 signal pathway. However, we suppose that PGE<sub>2</sub> does not affect the DA closure in oviparous animals because they do not have placentas that are the main organs produce PGE<sub>2</sub>.

**Methods and Results :** We used DAs from chick embryos at four stages [19 embryo days (e19), internal pipping (IP), external pipping (EP), and after birth (AB)]. We conducted mainly the Elastica van Gieson staining and the real-time PCR for EP4 and the other PGE<sub>2</sub> receptors. The neointima layer was observed after IP, and the DA was completely stuffed like mammals. However, the PCR data showed that all mRNA expression levels were not so high in the e19, IP and EP chick DAs.

**Conclusion :** Our present study demonstrated that chick DA closure occurs by the HA production toward the lumen and thickening the neointima layer. However, the signal pathway between PGE<sub>2</sub> and its receptors may not affect the chick DA closure unlike mammals. We should find other genes affect to the oviparous (especially chick) DA remodeling during hatching.

## 1PK-017

### High sensitivity Western blotting method revealed the developmental changes in the contractile proteins of rat embryonic hearts

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Our previous studies showed that Wistar rat heart begins to contract at embryonic day 9.99-10.13. Observation of embryos loaded with fluo-3AM revealed that the beginning of the calcium transient precedes the initiation of contraction before the appearance of the linear heart tube. In order to investigate the relationship between the calcium transient and contraction, we tried to detect the developmental change in the contractile protein amount of individual embryonic heart by standard Western blotting method. However, no band derived from individual heart was detected because the rat heart aged E 9.99-10.13 days is very small and has slight amount of proteins. Next, we tried to detect the contractile proteins with high sensitivity 3-step Western blotting method. In this method, increased Enhanced Chemiluminescent Light is achieved for detection of small quantities of protein by using a biotinylated second antibody that provides increased binding sites for streptavidin-conjugated HRP. Myosin regulatory light chain of embryonic heart increased around the period of the appearance of the heartbeat. The result indicated that the developmental increase in myosin regulatory light chain involves in the initiation of contraction and this high sensitivity Western blotting method has the advantages in researches into embryonic hearts.

## 1PK-018

### Mice Overexpressing Prostaglandin E Receptor EP4 in Vascular Smooth Muscle Cells Decreased Elasticity of the Aorta

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Mechanical properties of the arteries are mainly due to the elastin components. In the previous study, we found that prostaglandin E receptor EP4 signaling decreased elastic fiber formation in smooth muscle cells (SMCs) of the fetal artery the ductus arteriosus. In this study, therefore, we examined whether EP4 signaling inhibited elastic fiber formation and elasticity in the adult elastic artery. We created mice overexpressing human EP4 in vascular SMCs. A Cre-loxp system was utilized under SM22 promoter. EP4<sup>loxP/loxP</sup> or EP4<sup>loxP/+</sup> mice were intercrossed with SM22-Cre transgenic mice. EP4<sup>loxP/+</sup>/SM22-Cre animals (Transgenic) and EP4<sup>+/-</sup>/SM22-Cre animals (Control) were created. First, we confirmed that mRNA expression of human EP4 was detected in only transgenic mice by using quantitative RT-PCR. Immunohistochemistry revealed that protein expression of EP4 in SMC layers of the aorta was abundant in transgenic mice. Histological analysis showed the number or formation of elastic fibers in the aorta of both transgenic mice and control mice are not significantly different in Elastica van Gieson staining. However, elasticity of the vessel rings from the transgenic mice was lower than that from control mice by using wire myograph system (wall tension ; 8.29±0.70 (Transgenic) vs 6.25±0.20mN/mm (Control) when internal circumference was 3.7mm) p<0.05, n=4-7). These data suggest that EP4 signaling decreased elasticity of the adult aorta *in vivo*.

## 1PK-019

### Real-time imaging of Ca<sup>2+</sup> in cardiomyocytes in the beating heart

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The heart is a unique organ in which cardiomyocytes repeat contraction and relaxation in response to electrical stimulation, thereby ejecting blood. Despite numerous studies conducted thus far under various experimental settings, the molecular mechanisms of the cardiac excitation-contraction coupling still remain elusive *in vivo*. In the present study, therefore, we conducted an experimental system allowing for the real-time imaging of intracellular Ca<sup>2+</sup> in ventricular myocytes in the isolated beating heart of the mouse as well as in the isoflurane-anesthetized open-chest mouse. The isolated heart was continuously perfused with Tyrode's solution containing 3mM Ca<sup>2+</sup>. The heart or the mouse was mounted on the stage of a microscope with a confocal scanning unit, combined with an objective lens [20×(40×), numerical aperture 1.0 (0.8)] ; . Laser-excited fluorescence was detected by the EMCCD camera, and the signals were analyzed by using the ImageJ software. As reported previously (FujiwaraCirculation Research 2008), Ca<sup>2+</sup> waves were clearly observed at the cellular level in the isolated beating heart. Experiments are currently underway to image Ca<sup>2+</sup> waves/transients in the beating heart *in vivo*. At the meeting, we will discuss how the cardiac excitation-contraction coupling is organized *in vivo*.

## 1PK-020

### Donepezil, Therapeutic Acetylcholinesterase Inhibitor, Prevents Progression of Ventricular Dysfunction by Promoting Cardiac Glucose Metabolism in Rat with Chronic Heart Failure

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**Introduction :** Similarly to the vagal nerve stimulation, proactive intervention in the cholinergic system by donepezil, a therapeutic acetylcholinesterase inhibitor, prevented the cardiac remodeling after myocardial infarction (MI). However, the precise mechanism of donepezil-induced cardioprotection remains unclear. Since cardiac energy substrate switching is a potential therapeutic target for the pharmacological treatment of heart failure, we assessed the hypothesis that donepezil modulates the cardiac energy metabolism in the failing heart. **Methods and Results :** Rat MI model was constructed by left coronary artery occlusion. At the chronic phase of MI, resting heart rate was comparable between untreated and donepezil-treated (DPZ) groups. Nevertheless, left ventricular contractility evaluated with Langendorff-perfused hearts was significantly improved by donepezil treatment. At the same time point, cardiac expression of glucose transporter (GLUT) was higher in the donepezil-treated group than the untreated group. *In vitro* study with cultured cardiomyocytes showed that, compared to untreated cells, donepezil treatment up-regulated the protein expression of GLUTs and increased more than two-fold cellular glucose uptake. **Conclusion :** Donepezil exhibits cardioprotective action against ischemic heart failure independently of heart rate through the chronic modulation of the cardiac metabolism by facilitating the glucose utilization in failing heart.

## 1PK-021

### The Role of Brain Mineralocorticoid Receptor-mediated Sympathoexcitation in Salt-sensitive and Obesity-induced Hypertension

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We have shown previously that sympathoexcitation by brain oxidative stress mediates arterial pressure (AP) elevation in salt-sensitive and obesity-induced hypertension. We have also shown that mineralocorticoid receptor (MR) activation mediates oxidative stress-induced cardiac and renal dysfunction. Then, we hypothesized that brain MR activation could mediate AP elevation through brain oxidative stress-induced sympathoexcitation. We used high-salt (8%)-loaded Dahl-salt-sensitive rats (Dahl-S) as a salt-sensitive hypertension model, and high-fat (45% kcal as fat)-fed Sprague-Dawley rats (SD) as an obesity-induced hypertension model. Salt loading in Dahl-S and fat loading in SD significantly enhanced mRNA expression of Sgk-1 and PAI-1 in the hypothalamus, which suggested MR activation. Chronic intracerebroventricular (ICV) eplerenone, MR blocker, significantly reduced sympathetic nerve activity and AP both in salt-loaded Dahl-S and high-fat-fed SD. Reductions in renal sympathetic nerve activity and AP values elicited by acute ICV tempol, an antioxidant drug, and the hypothalamic oxidative stress level were significantly suppressed in chronic ICV eplerenone-treated rats, compared with vehicle-treated rats. In conclusion, brain MR activation can be a possible common pathogenic background of AP elevation through brain oxidative stress-induced sympathoexcitation in salt-sensitive and obesity-induced hypertension.

## 1PK-022

### Ischemia-Induced Intracellular Remodeling of Na Channels might be Responsible for the Proarrhythmia of Na Channel Blockade

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Recent experimental studies have reported that cardiac Na channels were redistributed within a myocyte located at the epicardial border zone of the 5-day infarcted canine heart, and that the subcellular Na channel remodeling contributed to conduction slowing. However, the impact of intracellular Na channel remodeling on the arrhythmogenicity in ischemic myocyte is unclear. We recently reported in simulations that the action potential propagation was greatly affected by the subcellular distribution of Na channels in physiological relevant myofiber model. Here, we extend the simulations into the investigation of combined effect of the subcellular Na channel remodeling and the Na channel blockade on conduction disturbance in the myofiber model. Then we found that the intracellular Na channel remodeling, lack of Na channels in the lateral membrane of surviving myocytes in the ischemic border zone, contributed to conduction slowing. In addition, conduction block tended to occur in the ischemic border zone when Na channel blockade was numerically administered to the myofiber model, but this was not the case when Na channels were distributed over the entire cell membrane. Ischemia-induced intracellular remodeling of Na channels might be responsible for the proarrhythmic effects of Na channel blockade in patients with old myocardial infarction.

## 1PK-023

### Mechanical and structural characteristics of cardiac muscle fibers with troponin-T mutant causing hypertrophic cardiomyopathy

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The E244D- and K247R-troponin-T (TnT) mutants, which are known to cause familial hypertrophic cardiomyopathy, have been shown to enhance calcium-dependent contraction on cardiac muscle fibers (Nakaura et al., 1999, Castro et al., 2003, Matsumoto et al., 2009). To clarify its mechanism, we conducted mechanical and X-ray diffraction experiments with skinned muscle fibers from which endogenous troponin-TIC complex had been removed with TnT-treatment (Hatakenaka and Ohtsuki, 1992). X-ray diffraction experiments were performed in the presence of 2,3-butane monoxime, which suppressed calcium-independent force development of the fibers lacking troponin I and C, at BL15A in the Photon Factory (Tsukuba) and BL45XU at Spring8 (Hyogo). Molecular dynamics simulation was performed with AMBER (ver.9) at the condition of constant pressure (1 atm) and temperature (310 K). When E244D-TnT was introduced into the troponin-free fibers of cardiac muscle, calcium-independent tension were almost similar to that of wild-TnT introduced fibers, but larger than K247R-TnT introduced fibers. The cause of the difference will be discussed in relation to the results from molecular dynamics simulation and X-ray diffraction experiment.

## 1PK-024

### Stimulation Frequency Dependence of Dynamic Vagal Control of Heart Rate in Rats

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**Purpose :** Dynamic heart rate control is important for moment-to-moment adjustments of cardiac function during daily activity. The present study examined the effects of stimulation frequency on the dynamic vagal control of heart rate in rats. **Methods :** In 10 normal control rats and 5 rats with chronic heart failure (CHF), the distal end of the sectioned right vagus was stimulated under anesthetic conditions. The stimulation frequency was changed among 10, 20 and 40 Hz in random order. Using each stimulation frequency, the stimulation was turned on and off every 500 ms according to a binary white noise signal for 15 min. **Results :** Transfer function from vagal nerve stimulation to heart rate approximated a first-order low-pass filter with pure dead time combined with a frequency-independent gain term. The steady-state gain, corner frequency, pure dead time, and the ratio of high-frequency gain to steady-state gain were estimated. In normal rats, the increase in the stimulation frequency increased the gain ratio from  $0.14 \pm 0.01$  at 10 Hz to  $0.22 \pm 0.02$  at 20 Hz ( $P < 0.05$ ) and  $0.41 \pm 0.05$  at 40 Hz ( $P < 0.01$ ). In contrast, the stimulation frequency did not affect the gain ratio in CHF rats ( $0.07 \pm 0.04$  at 10 Hz,  $0.07 \pm 0.05$  at 20 Hz,  $0.11 \pm 0.07$  at 40 Hz). **Conclusion :** The high-frequency stimulation improved the dynamic heart rate response in the higher frequency range in normal rats. In contrast, the ratio of high-frequency gain to steady-state gain remained low in CHF rats, suggesting the impairment of peripheral vagal control of heart rate in CHF.

## 1PK-025

### Intravenous infusion of hyperosmotic NaCl solution induces acute cor pulmonale in anesthetized rats

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Intravenous hyperosmotic NaCl infusion is an effective treatment for circulatory shock. However, a fast infusion rate (2 mL/kg at the rate of 1 mL/s) induces transient hypotension. This response has been reported to be due to decreased total peripheral resistance and/or decreased cardiac performance. Although the hypotension is transient and recovers within 2 min without detrimental consequences, it is important to understand the associated hemodynamics and mechanisms. We found that the hypotensive effect was larger with intravenous NaCl infusion than with intra-aortic infusion, indicating that change in cardiac performance played a more significant role than change in peripheral resistance. NaCl infusion induced an increase in pulmonary vascular resistance and central venous pressure and a decrease in right ventricular dP/dt max, suggesting acute cor pulmonale. Diastolic ventricular crosstalk-induced left ventricular failure was also observed. Hyperosmotic NaCl-induced hypotension was therefore mainly due to a combination of acute cor pulmonale and left ventricular failure.

## Poster Presentations Neuron, Synapse(1)

## 1PK-026

### Effects of egg-laying hormone on the activity patterns of jaw muscles that were induced by taste stimulation in *Aplysia kurodai*

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Egg-laying behavior in *Aplysia* is accompanied by behavioral changes such as feeding suppression. We previously showed that injection of egg-laying hormone (ELH) into the body cavity inhibited the intake of seaweed. Feeding motor programs in *Aplysia kurodai* can take the form of both preferred ingestion-like responses and non-preferred rejection-like responses. It is possible that the suppressive effect of ELH on food intake is associated with a reduction of the preferred ingestion-like responses and/or an increase of the non-preferred rejection-like responses. The activity patterns in the different portions (ma and mm) of the intrinsic buccal muscles I<sup>1</sup>+I<sup>3</sup> differed during the 2 types of responses. In this study, we performed extracellular recordings in the ma and mm portions of the jaw muscles to determine the feeding-related responses during ELH application in the semi-intact preparations. The rhythmic burst activity in both muscle portions and the rhythmic movements of the jaws were induced after the taste stimulation (*Undaria pinnatifida*). After ELH application, the ingestion-like burst numbers were significantly decreased, while the rejection-like burst numbers were significantly increased in comparison with the control. These results suggest that ELH-induced feeding suppression is associated not only with a reduction of the fraction of preferred ingestion-like responses, but also with an increase of the non-preferred rejection-like responses.



## 1PK-027

### Activation of IP<sub>3</sub> receptors during preconditioning stimulations determines the direction of synaptic plasticity in hippocampal neurons

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Low-frequency synaptic stimulation (LFS; 1-2Hz) reverses long-term potentiation (LTP) established by a prior high-frequency stimulation (HFS), which we previously named depotentiation (DP), while the same LFS suppresses following LTP induction if it applied prior to the HFS. In CA3 pyramidal neurons, mossy fiber HFS induces presynaptic LTP as well as the activation of postsynaptic CaMKII. Pharmacological blockade of mGluR-IP<sub>3</sub> receptor signals or Calcineurin (CN) during the following LFS ablated the induction of DP. Interestingly, blockade of CaMKII during the preconditioning HFS also attenuated the induction of DP by a following LFS, suggesting that both CaMKII and CN are involved in DP mechanisms. LFS induced CN activation also affected the plasticity if applied to the naive mossy fiber-CA3 synapse by attenuating the induction of following LTP (suppression of LTP). Similar results were obtained for roles of CaMKII, CN and the mGluR-IP<sub>3</sub> receptor signaling in the plasticity at the Schaffer collateral-CA1 synapse, while the NMDA receptor, which is absent from CA3 pyramidal neurons, also plays significant roles. Taken together, we propose that the activation of the mGluR-IP<sub>3</sub> receptor pathway by a preconditioning stimulation regulates phosphorylation/dephosphorylation signals including CaMKII and CN, which may determine the direction of following synaptic plasticity in hippocampal neurons.

## 1PK-028

### Coupling between Ca<sup>2+</sup> channels and endocytosis at the calyx of Held

Midorikawa, Mitsuharu; Sakaba, Takeshi (Graduate School of Brain Science, Doshisha University, Kyoto, Japan)

At the mammalian central synapse, influx of Ca<sup>2+</sup> from Ca<sup>2+</sup> channels trigger neurotransmitter release by exocytosis. Vesicular membranes fused to the presynaptic membranes are retrieved by the following endocytosis, and undergo recycling process for the next round of exocytosis. While the roles of Ca<sup>2+</sup> on exocytosis have been well established, its role on endocytosis and following cycling process remains relatively unclear. At the giant presynaptic terminal of the calyx of Held synapse in rat brainstem, it has been shown that synaptic transmission is mediated by N-type, P/Q-type and R-type Ca<sup>2+</sup> channels before postnatal day 10. However, role of each Ca<sup>2+</sup> channel subtype on endocytosis and following cycling has remained to be elucidated. Applying capacitance measurement technique to the presynaptic terminal of the calyx of Held, we measured the time course of endocytosis and examined the role of each type of Ca<sup>2+</sup> channels plays on the time course of endocytosis. Following endocytosis, synaptic vesicles have to be recycled. We made an attempt to look at the time course of synaptic vesicle cycling using optical technique, and examined how Ca<sup>2+</sup> channel subtype differentially mediate synaptic vesicle cycling at the calyx of Held synapse.

## 1PK-029

### Hippocampal metaplasticity underlying cognitive improvement in Alzheimer's mice

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Soluble amyloid  $\beta$  (A $\beta$ ), instead of insoluble A $\beta$  deposits, has been regarded to cause synaptic dysfunction in Alzheimer's disease (AD). However, soluble A $\beta$  toxicity is not targeted by any current therapies. We previously reported that intracellular soluble A $\beta$  suppresses activity of the big conductance calcium-activated potassium (BK) channel, which was recovered by electroconvulsive stimulation (ECS) (Yamamoto et al, J Neurosci, 2011). The present experiments showed that chronic transcranial magnetic stimulation (TMS), a magnetic version of ECS, improves spatial learning in 3xTg AD model mice, in which amyloid precursor protein, presenilin-1 and tau are genetically modified. Although TMS recovered long-term potentiation (LTP) in the AD mouse hippocampus, this alone is not the decisive factor for the recovery of spatial learning. We therefore examined the overall dependence of synaptic modification on the frequency of plasticity-inducing synaptic inputs. Such input-frequency profile of synaptic malleability, called the BCM curve, was abnormal in the AD model, indicating that a metaplastic change was introduced by the genetic manipulation intrinsic to the AD model. TMS altered this distorted BCM curve in AD mice, and the normal curve in wild-type mice as well. Significant interaction between the BCM profile and learning ability was detected. A particular profile observed in AD mice is suggested to correspond to learning inability, which was rectified by TMS to become compatible to learning.

## 1PK-030

### Deteriorated degradation of GAT1 by Ube3a deficiency causes a decrement of tonic inhibition in cerebellar granule cells and a cerebellar ataxia

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Angelman syndrome (AS) is a neurodevelopmental disorder caused by loss-of-function of *UBE3A* gene encoding the E3 ubiquitin ligase Ube3a (also known as E6-AP). Cerebellar ataxia is one of the characteristic feature in AS, however, mechanisms of which are still unclear. Here, we report that tonic inhibition is specifically decreased in cerebellar granule cells of maternal Ube3a deficient (*Ube3a<sup>m-/p+</sup>*) mice and that GABA transporter (GAT) 1 could be a substrate of Ube3a. We have found Ube3a controls degradation of GAT1 and its deficiency induces a surplus of GAT1 and hence of synaptic GABA uptake, resulting in a decrement of ambient GABA levels. Administration of low dose THIP, a selective agonist for extrasynaptic GABA receptors, e.g.  $\delta$  subunit, can improve aberrant proportion of firing patterns in Purkinje cells and ataxic motor dysfunctions in vivo. These results indicate that decreased tonic inhibition may underlie ataxia and thus be a potential therapeutic target.

### 1PK-031

#### Analysis of inhibitory presynaptic function by direct patch-clamp recording from a cerebellar Purkinje cell axon terminal

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Regulation of synaptic transmission between neurons is critical for function of nervous system. The  $\text{Ca}^{2+}$ -dependent regulation of transmitter release from presynaptic terminals and involving molecules have been clarified by direct electrophysiological recording from large axon terminals at excitatory synapses, such as Calyx of Held. However, the entire biophysical mechanism and the underlying molecular mechanisms mediated by complicated interactions of multiple proteins remain still elusive. Particularly, the functional property of inhibitory presynaptic release machinery remains largely unknown, due to the lack of direct measuring method from an inhibitory neuronal axon terminal. To enable the functional analysis of inhibitory presynaptic terminal, we have tried to directly perform patch clamp recording from an axon terminal of cerebellar Purkinje cell. Visualization of Purkinje cell axon terminals by EGFP transfection allowed us to precisely target a patch pipette on a tiny (1-2  $\mu\text{m}$ ) axonal varicosity. We will present data for the mechanism of transmitter release from a Purkinje cell axon terminal, such as voltage-dependent currents, membrane capacitance increase, and IPSCs mediated by  $\text{GABA}_A$ Rs. It will also be shown how inhibitory synaptic transmission is affected by repetitive stimulation of an axon terminal.

### 1PK-032

#### Organization of sensory synaptic inputs in mouse barrel cortex

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Neurons integrate thousands of synaptic inputs into spike output, and understanding the mechanism of this single neuronal computation is one of the most fundamental issues in neuroscience. To address this question, it is critical to uncover the spatiotemporal patterns of individual synaptic inputs in the intact brain. We used whole-cell patch-clamp recordings in combination with *in vivo* two-photon calcium imaging to visualize individual sensory synaptic inputs to layer 2/3 pyramidal neurons in the barrel cortex of mouse. Both spontaneous and sensory-evoked synaptic inputs were very sparse, consistent with very low firing rate in layer 4 and 2/3 neurons in the barrel cortex. We found that the spatiotemporal patterns of synaptic inputs were highly heterogeneous. Both spontaneous and sensory-evoked inputs were locally clustered, and vast majority of sensory inputs were assigned to a small fraction of spines. The results indicate that functionally coupled neurons, which may be synaptically connected or receive common inputs, tend to innervate nearby spines. Co-activation of nearby spines might be an efficient way for integrating synaptic inputs elicited by a given sensory stimulus.

### 1PK-033

#### Histochemical analysis of brain from neural differentiation factor Zfp521 deficient mice

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Embryonic stem (ES) cells are differentiating into any cell type of three germ lines with induction of specific molecules. Neural induction represents the earliest step in the determination of ectodermal cell fates. However, the mechanism of the neural induction step is still poorly understood. Recently, Kamiya et al was reported that zinc finger nuclear protein Zfp521, mouse homolog of human Znf521, is essential and sufficient for driving the intrinsic neural differentiation from mouse ES cell (Kamiya et al *Nature* 2011, 470 : 503-509). They showed the transition of ES cell differentiation from the epiblast state into neuroectodermal progenitors specifically depend on activator function of Zfp521. There is, however, little information related to the role of Zfp521 in brain development and neuronal cell differentiation in mouse model. To investigate the role of Zfp521 in the brain development and neuronal cell differentiation *in vivo*, we generated a Zfp521 knockout mice. Here we show the data which is the developmental phenotype analysis of Zfp521 knockout mouse brain and neuronal cell differentiation. From our results described here, it is found that Zfp521 defect is not serious to develop the brain *in vivo*.

### 1PK-034

#### The growing area of cultured astrocytes determines the axonal arborization and glutamatergic synapse formation for a given single neuron

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Astrocytes play a critical role in regulating information processing at synapses of the central nervous system. Moreover, in their presence, the number of synapses increases dramatically, suggesting that astrocytes provide neurons with the energy and secreted substrates they need for the formation, maturation and stabilization of functional synapses. However, whether and how the territory of astrocytes regulates synaptic function and maturation have not yet been thoroughly investigated. We newly developed custom-made microdot stamps designed to deposit adhesion substrate in 200, 300 and 500  $\mu\text{m}$  squares so that the growing area of cultured cerebral cortical astrocytes was regulated with different area. Using these microdot stamps, the axonal but not dendritic arborization was significantly increased in single neuron co-cultured with large area of astrocytes, where the densities of the astrocytes were unchanged among three devices. Number of excitatory synapses was decreased with an increment of the synapse size in larger area of astrocytes. Taken together, our data indicate that astrocytic spatial distribution would have a significant potential to modulate neural morphology and synaptic plasticity.

### 1PK-035

#### Developmental changes in the LTP in the neonatal rat corticostriatal projection : Optical imaging with the voltage-sensitive dye

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We applied voltage-sensitive dye imaging to neonatal rat cortical slice preparations, and analyzed developmental changes in synaptic plasticity, LTP, in the corticostriatal projection. Coronal slice preparations were dissected from P1-P21 rats, and the transmembrane voltage-related optical signals evoked by cortical stimulation were recorded using a 464ch optical recording system with the voltage-sensitive absorption dye. In the striatum, the optical signal was composed of a fast spike-like signal followed by a slow signal, which corresponded to an action potential and an EPSP, respectively. The slow signal could be detected at the P1 stage, suggesting that the EPSP is already expressed in the corticostriatal projection at least at early stages after birth. On the other hand, the slow signal was potentiated with a single shot of tetanic stimulation and the potentiation lasted at least one hour, which is considered to correspond to long-term potentiation. With ontogenetic examinations, we found that (1) the EPSP could be potentiated with tetanic stimulation from the P9 stage and that (2) after the LTP induction, the potentiation was maintained for a longer time in the postnatal 3W stage than in the 2W stage. These results suggest that characteristics of LTP change dynamically during postnatal development.

### 1PK-036

#### Effects of in ovo blockade of the spontaneous depolarization wave on functional synaptogenesis in the embryonic NTS

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We demonstrated previously that spontaneous activity in the embryonic brain exhibits a large-scale wave of neural depolarization, which propagates over a wide region of the CNS. This wave, referred to as the depolarization wave, is expressed during a specific period of embryogenesis, at which synaptic contacts are established and become functional between sensory nerves and postsynaptic neurons in the CNS. Such a developmental profile has led us to a hypothesis that the depolarization wave might serve as a regulator of synaptogenesis, which is known to be activity-dependent. In the present study, we tested this hypothesis by blocking the depolarization wave in ovo and examining its effects on functional synaptogenesis in the brainstem sensory nucleus, the nucleus of the tractus solitarius (NTS). Although chronic inhibition of the depolarization wave caused a retardation of the embryonic growth, the developmental time course, spatial distribution, and physiological characteristics of postsynaptic responses in the NTS were not significantly affected. The results seem not in favor of the idea that the depolarization wave plays an indispensable role in the formation of functional synaptic networks along the cranial sensory pathway.

### 1PK-037

#### Changes of spontaneous GABAergic currents after increasing pre- and post-synaptic activities in rat hippocampus

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GABA<sub>A</sub> receptor-mediated inhibitory postsynaptic currents (IPSCs) modify excitatory synaptic transmission by altering the activity of principal neurons. The plasticity of the inhibitory synapses has not been well characterized, comparing with that of the excitatory one. Postsynaptic depolarization-induced changes of GABA<sub>A</sub>ergic synaptic transmission have been reported, but both facilitation and inhibition were observed, and mechanisms of the plasticity have not been elucidated. In the present experiments, effect of the postsynaptic depolarization on spontaneous GABA<sub>A</sub>ergic synaptic transmission was examined in acute slices of neonatal rat hippocampus. Using whole cell patch-clamp recording method, spontaneous GABA<sub>A</sub>ergic IPSCs (sIPSCs) were recorded from CA3 neurons. Postsynaptic depolarization alone did not cause marked alteration of the frequency or amplitude of sIPSCs. Facilitation of the transmitter release by presynaptic depolarization increased frequency of the sIPSCs. Simultaneous activation of presynaptic and postsynaptic neurons, however, induced transient inhibition of the frequency of the sIPSCs. Though specific agonist of metabotropic glutamate receptors exogenously applied during postsynaptic depolarization did not mimic the inhibition of the sIPSCs, antagonists of the receptors partially suppressed the inhibition caused by the simultaneous pre- and post-synaptic stimulation. These results suggest that postsynaptic depolarization and simultaneous facilitation of presynaptic release of glutamate transiently inhibited the sIPSCs.

### 1PK-038

#### Long-lasting effects of chronic amphetamine treatment on miniature EPSCs in the mouse striatum

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Alteration of dopamine level in the basal ganglia produces neural and behavioral changes. The striatum, a major nucleus of the basal ganglia, is composed of two neurochemically, developmentally, and functionally distinct compartments : the striosomes and the surrounding matrix. The striosomes and dopaminergic neurons of SNc mutually innervate each other. Amphetamine is a psychostimulant drug which enhances dopamine neurotransmission and increases locomotor activity. Chronic administration of amphetamine induces repetitive behavior termed motor stereotypy. After the drug withdrawal period, enhanced stereotypy is still induced by the drug challenge. In this study, we examined whether the effects of chronic amphetamine treatment on the physiological activity of striatal neurons differ between each of the striatal compartments. Amphetamine (5 mg/kg) or saline was injected intraperitoneally twice daily (at 9 : 00 and 16 : 30) for 5 consecutive days. After withdrawal of 2-7 days, each mouse received a challenge with amphetamine or saline and was dissected after 1 hour from the final challenge. To visually identify the striosomes, we used a TH-GFP transgenic mouse strain expressing eGFP in a compartment-specific manner. Whole-cell recordings were made from medium spiny neurons in slices taken from the mouse striatum. We compared miniature excitatory postsynaptic currents (EPSCs) recorded between in the striatal compartments. These studies would help explain the roles of the striosomes in the activity of local neural circuits in striosome/matrix compartments.

## 1PK-039

### Long-term *in vivo* imaging of dendritic spines with microfluidic devices and open-dura surgery in the adult mouse cortex

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Structural plasticity and dendritic spine stability underlie learning and memory. Previous *in vitro* studies demonstrated that chronic blockade of NMDA receptors eliminated activity-dependent changes in spine volumes and reduced spine elimination rates, while sparing intrinsic fluctuations in spine volumes and baseline spine turnover. To characterize the spine baseline turnover *in vivo*, we developed a brain interface device for chronic imaging of dendritic spines in the cortical surface where a microfluidic system was used to locally apply various inhibitors. We removed the dura mater on the imaging area and chronically superfused the inhibitors whose concentrations were calibrated using a fluorescent probe. We have established the surgical procedures with which repeated two-photon imaging of dendritic spines can be performed in the adult mouse visual cortex for several days without inflammation. We observed that inhibitors of NMDA receptors and voltage-gated calcium channels successfully eliminated visual stimulation-evoked calcium transients and blocked the increases in spine formation by environmental enrichment, while the inhibitors spared baseline spine generation and only slightly reduced spine elimination rates. These results suggest that there is an intrinsic spine turnover even in the adult neocortex, which plays a key role in memory formation and retention.

## 1PK-040

### Relationship between the synchrony of climbing fiber activities and the aldolase C compartments in mouse cerebellar cortex

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The cerebellar cortex has an elaborate zonal organization extending rostro-caudally. Purkinje cells (PCs) located in these parasagittal stripes show synchronous complex spike (CS) activities in PCs. This functional structure is considered to arise from the anatomical organization of olivocerebellar projection and the electrical coupling among neurons in the inferior olive. The longitudinal compartments of aldolase C expression has been shown to correlate with anatomical climbing fiber innervation of PCs (Sugihara et al., 2001) and functional CS synchrony at the resolution of several hundred micrometers (Sugihara et al., 2007). To elucidate the relationship between the CS synchrony and the aldolase C compartments at much finer resolution, we performed two-photon calcium imaging in mice that express fluorescent protein in their PCs expressing aldolase C. We found a highly precise correlation between the CS synchrony and the aldolase C compartments. CS activities within aldolase C positive-PCs and those within aldolase C negative-PCs were both highly synchronized, and the border of high synchrony areas precisely corresponded to that of the aldolase C compartments at single cell resolution. These results suggest that CS synchrony precisely reflects CF innervation and the electrical coupling in the inferior olive, and that each aldolase C compartment represents distinct functional unit in cerebellar cortex.

## 1PK-041

### New pathway of feed-forward inhibition to the lateral amygdala uncovered by optical imaging

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Inhibitory GABAergic responses in the amygdala are known to control fear memory. Previous electrophysiological studies have suggested that the GABAergic input from the lateral intercalated nucleus (IITC) is a major source of inhibitory inputs to the lateral amygdala (LA) as well as the inputs from local GABAergic neurons in the LA, but the inhibitory effects of the medial intercalated nucleus (mITC) on the LA activities have not been reported. We have utilized optical membrane potential imaging to investigate a wide range of neuronal interactions among the amygdala. Using membrane potential imaging, we have previously reported that the external capsule stimulation induces synchronous large hyperpolarization in the LA of the mouse coronal slice preparation. However, the cellular inhibitory source of the large hyperpolarization in the LA remains unknown. In this report, by surgically eliminating the possible input pathways to the LA one-by-one, we found that the mITC plays a major role in controlling the large hyperpolarization in the LA. Our results suggest that the mITC neurons mediate feed-forward inhibition to the LA.

## 1PK-042

### Excitatory effects of GABA on synchronously oscillating neurons in slug olfactory center

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Laminar structure of synchronous oscillatory activity is common in the olfactory system of both vertebrates and invertebrates. In the terrestrial slugs, periodic oscillation is recorded from the surface of the laminar structure of procererebrum (PC) and its frequency changes are suggested to encode the olfactory information and memory. In the previous studies, glutamate have been found to function as the major inhibitory transmitter in the PC. On the other hand, our recent study showed that gamma-aminobutyric acid (GABA) acts as an excitatory neuromodulator in the PC. Classical neurotransmitters often have different actions on invertebrate neurons from those reported for vertebrate neurons. We thus examined what effect GABA has on the two type of PC neurons, bursting neuron (rhythm generator neuron) and nonbursting neuron (input-output neuron), in the present study. The GABA application reduced the amplitude of IPSCs and increased the number of spontaneous spikes in nonbursting neurons. The GABA-like immunoreactivity was observed in the neuropil layers of the PC, and the mRNAs for both GABAA and GABAB receptors were expressed in the PC. In particular, GABAB receptor mRNA, rather than GABAA, was found to be primarily expressed in the PC. These results suggest that GABA can function as an excitatory modulator for nonbursting neurons via mainly GABAB receptors activation and potassium conductance decrease.

### 1PK-043

#### Neuronal oscillations in the anterior cingulate cortex induced by kainic acid are modified by chronic ethanol treatment

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Neuronal oscillation is a prominent form of rhythmic activity occurring in the brain. Fast neuronal oscillations (30-100 Hz) are frequently observed in the thalamo-cortical structure during wakefulness and attentive behavior. Abnormalities in these oscillations in the anterior cingulate cortex (ACC), a caudal part of the prefrontal cortex, might underlie neuropsychiatric illnesses such as schizophrenia. Previously we revealed that chronic restraint stress facilitates synaptic plasticity in the ACC via disinhibition. In the current study, to investigate the role of GABAergic inhibition in neuronal oscillation in the ACC, we exploited *in vitro* model of neuronal oscillations in the slice including ACC from mice under chronic ethanol (EtOH) treatment. We found marked potentiations by bicuculline of theta (4-7 Hz), alpha (8-12 Hz) as well as beta (13-30 Hz) oscillations evoked by 50  $\mu$ M kainic acid in the slices from control animals. However, these potentiations was not observed in the slices from EtOH treated animals. Interestingly, there may be asymmetry in the oscillations between right and left hemispheres. Current results suggest GABAergic contribution to the oscillation and possible asymmetry in the oscillation generating circuit in the ACC.

### 1PK-044

#### Impaired water maze performance of TPH2-GFP BAC transgenic mouse with reduced serotonergic neurons by 5,7-DHT injection

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Brain serotonin is involved in mechanisms underlying learning and emotional behaviors. The injection of serotonergic toxin or serotonin reuptake inhibitor into brain results in the change of learning behaviors. However, there have been inconsistent results in learning behaviors after the injection of serotonergic toxin, some reports resulted in the enhancement of learning performance, while the other resulted in the reduction of it performance or no effect. These inconsistent results might come from the difficulties in evaluating the reduction of serotonergic neurons by the toxin. We have introduced the BAC transgenic mouse expressing green fluorescent protein under the control of promoter of the tryptophan hydroxirase 2, to clearly evaluate the reduction of serotonergic neurons in the midbrain raphe nucleus. Under barbiturate anesthesia, mice were injected serotonergic toxin, 5,7-DHT, or saline into the lateral cervical ventricle. After 1-4 weeks, mice were conducted with three different behavioral tests, open field test, prepulse inhibition and water maze test. The mouse with largely reduced serotonergic neurons in the midbrain raphe nucleus demonstrated impaired performance of water maze learning as compared with the mouse with intact serotonergic neurons. The mouse with largely reduced serotonergic neurons showed reduced prepulse inhibition in consistent with previous reports. The performance of open field tests was not different between two groups. These results suggest that serotonergic neuronal system enhances learning behavior.

### 1PK-045

#### GluN2B is essential for closing the critical period in corticospinal plasticity

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Some types of neuroplasticity are robust in young age but disappear after a short time window called "critical period (CP)". We studied mechanisms that close the CP of a specific type of plasticity in our *in vitro* corticospinal (CS) projection system. CS synapses are once formed throughout the spinal cord at 8 days *in vitro* (DIV), but later eliminated from the ventral side until 13 DIV. This elimination occurs only from 6 to 11 DIV, which is dependent on NMDA receptor and its subunit, GluN2B (2B). We focused on 2B declining toward the end of the CP. We found that the CP was elongated in GluN2A knockout mice where 2B was dominant even after the CP. Upregulation of 2B by application of MTEP or proBDNF re-opened the CP. Moreover TBB known to inhibit internalization of 2B also produced the elimination blocked by application of APV (6-11 DIV) after the CP. According to the previous studies in the visual cortex, excitatory-inhibitory balance was suggested to be important in controlling of the initiation and end of CP. Hence to see the effect of partial blocking of inhibitory input on closing the CP, we applied small amounts of bicuculline or strychnine. This partial block produced the elimination despite 2B decline after the CP, which might be due to increased Ca influx through 2B. These suggest that 2B is essential for closing the CP in the CS plasticity and the inhibitory inputs might act as its modulator.

### 1PK-046

#### Cannabinoid receptor-independent effect of 2-arachidonylglycerol on spontaneous synaptic currents in cultured hippocampal neurons

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Endocannabinoids, endogenous ligands for marijuana receptors (CB1 and CB2), play an important role in modulation of neural function. The endocannabinoid 2-arachidonylglycerol (2-AG) is released from post-synaptic neurons in an activity-dependent manner, and suppresses the transmitter release at various types of synapses throughout the brain. In some neurons, it also induces hyperpolarization and inhibits neuronal firing. These effects of 2-AG are mediated by CB1 cannabinoid receptors. Here we report a novel effect of 2-AG (or its metabolites), which is independent of cannabinoid receptors. Using cultured hippocampal neurons, we examined effects of 2-AG and the synthetic cannabinoid agonist WIN55,212-2 on spontaneous synaptic currents. WIN 55,212-2 suppressed the synaptic currents in most neurons. 2-AG decreased the frequency of synaptic currents in some neurons, but increased it in some other neurons. When neurons were pretreated with the CB1 antagonist AM281, 2-AG increased the frequency of synaptic currents in most neurons. This excitatory effect of 2-AG was insensitive to the CB2 antagonist AM630. These data show that 2-AG has dual effects on spontaneous synaptic events. One is inhibitory and mediated by CB1 receptors, and the other is excitatory and independent of CB1/CB2 cannabinoid receptors. Whether this excitatory effect is caused by 2-AG itself or 2-AG metabolites remains to be investigated.

## Poster Presentations

### Ionic Channel, Receptor(1)

#### 1PK-047

##### Functional diversity of TRPA1 channels among vertebrate species regarding noxious temperature and chemical sensitivity

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Pain is the sensation that enables animals to avoid potential injury by noxious stimuli, thus nociceptive receptors play important roles in survival in natural environments. Transient receptor potential ankyrin 1 (TRPA1) is a nonselective cation channel that is activated by noxious temperatures and chemicals thus serves as a nociceptive receptor in a wide range of animals. In mammals, TRPA1 is activated by irritative chemicals and potentially by noxious cold. To compare the channel properties among vertebrate species we characterized TRPA1 channel properties from western clawed frog (amphibians) and green anole lizard (reptiles) and found that TRPA1 was activated by mammalian TRPA1 agonists, while they were activated by not cold but heat. Since zebrafish TRPA1 has been reported to be insensitive to temperature stimulation, TRPA1 channel properties are diversified among different vertebrate species. To perform more detailed analysis, in the present study, we cloned chicken TRPA1 and characterized its channel property. We found that it was also activated by mammalian TRPA1 agonist and heat stimulations. In addition, we found a novel TRPA1 agonist and its sensitivity to TRPA1 differed among different vertebrate species. Here we present channel property of chicken TRPA1 and compare it to those of TRPA1 from other vertebrate species to show diversity of nociceptive receptor.

#### 1PK-048

##### Extracellular voltage gradients guide axons of retinal ganglion cells

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Extracellular electric fields direct growing axons towards the cathode under a voltage gradient as low as 7 mV/mm. However, it remains unknown whether embryonic nerve tissues generate such an electric field that guides growing axons to the correct path. The present study revealed that an extracellular electric field is standing in an embryonic retina and that axons of newborn retinal ganglion cells (RGCs) are guided by the pre-existing voltage gradient. The optic cup was isolated from a chick embryo. Extracellular potentials were recorded with a microelectrode from the dorsal, temporal, nasal, central, and ventral parts of the optic cup, just inside the inner limiting membrane, on which the RGC axons travel. Positive direct current (DC) potentials were recorded there, and the amplitude of the DC potential was largest at the dorsal part (8 mV), and almost null at the ventral part (<1 mV). It was 4-5 mV at the temporal and nasal parts, and 3-4 mV at the central part. Thus, the DC potential heads downhill towards the ventral part of the optic cup. The steepness of the voltage gradient was 14-15 mV/mm at the central part. RGCs are born first at the central part of the optic cup. Axons of the newborn RGCs head to the ventral part of the optic cup. Thus, the direction of the voltage gradient corresponds to the initial course of newborn RGC axons. The application of amiloride, a blocker for epithelial Na<sup>+</sup> channels, suppressed the positive DC potential, and also disturbed the correct travelling of RGC axons. It was suggested that the endogenous extracellular voltage gradient is essential for the correct travelling of RGC axons.

#### 1PK-049 (SPK-3)

##### The deltaC splice-variant of TRPM2 is the hypertonicity-induced cation channel(HICC)in HeLa cells, and the ecto-enzyme CD38 mediates its activation

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Hypertonicity-induced cation channels (HICCs) are key-players in proliferation and apoptosis. However, the actual molecular entity of HICCs has not yet been identified. We report that in HeLa cells, intracellular adenosine diphosphate ribose (ADPr) and cyclic ADPr, as activators of TRPM2, elicited cation currents the characteristics of which are identical to those of HICC currents activated by hyperosmolarity. Silencing of TRPM2 and CD38 (as the supposed source of ADPr and cADPr) inhibited hypertonicity- and nucleotide-induced currents and the regulatory volume increase. Systematic analysis of intracellular cADPr and extracellular application of nucleotides revealed that the outwardly directed gradient, rather than the intracellular activity, of ADPr and cADPr triggers activation of TRPM2. Cloning of TRPM2 verified the deltaC-splice variant as the molecular correlate of the HICC, which was supported by quantification of Ca<sup>2+</sup> selectivity. Pull-down and FRET/FLIM experiments revealed a close proximity of TRPM2 and CD38, and we thus propose a transport related to nucleotide export via CD38 as a novel mechanism of TRPM2 activation.

## 1PK-050

### Enhancement of the desensitization and resensitization in GABA<sub>A</sub> currents by deletion of PRIP-1/2 in pyramidal cells of the barrel cortex

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The desensitization of GABA<sub>A</sub> receptors (GABA<sub>A</sub>Rs) is mechanistically related to the unbinding of GABA from GABA<sub>A</sub>Rs. We show that the deletion of phospholipase C-related inactive proteins (PRIP-1/2) through the use of PRIP-1/2 double knockout (PRIP-DKO) mice, enhances GABA<sub>A</sub>Rs desensitization while it paradoxically induces their resensitization following removal of GABA in layer 2/3 pyramidal cells (PCs) of the barrel cortex. This resensitization of GABA<sub>A</sub>Rs was revealed as a hump-like tail current (tail-I) at the offset of GABA puff. The enhanced desensitization of GABA<sub>A</sub>Rs and the generation of hump-like tail-Is in PCs of PRIP-DKO mice were mediated by increases in [Ca<sup>2+</sup>]<sub>i</sub> and were largely abolished by calcineurin inhibition. These results suggest that the deletion of PRIP-1/2 enhanced the GABA<sub>A</sub>R desensitization and concomitantly reduced the GABA unbinding rate through activation of calcineurin, leading to a paradoxical potentiation of the overall GABA<sub>A</sub>R current and an enhanced lateral inhibition in layer 2/3 of the cortex.

## 1PK-051

### Role of the glial glutamate transporters for functional remodeling in contralateral hemisphere of somatosensory cortex after stroke

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After ischemic stroke, the corresponding area contralateral to the lesion may partly compensate for the loss of function. We previously reported the remodeling of neuronal circuits in the contralateral somatosensory cortex (SSC) during the first week after infarction for processing bilateral information, resulting in functional compensation. However, the underlying processes in the contralateral hemisphere after stroke have not yet been fully elucidated. Recent studies have shown that astrocytes may play critical roles in synaptic reorganization and functional compensation after a stroke. Thus, we aim to clarify the contribution of astrocytes using a rodent stroke model. In vivo Ca<sup>2+</sup> imaging showed a significantly large number of astrocytes in the contralateral SSC responding to ipsilateral limb stimulation at first week after infarction. The observation that application of (2S,3S)-3-[3-[4-(trifluoromethyl) benzoylamino] benzyloxy] aspartate (TFB-TBOA), a glial glutamate transporter blocker, disturbed the functional recovery. These findings indicate the involvement of glial glutamate transporters in functional remodeling/recovery in the area contralateral to the lesion. Our study has provided new insights into the mechanisms underlying synaptic remodeling after cerebral infarction, which contributes to the development of effective therapeutic approaches for patients after a stroke.

## 1PK-052

### Identification of the palmitoylation sites of HCN2 channel

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The S-palmitoylation is a posttranslational lipid modification of proteins. In this modification, palmitate is covalently attached to intracellular cysteine residues by thioester linkage in a reversible manner. Recent studies revealed that the S-palmitoylation regulates the trafficking and the function of ion channels. Therefore, we examined the possibility that hyperpolarization-activated cyclic nucleotide-modulated (HCN) channels may be the target of S-palmitoylation. To analyse the effects of 2-bromopalmitate (2BP; an inhibitor of palmitoylation) on the HCN channels, HCN1, 2 and 4 were expressed in *Xenopus* oocytes and the membrane currents were recorded using two-electrode voltage clamp method. After the treatment of 2BP, the current amplitudes of all HCN channels that we tested were significantly decreased. The amplitude of HCN1 were decreased from -10.14±0.79 μA (control) to -3.31±0.64 μA (2BP); HCN2, from -12.91±0.81 μA (control) to -6.79±0.53 μA (2BP); HCN4, from -15.26±1.50 μA (control) to -10.07±0.71 μA (2BP). We next carried out acyl-biotinyl exchange (ABE) assay, and biochemically confirmed that HCN1, HCN2, and HCN4 proteins were S-palmitoylated. By mutant analyses, we found that all five N-terminal cysteines (C63, C69, C82, C89, C104) of HCN2 are the targets of S-palmitoylation. Although our results raised the possibility that dynamic palmitoylation-cycle regulates the activity of HCN channels, further studies are indispensable to address how palmitoylation facilitates the current amplitudes of HCN channels.

## 1PK-053 (SPK-4)

### The sensor for the inner membrane lipid modulating the activation gating of the KcsA potassium channel

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The membrane lipids act as cofactor for the function of the ion channel proteins and specific lipid molecules are indispensable for maintaining channel activities. For the KcsA potassium channel, the presence of anionic phospholipids such as phosphatidylglycerol (PG) is prerequisite for the channel activity. Previously we demonstrated by means of single-channel current recordings in the asymmetric lipid bilayer that the PG molecule on the inner leaflet, rather than the outer leaflet, renders the KcsA channel highly active. In this study the lipid effect for the KcsA channel activity was further analyzed. The fluorescent method revealed that the helix-bundle gate is kept open in the PG liposome but not much in the liposomes made of neutral or cationic phospholipids. To elucidate the underlying mechanism of the interaction between anionic lipids on the inner leaflet and the activation gate, charge-neutralizing mutations to positively charged residues were introduced. Several amino acid residues lying at the inner boundary of the membrane were found to be sensitive to the PG effect on the gating. Mechanism underlying lipid-mediated regulation of the activation gating of the KcsA channel will be discussed.

## 1PK-054

### Extracellular zinc ion inhibits TRPM5 activation

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TRPM5 is a Ca<sup>2+</sup> impermeable cation channel activated by intracellular Ca<sup>2+</sup>. This channel is restrictedly expressed in taste cells, pancreas and brainstem, and might be involved in controlling membrane potentials. Its endogenous ligands are not well known. In this study, we found that extracellular Zn<sup>2+</sup> inhibits TRPM5 activation. In whole-cell patch-clamp recordings, extracellular application of 30 μM ZnCl<sub>2</sub> inhibited step-pulse-induced TRPM5 currents with intracellular 500 nM free Ca<sup>2+</sup>, and the inhibition was dose-dependent (IC<sub>50</sub>=3.6 μM). In addition, extracellular application of ZnCl<sub>2</sub> also inhibited temperature-dependent TRPM5 activation. Furthermore, we determined the amino acid residues required for inhibition of the Ca<sup>2+</sup>-evoked TRPM5 currents by Zn<sup>2+</sup>. These data indicate that TRPM5 might be inhibited by extracellular Zn<sup>2+</sup> under physiological conditions and its inhibition could be involved in the physiological function of TRPM5.

## 1PK-055

### Involvement of TRPA1 channel in the respiratory rhythm in the hypoxia during perinatal period

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Hypoxia shows the increase of respiratory rhythm which is attributed mainly to effects on peripheral chemoreceptor. However, the central effects of hypoxia on brainstem and spinal cord during developmental stage had not been understood. Our previous studies using isolated brainstem spinal cord preparations showed that respiratory rhythm was facilitated in hypoxic condition at embryonic day 18 (E18) and postnatal day 1 (P1) rats; in contrast, respiratory rhythm decreased in P2-4. In this study, we examined how TRPA1 channel agonist and antagonist effect on respiratory rhythm, and TRPA1 channels localize in the rostral ventrolateral medulla (RVLM) including the respiratory center using *in situ* hybridization. TRPA1 channel agonist excited the respiratory rhythm in E18-P1 rats, depressed in P2-4. TRPA1 channel was also seen to localize in the immediate vicinity of nucleus ambiguus in E20 and P1. In addition, we investigated extracellular recordings of respiratory neurons recorded from the RVLM. In hypoxic condition, the intraburst frequency of inspiratory neuron did not change but that of expiratory neurons was significant decrease. Tonic neurons in RVLM showed a marked depression in hypoxic condition. The central chemoreceptor might detect hypoxia mediated with tonic and expiratory neurons. These results showed TRPA1 channel might play an important role of modulation on respiratory network in hypoxic condition at perinatal period.

## 1PK-056

### Cross-communication between the L-type Ca<sup>2+</sup> channel and β-adrenergic receptor/adenylyl cyclase/cAMP pathway in mouse ventricular myocytes

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In cardiac myocytes, the voltage-dependent L-type Ca<sup>2+</sup> channel (LTCC) is regulated by local Ca<sup>2+</sup> signaling via Ca<sup>2+</sup>-dependent inactivation and facilitation mechanisms. In ventricular myocytes, Ca<sup>2+</sup>-inhibitable adenylyl cyclase (AC), such as AC5, plays a major role in the positive inotropic response to β-adrenergic stimulation. The aim of this study was to clarify the cross-communication via Ca<sup>2+</sup> signaling between LTCC and β-adrenergic receptor (β-AR)/AC5 system in regulating their activities. We compared the LTCC activity and its response to β-AR stimulation under high or low Ca<sup>2+</sup> buffering conditions by patch clamp technique. Basal current densities of LTCC were significantly larger under high Ca<sup>2+</sup> buffering condition compared to low Ca<sup>2+</sup> buffering condition. Response of LTCC current to β-AR stimulation was significantly larger under high Ca<sup>2+</sup> buffering condition than low Ca<sup>2+</sup> buffering condition. In contrast, such difference was not observed when AC5 was stimulated by an AC5-selective forskolin derivative (NKH477) or when Ba<sup>2+</sup> was used as a charge carrier. Interestingly, the loss of CICR by depletion of Ca<sup>2+</sup> stored in the SR attenuated the response of LTCC to the β-AR stimulation and effects of Ca<sup>2+</sup> buffering conditions. These results indicate that Ca<sup>2+</sup> signaling via LTCC exerts negative regulation of β-AR/AC5 pathway in mouse ventricular myocytes.

## 1PK-057

### Propofol-induced human TRPA1-mediated currents in HEK293T cells

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Propofol (2,6-diisopropylphenol) is one of the intravenous anesthetics, and commonly used for the induction and maintenance of general anesthesia. Additionally, propofol causes an intense pain upon injection. Recently, it was reported that transient receptor potential (TRP) receptors ankyrin 1 (TRPA1) is a candidate for propofol action target in rats and mice (Matta JA et al. PNAS 2008; 105: 25: 8784-8789, Fischer M et al. JBC 2010; 285: 45: 34781-34792). However, electrophysiological properties of propofol-induced responses of human TRPA1 are not fully investigated. To understand them, we performed whole-cell and single-channel patch-clamp recordings in HEK293T cells expressing human TRPA1. In whole-cell recordings, propofol caused a robust activation and desensitization of human TRPA1. In single-channel recordings, propofol activated human TRPA1, too. Our data show that propofol can directly activate human TRPA1.



## 1PK-058

### Searching for the molecular basis of maxi-anion channel

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The maxi-anion channel (Maxi-Cl) has been characterized as a major player for the release of the anionic signaling molecules, ATP and excitatory amino acids, from cells subjected to osmotic perturbation, ischemia or hypoxia. Its biophysical and pharmacological properties have been well elucidated. However, the molecular entity is unknown. Based on our molecular and functional studies, we have already excluded several genes (such as *Panx1*, *Panx2*, *Cx43*, *Slc35d2* and *Tmem63a*) as possible candidates (*Am J Physiol Cell Physiol* 2012, 303, in press; *J Physiol Sci* 2012, 62, S85). In the present study, we isolated a set of candidate genes for Maxi-Cl, based on proteomics analysis of Maxi-Cl-rich membrane blebs isolated from the plasmalemma of C127 cells (*J Physiol Sci* 2010, 60, S123). These genes include annexin family members (1, 3, 11), *Slc* family members (15a4, 25a11, 25a3, 25a4, 25a5, 2a3, 33a1, 44a1, 44a2) and *Tmem* family members (62, 65, 97, 138, 167b, 189). The siRNA-mediated gene knockdown strategy was performed using C127 cells, and maxi-anion channel currents were recorded using the patch-clamp method. Measured current amplitudes were not significantly different between siRNA- and mock-transfected C127 cells. Thus, above-mentioned genes can be excluded as a molecular entity of Maxi-Cl. Few additional genes (*Praf2*, *Tm4sf1*, *Ttyh2*) were also excluded, based on their heterologous expression study using Maxi-Cl-lacking HEK293T and C1300 cells.

## 1PK-059

### Using physiological temperature variations to perform a functional analysis of mutant HCN channels

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) currents, known as  $I_h$ , are implicated in the stabilization of resting membrane potential. In the human brain, HCN1 and HCN2 proteins make heterotetramer which, in turn, produces  $I_h$ . Recent studies report that HCN channels are involved in both absence and febrile seizures. We identified the HCN2 amino acid substitution (S126L) from two febrile seizure patients and examined the characterization of the HCN2 mutant. An in-vitro experiment using HEK293 cells showed little difference in the activation curve between wildtype and mutant at room temperature (25°C). However, when hyperthermic (mimicking a febrile situation), half maximal activation voltage ( $V_{1/2}$ ) shifted to depolarized side, making it larger in the mutant than in the wildtype. There was no remarkable difference between the wildtype and the mutant in the application of cyclicAMP. At this point, we decided to examine the effect of temperature raises in the physiological range (35-40°C). Doing so, led us to the determination that HCN2 mutation might contribute to neuronal hyperexcitability in a temperature-dependent manner, thus leading to febrile seizure in patients.

## 1PK-060

### Effects of interleukin on calcium channels in osteoblast

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The profile of interleukin (IL) in osteoblast have been studied using the whole cell configuration of the patch clamp technique. Murine osteoblastic MC3T3-E1 cells were cultured at 37 C in a 5% (v/v) CO<sub>2</sub> atmosphere with alfa-modified minimal essential medium. Fabricated recording pipettes (2-3 M ohm) were filled with the internal solution of the following composition (in mM) : 150 CsCl, 5 EGTA, 10 D-glucose, and 10 HEPES. The pH was adjusted to 7.3 with CsOH. Extracellular solution was a solution containing the following (in mM) : 115 BaCl<sub>2</sub> and 20 HEPES. The pH was adjusted to 7.4 with TEA-OH. 5 nM IL-1 beta facilitated voltage dependent calcium channels (VDCCs) current ( $I_{Ba}$ ), (21.3 plus-minus 8.1%). 5 nM IL-6 facilitated ( $I_{Ba}$ ) (35.6 plus-minus 10.6%). 50 pM IL-6 facilitated ( $I_{Ba}$ ) (15.8 plus-minus 8.1%). 500 pM IL-6 facilitated ( $I_{Ba}$ ) (22.9 plus-minus 11.1%). In contrast, 50 nM IL-6 inhibited ( $I_{Ba}$ ) (21.9 plus-minus 4.8%). These results suggest that IL modulates VDCCs in osteoblast.

## 1PK-061

### Analysis of basic electrophysiological properties of a new isoform of ClC-3, ClC-3d

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ClC-3 is a member of the ClC chloride channel/transporter family, which is expressed in many types of mammalian cells and may play roles in diverse cell functions. Four variants of mRNA of *clcn3* (*clcn3a*, *b*, *c* and *e*), encoding proteins of which have distinct N- and C-terminal amino acid sequences, have been reported, and the existence of two additional variants, *clcn3d* and *clcn3f*, have been predicted in mice. We previously reported the cloning of one of the predicted variants, *clcn3d*, from mouse liver (*J Physiol Sci* 61, S128, 2011). In that study, when overexpressed in HEK293T cells, a fraction of exogenous ClC-3d protein was found to be localized on the plasma membrane, whereas exogenous ClC-3a, a well-analyzed isoform which has the same N-terminal as and distinct C-terminal from ClC-3d, was predominantly localized at lysosomal membranes. In this study, we thus analyzed the channel properties of ClC-3d by whole-cell patch-clamp recordings under isotonic conditions. ClC-3d-transfected, but not mock-transfected, HEK 293 T cells exhibited a steeply outward-rectifying Cl<sup>-</sup> current evoked at over +40 mV with the ion permeability order of  $Cl^- = Br^- > I^-$ . No effects of DIDS, DCPIB and phloretin on ClC3d-mediated currents were observed. Activation of CaMKII and PKC never affected ClC-3d-mediated currents. A point mutation in the pore forming segment (E224A) altered the current to inwardly rectifying and ion permeability order to  $I^- > Br^- > Cl^-$ .

## 1PK-062

### Calmodulin potentiates IKs in guinea-pig SA node cells by activating Ca<sup>2+</sup>/calmodulin-dependent protein kinase II

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The slow component of the delayed rectifier K<sup>+</sup> current (*I<sub>Ks</sub>*) plays an important role in the repolarization process of cardiac action potentials and is regulated by various signalling pathways. In recent years, evidence has been presented that calmodulin (CaM) is involved in the modulation of diverse ion channels in cardiac myocytes under physiological and pathophysiological conditions. In the present study, we examined the regulation of *I<sub>Ks</sub>* by CaM in guinea-pig sinoatrial (SA) node cells using the whole-cell patch-clamp method. The density of *I<sub>Ks</sub>* was larger during intracellular dialysis with high Ca<sup>2+</sup> concentrations (10<sup>-7</sup> M) compared with that with low Ca<sup>2+</sup> concentrations (10<sup>-11</sup> M). Intracellular application of CaM (100 nM) markedly potentiated *I<sub>Ks</sub>* with a high Ca<sup>2+</sup> pipette solution but not with a low Ca<sup>2+</sup> pipette solution, thus showing that CaM potentiates *I<sub>Ks</sub>* in an intracellular Ca<sup>2+</sup>-dependent manner. This stimulatory action of *I<sub>Ks</sub>* by CaM is largely abolished by intracellular application of a specific Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CaMKII) inhibitor autocalmitide-2 inhibitory peptide (AIP) through a pipette. Furthermore, bath application of CaMKII inhibitor KN-93, but not its inactive analog KN-92, significantly reduced the stimulatory action of CaM on *I<sub>Ks</sub>*. Taken together, these observations suggest that CaM stimulates *I<sub>Ks</sub>* in guinea-pig SA node cells through the activation of CaMKII. This enhancement of *I<sub>Ks</sub>* by CaMKII may be involved in the modulation of SA node automaticity under some pathological conditions such as oxidant stress.

## 1PK-063

### Identification of a novel alternative splicing variant of mouse TRPA1 implicated in modifying TRPA1 activity under inflammatory and neuropathic pain

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TRPA1 is a nonselective cation channel activated by various pungent chemicals such as allyl isothiocyanate, allicin, cinnamaldehyde, and so on. TRPA1 channel is predominately expressed in sensory neurons and serves important function for nociception. Recently, TRPA1 was reported to be involved in inflammatory and neuropathic pain and critical for mechanical hyperalgesia. However, the detail mechanism of its involvement still remains unclear. In this study we identified a novel alternative splicing variant of mouse TRPA1. Both isoforms, TRPA1a (full length) and TRPA1b (splicing variant) could be translocated to the plasma membrane while physically interact with each other. Although TRPA1b did not respond to TRPA1 agonists, co-expression of TRPA1a with TRPA1b significantly increased TRPA1a plasma membrane expression as well as current density in response to TRPA1 agonists. Over-expression of TRPA1b in WT DRG neurons increased AITC responses. Moreover, expression of TRPA1a with TRPA1b produced larger AITC responses compared with expression of TRPA1a alone in TRPA1KO DRG neurons. Expression levels of TRPA1a and TRPA1b mRNAs changed dynamically in complete Freund's adjuvant-induced inflammatory and partial sciatic nerve ligation-induced neuropathic pain mouse models. These results indicate that TRPA1 channel could be involved in inflammatory and neuropathic pain conditions through an alternative splicing event.

## 1PK-064

### The improved effect of hypothermic cultivation on the functional expression of human cardiac Kv1.5 channels

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We herein investigated the role of low temperature exposure in the channel activity, expression, degradation and localization of human Kv1.5 (hKv1.5). In hKv1.5-expressing CHO cells, the Kv1.5 currents significantly increased at reduced temperature cultivation (28°C) in comparison to those observed at 37°C. The current densities doubled from 256.9±12.4 (control) to 528.1±21.1 pA/pF. Western blot analysis confirmed that the protein levels (immature and mature proteins) of hKv1.5 were significantly elevated at hypothermic conditions. Similar results were also obtained in cells cultured at even mild hypothermia (31°C~34°C). The treatment of cells with a proteasome inhibitor, MG132, significantly increased the immature (but not the mature) hKv1.5 protein at 37°C; there were no changes in either the immature or mature hKv1.5 proteins at low temperature conditions after MG132 exposure, indicating that the enhancement of the mature hKv1.5 protein may not result from the inhibition of proteolysis. Moreover, the hKv1.5 fluorescence signal in the HEK cells increased significantly on the cell surface at 28°C versus those cultured at 37°C. Finally, the low temperature treatment markedly shifted the subcellular distribution of the mature hKv1.5 lighter fractions, which showed considerable overlap with the trans-Golgi component. These results indicate that low temperature exposure stabilizes Kv1.5 protein in the cellular organs or on the plasma membrane, modulates its degradation and recycling trafficking, thus enhancing the functional hKv1.5 currents.

## 1PK-065

### Isoproterenol-induced spontaneous action potentials in the cardiac myocytes of transgenic mouse overexpressing HCN2

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Cardiac pacemaker channel, HCN2 & 4 are expressed in the ventricle of fetal heart, and is silenced during the development. It is well-known that these channels are reexpressed in the hypertrophied heart, and has been suggested to underlie arrhythmogenesis. To test this hypothesis, we generated a transgenic mice overexpressing HCN2 in the heart (HCN2-Tg). Contrary to our expectation, HCN2-Tg was not vulnerable to arrhythmia under physiological condition. We then analyzed the electrophysiological properties of cardiac myocytes isolated from HCN2-Tg using ruptured whole-cell patch method. The resting membrane potential (RMP) was not significantly different between wild-type (WT) and HCN2-Tg. No spontaneous action potential (SAP) was recorded in HCN2-Tg myocytes, although robust HCN2 current was recorded. However, 0.3 μM isoproterenol induced SAP in 73% of HCN2-Tg myocytes. In the rest of myocytes, RMP was significantly depolarized, most probably due to the up-regulation of HCN2 current (control, -71.3±1.7 mV; iso, -68.6±2.3 mV; n=8, P=0.036). When the myocytes were perfused with 3 mM K<sup>+</sup> bathing solution, RMP was -83.7±1.6 in WT, and -76.8±4.1 mV in HCN2-Tg. Furthermore, SAP was induced in 57% of HCN2-Tg myocytes. These finding suggested overexpression of HCN2 might increase the vulnerability to arrhythmia in a pathological condition.

## 1PK-066

### COX-2 selective blocker inhibits the TNF $\alpha$ -induced apoptosis in isolated rabbit articular chondrocytes

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Chondrocyte apoptosis contributes to the disruption of cartilage integrity in osteoarthritis (OA). Recently, we reported that activation of volume-sensitive Cl<sup>-</sup> current ( $I_{Cl,vol}$ ) mediates cell shrinkage triggering apoptosis in rabbit articular chondrocytes. On the other hand, COX blocker is frequently used for treatment of OA. However, it is not understood whether COX blocker affects metabolism of rabbit articular chondrocytes. In the present study, we examined in vitro effects of selective blockers for COX-2 and -1 on the TNF- $\alpha$ -induced activation of  $I_{Cl,vol}$  in rabbit chondrocytes using whole-cell patch-clamp technique. Exposure of isolated chondrocytes to TNF- $\alpha$  resulted in an obvious increase in the membrane Cl<sup>-</sup> conductance. The TNF- $\alpha$  evoked Cl<sup>-</sup> current exhibited electrophysiological and pharmacological properties similar to those of  $I_{Cl,vol}$ , such as outward rectification, prominent inactivation at large positive potentials (>+50 mV), inhibition by hyperosmotic cell shrinkage, and sensitivity to  $I_{Cl,vol}$  blockers. Pretreatment of cells with the COX-2 blocker etodolac markedly inhibited the  $I_{Cl,vol}$  activation by TNF- $\alpha$  as well as subsequent apoptotic events such as elevation of caspase 3 activity. In contrast, the COX-1 blocker sulindac had no effect on the increase in caspase 3 activity induced by TNF- $\alpha$ . Thus, the COX-2 selective blocker has an inhibitory effect on TNF- $\alpha$ -induced apoptotic events, which suggests the efficacy of this drug for the treatment of OA.

## 1PK-067

### Hyperbaric Oxygenation Treatment before Radiotherapy improves Radioresponse in a Xenograft mouse model of Glioblastoma

Katagiri, Chiaki; Matsushita, Masayuki (*Molecular and Cellular Physiology*)

Human glioblastoma is a primary malignant brain tumor in adult and is among the most aggressive malignancies. In brain tumor tissue, certain areas are coursed to hypoxic condition because of insufficient blood vessel supply. Such hypoxic condition area is also considered to induce resistance to radiation therapy. Molecular oxygen has been recognized an enhancement of radiation sensitivity. Hyperbaric oxygenation (HBO) improves the oxygen supply to hypoxic tumor cells. We examined the effect of radiotherapy after HBO breathing in experimental tumors using a tumor growth assay. U87-MG cells were transplanted into balb/c nu/nu mice leg. After the subcutaneous xenograft reached approximately 200mm<sup>3</sup>, mice were started radiation therapy, 2Gy/day for 10days, with or without HBO, 2.5 atmosphere with 100% oxygen for 40min. A significant growth delay was seen in the animals with radiation therapy after HBO, and the tumor size increased 7.0 fold in notreatment, 4.2 fold only radiation treatment, and 2.4 fold in radiation after HBO treatment. Next we analysed the changes of gene expression of tumor cells using mRNA differential display method, and will present our recent results on regulatory mechanisms of HBO treatment in glioblastoma.

## 1PK-068

### Requirement of autophagy in the development and ischemic tolerance of self-beating atypically-shaped cardiomyocytes (ACMs), a new subpopulation of heart cells

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Atypically-shaped cardiomyocytes (ACMs) are a new subpopulation of spontaneously beating heart cells with a peculiar morphology identified within a culture of cardiac myocyte-depleted fraction (CMDF) cells obtained from adult mouse cardiac ventricles. ACMs originate from small cells in CMDF and grow in size and start beating within ~ 3 days culture without appreciable proliferation or expression of stem cell marker proteins, but stay in the heart until elderly stages, while preserving the expression of fetal cardiac gene products, such as atrial natriuretic peptide (ANP). However, many of the characteristics are unclear. The present study examined whether pre-exposure of CMDF cells to severe ischemia abolished the ability of ACMs to develop into beating cells. Of ACMs that underwent ischemia, ~50% grew in size, changed the morphology and started beating during the subsequent culture under normoxia. ACMs displayed constitutively active autophagy during the culture. Under normoxic conditions, pre-treatment of CMDF cells with an autophagy inhibitor bafilomycin A1 significantly reduced the number of beating ACMs. Bafilomycin A1 also reduced the surviving ACMs underwent ischemia, thus indicating that ACMs require the autophagy, especially in the early period, not only to develop into beating cells but also obtain an ischemic tolerance. The results suggest the possibility that the development of beating ACMs could occur in injured heart, even the surviving cell population is small.

## Poster Presentations Cell Physiology, Molecular Physiology (1)

## 1PK-069

### Class II PI3 kinase-C2 $\alpha$ plays essential roles in endosomal Rac1 activation and cell migration in S1P-stimulated endothelial cells

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Recently, we showed that endothelial cell (EC)-specific targeted deletion of PI3K-C2 $\alpha$ , class II  $\alpha$ -isoform of PI3K family, in mice caused embryonic lethality due to defects in sprouting angiogenesis and vascular maturation. C2 $\alpha$  is mainly localized in endosomes, the trans-Golgi network and clathrin-coated vesicles. Lipid mediator, Sphingosine-1-phosphate (S1P) plays a crucial role in regulating EC migration. Therefore, we investigated the role of C2 $\alpha$  in S1P-induced EC migration and cell signaling. In human umbilical vein EC (HUVEC), G-protein coupled receptor S1P1 mediates cell migration and activation of Akt, ERK and Rac1 in response to S1P. Knockdown of either C2 $\alpha$  or class I p110 $\beta$  markedly inhibited S1P-induced migration, lamellipodium formation and tube formation whereas that of p110 $\alpha$  or Vps34 did not. FRET imaging showed that S1P-induced Rac1 activation occurs not only in the plasma membrane but also PtdIns-3-phosphate (PtdIns (3) P)-enriched endosomes. Knockdown of C2 $\alpha$  but not p110 $\beta$  abolished endosomal Rac1 activation. C2 $\alpha$  knockdown also inhibited S1P-induced S1P1 internalization into endosomes. Additionally, dynasore, an endocytosis inhibitor suppressed S1P-induced S1P1 internalization, Rac1 activation, migration and tube formation. These observations collectively indicate that C2 $\alpha$  is essential for S1P-induced S1P1 internalization and endosomal Rac activation and, thereby, migration in EC.

## 1PK-070

### IRBIT relieves the inhibition of NBCe1-B by intracellular Mg<sup>2+</sup>

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The electrogenic Na<sup>+</sup>-HCO<sub>3</sub><sup>-</sup> cotransporter NBCe1-B plays a key role in intracellular pH regulation and epithelial HCO<sub>3</sub><sup>-</sup> secretion. Our previous whole-cell patch-clamp study showed that bovine NBCe1-B (bNBCe1-B) currents heterologously expressed in mammalian cells are strongly inhibited by intracellular Mg<sup>2+</sup> (Mg<sup>2+</sup><sub>i</sub>), inhibition being relieved by truncation of the cytosolic NBCe1-B specific N-terminal region. Interestingly, NBCe1-B-like currents natively expressed in bovine parotid acinar (BPA) cells are much less sensitive to Mg<sup>2+</sup><sub>i</sub> inhibition compared to recombinant bNBCe1-B currents. Here, we hypothesized that this apparent discrepancy may involve IRBIT, a protein interacting to the N-terminal region of NBCe1-B. RT-PCR, Western blot and immunofluorescence confocal microscopy revealed that IRBIT was not only expressed in the cytosol, but also colocalized with NBCe1-B in the region of plasma membranes of BPA cells. IRBIT was coimmunoprecipitated with NBCe1-B from bovine parotid cell lysate. Heterologous coexpression of IRBIT reduced the Mg<sup>2+</sup><sub>i</sub> sensitivity of bNBCe1-B currents in HEK293 cells. Collectively, these results suggest that IRBIT may relieve the inhibition of NBCe1-B by Mg<sup>2+</sup><sub>i</sub>.

## 1PK-071

### Effects of UVA light irradiation on cultured RAW264.7 cells

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We studied effects of ultraviolet A (UVA) irradiation using light-emitting diode on growth of RAW 264.7 cells. Cells were plated on 96 well plates at a density of 10<sup>5</sup>-10<sup>6</sup> cells/ml. After 24 hr, these cells were irradiated for 0-5 min and maintained for 0-72 hr in a CO<sub>2</sub> incubator. Irradiation for more than 2 min significantly suppressed the cell growth. Addition of N-acetyl cysteine (NAC) as a scavenger perfectly recovered from the cell growth inhibition and removed almost ROS induced by the irradiation. Intracellular glutathione content was not so strongly decreased, but the activity of glutathione reductase was significantly affected by the irradiation. Finally, to detect ROS induced in the culture medium irradiated by UVA light, we measured EPR signals in the presence of spin trapping agents (TPC and DMPO) by EPR spectrometer. NaN<sub>3</sub> decreased the spin peaks formed by TPC and histidine decreased the peaks by DMPO. This measurement indicates that singlet oxygen is initially induced and the singlet oxygen is converted into hydroxyl radicals. These results suggest that these ROS induced in cytoplasm or cultured medium inhibit the growth of RAW cells.

## 1PK-072 (SPK-5)

### Mitochondrial NCX controls directional migration of B lymphocyte

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To clarify the roles of mitochondria Ca<sup>2+</sup> handling on chemotaxis of B lymphocyte, we studied CXCL12-induced migration of A20 B lymphocytes. CXCL12 (100 ng/ml) increased transwell migration from 4.6 $\pm$ 0.5% to 12.6 $\pm$ 0.6%. This increase was dose-dependently inhibited by CGP-37157 (an inhibitor of mitochondrial Na-Ca exchange (NCX<sub>m</sub>, NCLX)). In cells which NCLX was knocked down by siRNA, the transwell migration was inhibited (control siRNA : 7.3 $\pm$ 0.2% vs. NCLX siRNA : 1.8 $\pm$ 0.2%). These data suggest that NCX<sub>m</sub> is related to chemotaxis of A20 B lymphocytes. In 8 hrs observation of cell migration without CXCL12, mean displacement of NCLX siRNA transfected cells was larger (21.4 $\pm$ 1.5  $\mu$ m) than control cells (13.5 $\pm$ 2.2  $\mu$ m). Applying CXCL12 gradient did not increase mean displacement and directional migration to chemokine in NCLX siRNA cells. Above data indicate that NCLX knock down accelerates random migration and inhibits directional migration. Intracellular Ca<sup>2+</sup> was higher in NCLX siRNA cells (fura-2 ratio 0.49 $\pm$ 0.01) than the control cells (0.45 $\pm$ 0.01, P<0.05) in the absence of CXCL12. After 2 hrs CXCL12 stimulation, the intracellular Ca<sup>2+</sup> increased in control (0.51 $\pm$ 0.01) but not in NCLX siRNA cells. Mitochondria redistributed at the rear side (uropod) during migration in control but not in NCLX siRNA cells. Mitochondrial and cytosolic Ca<sup>2+</sup>, and localization of mitochondria maybe related to NCLX mediated control of migration.

### 1PK-073

#### A role of $K^+-Cl^-$ cotransporter in the cell cycle regulation of breast cancer MDA-MB-231 cells

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Our previous studies indicate that the intracellular  $Cl^-$  could act as a signal regulating cell cycle. The intracellular  $Cl^-$  concentration is basically regulated through a balance between uptake and release of  $Cl^-$  through  $Cl^-$  transporters and  $Cl^-$  channels, suggesting that the activities and expression of  $Cl^-$  transporters such as  $Na^+-K^+-2Cl^-$  cotransporter (NKCC) and  $K^+-Cl^-$  cotransporter (KCC) may affect cell proliferation and cell cycle. In this study, we investigated a physiological role of KCC on the regulation of cell cycle in the synchronized breast cancer MDA-MB-231 cells by blocking the activity of KCC with a KCC inhibitor, dihydroindenyl-oxy-alkanoic acid (DIOA). Incubation of the cells with 200  $\mu M$  DIOA led the cell to the  $G_1$  phase arrest. In the normal cell cycle, the degradation of p21 was observed in the S phase and cyclin E was synthesized during the late  $G_1$  phase in a cyclin D-dependent manner. In contrast, treatment of the cell with DIOA broke this cell cycle regulation. Furthermore, the intracellular  $Cl^-$  concentration was significantly decreased in MDA cells treated with DIOA for 48 h. Taken together these results, it is suggested that 1) DIOA suppressed cyclin D-dependent cyclin E synthesis leading to the  $G_1$  arrest, 2) the reduction of nuclear  $Cl^-$  concentration plays a crucial role in the DIOA-induced  $G_1$  phase arrest in MDA cells, and 3) the cell cycle progression might be regulated by KCC controlling intracellular  $Cl^-$  concentration.

### 1PK-074

#### Diffusion of manganese chelates in the rat brain measured by $T_1$ -weighted MRI

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In order to optimize manganese-enhanced MRI (MEMRI) in thalamic and hypothalamic nuclei, diffusion of manganese in the brain followed by intravenous infusion of Mn-bicine, Mn-citrate and Mn-HIDA. The 3 Mn-chelates could not cross the intact BBB and appeared in the CSF instantaneously, and then diffused into the brain parenchyma through the ependymal layer. No statistically significant differences were detected between the 3 Mn-chelates, suggesting that the Mn-chelates themselves enter into the parenchyma, and not only the free  $Mn^{2+}$  ion. In order to obtain diffusion constant, intensities of  $T_1$ -weighted MRI, 9 ROI in the hypothalamus perpendicular to the third ventricle, were measured during continuous infusion of Mn-bicine in the lateral cerebroventricle. Image intensity of  $T_1$ -weighted MRI was converted into Mn concentration using  $T_1$  relaxation time. On an assumption of the simple diffusion process, apparent diffusion coefficient ( $D_{ap}$ ) of manganese ( $4.2 \times 10^{-5} \text{ mm}^2 \text{ s}^{-1}$ ) is much slower than that of water ( $6 \times 10^{-4} \text{ mm}^2 \text{ s}^{-1}$ ), and the  $D_{ap}$  tended to decrease when the distance from the third ventricle increased. These results suggest i)  $Mn^{2+}$  ion enters into neural cells, and ii) some parts of Mn efflux from the brain via veins.

### 1PK-075

#### Functional expression of transfected ROMK potassium channels in polarized and non-polarized membranes of cultured M1 cells

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Potassium secretion along the collecting duct is indispensable for the body fluid potassium homeostasis, and apical ROMK  $K^+$  channels are the major candidate for the route of this secretion. Recently, we have cloned ROMK1 (Kir 1.1)  $K^+$  channel and examined the exogenous expression of the cloned ROMK1 fused with EGFP in cultured M1 collecting duct cells. We used two types of culture dishes. One was conventional glass dishes, and the other is dishes with membrane inserts to expose the polarized apical membrane of M1 cells. Visual expression of ROMK-EGFP in the cells was confirmed with a fluorescent microscope, and the functional expression was examined using the cell-attached mode of the patch-clamp technique. Without the exogenous expression, ROMK-like  $K^+$  channel has not been detected. The current identical to ROMK  $K^+$  channel was observed in the ROMK-EGFP-expressing M1 cells on both glass dishes and membrane inserts. Although no appreciable difference in fluorescence intensity and the localization of ROMK-EGFP was observed between the two conditions, there was a significant difference of the frequency of the ROMK current acquisition. Namely, ROMK current was detected in 70.6% of the cells on the membrane inserts, but was observed only in 8.3% on the glass dishes. It is strongly suggested that ROMK1 has a high affinity to polarized apical membrane to open the channel in cultured M1 cells. The factor of this affinity remains to be investigated.

### 1PK-076

#### Measurement of $Ca^{2+}$ in the endoplasmic reticulum during $Ca^{2+}$ oscillations in mammalian eggs

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In mammalian egg, repetitive increases in cytosolic  $Ca^{2+}$  concentration ( $[Ca^{2+}]_{cyt}$ ), or  $Ca^{2+}$  oscillations, are induced by the fusing spermatozoa, and trigger a series of events for egg activation. Each  $Ca^{2+}$  transient in the oscillations is due to  $Ca^{2+}$  release from the endoplasmic reticulum (ER) through inositol 1,4,5-trisphosphate receptor/ $Ca^{2+}$  channels. Therefore, the information about  $Ca^{2+}$  concentration in the ER lumen ( $[Ca^{2+}]_{ER}$ ) is essential for understanding the mechanism of  $Ca^{2+}$  oscillations. In the present study, we tried to apply a genetically coded  $Ca^{2+}$  probe, D1ER, which is based on fluorescence resonance energy transfer (FRET) between two GFP variants, on analyzing the changes in  $[Ca^{2+}]_{ER}$  during  $Ca^{2+}$  oscillations in mouse eggs. When immature oocytes were injected with cRNA and matured in vitro, D1ER expressed in the eggs was targeted to the ER and successfully reported the changes in  $[Ca^{2+}]_{ER}$  as those in FRET signals, as confirmed by the experiments where  $[Ca^{2+}]_{ER}$  were forced to change by thapsigargin and/or ionomycin. By simultaneous measurement of  $Ca^{2+}$  oscillations with D1ER and fura-2, it was revealed that the recovery of  $[Ca^{2+}]_{ER}$  was much slower than the decrease in  $[Ca^{2+}]_{cyt}$  after  $Ca^{2+}$  release at each  $Ca^{2+}$  transient. Interestingly, the rate of recovery, as well as the frequency of  $Ca^{2+}$  oscillations, was dependent on extracellular  $Ca^{2+}$  concentration. Some results of modifications in D1ER to improve the performance will also be presented.

## 1PK-077

### Tumor necrosis factor $\alpha$ stimulates activity of an inwardly rectifying $K^+$ channel in human kidney proximal tubule cells

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Several cytokines have been suggested to affect  $Na^+$  reabsorption in the renal tubular epithelia. Since the driving force of the transepithelial  $Na^+$  reabsorption is dependent on the  $K^+$  channel activity, as well as  $Na^+-K^+-ATPase$ , it is important to know whether cytokines would affect the  $K^+$  channels. We have previously reported that interferon- $\gamma$  possessed a time dependent biphasic effect on activity of an inwardly rectifying  $K^+$  channel with an inward conductance of 40 pS in cultured human proximal tubule cells. We also found that interleukin-1 $\beta$  suppressed the activity of this  $K^+$  channel. In this study, we investigated the effect of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) on the activity of the 40 pS  $K^+$  channel, using the patch-clamp technique. In cell-attached patches, TNF- $\alpha$  (20 ng/ml) stimulated channel activity in a few minutes. This stimulatory effect was blocked by a soluble TNF receptor analog, etanercept (10  $\mu$ g/ml). It has been reported that TNF- $\alpha$  stimulated activity of a 70 pS  $K^+$  channel in the thick ascending limb of the rat kidney through activation of protein tyrosine phosphatase (PTP). However, a PTP inhibitor, phenylarsine oxide (1  $\mu$ M) did not affect the stimulatory effect of TNF- $\alpha$  on the 40 pS  $K^+$  channel in human proximal tubule cells. In contrast, an inhibitor of protein kinases, K252a (1  $\mu$ M), abolished the effect of TNF- $\alpha$ . These results suggested that the stimulatory effect of TNF- $\alpha$  on  $K^+$  channel activity in human proximal tubule cells was mediated through its specific receptor and dependent on protein phosphorylation processes.

## 1PK-078

### Establishment and analysis of MMR2 knockout mice : the roles of Monocyte/Macrophage MHC receptor (MMR)1 and 2 in allograft rejection

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Our previous studies have shown that we isolated two novel receptors on macrophages or monocytes for H-2D<sup>d</sup> and H-2K<sup>d</sup> (mouse MHC), MMR1 and MMR2. In the present study, we investigated the roles of MMR1 and MMR2 genes in allograft rejection by establishment of MMR2 knockout (MMR2<sup>-/-</sup>) C57BL/6 mice [H-2D<sup>b</sup>K<sup>b</sup>]. The MMR2<sup>-/-</sup> mice showed normal body growth and fertility, but showed loss of the expression of not only MMR2 but also MMR1 mRNA or protein in peripheral blood mononuclear cells. The transplantation of skin grafts from various mice to MMR2<sup>-/-</sup> mice revealed that the skin grafts from H-2D<sup>d</sup>- and H-2K<sup>d</sup>- or H-2D<sup>d</sup>K<sup>d</sup>-Tg mice onto MMR2<sup>-/-</sup> mice were tolerated, but that the skin grafts from C3H [third-party MHC], B10D.2 [allogenic MHC class II] or BALB.B [allogenic minor histocompatibility antigen] mice onto MMR2<sup>-/-</sup> mice were rejected. Meanwhile, intradermally injected H-2D<sup>d</sup>, H-2K<sup>d</sup>- or H-2D<sup>d</sup>K<sup>d</sup>-transgenic EL-4 cells (lymphoma of C57BL/6 origin) into MMR2<sup>-/-</sup> mice were rejected in a transgene-number dependent manner. <sup>51</sup>Cr release assays showed that intraperitoneally injected H-2D<sup>d</sup>- and/or H-2K<sup>d</sup>-EL-4 cells into MMR2<sup>-/-</sup> mice were lysed by CD8<sup>+</sup> population and that the cytotoxic activity was inhibited by anti-TCR $\alpha\beta$  antibody. These results indicated that MMR1 and MMR2 on macrophages or monocytes play a role in rejection of skin grafts bearing H-2D<sup>d</sup> and/or H-2K<sup>d</sup> but not lymphoma bearing them.

## 1PK-079

### Simulation of energy metabolism in fetal and adult ventricular cells

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Recapitulation of the fetal cardiac gene program has been regarded as a feature of hypertrophied and failing hearts and considered to underlie electrical remodeling in diseased heart. In our previous study, we modeled developmental changes in action potentials of rodent ventricular cells via integration of quantitative changes in individual ionic components both on cellular membrane and sarcoplasmic reticulum into a comprehensive electrophysiological model of guinea pig ventricular cell: the Kyoto model. In this study, we further modified the model to include developmental changes in energy metabolism of the ventricular cells. Compared to adult guinea pigs, fetal guinea pigs have higher anaerobic glycolytic capacity and lower activities of mitochondrial enzymes, due to the relatively hypoxic condition in fetus. We modeled the changes in activities of the enzymes and individual ionic components, and constructed a ventricular cell model which represents the changes in energy metabolism, on the basis of the activities of the enzymes at specific stages in development as reported in literatures. Consequently, the simulation results of our models between fetal and adult stages were roughly consistent with the developmental changes in concentrations of intermediate metabolites in glycolytic pathway. This model will aid us to simulation of certain stresses that may occur during birth asphyxia.

## 1PK-080

### Involvement of AQP5 in carbachol-induced volume decrease and in reduction of the activation energy for water transport by rat parotid acinar cells

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Aquaporin-5 (AQP5) is highly expressed in the salivary gland, and plays an important role in the fluid secretion. A naturally occurring point mutation of AQP5 (AQP5-G103D) was earlier found in the rat; this mutant molecule has almost the same water permeability and less AQP5 protein expression compared with wild-type (WT) in the salivary gland acinar cells. In this study, we measured the cell volume and activation energy ( $E_a$ ) for water transport using WT and mutant acinar cells. In the WT rat, cholinergic agonist carbachol (CCh) caused a transient swelling of the acinus, followed by a rapid agonist-induced cell shrinkage, reaching a plateau at 30 s. In the mutant rat, the acinus did not swell by CCh stimulation, and the agonist-induced cell shrinkage was delayed by 8 s, reaching a transient minimum at around 1 min, and recovered spontaneously even though CCh was persistently present. In the resting WT acinar cells,  $E_a$  value showed no detectable change after CCh stimulation. In the resting mutant acinar cells, high  $E_a$  value was detected which was decreased a bit after CCh stimulation. These results suggest that AQP5 was the main pathway for water transport in the acinar cells and that the rapid agonist-induced acinar cell shrinkage as well as continuous reduction of the acinar cell volume are necessary for the steady salivary secretion in the WT rat.

## 1PK-081

### Cl<sup>-</sup> channels/transporters as new targets for cancer therapies based on disruption of autophagy ability via modification of lysosome acidification

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Due to hypoxic and hypo-nutrient conditions, cancer cells elevate autophagy ability to use recyclable materials within their own cells. Autophagy is a catabolic process degrading their own components mediated through lysosomal machineries. Activity of lysosomal enzymes depends on the intra-lysosomal acidity primarily generated by V-type H<sup>+</sup>-ATPase co-operating with Cl<sup>-</sup> movements via Cl<sup>-</sup> channels/transporters. The purpose of the present study was to identify roles of cytosolic and intra-lysosomal H<sup>+</sup> and Cl<sup>-</sup> on autophagy using a model cancer cell line (MKN28). MKN28 cells were cultured for 48 h in a medium with normal, low concentration of Cl<sup>-</sup> replaced with NO<sub>3</sub><sup>-</sup> or low pH condition (pH<sub>i</sub>=6.8). Culture under a low pH condition caused suppression of proliferation associated with an increase in doubling time without arrest at any specific cell cycle phases but via elongation of all cell cycle phases, increases in intra-lysosomal and cytosolic concentrations of H<sup>+</sup> (a decrease in pH) without changes in [Cl<sup>-</sup>]<sub>c</sub> or expression of LC3/p62. On the other hand, culture under a low Cl<sup>-</sup> medium caused suppression of proliferation associated with G<sub>0</sub>/G<sub>1</sub> arrest, decreases in [Cl<sup>-</sup>]<sub>c</sub> and intra-lysosomal and cytosolic concentrations of H<sup>+</sup> (an increase in pH), and increases in expression of LAMP1 and LC3/p62. These results suggest that cytosolic Cl<sup>-</sup> is one of potent regulators of autophagy.

## 1PK-082

### Two-photon FRET/FLIM imaging of SNARE-dependent exocytosis

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When cells release hormones and neurotransmitters through exocytosis, cytosolic Ca<sup>2+</sup> triggers the fusion of secretory vesicles with the plasma membrane. This fusion requires assembly of a SNARE protein complex. However, the initial conformations of SNAREs from which Ca<sup>2+</sup> triggers exocytosis has not been well understood. We have therefore constructed a various probe that detects the assembly of SNAREs, SNAP25b, syntaxin-1A and VAMP2 through fluorescence resonance energy transfer (FRET) using two-photon microscope. We used both the fluorescence ratios of intramolecular FRET probe of SNAP25 (SLIM) and the fluorescence lifetime of various intermolecular FRET probes. We found that assembly of t-SNAREs, SNAP25b and syntaxin1a was regionally variable in the islet of Langerhans, and affected the readiness for exocytosis. We could detect neither ternary SNARE complex formation nor oligomerization of SNAREs in the plasma membrane of beta-cells. In contrast, SLIM probe exhibited an abnormally large FRET signal in the active zone in the synaptic terminals, where ternary SNARE complex prevailed, suggesting that SNAREs are oligomerized. Interestingly, our results with intermolecular FRET of SNAP25 probes strongly suggested that two SNARE domains of one SNAP25 molecule was assembled not in the same SNARE complex, but with two neighboring SNARE complexes in an oligomer. The domain swap model has been controversial, but our new data show that this is the case for the active zone, but not for the endocrine cells.

## 1PK-083

### Cyclic Stretch Promotes Elastic Fiber Formation in Rat Aortic Smooth Muscle Cells

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[Background] Vessels are exposed to various stresses including pulsatile flow. These stresses are known to be critical factors for construction of vascular elastic fiber. In particular cyclic stretch is considered to change the elastic modulus. However, the relationship between stretch and elastic fiber formation remains unknown. [Methods] The aortic Smooth muscle cells (SMCs) of rat at embryonic day 21<sup>st</sup> were plated on a silicone chamber coated with fibronectin. The chamber was set on a stretch machine, and stretched for 1day-14 days. We assessed the elastic fiber formation by immunohistochemistry and the expression levels of elastic fiber associated genes, and differentiation markers of SMCs. [Results] Stretch promoted elastic fiber formation after 10days and 14days. The expression levels of mRNAs of elastic fiber-associated genes such as elastin, lysyl oxidase, lysyl oxidase like protein 1, fibrillin1, fibulin4 and 5 and latent TGF-β binding protein 1 and 2 were increased by stretch, although the timing of increasing these genes'expression was different. qRT-PCR analysis showed that SMCs differentiation markers such as h-caldesmon and SM1 were increased after 14day-stretch. [Conclusion] Cyclic stretch promoted elastic fiber formation probably through the alignment of elastin and the repeat of deposition and decomposition by changing in the gene expression and differentiation of aortic SMCs.

## 1PK-084

### S100 PROTEINS MODULATE PROTEIN PHOSPHATASE 5 FUNCTION : A LINK BETWEEN Ca<sup>2+</sup> SIGNAL TRANSDUCTION AND PROTEIN DEPHOSPHORYLATION

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PP5 is a unique member of serine/threonine phosphatases comprising a regulatory tetratricopeptide repeat (TPR) domain and functions in signaling pathways that control many cellular responses. We previously reported that Ca<sup>2+</sup>/S100 proteins directly associate with several TPR-containing proteins and lead to dissociate the interactions of TPR proteins with their client proteins. Here, we identified PP5 as a novel target of S100 proteins. In vitro binding studies demonstrated that S100A1, S100A2, S100A6, and S100B proteins specifically interact with PP5-TPR and inhibited the PP5-Hsp90 interaction. In addition, the S100 proteins activate PP5 by using a synthetic phosphopeptide and a physiological protein substrate, tau. Overexpression of S100A1 in COS-7 cells induced dephosphorylation of tau. However, S100A1 and permanently active S100P inhibited the apoptosis signal-regulating kinase 1 (ASK1) and PP5 interaction, resulting the inhibition of dephosphorylation of phospho-ASK1 by PP5. The association of the S100 proteins with PP5 provides a Ca<sup>2+</sup>-dependent regulatory mechanism for the phosphorylation status of intracellular proteins through the regulation of PP5 enzymatic activity or PP5-client protein interaction.

## Poster Presentations Sensory Function(1)

### 1PK-085

#### Do peripheral mechanisms contribute to the persistent muscular hyperalgesia by repeated cold stress(RCS)?

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There are many patients suffering from chronic muscle pain. However its mechanisms were poorly understood so far. Previously we reported that rats exposed to RCS showed muscular mechanical hyperalgesia (Nasu et al., 2010). Despite of many studies on the central mechanisms for RCS, almost nothing is known on peripheral changes. Therefore, we investigated effects of intramuscular lidocaine on the muscular hyperalgesia after RCS and changes in the blood flow (BF) response to exercise. RCS was underwent by moving rats every 30 min from the -3°C room to the 22°C room, and kept in the -3°C room during the night. This cycle was repeated for 5 days. After RCS, we firstly studied the effect of lidocaine. Four days after RCS lidocaine (i.m., 30 ul) increased for 1 hr the lowered muscular withdrawal threshold not only of the injected side but also of the contralateral side. This result suggests that peripheral afferent contributes to maintaining the hyperalgesic state after RCS. Next, we examined BF response to exercise (rhythmic isotonic contraction for 10 min) of lower leg flexors. Regional BF was measured with fluorescent microsphere method. Exercise-induced increase in BF of the tibialis anterior muscle was enhanced after RCS, but that of extensor digitorum longus muscle was decreased. These changed regional BF distribution may have some influence in pain state.

### 1PK-086

#### Anatomically Structured Features of Burst Spiking in Thalamic Reticular Nucleus Cells Projecting to Visual Thalamic Nuclei

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The thalamic reticular nucleus (TRN) plays a pivotal role in gain and/or gate control of sensory input by providing inhibition to thalamic nuclei. The visual sector of the TRN contains two groups (TRN-DLG and TRN-LP) of cells projecting to the dorsal lateral geniculate (DLG) and lateral posterior (LP) nuclei, which subserve geniculate and extra-geniculate visual processing. TRN cell activity is characterized by robust burst spiking that imposes tremendous impacts on thalamic cell activity. The features of burst spiking in visual response and spontaneous activity were examined with regard to the locations of cell body and terminal field in anesthetized rats, using juxta-cellular recording and labeling technique. The results indicated correlations between the diversity of burst spiking and the topography of TRN cell projections to the DLG and LP in addition to the previously reported dichotomy in the features of burst spiking (TRN-DLG and TRN-LP groups have high and low propensities for burst spiking, respectively). Also noteworthy is that sustained effects of visual input on burst spiking were recognized in recurrent activation of TRN-DLG but not of TRN-LP group. The dichotomized features of burst spiking are presumed to have differential influences on thalamic cell activity in the DLG and LP for attentional gating function. Further, there may be complex neural organizations that shape thalamic cell activity through the diversity of burst spiking structured in efferent connectivity of the TRN.

### 1PK-087

#### Peripheral nociceptors of the muscle fascia and their spinal projection : a novel physiological role of fascia as a nociceptive organ

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Muscle fascia has long been assumed as an important source of pain/nociception while little documentation is available. Here we studied characteristics of fascial thin-fiber afferents, the distribution of presumptive nociceptive nerve fibers from the rat crural fascia (CF) and their spinal projection. Thin-fiber afferents were identified from the peroneal nerve in vivo under deep anesthesia by the teased fiber technique. Forty-three % of the fascial C-fibers were polymodal receptors (nociceptors) responding to mechanical, chemical, and heat stimuli while almost all A $\delta$ -fibers responded only to mechanical stimulus. Nerve fibers with calcitonin gene-related peptide (CGRP) and peripheral immunoreactivity densely distributed in the distal one third of the CF. Repetitive pinching to the CF induced c-Fos immunoreactive neurons in the superficial dorsal horn at L2-L4 peaking at L3. Taken together, 1) peripheral afferents responding to noxious stimuli did exist in the fascia, 2) peptidergic and non-peptidergic axons of C-fibers distributed in the CF, and 3) nociceptive information from the CF was mainly processed in the spinal dorsal horn at L2-L4. These results clearly demonstrated that the "muscle fascia" is an important tissue for nociception.



## 1PK-088

### Effects of restraint stress on glial activity in the rostral ventromedial medulla

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Stress affects brain activity and promotes long-term changes in multiple neural systems. Exposure to stressors causes substantial effects on the perception and response to pain. Postmortem studies of stress-related psychiatric disorders have demonstrated a decrease in the number of astrocytes and the level of glial fibrillary acidic protein (GFAP), a marker for astrocyte, in the cerebral cortex. In the present study we examined GFAP, S100 $\beta$  and CD11b protein levels in the rostral ventromedial medulla (RVM) after the subacute and chronic restraint stresses to clarify changes in descending pain modulatory system in the rat with stress-induced hyperalgesia. Chronic restraint stress (6h/day for 3weeks), but not subacute restraint stress (6h/day for 3days), caused a marked mechanical hypersensitivity. Subacute and chronic restraint stresses induced a significant decrease of GFAP protein level in the RVM (21.9 $\pm$ 3.6%,  $p$ <0.01 and 18.2 $\pm$ 5.1%,  $p$ <0.05 vs. control group, respectively). The immunohistochemical analysis revealed that chronic restraint stress induced a significant decrease in GFAP-immunoreactivity in the nucleus raphe magnus (NRM), a part of the RVM, compared to subacute restraint stress. In contrast there was no significant difference in the S100 $\beta$  and CD11b protein levels between the control and stress groups. These findings suggest a damage of astrocytes in the RVM after the chronic restraint stress, which may be involved in a dysfunction of the RVM that plays pivotal roles in pain modulation.

## 1PK-089

### Voltage-gated sodium channel expressed in the rat retina

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In the retina, only ganglion cells (RGCs) and subsets of amacrine cells, including AII amacrine cells and dopaminergic amacrine (DA) cells, generate TTX-sensitive action potentials evoked by light stimulus. In RGCs, various types of Na $_v\alpha$  (voltage-gated sodium channel  $\alpha$  subunit) mRNAs (Na $_v$ 1.1-1.3, and Na $_v$ 1.6) are expressed. On the other hand, we detected only Na $_v$ 1.1 in AII amacrine cells [Kaneko and Watanabe, 2007]. Difference in expression pattern of Na $_v\alpha$  subtypes might reflex functional difference of action potentials between amacrine and ganglion cells. It is still unknown what types of Na $_v\alpha$  are expressed in DA cells. To examine the specific Na $_v\alpha$  subtypes expressed in DA cells, we applied *in situ* hybridization (ISH) and immunohistochemistry (IHC) using anti-tyrosine hydroxylase (TH) antibody on the rat retina. TH-labeled DA cells are located nearby INL (inner nuclear layer)/IPL (inner plexiform layer) border. ISH results showed that there were many Na $_v$ 1.1-expressed cells (about 300 cells/section), few Na $_v$ 1.2-expressed cells (75 cells/64 sections), and few Na $_v$ 1.6-expressed cells (25 cells/21 sections) located nearby INL/IPL border. The results of double staining using ISH and IHC showed that part of TH-labeled DA cells expressed Na $_v$ 1.2 or Na $_v$ 1.6 (27 Na $_v$ 1.2-expressed cells/287 DA cells, 4 Na $_v$ 1.6-expressed cells/97 DA cells). Almost all TH-labeled DA cells did not express Na $_v$ 1.1 (0 Na $_v$ 1.1-expressed cells/278 DA cells). Our results suggest that Na $_v$ 1.2 and perhaps Na $_v$ 1.6 might be candidates for the specific Na $_v\alpha$  subtypes expressed in DA cells.

## 1PK-090

### Impaired Olfactory Functions and Lesion of Dopaminergic Neurons in the Olfactory Bulb Induced by Intranasally Administrated Rotenone

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The brain is protected by the blood-brain barrier (BBB) from the invasion threat of chemicals as well as infectious beings. An olfactory transport pathway from the olfactory epithelium to the olfactory bulb is useful to bypass the BBB. Rotenone, an organic pesticide, functions as an inhibitor of mitochondria complex-I. It has been reported that continuous intravenous administration of rotenone impairs dopaminergic neurons at the nigra. There are dopaminergic interneurons (TH-positive neurons), whose function is not fully clarified, at the glomerular layer in the olfactory bulb (OB). In the present study, we administered rotenone intranasally to explore physiological functions of TH-positive neurons in the OB of female mice. Intranasally administrated rotenone induced parkin, a mitochondrial stress marker, in the OB. It was also revealed that the rotenone treatment decreased number of TH-positive neurons at the OB. In addition, untreated mice showed avoidance to odor of butyric acid, while rotenone-treated mice did not. These results suggested that intranasally administrated rotenone delivered to the OB via the olfactory transport pathway and impaired olfactory functions by decreases of TH-positive neurons.

## 1PK-091

### Different anesthetics differently affect the spreading pattern of evoked and spontaneous neural activity in the rat sensorimotor cortex detected by the multiple-site optical recording

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Our previous study revealed that the propagation of depolarization as detected by the multiple-site optical recording system, either evoked response following electrical stimulation or spontaneous activity, spread over the whole sensorimotor cortex. We report here that the propagation patterns of this wave differ depending on anesthetics. Two different anesthetic conditions were compared: single use of urethane (1.5 g/kg) and a mixture administration of urethane (800 mg/kg) and  $\alpha$ -chloralose (80 mg/kg). The sensorimotor cortex including the hindlimb region of a rat was exposed and stained with a voltage sensitive dye (RH-414). An evoked activity was induced by the electrical stimulation to hindlimb. Firstly, we constructed the isochrone map and compared the propagation speed of depolarization waves between the two conditions. Speeds of both the evoked and spontaneous activities were significantly larger under a mixture administration. Secondly, we measured the parameters in the optical signal; amplitude, the full width at the half maximum (FWHM) and the slope of rising phase. No significant difference was observed in the amplitude. The FWHM, for both the activities, was significantly larger under urethane only. Thus, the two anesthetics generally affected the evoked and spontaneously activity in the same way. The slope of rising phase, by contrast, showed activity-specific difference; under urethane only, the slope was more gradual only in spontaneous activities.

## 1PK-092

### Electrochemical analysis of the cochlear lateral wall crucial for inner ear function

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Endocochlear potential (EP) of +80 mV in K<sup>+</sup>-enriched endolymph is crucial for hearing and achieved by unique electrochemical property in lateral cochlear wall comprising inner and outer epithelial layers. Intrastrial space (IS), an extracellular space between the two layers, shows low [K<sup>+</sup>] and +90 mV. This intrastrial potential (ISP) dominates the EP and is formed by K<sup>+</sup>-diffusion that depends on a large K<sup>+</sup>-gradient across the apical sides of the outer layer. Our experiments previously suggested that K<sup>+</sup> transport across the basolateral sides of outer layer was involved in maintaining the apical K<sup>+</sup>-gradient and thus the EP. In this study, we intended to verify the hypothesis by detailed physiological assays. K<sup>+</sup>-sensitive electrode was advanced across the lateral wall. In outer layer region under normal conditions, the electrode detected spike-like elevations of [K<sup>+</sup>], which mirror intracellular milieu of the layer, with slightly positive potential. Blockage of the K<sup>+</sup> transport across the outer layer suppressed the amplitude of the spikes, resulting in decline of the apical K<sup>+</sup>-gradient. Unexpectedly, the potential in the layer significantly became negative. These results confirm our previous hypothesis and also indicate that the potential of the outer layer might contribute to the ISP and the EP.

## 1PK-093

### Compression on myofascial trigger points in patients with neck pain immediately affected cerebral hemodynamics in the prefrontal cortex and autonomic nervous activity

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Ischemic compression (IC) on myofascial trigger point (TP) has been reported to provide immediate relief of musculoskeletal pain. In the present study, we investigated effects of IC on TP in patients with neck pain on cerebral hemodynamic response and autonomic nervous activity using near infrared spectroscopy (NIRS) and ECGs. Seventeen female subjects with musculoskeletal pain of the trapezius muscles were randomly assigned to two groups: TP (N=8) and non-TP (N=9) compression. Each subject received compression for 30 sec 4 times. During the experiment, cerebral hemodynamic responses (changes in Oxy-Hb, Deoxy-Hb, and Total-Hb concentration), and autonomic nervous activity based on heart rate variability (HRV) were monitored. TP compression significantly reduced subjective pain rating score compared with non-TP compression. Furthermore, TP compression significantly decreased oxy-Hb concentration in the prefrontal cortex (BA 10) and sympathetic activity compared with non-TP compression, while parasympathetic activity was increased. The changes in autonomic nervous activity were significantly correlated with changes in cerebral hemodynamics in the anterior dorsomedial PFC and subjective pain rating score. The results suggest that TP compression reduced neck pain through its effects on the PFC.

## 1PK-094

### Protein kinase M<sub>ζ</sub>-dependent late phase of long-term potentiation at synapses in the mouse accessory olfactory bulb

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In mice, some urinary pheromones of the male induce implantation failure in the pregnant female. The inability of the mating male to disrupt the pregnancy depends on the memory of his pheromones formed by the female. The pheromonal memory is based on the neural changes in the accessory olfactory bulb (AOB), the first relay in the vomeronasal system. Microcircuits in the AOB include the prominent reciprocal dendrodendritic synapse between mitral cell projection neurons and granule cell interneurons. Long-term potentiation (LTP) at the AOB synapse is expected to underlie the pheromonal memory. In the previous meeting, we showed that antidromic tetanic stimulation of mitral cell axons induces long-term potentiation (LTP) at the mitral-to-granule cell synapse in slice preparations of the AOB, and that the late-phase LTP (L-LTP) is protein synthesis-dependent. Here we examined whether the protein synthesis-dependent L-LTP requires protein kinase M<sub>ζ</sub> (PKM<sub>ζ</sub>) activity, which is suggested to play an important role in LTP maintenance in the hippocampus as well as several forms of memory. Using AOB slices, we measured field EPSP derived from granule cells to examine the effects of PKM<sub>ζ</sub> inhibition on the L-LTP. Under bath application of a peptide inhibitor of PKM<sub>ζ</sub>, ZIP (5 μM), the tetanic stimulation failed to induce L-LTP; scrambled ZIP (5 μM) did not affect it. The results are consistent with the hypothesis that PKM<sub>ζ</sub> is required for LTP maintenance at the AOB synapse.

## 1PK-095

### Regulation of Channel Opening of Electrical Synapses between Retinal Neurons Is Present in the Cytoplasmic Sites of Gap Junction Connexins

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Electrical synapses are present in retinal neurons expressing the gap junction channel subunit, connexin (J. Neurosci., 2004). Our studies demonstrated cellular components in amacrine cells performing lateral interaction (J Intgra Neurosci, 2009). Electrical current spread through connections of amacrine cells is expected to synapse certain inhibitions. My recent studies revealed channel opening of gap junctions is regulated by high level of intracellular cyclic AMP as well as intracellular Ca<sup>2+</sup> concentration (Brain Res., 2012). In the present study, I investigated regulatory sites of connexins in electrical synapses by intercellular messengers under dual whole-cell patch clamp recordings of the cells. I examined how passage currents through electrical synapses are modulated by specific antibodies against connexin36 as well as intracellular application of cyclic nucleotides and by change of Ca<sup>2+</sup> concentration. Chelating intracellular Ca<sup>2+</sup> led us to observe large passage currents and transjunctional conductance (G<sub>j</sub>) between the cells (2.43 ± 1.21 nS, n=7). G<sub>j</sub> between the cells suppressed to 0.23 nS by intracellular application of cyclic AMP in pipette with 5mM concentration, compared with that of control condition. Intracellular application of an antibody against the cytoplasmic loop reduced G<sub>j</sub> (0.98 ± 0.23 nS, n=4). Application of another antibody against the extracellular loop leaves G<sub>j</sub> as in the control level (1.79 ± 0.51 nS, n=4). These results demonstrate that regulation of channel opening of gap junctions is present in the cytoplasmic sites of connexins.

## 1PK-096

### Inhibitory action of levobupivacaine on TTX resistant (Na<sub>v</sub>1.8)sodium channel

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Levobupivacaine (popsaine<sup>®</sup>), S-isomer of bupivacaine, is clinically used as a local anesthetic and less cardiotoxic than racemic bupivacaine. Our previous studies have shown that levobupivacaine selectively inhibited action potentials elicited in noxious C and A $\delta$  fibers. Little is known, however, which kinds of Na channels are preferentially suppressed by levobupivacaine. In this study, we examined inhibitory action of levobupivacaine on TTX resistant Na<sub>v</sub>1.8 sodium channel and compared the inhibitory action of levobupivacaine with those of other local anesthetics. Na<sub>v</sub>1.8 channel was expressed in a dorsal root ganglion-derived cell line, ND7/23. In the presence of TTX, depolarizing voltage-clamp pulses elicited an inward current and the voltage-current relationship was identical to those for Na<sub>v</sub>1.8 sodium channel shown in previous studies. Levobupivacaine, ropivacaine, bupivacaine and lidocaine all inhibited the Na<sub>v</sub>1.8 sodium current with IC<sub>50</sub>s of 178, 260, 140 and 318  $\mu$ M, respectively. Levobupivacaine showed a potent use-dependent block of Na<sub>v</sub>1.8 sodium currents. The present and our previous results suggest that S-isomer of local anesthetics, especially levobupivacaine, selectively inhibit noxious information by conduction block in C and A $\delta$  fibers expressing Na<sub>v</sub>1.8 channel.

## 1PK-097

### In vivo analysis of synaptic responses evoked in parasympathetic preganglionic nucleus in the rat lumbosacral spinal cord

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Coordinated movement of the bladder, urethra and external urethral sphincter in the lower urinary tract (LUT) is essential for the bladder filling and voiding. The activations of these organs are precisely controlled by the central nervous system including the spinal cord. Parasympathetic preganglionic nucleus (PGN) in the lumbosacral spinal cord plays an important role of in regulating different pelvic organ function including micturition and defecation. Although pharmacological and behavioral approaches have been well utilized to study the spinal control of LUT functions, the cellular mechanism, however, is still unclear. In this study, we developed an in vivo extracellular recording and whole-cell patch-clamp recording techniques to investigate how the spinal cord controls the LUT function at the synaptic level. The firing frequency of spinal dorsal horn neurons in the PGN was synchronously changed with the intravesical pressure. The electrophysiological properties of PGN in L6 spinal cord slices with an attached dorsal root were also studied. Monosynaptic excitatory postsynaptic currents mediated through A $\delta$  and C fiber were elicited in PGN neurons. The newly developed in vivo recording techniques in addition to the lumbosacral slice-patch recording are useful for elucidating the detailed mechanism for spinal control of LUT function.

## 1PK-098

### The mechanism of oral mucositis-induced pain hypersensitivity in rats

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Head and neck cancer patients under the chemo-radiation-therapy frequently suffer from oral mucositis, inducing severe pain due to stimulatory taste and touch of foods. However, pain mechanisms in oral mucositis have not been well clarified. In this study, we investigated pain sensitivities to taste and mechanical stimulations in oral mucositis in rats using our newly-developed assays. On day 2 after 50% acetic acid treatment for 30 s, the oral mucosa in the labial fornix region of inferior incisors showed severe infiltration of inflammatory cells and epidermolysis, resulting high tissue permeability, while it was cured within one week. The orofacial nocifensive behavior spontaneously was significantly increased and enhanced by sour taste stimulation to oral mucosa on day 2 after oral mucositis treatment. Furthermore, the withdrawal threshold of direct mechanical stimulation to oral mucosa was significantly decreased on days 2-5 after treatment. The withdrawal threshold was increased to cut off value with topical anesthesia of xylocaine in the oral mucosa. However, the withdrawal threshold after the topical anesthesia did not reach to the cut-off value in oral mucositis model. These results suggest that oral mucositis leads pain hypersensitivities to sour and mechanical stimulations, due to inflammation and destruction of mucosal barrier with high permeability in oral mucosa.

## 1PK-099

### The property of the Corneal Dry-responsive Neurons in the Trigeminal Nucleus in the Rat

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Corneal primary afferent neurons that respond to drying of the ocular surface have been previously characterized and found to respond to innocuous cooling, menthol, and hyperosmotic stimuli. The purpose of the present study was to examine the receptive field properties of second-order neurons in the trigeminal nucleus that respond to drying of the ocular surface. Single-unit recordings were performed in anesthetized rats. Dry-responsive corneal units were isolated in the brainstem, at the transition zone between the spinal trigeminal subnucleus caudalis and interpolaris. All dry-responsive corneal units responded to innocuous cooling of the ocular surface. In addition, these neurons responded to hyperosmotic stimuli and menthol application. Half of the neurons were also activated by noxious heat and low pH. Furthermore, neurons that were activated by noxious stimulation had a significantly lower response to cold and menthol stimulation. Drying of the ocular surface activates primary afferent and second-order neurons involved in basal tearing. Many of the dry-responsive neurons recorded in the trigeminal nucleus receive input selectively from cold sensitive primary afferents. However, an additional subset of dry-responsive neurons receives convergent input from cold cells and polymodal neurons. Alterations in the properties of dry-responsive neurons located in the trigeminal nucleus may contribute to dry eye syndrome.

## 1PK-100

### Ca<sup>2+</sup> concentration in endolymphatic surface cells modulates the endocochlear potential and transepithelial resistance in the guinea pig cochlea

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Using electrophysiological methods, we measured the changes in endocochlear potential (EP) and cochlear transepithelial voltage deflection (CoTVD) by current injection with or without asphyxia. Furthermore, we analyzed the data of CoTVD fitted to the exponential curve. We obtained the two separate exponential curves, one is a first cochlear transepithelial voltage deflection (fCoTVD, time constant ( $\tau_1$ ) is 17 ms), and the other is a slower cochlear transepithelial voltage deflection (sCoTVD, time constant ( $\tau_2$ ) is 400-600 ms). From this analysis, the present study demonstrated that (1) endolymphatic perfusion of nifedipine caused an increase in EP to +75-+95 mV with a significant decrease in fCoTVD, (2) endolymphatic perfusion of Gd<sup>3+</sup> produced a significant decrease in  $\tau_2$  without significant change in sCoTVD, (3) endolymphatic perfusion of (S)-Bay K8644 or 10 mM Ca<sup>2+</sup> produced the decrease in EP and a significant increase in  $\tau_2$  without significant change in sCoTVD. In all experimental condition including asphyxia,  $\tau_1$  is always constant at 16-17 ms. These findings suggest that 1) the change in cytosolic Ca<sup>2+</sup> concentration of endolymphatic surface cells may induce the change in conductance, capacitance, or positive potential in basolateral membrane in marginal cells, resulting in the change in EP.

## Poster Presentations Behavior Science, Biorhythm(1)

## 1PK-101

### Orexin neurons consolidate wakefulness and inhibit cataplexy in narcoleptic mice through two distinct pathways

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Loss of orexin neurons in humans is associated with narcolepsy, a sleep disorder characterized by excessive daytime sleepiness and cataplexy. Mice lacking orexin peptides, as well as those lacking orexin receptors (OX1R<sup>-/-</sup>; OX2R<sup>-/-</sup> mice), display a phenotype similar to narcolepsy, highlighting a critical role of orexin signaling in the maintenance of wakefulness. However, the precise neural mechanisms downstream of orexin neurons remain uncertain. We found that targeted restoration of orexin receptor expression in noradrenergic neurons of the locus coeruleus and in serotonergic neurons of the dorsal raphe in OX1R<sup>-/-</sup>; OX2R<sup>-/-</sup> mice differentially inhibited pathological fragmentation of wakefulness (i.e., sleepiness) and direct transitions from wakefulness to REM sleep (cataplexy-like episodes), respectively. Furthermore, pharmacogenetic activation of these neurons using DREADD technology significantly ameliorated narcolepsy of mice lacking orexin neurons. These results suggest that orexin neurons consolidate wakefulness and suppress cataplexy by activating locus coeruleus noradrenergic and dorsal raphe serotonergic neurons, respectively.

## 1PK-102

### An animal model of human sleep-wake cycle : methamphetamine - induced oscillation and behavioral rhythms in rats

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Chronic Methamphetamine (MAP) treatment to rats and mice induces behavioral rhythms independent of the suprachiasmatic nucleus (SCN), a master circadian clock. The MAP-induced behavioral rhythm is regarded as an animal model of human's sleep-wake cycle because of the several common features such as an internal desynchronization. The MAP-induced rhythm is considered to be based on the brain oscillatory system called as MAP-induced oscillation (MAO). But neither the site nor mechanism of MAO has been uncovered.

In this study, we treated *Period2-dLuciferase* transgenic rats with water containing MAP (0.005%) for 4 h daily from 10 : 00 (restricted-MAP : R-MAP) under LD cycles (lights on 06 : 00-18 : 00) for 14 days. Control rats were restricted water supply to 4 h. The behavioral rhythm was set by R-MAP and started to free-run from this phase after the termination of R-MAP, suggesting the entrainment of MAO. Circadian *Per2* bioluminescent rhythms were measured in the cultured brain tissues containing one of the following structures : the olfactory bulb ; caudate-putamen ; parietal cortex ; substantia nigra ; and SCN. The *Per2-dLuc* rhythms in the extra-SCN brain areas were not significantly phase-shifted by R-MAP. On the other hand, the rhythms in the SCN-lesioned rats phase-shifted to the treatment time. These results suggest that the *Period2* in the extra-SCN brain areas examined were regulated by the SCN more strongly than by MAO under R-MAP, and they could not be the site of MAO.

### 1PK-103

#### Temperature sensitivity of circadian oscillator in the suprachiasmatic nucleus *in vivo*—Ambient temperature as a time cue for maternal entrainment

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Maternal rhythms entrain the prenatal and neonatal circadian clock in the suprachiasmatic nucleus (SCN) before light entrainment is established. Ambient temperature is one of plausible time cues of the entrainment. Recent report demonstrated resistance of cultured SCN to temperature cue by keeping neuronal networks. However, little is known about the effect of temperature on the circadian clock in the SCN *in vivo*. In the present study, we examined the effects of cyclic changes of ambient temperature on the circadian clock of new born rats to identify the responsible time cues associated with maternal entrainment. Blinded neonatal rats carrying a clock gene, *Per2*, bioluminescence reporter were exposed to 10, 20, 15 or 30 °C during 6-h maternal separation at early light phase from postnatal day 1 (P1) to P5. On P6, the SCN was cultured for photometric monitoring of the *Per2* expression rhythm. The peak phase of the rhythm was delayed in a temperature dependent manner, suggesting a direct effect of temperature to the circadian clock. However, there was no difference in the phase-shifts between 20 and 30 °C, indicating that ambient temperature is not the sole factor for the phase-shift over 20 °C.  $Q_{10}$  values of the rhythm calculated based on the hypothalamic temperature during the maternal separation suggest that temperature compensation is an important physiological function for young pups whose body temperature is easily affected by maternal care.

### 1PK-104

#### Locomotor activity, memory and anxiety in diabetic rat with dementia

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Recently, it is noted that there exists a close relation between diabetes (DM) and dementia (DT). Therefore, we studied the effects of dementia on locomotor activity, memory and anxiety of diabetic rat. DM was induced by streptozotocin (60 mg/kg) and DT, by scopolamine (1.5 mg/kg). Four groups of rat were used; normal rat (C rat), DM rat, DT rat and diabetic rat with dementia (DM+DT rat). In these rats, locomotor activity, memory and anxiety were examined. As for the locomotor activity, the activity in both DM rat and DM+DT rat was significantly decreased, compared with that in C rat, while there was no significant difference between C rat and DT rat, or between DM rat and DM+DT rat. In the memory experiment using passive shuttle avoidance system, the avoidance from electric shock in DM+DT rat significantly decreased, compared with that in both C rat and DM rat, resulting in raised "no-reaction". Next, in the experiment of anxiety using elevated plus-maze, DT rat and DM+DT rat had a shorter time to stay on the open arm than C rat, suggesting that dementia may be responsible for the anxiety, whereas no significant difference was observed in between C rat and DM rat or in between DT rat and DM+DT rat. These results suggest that DM leads to the decreased activity in locomotion, and that in the memory the disease worsens the condition of dementia.

### 1PK-105

#### The behavioral development in the GlyT2-deficient mice

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Glycine transporter 2 (GlyT2) is present in axons and presynaptic terminals of inhibitory neurons that secrete glycine. Moreover, the GlyT2 gene was identified as a candidate disease gene in human hereditary hyperekplexia. GlyT2-deficient mice were born normally but they died during the second postnatal week, displaying severe motor alterations at P10. To investigate the effects of the GlyT2 gene deletion on the behavioral development focused on early developmental stage, in this study we performed the behavioral tests of GlyT2<sup>-/-</sup> mice and their littermates, GlyT2<sup>+/-</sup> and GlyT2<sup>+/+</sup> mice at P0-10. The behavioral tests were as following: rolling-over; pivoting; creeping; walking and swimming. There were no differences in the results of behavioral tests and body weight between GlyT2<sup>+/+</sup> and GlyT2<sup>+/-</sup> mice. On the other hand, the body weight was lower in GlyT2<sup>-/-</sup> mice than in GlyT2<sup>+/-</sup> and GlyT2<sup>+/+</sup> mice at P3. Rolling-over, pivoting and creeping of GlyT2<sup>-/-</sup> mice were poorer outcome than those of GlyT2<sup>+/-</sup> and GlyT2<sup>+/+</sup> mice. The motor coordination in swimming of GlyT2<sup>-/-</sup> mice were worse than that of GlyT2<sup>+/+</sup> and GlyT2<sup>+/-</sup> mice after P7. The excessive muscle-tone was observed at P5 GlyT2<sup>-/-</sup> mice. These results suggested that the GlyT2<sup>-/-</sup> mice could not coordinate between forelimb and hind legs because of the excessive muscle-tone and/or failure of the coordination.

### 1PK-106

#### Somatostatinergic clock gatekeeping in the suprachiasmatic nucleus controls entrainment to photoperiod

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Somatostatin (SST) neuron locates dorsomedial compartment and projects to ventrolateral compartment (VL) of the mammalian core clock, suprachiasmatic nucleus. SST is supposed to control clock resetting by light because VL receives the light signal for clock resetting from the retina via retinohypothalamic tract. Here, we report SST depleted mutant mice entrained to long photoperiodic condition with delayed phase relationship to environmental light. In delayed entrained SST mutant mice, irregular mPer1 expression is observed at the dusk in VL. Single 4 hours evening light restriction normalized this VL mPer1 expression and delayed activity rhythm phase. Our results indicate SST contributes to entrainment to natural photoperiod by controlling VL evening light responsiveness specifically under long photoperiod.

## 1PK-107

### Localization and circadian expression rhythm of tryptase in the suprachiasmatic nucleus of mice

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Circadian rhythms are endogenous self-sustained oscillations with periods of 24 h that regulate physiological and behavioral processes and respond to environmental cues such as the light/dark cycle. In mammals, the suprachiasmatic nucleus (SCN), which comprises a pair of structures, in the hypothalamus regulates most circadian behaviors. Vasoactive intestinal peptide (VIP) is a neuropeptide that contributes partly to the mechanism by which SCN neurons are coupled to each other. Neurons containing VIP are mostly localized in the ventral (core) region of the SCN, and the efferent fibers are distributed in the lateral (shell) region or the subventricular zone. Tryptase is the most abundant secretory granule-derived serine protease contained in mast cells. In vitro experiments suggested that VIP was cleaved by tryptase specifically at 2 sites. In this study, we investigated the localization and circadian expression rhythm of tryptase in the SCN of mice. Tryptase is strongly expressed in the SCN and lungs. Fluorescence immunocytochemistry showed that the antibody against tryptase stained some cells positive for glial fibrillary acidic protein and indicated that tryptase is located in the shell region of the SCN. The tryptase expression levels exhibited a circadian rhythm in the SCN and lung with peak levels at circadian time (CT) 0 and CT 12, respectively. These data suggest that tryptase is closely related to the cleavage and/or degradation of VIP in the SCN and may be a crucial component of the mammalian circadian timekeeping machinery.

## 1PK-108

### The effects of the first night on sleep parameter measured in the home of subjects

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This study is to investigate the effects of the first night on sleep parameter measured in the home of subjects compared with the following night. The fourteen healthy women aged 19-49 years participated in this study for two nights during sleep. We examined both the RR interval variability for two and a half hours from onset of the sleep and the subjective evaluation about sleep quality. The subjects answered the questionnaire about sleep quality, such as sleep onset latency, awaked time during sleep, subjective evaluation using visual analog scale. Autonomic function was estimated by the Lorenz plot and time domain analysis for RR interval. The hours of sleep and sleep onset latency in the first night and the following night were 389.8±73.0 min and 398.5±81.7 min, 25.3±16.5 min and 24.1±18.1 min, respectively. The RR interval during sleep increased in the beginning, and decreased, and then increased again in both cases of the first night and the following night. The periods of the ultradian rhythm of RR interval were 95.1±21.3 min and 92.7±23.0 min in those nights, respectively. There was no difference in the subjective evaluation about the sleep quality between the first night and the following night. The results of heart rate variability also did not show any significant difference in those nights. We concluded that we observed absent first night effect compared with the following night in the home of subjects.

## 1PK-109

### Influence of testosterone administered during the sexual pre-maturation period on retention of extinction memory after conditioned taste aversion in male mice

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After acquisition of the CTA memory by the application of a novel taste as the conditioned stimulus (CS) and a malaise as the unconditioned stimulus (US), mice acquire the extinction memory by repeated presentation of CS without US. When sexually mature or immature male mice (C57BL/6) underwent the conditioning period followed by the extinction period, mature mice showed higher retention of extinction memory than immature mice. We castrated sexually immature males and chronically administered testosterone at the sexual pre- or post-maturation period. As a result, the pre-administration enhanced the retention of extinction memory but the post-administration did not. In addition, sexually immature males showed the higher expression level of androgen receptor gene than mature males in the ventral medial prefrontal cortex and amygdala, which are related to the extinction of CTA memory. These results suggest that the testosterone acting during sexual pre-maturation period plays an important role for the maturation of mechanism of retention for extinction memory. We will report the difference of testosterone action on the vmPFC or amygdala between pre- and post-maturation periods, using the cultured brain slice.

## 1PK-110

### Induction mechanism of thirst in hangover : Drinking behavior and central c-Fos expression by intraperitoneal injection of ethanol in rats

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We sometimes experience thirst sensation, as well as headache, vomiting and shivering in hangover. It is understood that the thirst is caused by alcohol diuresis due to decrease of vasopressin from the posterior pituitary. However, the whole mechanism of ethanol action on induction of thirst in hangover is not well-understood. We designed to investigate action of ethanol and the metabolite acetaldehyde on water and salt intake, by using rats. In two-bottle test of tap water and 0.3M NaCl, water intake was significantly increased 3-7 hrs after intraperitoneal injection of ethanol (2.5g/kg) and ethanol+cyanamide (10mg/kg), compared to saline injection. The amount by ethanol+cyanamide was higher than by only ethanol. The salt intake was also increased after ethanol and ethanol+cyanamide, although not significantly. The number of c-Fos immunopositive neurons after the same injection was tested in several nuclei in the central nervous system, which are related to the body fluid balance, such as the organum vasculosum laminae terminalis, the medial nucleus of preoptic area, the subfornical organ, the supraoptic nucleus and the paraventricular nucleus. The number of c-Fos positive neurons was increased after the injection of ethanol and ethanol+cyanamide. These suggest that ethanol and acetaldehyde induce water and salt intake through the thirst center in the brain.

## 1PK-111

### Development of a tympanic thermometer and the profiles of human circadian rhythms

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Many reports have demonstrated that discordance between circadian rhythms and the work-sleep schedule causes various diseases such as sleep disorder, affective disorder, hyperlipidemia and hypertension, and increase the ratio of breast and prostate cancer. The demand to know the profile of individual circadian rhythm is increasing but there has been no handy apparatus to know the phase of individual circadian rhythm at home. Because tympanic temperature corresponds to the core body temperature that reflects the main clock in the circadian center, one day monitoring of the deep temperature would show the phase of the center of the circadian clock. Here we developed a tympanic thermometer equipped with two directional infrared sensors. One sensor faces the tympanic membrane, and the other faces the external auditory canal. There was a difference in the values measured by the two sensors, high in the former and low in the latter, which suggests that the former sensor nicely faces the tympanic membrane. The thermometer showed the diurnal variation with a trough during the night and a peak during the day. The findings suggest that the temperature circadian rhythm shown by the apparatus seems to be in phase with the circadian rhythm of core body temperature and that the thermometer is a easy to use apparatus to reveal the phase of the circadian clock.

## 1PK-112

### Pharmacogenetic stimulation of preoptic area GABAergic neurons increase NREM sleep

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Neurons in the preoptic area (POA), especially the ventral lateral preoptic area (VLPO) and the median preoptic nucleus (MnPO), fire rapidly during sleep and cease firing during wakefulness. These neurons carry GABA, and thought to play an important role in initiation and maintenance of sleep by sending inhibitory projections to the arousal systems that reside in the brain stem. Recently, several evidence have suggested that orexinergic neurons in the hypothalamus, which play a critical role in maintaining arousal, are also influenced by these neurons.

To elucidate the roles of these neurons in regulation of orexin neurons, we pharmacogenetically stimulate these sleep-active neurons. We used Gad1-Cre knock-in mice, in which Cre recombinase is exclusively expressed in GABAergic neurons. And we used an adeno-associated viral vector to deliver hM3Dq or hM4Di to Cre-expressing GABAergic neurons in the POA.

Pharmacogenetically stimulation of POA GABAergic neurons resulted in increase of NREM sleep. And optogenetic stimulation of POA GABAergic neurons inhibited activity of orexin neurons. These observations suggest that the POA GABAergic neurons are important inhibition of arousal regions including hypothalamic orexin neurons.

## 1PK-113

### Effects of ethanol and acetaldehyde on subfornical neurons in rats

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Thirst sensation may be caused by central action of ethanol and the metabolite acetaldehyde on the thirst center in the brain, as well as by alcohol diuresis due to decrease of vasopressin from the posterior pituitary. To answer this question, we investigated effects of ethanol and acetaldehyde on neurons in the subfornical organ (SFO), which is known to be a thirst center in the brain, by using slice preparation in rats. Extracellular (Multichannel Systems, Germany) and patch clamp recording were performed. In half of SFO multi-units, ethanol (5-200 mM) increased firing rates in a dose-dependent manner. In a small number of the multi-units, the firing rates were decreased. In current clamp recording, ethanol induced membrane depolarization with the increased firing rates. The depolarization was still observed in TTX-containing solution. Meanwhile, acetaldehyde (3-100  $\mu$ M) showed inhibitory action on most SFO multi-units in a dose-dependent manner. Excitatory responses prior to the inhibitory responses were sometimes observed. The excitatory responses were suppressed by the application of glutamatergic and GABAergic receptor blockers. These suggest that ethanol excites and acetaldehyde inhibits SFO neurons, modulating water and salt intake in rats.

## 1PK-114

### A critical role of serotonergic inhibitory projections to orexin neurons in regulation of sleep/wakefulness states

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Orexins are a pair of neuropeptides implicated in regulation and maintenance of sleep/wake states. We previously reported that virtually all orexin neurons were directly and potently inhibited by serotonin via an activation of 5HT1A receptors. Since serotonin plays an important role in the sleep/wake regulation, serotonin-mediated inhibition of orexin neurons might be important for physiological regulation of sleep/wake states. To address this hypothesis, we generated a mice in which the genes encoding 5HT1A receptors are specifically deleted in orexin neurons (5HT1A flox/flox ; orexin-Cre mice). Histological analyses as well as patch clamp recordings of orexin neurons revealed that serotonin failed to hyperpolarize most of orexin neurons in 5HT1A flox/flox ; orexin-Cre mice, while it strongly inhibited these cells in control slices. These mice showed several abnormalities in sleep architecture. During light period, these mice showed decreased episode duration of both wakefulness and NREM sleep, shortened inter-REM interval, and increased numbers of each episode as compared to control groups. Conversely, sleep/wake states of these mice appeared almost normal during dark period. These results suggest that the inhibitory, feedback projection of serotonergic neurons to orexin neurons plays an important role in regulation of sleep/wake state, especially during light period.

## 1PK-115

### Role of the *Bmal1* gene in the regulation of cardiac function in mice

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Cardiac function is subject to the diurnal regulation governed by the circadian clock system in mammals. Dysfunction of the circadian system has been shown to induce a malfunction of many organs including the heart. However, little is known about how the circadian clock modulates physiological processes required to maintain cardiac function. Here we show that a heart-specific knockout of *Bmal1*, a core circadian clock gene, leads to age-dependent cardiomyopathy with alteration in cardiac energy metabolism in mice. Genome-wide gene expression analysis revealed that expression of genes controlling energy metabolism such as mitochondrial biogenesis and ATP production is decreased in the heart tissue of knockout animals. In addition, both function and morphology of mitochondria in cardiomyocytes are altered in heart-specific *Bmal1* knockout mice. Taken together, our results indicate that the circadian clock in the heart plays an important role in regulating mitochondrial energetics, and thereby maintains cardiac function in mammals.

## 1PK-116

### Identification of novel cell-penetrating peptides for targeting glioblastoma

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Glioblastoma (GBM) is the most common intractable malignant brain tumor. Despite recent progress of treatment technologies, prognosis in this disease remains very poor. Therefore, there is an urgent need to develop of more effective new diagnosis and treatment approach. In the previous study, Cell-penetrating peptides (CPPs) have attracted the attention as a potential biomedical tool. However, there are few reports about CPPs, which delivers its payload such as drugs and imaging agents to target cells specifically in cancer treatment. This study aims to develop novel CPPs targeted for GBM. Here we conducted screening of CPPs targeting human GBM cell lines U87MG using random peptides library constructed from mRNA display. Based on amino-acid sequence of the candidate CPPs obtained from screening, we synthesized fluorescent peptides and examined transduction efficiency for various cell lines by confocal laser scanning microscopy. We found candidate CPPs that incorporated into GBM cell lines higher selectively and efficiently in comparison with cell lines derived from histologically different types. In addition, modification of amino-acid of the CPPs resulted in increase of cell-penetrating activity. The present study, it suggested that the selected peptides might act as tumor-specific delivery tool for GBM. Further studies are required to evaluate the distribution of these CPPs in tumor tissue of GBM in vivo.

## 1PK-117

### PI3 kinase/Akt/HIF-1 $\alpha$ pathway is associated with hypoxia-induced EMT in fibroblast-like synoviocytes

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Invasion of fibroblast-like synoviocytes (FLSs) is considered to play a critical role in the pathogenesis of rheumatoid arthritis (RA). However, its effect on invasion of RA-FLSs remains elusive. Here, we found that with exposure to hypoxia, FLSs experienced epithelial-mesenchymal transition (EMT), with increased cell invasion. We demonstrated that hypoxia-induced EMT was accompanied by increased hypoxia-inducible factor (HIF) -1 $\alpha$  expression and activation of Akt. After knockdown or inhibition the expression of HIF-1 $\alpha$  by small interfering RNA (siRNA) or genistein (Gen), the EMT and invasion ability of FLSs were obviously receded. Furthermore, we showed that inhibition of PI 3K/Akt and HIF-1 $\alpha$  could alleviate the outcomes, radiology progression, synovial hyperplasia and inflammatory cells infiltration in collagen-induced arthritis (CIA) rats. These results suggest that PI3 K/Akt/HIF-1 $\alpha$  pathway plays a pivotal role in hypoxia-induced EMT and invasion of RA-FLSs.

## Poster Presentations Pathophysiology(I)



## 1PK-118

### Clinical significance of expression levels of PTENmRNA and hTERT, DNA-PKcs in bone marrow for childhood acute leukemia

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**Objective :** To study the expression and clinical significance of tumor suppressor gene, DNA repair gene and telomerase component in acute leukemia. **Methods :** 45 children with acute leukemia were studied including 25 cases of first diagnosed and 20 cases of remission ; 20 cases in control group. (1) Detect the expression levels of PTENmRNA by in situ hybridization and the expressions of hTERT and DNA-PKcs proteins by immunohistochemistry on paraffin-embedded sections of marrow biopsy samples. Compare the differences of three groups. (2) Observe the relationship between curative effects and the expression levels of three items. **Results :** (1) In first diagnosed group, positive rates of PTENmRNA, hTERT and DNA-PKcs are 60.0%, 84.0%, 72.0%. Differences of the expressions between first diagnosed group and remission group or control group are notable ( $P < 0.05$ ). (2) After treating for 4 weeks in 20 cases of first diagnosed children, 5 cases cannot get complete remission (CR) ; Among them, there are 4 cases with loss expression of PTENmRNA. **Conclusions :** (1) Childhood acute leukemia shows loss expression of PTENmRNA and DNA-PKcs protein, high expression of hTERT, indicating that tumor suppressor gene, DNA repair gene and telomerase have an important role in the occurrence of childhood acute leukemia. (2) Expression levels in first diagnosed group and remission group are great different, which is beneficial to judging curative effect.

## 1PK-119

### The endocannabinoid 2 - arachidonoylglycerol suppresses excitatory synaptic input to the dentate gyrus and ameliorates seizures

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More than 30 percent of patients with epilepsy have intractable seizures. Previous studies demonstrated that endocannabinoids cause retrograde suppression of excitatory synaptic transmission in the hippocampus during seizures, which oppose to further development of seizures. However, it is unclear which endocannabinoid suppresses seizures and how endocannabinoid signaling changes excitability in the hippocampus during seizures. We have recently demonstrated that 2-arachidonoyl glycerol (2-AG) produced by diacylglycerol lipase  $\alpha$  (DGL $\alpha$ ) is the endocannabinoid that mediates retrograde synaptic suppression. The present study aimed at elucidating roles of 2-AG during seizures in the dentate gyrus. Stimulus and recording electrodes were implanted into the angular bundle and the dentate gyri, respectively, of adult DGL $\alpha$  knockout mice and wild-type littermates. Current source density in the dentate gyrus was calculated from local field potentials. We found that seizures evoked by perforant path stimuli were significantly longer in DGL $\alpha$  knockout mice than in wild-type littermates. Furthermore, current sink around the inner molecular layer of the dentate gyrus was significantly larger during seizures in DGL $\alpha$  knockout mice than in wild-type mice. These results suggest that 2-AG suppresses excitatory input around the inner molecular layer and ameliorates seizures in the dentate gyrus.

## 1PK-120

### Corticosteroids provide suppressive signals for microglial activation during acute stress

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Our previous studies demonstrated that exposures of animals to acute stress immediately induce microglial morphological activation in the brain. The mechanism of the stress-induced microglia activation remains to be elucidated. Here we investigated the effects of adrenal corticoids on microglial activation following acute stress. We compared microglial activation using in-vivo experiment models, such as adrenalectomized, sham-operated, and adrenalectomy plus corticosterone administered rats, following 2 h period of acute water restraint stress. We found that : 1) Acute stress induced microglial activation in sham-operated rats ; 2) Acute stress in adrenalectomized rats robustly enhanced microglial activation ; 3) Corticosterone treatment in adrenalectomized rats significantly reduced the effects of adrenalectomy. Thus, the present study demonstrates that, under the physical/emotional stress, corticosteroids may provide suppressive signals for microglial activation in the brain, contributing to the suppression of inflammatory changes in the brain.

## 1PK-121

### Decreased urate excretion from intestine is a common cause of hyperuricemia

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ABCG2/BCRP is identified as a high-capacity urate exporter and its dysfunction raises gout/hyperuricemia risk. Generally, hyperuricemia has been classified into urate "overproduction type," and/or "underexcretion type," based on only renal urate excretion without considering extra-renal pathway such as gut excretion. Here we show that decreased extra-renal urate excretion caused by ABCG2 dysfunction is a common mechanism of hyperuricemia. Clinical parameters, including urinary urate excretion (UUE), were examined in 644 Japanese male outpatients with hyperuricemia. Paradoxically, UUE and risk ratio of urate overproduction were significantly increased by ABCG2 dysfunction. In *Abcg2*-knockout mice, serum uric acid levels and renal urate excretion were increased, while intestinal urate excretion was decreased. Together with high ABCG2 expression in extra-renal tissues, these results suggest that the "overproduction type" in the current concept of hyperuricemia should be renamed "renal overload type," which consists of two different mechanisms, "extra-renal urate underexcretion" and genuine "urate overproduction." This new concept will elucidate the pathophysiology of hyperuricemia and gout.

## 1PK-122

### The effects of increase of BDNF in hippocampus on recovery of cognitive function in cerebral infarction model rats

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We investigated the effectiveness of exercise in acute stage on recovery of cognitive function after stroke. The injection of 3,000 particles of microsphere (MS, 45µm) through the right internal carotid artery induced small infarctions in cortex, striatum and hippocampus in rats. The operated animals presented mild motor paralysis at 24 hours after MS injection. Mild exercise was started at the 24 hours after MS injection by a treadmill (15 m/min×30 min/day for 7 days) in the acute-phase exercise group. A late-phase exercise group started exercise at 8 days after MS injection, and non-exercise group and sham operated group were also examined as control groups. The cognitive function was evaluated by Morris water-maze test and significantly improved in the acute-phase exercise group compared with the other groups. On the other hand, both acute-phase and late-phase exercise groups showed an elevation of brain-derived neurotrophic factor (BDNF). The rats that were infused BDNF into their hippocampus continuously by osmotic pump in acute-phase after stroke showed significant recovery in cognitive function, however, the rats that were infused BDNF in late-phase showed no recovery. These results suggest that exercise in acute-phase after onset of cerebral infarction and the resultant elevation of BDNF level is critical to improve cognitive function.

## 1PK-123

### Continuous grasping of polyethylene-fiber cushion induced prompt recovery of hand contracture and rigidity due to cerebral infarction

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3-D high repulsion cushion (cushion made by tangled fine polyethylene fibers) has a specific repulsive power. The blood flow of the prefrontal cortex was significantly increased by simple stepping on the cushion. We paid attention to the specific nature of the this cushion: high repulsive power, elasticity and ventilation, and devised a special grip cushion for treatment of hand contracture and rigidity, as hands are more rich in sensory receptors and afferents, thus expected to be stimulated more significantly. Continuous grasping of the cushion made the recovery from contracture and rigidity of fingers and hands of patients of cerebral infarction in a short duration. It also stimulated the recovery of infection and erosion of the skin of hands. These beneficial effects of the cushion may due to continuous relaxation of flexors and continuous stimulation of extensors that work similar to continuous rehabilitation for 24 hours unconsciously. The further mechanism of recovery from contracture and rigidity will be discussed.

## 1PK-124

### Investigation of the molecular mechanisms governing muscle contracture caused by immobilization

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To investigate the mechanisms underlying muscle contracture caused by prolonged immobilization. We immobilized rats in shortened positions for 1, 2 and 4 wk, and the soleus muscle was used for the evaluations. The mRNA expression of HIF-1 $\alpha$ , TGF- $\beta$ 1,  $\alpha$ -SMA, type I collagen (COL1) and type III collagen (COL3) was detected by RT-PCR, and the presence of myofibroblasts (MF;  $\alpha$ -SMA positive cell) was detected by immunostain. The results showed that the mRNA level of HIF-1 $\alpha$  was significantly higher in the 4-wk immobilized rats than in the controls, whereas the mRNA levels of TGF- $\beta$ 1,  $\alpha$ -SMA, COL1 and COL3 were significantly higher in the immobilized rats than in the controls, irrespective of the duration of immobilization. Furthermore, the mRNA levels of HIF-1 $\alpha$  and COL1 and the number of MF were significantly higher in the 4-wk immobilized rats than in the 1 and 2-wk immobilized rats. Collectively, these results suggest that the upregulation of TGF- $\beta$ 1 may activate fibroblasts and promote the differentiation of fibroblasts to MF, and these changes may lead to an increase in COL1 and COL3. In addition, we observed that the soleus muscle became hypoxic after immobilization for 4 wk. Therefore, we hypothesize that these alterations in the skeletal muscle may influence the progress of muscle contracture.

## 1PK-125

### Effect of forced-use of impaired forelimb on cortical map reorganization after capsular hemorrhage in rats

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Forced impaired limb use (FLU) is an effective rehabilitative strategy after stroke. FLU effect on motor cortex reorganization after internal capsule hemorrhage (ICH) was investigated in ICH rats. Wistar rats received collagenase type IV (15 Units/ml, 1.4µl) into the internal capsule and a one-sleeve cast was fitted at 1 day after ICH to enhance the impaired forelimb use in their daily activities for 7 days. We carried out serial measurements of motor map in ipsilesional motor cortex by intracortical microstimulation (ICMS) at 5 day before and 1, 10 and 26 day after ICH. Motor functions (reaching, stepping) were assessed at 12 and 28 day. Forelimb movements were not elicited at all by ICMS in the ipsilesional motor cortex on day 1 after ICH. On day 10, ICMS-evoked forelimb movements were detected in small area of caudal forelimb area (CFA) of the ipsilesional motor cortex in ICH rats. FLU led to expansion of the forelimb area in CFA and rostral forelimb area (RFA) compared to the non-treated control. The forelimb motor maps were further extended in FLU group at day 26. Motor assessments showed the better recovery in FLU-treated ICH rats than non-treated ICH rats. Injection of muscimol (1µM, 1µl) into the newly-emerged forelimb areas of CFA and RFA resulted in impairment of the recovered movements. These data suggested that FLU promoted the reorganization of ipsilesional motor cortex as a substrate for functional recovery.

## 1PK-126

### Neuroprotective effects of a water-soluble $\alpha$ -lipoic acid derivative, DM-HisZn, on rat brain tissue after ischemia-reperfusion injury : spin resonance analyses study

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We examined neuroprotective effects of a new  $\alpha$ -lipoic acid derivative with water-solubility, sodium zinc histidine dithiooctanamide (DM-HisZn) after ischemia-reperfusion injury (IRI). Rat brain slices were superfused with well-oxygenated artificial cerebrospinal fluid. IRI consisted of halt of superfusion for 1 hour followed by reperfusion for 2 hours. We evaluated energy metabolism by measuring phosphocreatine (PCr) in brain slices before, during and after IRI using phosphorous nuclear magnetic resonance (<sup>31</sup>P-NMR) spectroscopy. Radical scavenging activity of DM-HisZn was assessed by electron spin resonance (ESR) spectroscopy using DMPO or CYPMPO as spin traps. Antioxidant activity of DM-HisZn on peroxidation of brain homogenate was evaluated by thiobarbituric acid reactive substances (TBARS) assay. In <sup>31</sup>P-NMR study, better recovery of PCr level after IRI was demonstrated as the concentration of DM-HisZn increased. In ESR study, DM-HisZn effectively scavenged DPPH, superoxide anion and ascorbate free radicals with EC<sub>50</sub> of 0.1 mM, 2 mM, and 1 mM, respectively, but no scavenging activity was observed for hydroxyl radicals. DM-HisZn dose-dependently inhibited peroxidation of brain homogenate by hydroxyl radicals (EC<sub>50</sub> 0.7 mM) and by carbon-center radicals (EC<sub>50</sub>>1mM). It is speculated that DM-HisZn might be neuroprotective against IRI through its antioxidant activity.

## 1PK-127

### Neuroprotective effects of acetyl-L-carnitine on rat brain after ischemia-reperfusion injury : spin resonance analyses study

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Acetyl-L-carnitine (ALCAR), a carnitine ester, is a compound which exists in organisms. It provides acetyl-CoA to TCA cycle and maintains aerobic energetics in electron-transport system. In addition, it is speculated that ALCAR inhibits peroxidation of tissue as an antioxidant. We assessed the recovery of brain energy metabolism of rat brain slices from ischemia-reperfusion injury (IRI) to evaluate neuroprotective effects of ALCAR using phosphorous nuclear magnetic resonance (<sup>31</sup>P-NMR) spectroscopy. Also radical scavenging activity of ALCAR was examined by electron spin resonance (ESR) spectroscopy. Antioxidant activity of ALCAR on peroxidation of brain homogenate was evaluated by TBARS (thiobarbituric acid reactive substance) assay. <sup>31</sup>P-NMR showed no significant difference in recovery levels of phosphocreatine between ALCAR (3 mM) and control, indicating no neuroprotective effect in energetics. TBARS assay demonstrated that ALCAR inhibited peroxidation of brain homogenate by carbon-centered radicals up to 32-49% at concentrations of 0.1  $\mu$ M-1 mM, whereas no inhibition was observed against hydroxyl radicals. Direct radical scavenging activity was observed for DPPH, superoxide anion, and ascorbate free radicals, but not for hydroxyl radicals by ESR. These results might suggest that the antioxidant activity be the primary mechanism of neuroprotective effects of ALCAR, and its energetic property be secondary.

## 1PK-128

### Human erythrocytic and megakaryocytic lineages cell lines actively convert the oxidized form of albumin to the reduced form : affect of redox state of albumin and physiological significance

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Human serum albumin (HSA) is composed of human mercaptoalbumin (HMA) with cysteine residue at position 34 (Cys-34) having reducing power, reversibly oxidized human non-mercaptoalbumin (HNA-1), and strongly oxidized human non-mercaptoalbumin (HNA-2). It is already known that the percentage of oxidized albumin increases in several diseases, such as diabetes mellitus, chronic renal failure, and hepatic disease. And it is known that the percentage of oxidized albumin increases with age. We have recently revealed that human aortic endothelial cells and human leukocyte cell lines showed conversion of HNA to HMA using by HPLC system. And we showed that reduced albumin is considered to participate in redox regulation of blood vessel systems. Hemangioblast is defined as a precursor cell with the potential of to differentiate into both endothelial and hematopoietic cell lineages. We investigated whether hematopoietic lineage cells, especially erythrocytic and megakaryocytic lineages, have reductive activity or not using cell lines (HEL.92.1.7, MEG-01). In the absence of cells, the HMA content decreased because of auto-oxidation. However, HMA content of HEL.92.1.7 and MEG-01 cells gradually increased until 24h. These results showed that reductive activity is probably common function among hematopoietic and endothelial cell lineages, and these cells are considered to participate in redox regulation in blood serum or bone marrow.

## 1PK-129

### Novel treatment for sepsis and systemic inflammatory response syndrome with bromvalerylurea

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Bromvalerylurea (BU) is an outdated hypnotic/sedative and currently is rarely used in clinics. Recently we found that BU suppressed nitric oxide (NO) production by LPS-stimulated microglia and macrophages, suggesting that BU treatment is useful for peripheral inflammatory disorders mediated by macrophages. In this study, BU was used to treat a rat model of sepsis induced by cecum-ligation and puncture (CLP) with symptoms of systemic inflammatory response syndrome (SIRS). Peritonitis progressed rapidly with marked swelling of the ileum 24 h after CLP. The swollen ileum contained a large number of leukocytes that accumulated in the Peyer's patches. Measurement of serum interleukin (IL) -6 protein levels in septic rats by ELISA was used as an index of severity of sepsis, and was elevated 20 fold. The increase of serum creatinine levels and deterioration of arterial blood gas data in septic rats suggested they had developed multiple organ failure (MOF) as a consequence of SIRS. The mortality rate of sepsis rats was 80% 1 week after CLP. BU was injected subcutaneously twice a day to treat sepsis in rats. BU decreased serum IL-6 levels 10-fold in 24 h and the mortality rate was suppressed by 50% at 1 week. Swelling of the ileum was ameliorated and accumulation of leukocytes was almost abolished. These data indicate that BU may be a novel effective treatment for sepsis-induced SIRS.

## Poster Presentations Environmental Physiology(1)

### 1PK-130

#### “Rose essential oil” inhalation inhibits stress-induced skin-barrier disruption in rats and humans

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Animals exposed to stressful stimuli exhibit neuronal activation of several brain regions such as the hypothalamic paraventricular nucleus (PVN), thereby increasing plasma concentration of ACTH and glucocorticoids. So called “green odor” has been reported by us to inhibit stress-induced activation of the HPA axis, leading to the prevention of the chronic stress-induced disruption of the skin barrier. We investigated whether rose essential oil, another sedative odorant, has inhibitory effects on the stress-induced increases in plasma concentration of glucocorticoids [corticosterone (CORT) in rats and cortisol in humans] and in the rat’s neuronal activity of the PVN, and stress-induced disruption of the skin-barrier in rats and humans. The results showed that in rats acute restraint stress evoked increases in plasma CORT that was significantly inhibited by inhalation of rose essential oil. Inhalation of the same odor reduced the acute stress-induced increases in the number of c-Fos-positive cells in the PVN. In both rats and humans, chronic stress-induced elevation of transepidermal water loss, an index of the disruption of skin-barrier function, was significantly inhibited by inhalation of rose essential oil, as were the increases in salivary concentration of cortisol induced in humans by chronic stress-exposure. The present results suggest that chronic stress-induced disruption of the skin barrier is prevented by the rose essential oil, possibly through its inhibitory effect on the HPA axis in rats and humans.

### 1PK-131

#### Responses in arterial pressure oscillation and muscle sympathetic nerve activity after long exposure to simulated microgravity

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It has been known both spectral power within the low frequency component of 0.04 to 0.15 Hz of systolic pressure (SAP\_LF) and muscle sympathetic nerve activity (MSNA) are increased during head-up tilt (HUT). MSNA during HUT is altered after space flight and after exposure to simulated microgravity. In the present study, correlation of SAP\_LF and MSNA were analyzed before and after 20days of -6 degrees of head down bed rest. Measurements were performed during head-up tilt (HUT) of -6, 0, 30, and 60 degrees. Mean AP did not change during HUT in pre-and post-bed rest, but MSNA in post-bed rest condition significantly increased during -6, 0, 30, and 60 degrees, compared to those in pre-bed rest condition. SAPLF also significantly increased in post-bed rest condition, compared to pre-bed rest condition during -6, 0, 30, and 60 degrees. SAPLF during 60 degrees of HUT also significantly increased, compared to that during -6 degree in post-bed rest condition. MSNA and SAP\_LF were both linearly and quadratically correlated with SAP\_LF ( $r^2=0.52$  and  $0.60$  for linear and quadratic regression, respectively). Thus, SAP\_LF could be an index of MSNA during HUT for both pre- and post-bed rest condition.

### 1PK-132

#### The molecular mechanism of early life stress for synaptic instability in somatosensory cortex

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Maternal-separation (MS) is one of the most important models of chronic stress-induced effects on neuronal development with implications for humans. It has previously been shown that MS causes not only the change in serum concentration of stress-induced hormones but also significant structural changes of neurons in several areas. We have recently reported that the stability of mushroom spine, which is one of the most stable structures in brain, was decreased in male mice following MS, using in vivo imaging (Takatsuru et al., 2009). Such male mice also showed the hypersensitivity to sensation test (von Frey test; Takatsuru et al., 2009). However, underlying mechanisms, especially, MS-induced molecular changes were not fully understood. In this study, we showed the relationship between somatosensory function and cortical field potential in MS mice. We also found that the release of glutamate in MS mice was significantly increased in SSC. Finally, we investigated the effect of MS on expression of glutamate receptor subunit proteins that are related to synaptic function, to clarify the mechanism of MS induced synaptic instability.

## 1PK-133

### Maternal behavior of female mice exposed to early life stress

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Child abuse has become serious problem throughout the world. It is said that many child abuse survivors suffer from negative outcome in later childhood and early adulthood. However, the mechanism underlying psychopathological problem has not yet fully been understood. Maternal deprivation (MD) model is one of the most important models for chronic stress-induced effects on neuronal development with implications for humans. It has previously been shown that MD causes not only the change in serum concentration of stress-induced hormones but also significant structural changes of neurons in several areas. In this study, we examined the maternal behavior in MD exposed female mice. We found that MD exposed mice showed low possibility of pregnant and high possibility of neglecting their pups. We also found that the body weight of pups from MD suffered mice significantly smaller than those from control mice. We also compared the hormonal level during lactating period (at post natal day 14) both in pups and mothers.

## 1PK-134

### Comparison of body temperature, skin blood flow and subjective thermal sensations during gradual decrease of ambient temperature between women in follicular and luteal phase and men

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The purpose of this study was to compare changes in body temperature, skin blood flow and subjective thermal sensations during gradual decrease of ambient temperature between women in follicular (FP) and luteal (LP) phase and men (M). Ten eumenorrheic women and ten men sat on chairs in a climatic chamber set at 30°C with relative humidity of 50% for 30 min. After thermal equilibrium was attained, room temperature was lowered to 24°C for 20 min and then reversed to 30°C for 20 min. Tympanic (Tty) and skin temperatures (Tsk), skin blood flow (SBF) of finger-tip and toe and ratings of thermal sensations were recorded throughout the experiment. Tty, Tsk and SBF decreased in almost all subjects with decreasing room temperature. Tty tended to be higher in LP than in FP throughout the experiment. There were no differences in degree of changes in Tty, Tsk, SBF and ratings of subjective thermal sensations between FP and LP in women. Tty tended to decrease more in M as compared with FP and LP, while changes in rating of thermal sensations tended to be smaller in M. It was suggested that body core temperature might be well maintained in response to gradual cold exposure, because of the enhanced sensation of cold, in women as compared with in men.

## 1PK-135

### The Correlation Between Score of Emotional Sweating and Menstrual Cycle by The Differences in Life Styles in Healthy Female Students

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Purpose : The purpose of this study was to examine the correlation between the grade of healthy lifestyle and the functional modulation of the autonomic nervous system and the menstrual cycle. Methods : Subjects were comprised of 79 healthy female students (21.8±5.2 years old) with regular menstrual cycles. The Scores of emotional sweating and healthy life habit (DIHAL..2) were compared among the follicular, luteal and menstrual phases. The Japanese version of the Profile of Mood State (POMS) was also examined. Results : Score of emotional sweating did not change during the whole menstrual cycle. However, there was correlation between score of emotional sweating and satisfaction with the present condition. In addition, there found correlation between the life habit and POMS score. Among all of these life habit, there was strong correlation between POMS score and sleep-related scores. For the healthy life style, there was correlation between POMS score and physical, mental and social health. Conclusion : Score of emotional sweating did not change during the whole menstrual cycle. This result supports earlier reports but there found a possibility that dissatisfaction for the present health condition influences the function of the autonomic nervous system in female students. Furthermore, these results suggest that repose factor and physical and mental healths influence the mood conditions.

## 1PK-136

### Chronic exposure to extremely low-frequency magnetic field stimulates corticosteroids secretion in both mice and Y-1 cell line

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An extremely low-frequency magnetic field (ELF-MF) is generated by power lines and by household electrical devices. Many studies have investigated whether ELF-MF has biological effect using cell line, animal model, human subjects and epidemiological study, but the effects remain controversial. We previously reported that chronic ELF-MF exposure affects corticosterone synthesis and depression-like behavior without enhancement of hypothalamic-pituitary-adrenal axis in mice. Here, we investigated the effect of 200 hours ELF-MF exposure (3 mT intensity) on plasma level of aldosterone, another corticosteroid which is downstream of corticosterone, and blood pressure in mice. As the results, plasma aldosterone significantly increased in ELF-MF-exposed mice compared with sham-exposed group. Besides that, the diastolic and mean blood pressure also increased. On the other hand, plasma norepinephrine and mRNA expression of adrenal tyrosine hydroxylase and pituitary vasopressin did not change. We also estimated the effect of 24 hours ELF-MF exposure (3 mT intensity) on mouse adrenal cortex-derived Y-1 cell line. ELF-MF exposed Y-1 cells showed significant increase of proliferative rate and the amounts of corticosteroids in medium compared with sham group. Our evidence suggests the possibility that high-intensity and chronic exposure to ELF-MF stimulates adrenal cortex directly both in vivo and in vitro.

## 1PK-137

### Impairment of rearing behaviors in CIN85-deficient mice

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Background : Cbl-interacting protein of 85 kDa (CIN85) is a scaffold/multi-adaptor protein implicated in the regulation of receptor endocytosis, cell division and the cellular cytoskeleton. Recently, we reported that mice deficient of CIN85 expression show hyperactive phenotypes. As a molecular explanation of this phenotype, the absence of striatal CIN85 causes decreased dopamine receptor endocytosis in striatal neurons in response to dopamine stimulation. Observation : We show here another phenotype besides the hyperactivity of CIN85 knockout (KO) mice that of maternal neglect of the newborns. Even though there is no difference in the number of live births from CIN85 KO homozygote, heterozygote and wild-type mothers, respectively, almost all pups born to CIN85 KO homozygote mothers have died within two days of birth. Moreover, despite of the fact that no defect in the mammary glands of CIN85 KO mother mice was found, milk was not detected in the stomachs of most pups. Importantly, when measuring the plasma levels of prolactin (PRL), we detected significantly decreased PRL levels in CIN85 KO mice compared to heterozygote and wild-type mice. PRL injection in CIN85 KO mice could however partially rescue the defect in maternal behavior. Conclusions : Our findings indicate an important role of CIN85 in the regulation of the dopamine-PRL system in the brain and provide new insight into a molecular explanation for maternal behavior.

## 1PK-138

### Cutaneous vascular responsiveness to carbon dioxide during heat stress

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In normothermic condition, extra-cranial cerebral blood flow (CBF) is not or less sensitive to changes in arterial carbon dioxide (CO<sub>2</sub>). Heat stress increases skin blood flow (SkBF) but not intra-cranial CBF. Skin blood flow responsiveness is capable of changing when SkBF is increased. We hypothesized that cutaneous vasodilation due to heat stress enhances SkBF responsiveness to change in CO<sub>2</sub>. Seven healthy male subjects (age : 21.4±1.1 yr) rested supine position with wearing a water-perfused suit. End-tidal CO<sub>2</sub> (P<sub>ET</sub>CO<sub>2</sub>) was modulated via standardized hypoventilation and hyperventilation. SkBF at forehead, cheek, and forearm was measured via laser-Doppler flowmetry and represent as cutaneous vascular conductance (CVC). In normothermic condition, CVC at all sites did not change during the perturbation. Heat stress was increased esophageal temperature from 37.1±0.3 to 38.6±0.3°C and decreased PETCO<sub>2</sub> from 40.5±2.2 to 34.3±5.7 mmHg. Then, the hypoventilatory challenge (P<sub>ET</sub>CO<sub>2</sub> : 42.7±3.1 mmHg) did not change CVC at the all sites. However the hyperventilatory challenge (P<sub>ET</sub>CO<sub>2</sub> : 22.4±2.5 mmHg) decreased CVC at forehead (2.20±0.48 to 2.08±0.44 au/mmHg) and cheek (3.38±0.67 to 3.13±0.60 au/mmHg), but not at forearm (2.09±0.54 to 2.01±0.65 au/mmHg). These results suggested that hypocapnia while hyperthermic individuals decreases skin blood flow at the extra-cranium but not forearm.

## 1PK-139

### The effect of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in primary culture of rat cerebellum

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We have previously reported that hydroxylated polychlorinated biphenyl (OH-PCB) and polybrominated diphenyl ether (PBDE) disrupt brain development through suppression of thyroid hormone (TH) receptor (TR)-mediated transcription. Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are degradation products of fluorotelomer alcohol and have been used in a wide variety of industrial and commercial products such as textiles and leather products ; fire fighting foams, metal plating, the photographic industry, photolithography, semi-conductors, paper and packaging, coating additives, cleaning products, and pesticides. The adverse effects in the developing brain are becoming of a great concern. Thus, we investigated the effect of PFOS/PFOA on nuclear hormone receptors-mediated transcription. Either PFOS or PFOA did not affect TR- and glucocorticoid receptor-mediated transcription in CV-1 monkey kidney fibroblast-derived clonal cells. PFOA but not PFOS suppressed steroid and xenobiotic receptor-mediated transcription. The change in dendrite arborization of Purkinje cells in primary culture of newborn rat cerebellum was also examined. PFOS but not PFOA suppressed TH-induced dendrite arborization of Purkinje cells in primary cerebellar culture. In conclusion, PFOS may disrupt TH-mediated cerebellar development but the mechanism of action may be different from those of OH-PCB or PBDE.

## 1PK-140

### Responses of serotonin release in the amygdala to cutaneous stimulation are mediated by corticotropin releasing factor in the dorsal raphe nucleus

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Our previous study showed that responses of serotonin (5-HT) release in the central nucleus of the amygdala (CeA) to cutaneous stimulation were abolished after intracerebroventricular administration of a corticotropin releasing factor (CRF) receptor antagonist,  $\alpha$ -helical-CRF (9-41), in anesthetized rats ; however, the site of its action has not been determined. In the present study we examined the contribution of CRF receptors in the dorsal raphe nucleus (dRN) where cell bodies of the 5-HT neurons to the amygdala are located. A coaxial microdialysis probe was stereotaxically implanted in the CeA and perfused with modified Ringer's solution at a speed of 1  $\mu$ l/min in anesthetized rats. Pinching and stroking stimulation were applied to the back for 10 min.  $\alpha$ -helical-CRF (9-41) was microinjected into the dRN. After vehicle injection, pinching stimulation increased the 5HT release, and stroking stimulation decreased the release. The responses of 5-HT to pinching and stroking were both abolished after injection of  $\alpha$ -helical-CRF (9-41). These results suggest that responses of 5HT release in the CeA to sensory stimulation of the skin are regulated via the CRF neurons terminated at the dRN.

## 1PK-141

### Lowering barometric pressure induces c-Fos expression in neurons of rat vestibular nucleus

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#### [Background & Aim]

Weather changes are suggested to aggravate vertigo. We previously reported that distinct neurons in the vestibular nucleus as well as trigeminal nucleus caudalis responded to lowering barometric pressure by electrophysiological study (Headache 2010; 50: 1449-1463). Here, we aim to examine whether the vestibular nucleus neurons of conscious rats are affected by lowering barometric pressure.

#### [Methods]

Awake rats were kept under the following two barometric pressure conditions; the lowering of barometric pressure by 20 hPa from the atmospheric pressure over a period of 10 min (LP; N=3), and normal atmospheric pressure in the decompression chamber for the same period as LP (control; N=5). After 120 min, the rats were sacrificed, and brainstem frozen sections were prepared. C-Fos expression was examined in the vestibular nucleus by immunohistochemistry.

#### [Results & Conclusion]

C-Fos expression was examined in the vestibular nucleus by immunohistochemistry. The number of c-Fos immunoreactive (IR) neurons in the vestibular nucleus was 21.2±19.7 in control groups. Meanwhile, in LP groups, the number of c-Fos-IR neurons was 332.7±73.4, and showed a significant increase compared with control (p<0.001, student's t-test). The data imply that lowering barometric pressure could affect the vestibular system.

## 1PK-142

### Estrogen replacement attenuates intermittent hypoxia-induced hypertension in ovariectomized rats

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Clinical studies suggest that obstructive sleep apnea is associated with systemic hypertension. Intermittent hypoxia (IH) is used to mimic the hypoxemia that occurs during sleep apnea. We examined whether chronic estrogen replacement in ovariectomized rats affects the blood pressure (BP) elevation induced by IH. Female Wistar rats aged 9 wk were ovariectomized and implanted with radiotelemetry devices for BP and heart rate measurements. After 4 wk, the rats were assigned either to a placebo-treated (Pla; n=15) group or a group treated with 17β-estradiol (E2; n=15). Rats were exposed to IH for 2wk (90 s at 4% O<sub>2</sub> every 3 min, 8 h day<sup>-1</sup> during the light phase). Two wk later, the reactivity to vasoactive substances was examined in mesenteric arteries isolated from IH-exposed and control rats. Estrogen replacement could not prevent the elevation of BP during IH exposure, but inhibited the increase of BP during the dark phase induced by 1-wk IH exposure. Sensitivity of Ach-induced endothelium-dependent nitric oxide (NO) in mesenteric arteries was greater in E2 group than Pla group of IH rats. This study suggests that enhanced sensitivity to endothelium-dependent NO in mesenteric arteries may contribute to suppression of IH-induced BP elevation in E2 group.

## Poster Presentations

### Autonomic Nervous System(1)

## 1PK-143

### A decerebrated, arterially-perfused *in situ* rat preparation for studies of breathing, chewing, and swallowing behaviors

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Most recent studies of trigeminal respiratory motor behavior use *in vitro* brainstem-spinal cord of the neonatal rat (P0-2 days). However, the relevance of these studies to the neural mechanisms of adult breathing, chewing and swallowing is unclear, because the neonatal brainstem-spinal cord is immature and most studies have been performed before the primitive reflexes disappear. To overcome these difficulties in the rat, we have adapted a decerebrate, artificially-perfused *in situ* preparation first developed by Pickering et al. (J. Neurosci. Meth. 2006). For this purpose, the rat was decerebrated (P9-P24) and survived by a type of total artificial cardiopulmonary bypass as a means of extracorporeal circulation to deliver oxygen to the tissues of the entire body. To monitor the viability of the preparation we recorded respiratory discharge from the phrenic nerve. Simultaneously, we recorded respiratory-related discharge from the trigeminal, facial, and hypoglossal nerves. All respiratory-related discharges synchronized to the phrenic discharge. The body temperature of the preparation was set to room temperature. This time, we report the effect of various perfusion flow rates on neuronal discharge. Experiments are currently in progress to establish the utility of this preparation for studies of adult trigeminal respiratory motor behavior generated by the mature brainstem-spinal cord.

## 1PK-144

### Heart Rate Variability is Under Regulation of Sympathetic and Parasympathetic Nervous Activities in Adult Medaka

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The physiological nature and function of the vertebrate autonomic nervous system (ANS) in fish has traditionally been investigated in larvae or in anesthetized adults, in spite of the incomplete development of ANS in larvae and the possible disturbance of ANS activity under anesthetics. Here, we present a new method, which is based on the observation of the heart movement in high-speed movie images, to evaluate cardiac regulation by ANS in unanesthetized adult medaka (*Oryzias latipes*). We also determined the heart rates of medaka and examined the spectral analysis of heart rate variability (HRV) and the frequency characteristics of HRV modulated by ANS using the method. The steady-state heart rate fluctuates in controls, and HRV was significantly reduced by atropine in the middle range or propranolol at high range, suggesting that HRV in adult medaka is modulated by both the parasympathetic and sympathetic nervous systems within these frequency ranges. Such modulations of HRV by ANS was remarkably suppressed in anesthetized adult medaka, while constant light suppressed HRV in only the middle Hz range indicating parasympathetic withdrawal. Therefore, the method described herein renders medaka as an excellent model system to investigate physiological effects on vertebrate ANS activities.

## 1PK-145

### Orexin-A inhibits reflex swallowing via orexin-1 receptors situated in the commissural part of nucleus tractus solitaries

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Our previous study demonstrated that fourth ventricular administration of orexin-A (OX-A) inhibited reflex swallowing via orexin receptor 1 (OX1R). In the present study, we examined the effect of microinjection of OX-A into the three different sites of the dorsal vagal complex to identify the effective site of OX-A to inhibit reflex swallowing. Swallowing was induced by the electrical stimulation (20 Hz, 20 sec) of the central cut end of the superior laryngeal nerve and was identified by the electromyogram lead penetrated the mylohyoid muscle through bipolar electrodes. OX-A was injected into one of the following sites: 1) the lateral part of the nucleus tractus solitarius (NTS) around the solitary tract where the dorsal swallowing group is housed, 2) the medial part of the NTS and the adjacent area, 3) the area postrema (AP) and adjacent NTS. The microinjection of OX-A but not vehicle into the AP and adjacent NTS significantly decreased swallowing frequency. The microinjection of OX1R antagonist (SB334867) into the AP and its vicinity disrupted the inhibitory response induced by the fourth ventricular administration of OX-A. To determine precise region to induce response, the parenchymal lesion were done. The electric lesion of the commissural NTS, but not the ablation of the AP, abolished the inhibition of reflex swallowing induced by the fourth ventricular administration of OX-A. These results suggest that OX-A inhibits reflex swallowing via OX1R situated in the commissural NTS.

## 1PK-146

### Developmental differences in brain neuronal nitric oxide synthase(nNOS)activity between Dahl salt-sensitive and -resistant rats

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We previously demonstrated that brain nNOS activity is enhanced in hypertensive Dahl salt-sensitive (DSS), but not in Dahl salt-resistant (DSR) rats. This difference might be due to genetic-related developmental differences in brain nNOS activity between these two strains, or to differences in their susceptibility to high-salt with development. In the present study, 1) Developmental changes in brain nNOS activities between DSS and DSR rats under a regular diet were compared. 2) The effects of the age at which high-salt loading was started on brain nNOS activities between these two strains were compared. In both strains, nNOS activities slightly decreased with age in the brainstem and diencephalon, but significantly increased with age in the cerebellum. No remarkable difference was observed in the trend of the developmental changes in the activities between these two strains. The enhancement of brain nNOS activity by high-salt was region-dependent. Brainstem nNOS activity was enhanced regardless of the developmental stage in hypertensive DSS rats, but not in DSR rats even in the immature susceptible period (critical period). High-salt stimuli in the critical period did not affect diencephalon nNOS activity, but enhanced cerebellar nNOS activity in both strains. These results suggest that differences in brain nNOS activity between DSS and DSR rats might not be due to developmental differences, but rather due to their different susceptibilities to high-salt.

## 1PK-147

### Role of the visceral afferent vagus nerves on feeding behavior induced by ghrelin, CCK-8, or Lipopolysaccharide in rats

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Lipopolysaccharide (LPS), a proinflammatory substance, triggers anorexia via the afferent vagus nerves. CCK and Ghrelin (Ghr) secreted from the gastro-intestinal tracts evoke satiety or hunger sensation respectively, via the afferent vagus nerves. However, it remains unclear which branches of the visceral vagus and how take part in the transmission of such sensation. To address this issue, we determine 24 hr- or 3 hr-food intake after injection of such anorexic or appetite substances in rats with pharmacologic pretreatment (employed histamine or 5-HT receptor antagonists) or with surgical deafferentation of the gastric, hepatic, or celiac branches of the vagus. Suppression of 24hr-feeding induced by LPS (100µg/kg, ip) was attenuated by 5-HT<sub>3r</sub> antagonist or combined resection of the hepatic and gastric vagus. H<sub>2</sub>-histaminergic antagonist, but not H<sub>1</sub>-antagonist, attenuated the appetite induced by Ghr (20 µg/kg, ip; 3 hr-feeding after light on). Satiety induced by CCK-8 (12 µg/kg, ip; 3 hr-feeding after light off) was abolished by 5-HT<sub>3r</sub> antagonist or by celiac vagotomy, but not by gastric or hepatic vagotomy. These data suggest a possibility that 1) feed-suppression induced by LPS or CCK-8 involves 3-type 5-HT receptors, but anorexic sensation induced by them are mediated via the hepatic or celiac afferent vagus, respectively; 2) appetite induced by Ghr is mediated via the gastric and hepatic afferents vagus and involves 2-type histaminergic receptors.



## 1PK-148

### Contribution of central motor commands and cognition of visual cues to the skin sympathetic nerve activity evoked by voluntary muscle contraction

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**Background** ; Skin sympathetic nerve activity (SSNA) are evoked by the voluntary muscle contraction. By comparing with latencies of SSNA burst in voluntary handgrip and evoked handgrip, we have reported that the central motor command output rather than somatosensory afferents by muscle contraction contributes to the burst of SSNA accompanied by voluntary handgrip (Tsukahara 2010). The mechanism how sympathetic outflow to the skin is activated by the voluntary muscle contraction has not been well elucidated. **Objective** ; The aim of this study is to clarify how much contribution is distributed among motor command and cognition of visual cue to the skin sympathetic nerve activity (SSNA) evoked by voluntary muscle contraction. **Methods** ; We microneurographically recorded SSNA from tibial nerve simultaneously with sympathetic skin response (SSR) and skin blood flow (SBF) reduction response on the sole. Electroencephalogram on the scalp was recorded and averaged to figure out the movement-related cortical potential (MRCP) and event-related potential (ERP). We suggested how the central motor command and cognition of visual cue contribute to the skin sympathetic outflow on glabrous skin areas for preparing voluntary muscle contraction.

## 1PK-149

### Differentiation of sudomotor and vasoconstrictor activity of skin sympathetic nerve activity(SSNA) on time dependent analysis

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Skin sympathetic nerve activity (SSNA) can be differentiated into sudomotor and vasoconstrictor activity by simultaneous measurement of sweat expulsion and skin blood flow. Analyze of characteristics of SSNA will help to elucidate the thermoregulatory function. In previous research, the analysis has been quantified on the basis of burst per heartbeat. However, there are interactions of some factors not depend on heartbeat. The aim of study is to clarify the new regulation in the difference between sweating and SSNA. Sweating is also induced by mental activity. We hypothesized that the sudomotor activity mainly relates to function of event-related potential and vasoconstrictor to somatosensory potential because mental sweat activities is contributed by feed-forward system correlated to cognitive function. The SSNA was recorded by microneurography from tibial nerve at popliteal fossa during voluntary handgrip task. SSNA signals were fed into high impedance bioamplifier, band pass filter and integrator. Sweat expulsion was measured by capsule ventilation method. Skin potential was measured by skin electrode and bioamplifier. By using template matching on identification of sudomotor and vasoconstrictor impulses, coefficient of determination in SSNA was calculated. We concluded that consideration for time constant is necessary to better differentiation of SSNA.

## 1PK-150

### Changes in regional blood flow of the mesencephalic ventral tegmental area during spontaneous fictive motor activity in decerebrate rats

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We have provided evidence suggesting that mesencephalic ventral tegmental area (VTA) plays an important role in the generation of the cardiovascular response to exercise (Nakamoto et al. J Appl Physiol 2011), but whether the neural activity in the VTA increases accompanying the cardiovascular response during exercise remained unclear. The present study aimed to investigate whether the regional blood flow (RBF) in the VTA, which is ascribed to the neural activity, changes in association with the cardiovascular responses during spontaneous fictive motor activity. Rats were decerebrated at the pre-mammillary and precollicular level and then paralyzed. A probe of laser Doppler flowmetry was inserted into the right VTA, and the RBF was measured during 1) fictive motor activity, 2) transient occlusion of the abdominal aorta, and 3) intravenous administration of phenylephrine. The tibial motor activity was measured to confirm the fictive motor activity. The RBF of the VTA increased accompanying a pressor response in all of the three conditions. Importantly, the extent of increment of RBF and vascular conductance in the VTA was much larger ( $P < 0.05$ ) during fictive motor activity as compared to that during aortic occlusion or phenylephrine administration, despite the similar pressor response among these conditions. The present results suggest that the neural activity in the VTA contributes to the cardiovascular response during spontaneous fictive motor activity in decerebrate rats.

## 1PK-151

### The Effect of Aortic Depressor Nerve Stimulation on the Neurons in the Rostral Nucleus Tractus Solitarius

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We investigated the effect of the electrical stimulation of the aortic depressor nerve (AND) on the neurons in the rostral nucleus tractus solitarius (rostral NTS). Extracellular recordings were taken from neurons in the rostral NTS that showed spontaneous firing in urethane-chloralose anaesthetized rats. We also tested whether these neurons respond to stimulation of the lingual nerve. Many of the neurons in the rostral NTS with spontaneous activity decreased their activity to AND stimulation, and a majority of them were found to exhibit a pulse-related activity in ECG-triggered correlation histogram, indicating that they received cardiac input. Neurons that decreased their activity to AND stimulation also altered their firing rate to lingual nerve stimulation. Thus, AND stimulation exert inhibitory effect on the many of the rostral NTS neurons that showed spontaneous activity, and these neurons also receive lingual nerve inputs.

## 1PK-152

### The Regulatory Mechanism of Distention-evoked ATP Release from Oesophageal Epithelium

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Gastro-oesophageal reflux disease (GERD) involves oesophageal hypersensitivity to mechanical or heat stimulus as well as acids. ATP release from the epithelium of gastro-oesophageal tract in response to mechanical/chemical stimuli contributes to visceral sensation such as distention, pain and/or discomfort. However, molecular mechanism underlying the regulation of ATP release from oesophageal epithelium remains to be understood. In this study, we investigated the regulation of ATP release from rat oesophageal mucosa using customized Ussing chamber. We applied the hydrostatic pressure (45 cmH<sub>2</sub>O) to the serosal side in order to distend opened oesophagus, and measured ATP content in mucosal side of chamber by a luciferin-luciferase assay. Although high-dose of capsaicin (1 mM) significantly evoked ATP release without distention, distention-evoked ATP release was not blocked by capsazepine (10 μM), a TRPV1 antagonist. This data indicates that oesophageal distention could not activate TRPV1 channels. On the other hand, a PGE<sub>2</sub> receptor EP1 antagonist, ONO-8711 (1 μM), significantly reduced distention-evoked ATP release from oesophageal mucosa to approximately 24%. In addition, a nitric oxide synthase inhibitor L-NAME (30 μM) significantly increased distention-evoked ATP release. Thus, our present study showed that distention-evoked ATP release was enhanced by PGE<sub>2</sub>-EP1 but suppressed by nitric oxide. These signaling pathways might be potential drug targets for the treatment of oesophageal hypersensitivity in GERD.

## 1PK-153

### The effect of estrogen on biological rhythms in female rats

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**Purpose** We assessed the influence of plasma estrogen level on circadian body temperature rhythm, together with the rhythms of heart rates and physical activity. **Methods** Wistar rats were bilaterally ovariectomized, and implanted a telemetry device for the measurements of body temperature (T<sub>b</sub>), physical activity (Act), and ECG in the abdominal cavity. They were divided into two groups: one was subcutaneously placed two silicon tubes containing 17β-estradiol (o.d. 1.57mm and length 3cm; E<sub>2</sub> (+)) and the other two empty tubes (E<sub>2</sub> (-)). Nine days after the surgery, the tubes were removed. **Results** Before the removal of the tubes, T<sub>b</sub> in E<sub>2</sub> (-) group was lower (P<0.05) than that in E<sub>2</sub> (+) group at 0:30-1:00 (37.4±0.1°C and 38.0±0.3°C, respectively). However, there was no difference in T<sub>b</sub> 15 days after the removal. Heart rates in E<sub>2</sub> (-) group were greater (P<0.05) than those in E<sub>2</sub> (+) group (387±14 and 336±13 beats/min (bpm) in the light phase, and 450±11 and 389±11 bpm in the dark phase, respectively) before the removal. After the removal, heart rates in E<sub>2</sub> (+) group gradually increased (349±11, 375±13, and 377±13 bpm in the light phase; and 422±13, 436±13, and 425±16 bpm in the dark phase 1, 7, and 15 days after the removal, respectively). There was no difference in heart rates ten days after the removal. **Conclusion** A reduction of plasma estrogen level may affect both circadian body temperature and heart rates rhythms; however, such influence might be induced by independent mechanisms.

## Poster Presentations Muscle Physiology(1)

## 1PK-154

### Low ionic strength enhances force in individual myosin heads without changing ATPase activity in skinned rabbit psoas muscle fibers

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Although evidence has been accumulated that, at low ionic strength, myosin heads in relaxed skeletal muscle fibers form linkages with actin filaments, the effect of low ionic strength on relaxed and Ca<sup>2+</sup>-activated muscle fibers are not yet thoroughly investigated. We have studied the effect of low ionic strength on the mechanical properties and the contraction characteristics of skinned rabbit psoas muscle fibers. By progressively reducing the external KCl concentration from 125mM to 0mM (corresponding to decrease of ionic strength from 170 to 50mM), relaxed fibers showed changes in mechanical properties consistent with actin-myosin linkage formation. The maximum unloaded shortening velocity of Ca<sup>2+</sup>-activated fibers, however, did not change appreciably at low ionic strengths, indicating the disappearance of actin-myosin linkages on Ca<sup>2+</sup> activation. Though the maximum Ca<sup>2+</sup>-activated isometric force increased twofold at 0mM KCl, the Mg-ATPase activity of the fibers showed no significant changes. These results strongly suggest that the force generated by individual myosin head increases up to twofold at low ionic strength.

## 1PK-155

### Effects of dilated cardiomyopathy and aging on human myocardial SPOC

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SPOC (SPontaneous Oscillatory Contraction) is a phenomenon observed in the contractile system of striated (skeletal and cardiac) muscles at intermediate levels of activation between contraction and relaxation. We have investigated the molecular mechanisms of SPOC, and reported that the period of resting heart rate is well correlated with that of SPOC in skinned left ventricular preparations of various animal species (for review, see Ishiwata et al., Prog. Biophys. Mol. Biol. (2011) 105 : 187-198). We recently found that SPOC indeed occurs in human myocardial preparations, and reported the properties of SPOC, such as the period and propagation velocity of the SPOC wave (47th Ann. Meeting of Biophys. Soc. Japan (2009) : S122). In the present study, we examined the SPOC properties by using single human cardiac myofibrils from the left ventricle of non-failing (19-65 years) and dilated (DCM, 15-63 years) hearts. The use of single myofibrils allowed us to observe the movements of individual sarcomeres with no influence of connective tissues or intercalated disks. We found that sarcomeric shortening was slower and accordingly the SPOC period was longer in myofibrils from DCM hearts than those from non-failing hearts. At the meeting, we will discuss the effects of DCM and aging on the properties of human myocardial SPOC at the molecular level. This research is approved by Human Ethics Committee at Waseda University.

## 1PK-156

### Effect of intracellular Ca<sup>2+</sup> on myosin heavy chain I, HSP 70, and IL-6 mRNA levels in C2C12 cells

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Maintenance of skeletal muscle mass is essential for overall health, functionality and quality of life, and it is critical to elucidate the fundamental mechanisms underlying the maintenance. Skeletal muscle has been known to cause disuse atrophy during long-term recumbence and to recover by exercise. Although there are many studies about skeletal muscle regulatory factors that are known to affect skeletal muscle mass, mechanism of exercise on muscle cell proliferation is still unclear. Our previous study using rat soleus muscle indicated that myosin heavy chain (MHC) and HSP 70 proteins were significantly increased by the exercise after disuse atrophy. Therefore we examined effects of increase in intracellular Ca<sup>2+</sup> on MHC I, HSP 70, and IL-6 mRNA expression level using real-time PCR method in C2C12 skeletal myoblasts. The C2C12 cells which were differentiated into myocyte in D-MEM containing 2% FBS was incubated with 1 μM ionomycin in 6 or 24 hr. First, we measured MHC I mRNA level and it was significantly increased by the 6 hr incubation with ionomycin compared with control. 24 hr incubation with ionomycin significantly upregulated HSP 70 mRNA level, although 6 hr incubation with it did not affect the mRNA level. Additionally, IL-6 mRNA level was significantly increased by the 6 hr incubation of ionomycin. These results suggest that increase in intracellular Ca<sup>2+</sup> could act as a major factor upregulating MHC I, HSP 70, and IL-6 mRNA levels.

## 1PK-157

### Effect of vagotomy on fasted and postprandial motility of the suncus stomach

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During the fasted state, the stomach and small intestine undergo a temporally coordinated cyclic motor pattern known as the migrating motor complex (MMC) in dogs and humans. Feeding replaces the MMC with a pattern of noncyclic, intermittent contractile activity called postprandial contractions. Recent studies have shown that ghrelin, a member of the motilin family, also regulates the MMC as well as motilin. In this study, we investigated the effect of vagotomy on gastric motility using the conscious suncus, a motilin- and ghrelin-producing small animal. During the fasted state, cyclic MMC consisting of phase I, II, and III was observed in both sham-operated and vagotomized suncus, but the duration and motility index of phase II were significantly decreased in vagotomized suncus. Ghrelin infusion enhanced the amplitude of phase II in sham-operated suncus but not in vagotomized suncus. Motilin infusion during phase I induced phase III-like contractions in both sham-operated and vagotomized suncus. After feeding, phase I was replaced by postprandial contractions and motilin infusion did not induce phase III-like contractions in sham-operated suncus. However, in vagotomized suncus, feeding did not interrupt phase I, and exogenous motilin induced phase III-like contractions as in the fasted state. These results suggest that the vagus nerve is important for the regulation of phase II of the MMC and postprandial contractions, but not for the regulation of phase III of the MMC.

## 1PK-158

### A novel experimental system for the measurement of single sarcomere motion in rat neonatal cardiomyocytes

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In the present study, we developed a novel experimental system allowing for the measurement of the movement of single sarcomeres in rat neonatal cardiomyocytes expressed with AcGFP at Z-lines (spatial resolution, 3 nm at 50 fps). In this system, we successfully induced transient sarcomeric contractions upon electrical stimulation (1-5 Hz) and spontaneous sarcomeric oscillations (Cell-SPOC) after treatment with ionomycin in the presence of ryanodine and thapsigargin. Cell-SPOC was observed in the presence of high concentrations of Ca<sup>2+</sup>-EGTA (e.g., 10 mM), supporting our view that the phenomenon is intrinsic to sarcomeres. Indeed, the measurement of intracellular Ca<sup>2+</sup> with Fluo-4 confirmed that Ca<sup>2+</sup> oscillations did not occur under our experimental conditions. As found in adult cardiomyocytes, the sarcomeric oscillations consisted of quick lengthening and slow shortening during Cell-SPOC. Studies with electric stimulation revealed that the waveform properties at physiologically high electric frequencies (4-5Hz) were statistically indistinguishable from those obtained during Cell-SPOC occurring at the similar frequencies. These results suggest that the auto-oscillatory properties of cardiac sarcomeres may be involved in the regulation of cardiac beat in neonates.

## 1PK-159

### Effect of junctophilin knockdown on the localization and function of L-type calcium channels and ryanodine receptors in skeletal myotubes

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Ca<sub>v</sub>1.1 L-type calcium channels (LTCC) form a signaling complex with ryanodine receptors (RyR) at the junctional membrane (JM) in skeletal myocytes. Although junctophilins (JPs) are known to contribute to the stabilization of the JM complex by bridging the plasma membrane and sarcoplasmic reticulum, the roles of JPs in the JM-targeting and function of LTCC and RyR are unknown. To clarify these issues, we treated C2C12 and GLT myotubes with Ca<sub>v</sub>1.1 transfected with siRNA against JP1 or JP2 and assessed the JM-targeting of LTCC and RyR with immunocytochemistry, LTCC currents with the patch-clamp technique and intracellular Ca concentration with Fluo-4. JP1 or JP2 knockdown (KD) significantly inhibited the JM-targeting of LTCC whereas JP2 but not JP1 KD dramatically inhibited the JM-targeting of RyR. JP2 but not JP1 KD significantly decreased the peak LTCC current amplitude whereas JP1 or JP2 KD significantly decreased the number of myotubes exhibiting Ca<sup>2+</sup> transient in response to electrical stimulation. Neither JP1 nor JP2 KD affected the expression or JM-localization of JP2 or JP1, respectively, indicating that these manipulations did not disrupt JM structures per se. These results suggested that JP1 contributes to the normal JM-targeting of LTCC and intracellular Ca transient but not the LTCC current amplitude. In contrast, JP2 may be involved in the JM-targeting and function of both LTCC and RyR.

## 1PK-160

### Acupuncture treatment improved the skeletal muscle atrophy induced by spiral wire immobilization procedure in mice

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Exploring the mechanisms involved in skeletal muscle atrophy and developing a new therapeutic approach for preventing loss of muscle mass are necessary in our rapidly aging society. We hypothesized that acupuncture is an effective method to improve skeletal muscle atrophy. After two weeks of disused immobilization, we applied electroacupuncture (EA) intervention against skeletal muscle atrophy induced by spiral wire immobilization (SWI) and examined the effect of EA compared to the non-treatment group. Muscle wet weights of the SWI group (S) were significantly reduced in soleus (p<0.001), plantaris (p<0.05), and gastrocnemius (p<0.001) compared to the two weeks of the control group (C). After removing the casting, we applied acupuncture treatment for seven days. SWI induced a significant reduction in soleus muscle was significantly increased by the seven days EA treatment (p<0.05). We also found the significant increase in the mass of soleus muscle in the SEA7 group than that in the one day of EA treatment group (SEA1) group. These data indicated that EA treatment of seven days is effective method to improve the phenotype of soleus muscle weight. We conclude that EA is effective procedure to improve skeletal muscle atrophy induced by spiral wire cast immobilization.

## 1PK-161

### Partial depression of skeletal muscle hypertrophy in heat shock transcription factor 1-null mice

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Hypertrophic stimuli up-regulate heat shock proteins (HSPs) that are mediated by heat shock transcription factor 1 (HSF1)-associated stress response in mammalian skeletal muscles. Recently, we have reported that absence of HSF1 partially depressed the regrowth of atrophied soleus muscle. However, a physiological significance of HSF1-associated stress response in skeletal muscle hypertrophy are still unclear. The purpose of this study was to investigate the effects of HSF1-deficiency on loading-associated skeletal muscle hypertrophy by using HSF1-null mice. Functional overloading on left soleus was performed by cutting the distal tendons of gastrocnemius muscles for 4 weeks. Hypertrophy of soleus muscle in HSF1-null mice was partially depressed, compared with that in wild type mice. Evidences from this study suggested that HSF1 and HSF1-associated stress response may play an important role in loading-induced muscle hypertrophy, in part. This study was supported, in part, by KAKENHI (22240071, 24650411, 24650407) from Japan Society for the Promotion of Science and the Science Research Promotion and the Promotion and Mutual Aid Corporation for Private Schools of Japan.

## 1PK-162

### Absence of heat shock transcription factor 1 depresses regenerative potential of injured skeletal muscle in mice

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Hypertrophic stimuli, such as mechanical stresses, facilitate the regeneration of injured skeletal muscle. Mechanical stress induces stress response, which is mediated by heat shock transcription factor 1 (HSF1), and up-regulates heat shock proteins (HSPs) in mammalian skeletal muscles. Therefore, HSF1-associated stress response may play a key role in the regeneration of injured skeletal muscle. The purpose of this study was to investigate the effects of HSF1-deficiency on the regeneration of injured skeletal muscle. Cardiotoxin was injected into left soleus muscle of mice. Soleus muscle from both hindlimbs was dissected 2 and 4 weeks after the injection. HSF1-deficiency inhibited the recovery of muscle wet weight and muscular protein content of injured soleus muscle. Up-regulation of HSPs was also depressed in HSF1-null mice, compared with wild type mice. HSF1-associated stress response may play a key role in the regeneration of injured skeletal muscle. This study was supported, in part, by KAKENHI (22240071, 24650411, 24650407) from Japan Society for the Promotion of Science and the Science Research Promotion and the Promotion and Mutual Aid Corporation for Private Schools of Japan.

## 1PK-163

### Effect of thermal therapy on muscular mechanical hyperalgesia induced by lengthening contraction of rat gastrocnemius muscle

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Thermal therapy including icing or heating of the muscle has been used as an effective intervention for muscular pain. However, their mechanisms remain unclear. The aim of this study was to investigate effects of icing and heating of the gastrocnemius muscle on mechanical hyperalgesia induced by lengthening contraction (LC). Six week-old male SD rats at the beginning of experiments were used. Following the adequate handling, LC was applied to the left gastrocnemius muscle. Animals were randomly assigned to three groups, icing, heating or control (non-thermal stimulation) groups. Thermal stimuli were applied by putting iced (10°C) or heated (42°C) pack on the skin over the muscle just after LC for 20 min. The withdrawal threshold measured by Randall-Selitto apparatus, but not von Frey hairs, decreased after LC (i.e. muscular mechanical hyperalgesia) in the control rats. In contrast, withdrawal threshold did not change in the heating group, but did decrease in the icing group. These results demonstrated that heating but not icing the exercised muscle ameliorated muscular mechanical hyperalgesia following LC, and this model should be useful to understand mechanisms of thermal therapy.

## 1PK-164

### Functional role of the connection between skeletal muscles by fasciae

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The present study was investigated to know the functional role of the connection between skeletal muscles by fasciae in knee joint. We measured the length-force (L-F) relationship, *in vivo*, in whole muscle preparations in knee extensor, triceps femoris muscle (TFM), of the frog, *Rana catesbeiana*. TFM consists of three muscles, rectus femoris (RFM), vastus medialis (VMM) and vastus lateralis (VLM). Frogs were anesthetized by injecting urethane intraperitoneally. Then All the branches of sciatic nerve except for the one innervating VMM and VLM were cut. The active isometric force of VMM and VLM was measured at various muscle lengths at 20±0.5°C. Experiments were performed on three different preparations; in one preparation, surface of TFM was totally covered with fascia (CEF), in the second one, hamstrings was removed from TFM (CCH), and in the third one, RFM was removed from CEF (CCR). L-F curve of CCR was nearly same as that in CEF. But L-F curve in CCH is located at the longer length-range than that in CEF and CCR. The physiological length-range of TFM *in vivo* existed in the ascending limb in L-F curve, and it was nearly same in L-F curve of CEF and CCR. The results indicate that connection between TFM and hamstrings have remarkable influence on active force outputs than that between TFM and RFM, suggesting that one of the functional roles of connection between muscles is to produce stronger force outputs.

## Poster Presentations Neurochemistry(I)

## 1PK-165

### Neurotrophic effects of a novel nucleic acid analogue COA-CI(2CI-C.OXT-A)

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COA-CI (2CI-C.OXT-A) is a synthesized nucleoside analogue with the molecular weight of 284. We have reported that COA-CI significantly stimulated tube formation of human umbilical vein endothelial cells (HUVEC). Immunoblot analyses suggested that the angiogenic responses in HUVEC induced by COA-CI are mediated by a MAP kinase cascade comprising MEK and ERK1/2. We also found that COA-CI promoted the synthesis and secretion of VEGF, the most promotive factor for angiogenesis, in human fibroblast.

In this study, we examined the effects of COA-CI on the properties of PC12 cells. COA-CI revealed neurotrophic potencies. It increased neurite outgrowth and activity of acetylcholine esterase, a marker enzyme for the differentiation of PC12 cells. It also promoted the phosphorylation of ERK1/2 and tyrosine hydroxylase (TH), the rate limiting enzyme in the biosynthesis of catecholamines. These potencies are similar to those of an endogenous neurotrophic compound, nerve growth factor (NGF). A MEK inhibitor PD98059 inhibited neurite outgrowth as well as the promoted phosphorylation of ERK1/2 and TH caused by COA-CI and/or NGF. However, K252a, a specific inhibitor of NGF receptor, TrkA, selectively inhibited the effects of NGF but not COA-CI. These results indicate that COA-CI induces neurotrophic responses in PC12 mediated by a MAP kinase cascade comprising MEK and ERK1/2, but independently of NGF receptor TrkA.

## 1PK-166

### Cutaneous mechanical hyperalgesia induced by a high-fat-cholesterol diet in SHRSP5/Dmcr rats : upregulation of Mss4 in dorsal root ganglion cells

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Arteriollipidosis-prone (AL, SHRSP5/Dmcr) rats have been developed as an animal model of a high-fat-cholesterol (25% palm oil and 5% cholesterol) (HFC) diet-induced non-alcoholic steatohepatitis and hepatic fibrosis (Kitamori et al., 2012). We found that when AL rats were fed with HFC diet for 10 days, the thresholds for cutaneous mechanical pain responses (hind paw lifting in the pinprick test with von Frey filaments) were significantly decreased from 3 to 10 days after the start of HFC diet (n=6, p<0.05 vs control diet by Student's t-test), while the blood pressures were unaffected. To define genes upregulated by HFC diet, we performed PCR-based cDNA subtraction, "suppression subtractive hybridization" of mRNA in dorsal root ganglion cells (DRGs) of AL rats fed HFC diet for 10 days. We detected 6 different genes upregulated. Among them, we focused on Mss4, which was recently reported to be involved in the regulation of stress response. Mss4 mRNA upregulation was confirmed by RT-PCR using  $\beta$ -actin as an internal standard. Expression level of Mss4 in the DRGs was slightly, but significantly increased by HFC diet for 10 days (n=6, p<0.05 vs control diet by Student's t-test). These findings suggest that Mss4 is involved, at least in part, in the mechanical hyperalgesia induced by HFC diet. [Research funds : KAKENHI 23590724]

## 1PK-167

### Effect of ATBF1 on APP processing and A $\beta$ production

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Accumulation of the amyloid  $\beta$  peptide (A $\beta$ ), derived from the amyloid precursor protein (APP), is a characteristic hallmark of Alzheimer disease (AD) and play a central role in the pathogenesis of AD. Here we have identified a novel APP binding protein, AT-motif binding factor 1 (ATBF1), that binds to APP cytoplasmic domain. We found that the level of ATBF1 was increased in the cytoplasm of hippocampal neurons of AD brains compared with those of non-AD brains. Furthermore, cotransfection of HEK293T and SH-SY5Y cells with ATBF1 and APP695 increased cellular APP level through the binding of ATBF1 to APP cytoplasmic domain, which led to the increased in soluble APP $\alpha$  (sAPP $\alpha$ ) and A $\beta$  production without the expression changes of APP processing enzymes ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -secretase). Conversely, knockdown of endogenous ATBF1 expression reduced levels of cellular APP, sAPP $\alpha$ , and A $\beta$  production in HEK293 cells stably overexpressing human APP695. Immunofluorescence staining showed a colocalization of ATBF1 and APP after cotransfection of HEK293T and SH-SY5Y cells with ATBF1 and APP. Thus, the identification of ATBF1 as a novel APP-binding protein highly expressed in the cytoplasm of hippocampal neurons of AD brains should provide the understanding of relationship between APP processing and A $\beta$  production.

## 1PK-168

### Specific expression of n-acetylglucosaminyltransferase V in mouse neural stem cells

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Neural stem cells (NSCs) express variety of oligosaccharides on cell surface which are classified as hybrid-type and complex-type Asparagine-like oligosaccharide chains, called N-glycans. Generally, formation of hybrid-type N-glycans is regulated by N-acetylglucosaminyltransferase-V (GnT-V), however, the expression of this regulating enzyme in NSCs remains unknown. We prepared cultured NSCs from adult or embryo cortex, and maintained these cell either as proliferating cells or differentiated cells in vitro. Reverse-transcriptase polymerase chain reaction (RT-PCR) analysis and Western blot analysis revealed that GnT-V dominantly expressed in proliferating cells, and its expression diminished clearly in differentiated cells. In contrast, GnT-Vb and GnT-III, which regulate hybrid-type and complex-type N-glycan, respectively, expressed in both cells. In addition, L-PHA binding to hybrid-type N-glycan is not altered during differentiation. These results suggest that specific GnT-V expression could regulate the molecules which are necessary for NSCs proliferation and/or cell migration.

## 1PK-169

### Effect of Neurotrophin on axonal damage induced by lidocaine in rats

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This study examined the effects of neurotrophin on lidocaine neurotoxicity. Methods : Rats were divided into four groups. The first (L-N group) received 0.12  $\mu$ l/g BW of 20, 10, 3.6 neurotrophin units (NU) of neurotrophin solution mixed with 7.5% lidocaine. The second (L group) received 0.12  $\mu$ l/g BW of 7.5% lidocaine dissolved in distilled water. The third group (DW group) was injected with distilled water, and the last (20NU group) received 20 NU neurotrophin alone. Seven days after the intrathecal injection, the L2 spinal cord were removed for histological examination. Hind limb function was evaluated by walking behavior and sensory threshold. Results : Histological abnormalities were observed in the posterior roots and posterior column in 3 out of 6 rats of the L group, and in the posterior roots in 3 out of 7 rats of the L-20 NU group, although the histological lesions were milder in the L-20NU group than the L group. No histological abnormalities were detected in the L-3.6N, L-10NU, 20NU, and DW groups. Rats of the 20NU and DW groups recovered completely to normal ambulation within 15 min, while those of the L, L-3.6NU and L-10NU showed significantly slower recovery of ambulation than the 20NU and DW groups. Conclusion : Neurotrophin, at dose close to that used clinically (3.6 NU), provides neuroprotection and antagonizes lidocaine-induced neurotoxicity.

## 1PK-170

### Comprehensive Odor mapping in the Mushroom Body neurons in *Drosophila melanogaster*

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Mushroom body (MB) is third-order olfactory neurons in the insects and it contains over 2000 cells. It has a pivotal role to integrate odor information and reward or punitive information and is necessary for olfactory learning and memory. MB is hierarchical equivalent of piriform cortex that is third-order olfactory neurons in mammal. Piriform cortex is thought to process odor information and odors are coded sparsely in the region. In insects, although it was indicated that MB also codes odors sparsely, the odor mapping in the region is still an open question. It is thus necessary to observe the entire MB cell population to understand the odor representation. In the previous studies in *Drosophila*, odor responses of MB cell bodies were detected in a single z section. In this study, we observed odor responses of all MB cell bodies located three-dimensionally at one time by using fast z scanning under two-photon microscope with a green fluorescence calcium indicator GCaMP7. We observed the population of the MB cells responding to some odors and their mixtures with various intervals. This 4D observation will reveal the comprehensive patterns of odor coding. This work will lead us to understand how flies recognize different odors and their mixtures, and it is necessary to elucidate how they distinguish and learn odors and form the memory.

## 1PK-171

### Roles of CD200/CD200R expressed by macrophages in ischemic core of rat brain

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Results from our previous study have shown that marrow-derived macrophages expressing NG2 chondroitin sulfate proteoglycan (NG2) massively accumulate in the ischemic core, and ameliorate traumatic brain injuries. Since many of such macrophages express CD200 that has been recognized as an inhibitory molecule for myeloid leukocytes, it was postulated that the interaction of CD200 with its receptor CD200R may be involved in the regulation of functions of macrophages in the ischemic core. CD200, expressed on neurons in the normal brain, mediates inhibitory signals through CD200R. In this study, we investigated expression of CD200 and its receptor CD200R, and the roles of interaction of CD200-CD200R in the ischemic core. Macrophages isolated from ischemic core expressed mRNAs encoding CD200 and CD200R. As revealed by immunohistochemical staining, most macrophages were CD200<sup>+</sup>/CD200R<sup>+</sup>. When incubated with lipopolysaccharide (LPS), isolated macrophages elevated CD200-mRNA expression. To further investigate the effects of CD200 on macrophages, C6 glioma cells were established that stably expressed CD200 by transfection were cocultured with isolated macrophages. Consequently, the coculture enhanced expression of mRNAs encoding iNOS or inflammatory cytokines by macrophages increased and suppressed those for neuroprotective factors. Thus, activated macrophages expressed CD200, and CD200 accelerates activation to macrophages in spite of the prevalent notion.

## 1PK-172

### GDNF promotes neurite outgrowth and upregulates galectin-1 through the RET/PI3K signaling in cultured adult rat dorsal root ganglion neurons

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Galectin-1 (GAL-1), a member of a family of  $\beta$ -galactoside binding animal lectins, is predominantly expressed in small non-peptidergic (glial cell line-derived neurotrophic factor (GDNF)-responsive) sensory neurons and Schwann cells in the sections of adult rat dorsal root ganglia (DRG), but its functional role and the regulatory mechanisms of its expression in the peripheral nervous system remain unclear. In the present study, recombinant GDNF family of ligands (GFL), such as GDNF, neurturin (NRTN) and artemin (ARTN), promoted neurite outgrowth and increased the relative protein expression of GAL-1 in the neuron-enriched culture of DRG. The GAL-1 expression in immortalized adult rat Schwann cells IFRS1 was unaltered by treatment with GDNF, which suggests that GDNF acts on neurons, rather than Schwann cells, to upregulate GAL-1. The GDNF-induced neurite outgrowth and GAL-1 upregulation were attenuated by anti-GDNF family receptor (RET) antibody and phosphatidylinositol-3-phosphate-kinase (PI3K) inhibitor LY294002, but not by mitogen-activated protein kinase kinase (MEK) inhibitor U0126. Taking these findings together, the neurite-outgrowth promoting activity of GDNF may be attributable, at least partially, to the upregulation of GAL-1 through RET-PI3K pathway.

## 1PK-173

### Two dimensional phospho-peptide map analysis of tau phosphorylation catalyzed by multiple kinases

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Neurofibrillary tangles (NFTs), one of pathological hallmarks in Alzheimer's disease (AD) patient brain, are composed of hyperphosphorylated forms of the microtubule-associated protein tau. Unlike normal tau, the hyperphosphorylated tau proteins exist as paired helical filaments (PHF-tau). We focus on the regulatory mechanisms to accelerate tau phosphorylation by multiple tau kinases in AD brains. We conducted *in vitro* kinase assay using recombinant human tau, CDK5, and GSK3 $\beta$ . Degree of tau phosphorylation and identification of phosphorylatable sites were analyzed by two dimensional phospho-peptide map. Two spots had been shown to increase in density by the specific application of kinases, the sequential application of CDK5 following GSK3 $\beta$ . We had verified the sequences of tau peptides by the positions of spots on the phospho-peptide map. One of the sites had been found the spot with the doubly phosphorylated Ser202/Thr205, which had been shown to increase the phosphorylation on the early stage of AD patient brains. The other spot has never been reported and we will discuss this using point mutant of tau.

## Poster Presentations

### CNS Function(I)

#### 1PK-174

##### Physiological analysis of coffee flavor to the nervous system by means of contingent negative variation

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In order to investigate coffee flavor to physiological effects, we measured the contingent negative variation (CNV) of EEG and a reaction time to push button against a target sound stimulus. Activities of the autonomic nervous system were analyzed by measuring heart rate and heart rate variability. Psychological conditions were monitored by describing two psychological tests, i.e. the multiple mood scale and the General Arousal Checklist (GACL). Subjects were young healthy 6 men and 4 women. Coffee was filtrated from 20 g of powder to 200 ml of beaker and was kept warm by using a alcohol lamp to continuously evaporating coffee flavor. Areas of CNV in central scalp positions, i.e. Fz, Cz and Pz, of EEG were increased during the period of smelling coffee flavor compared to the control tap water experiment. Power spectral density of EEG was suppressed in the later period of CNV during the period of coffee smelling. Almost no change was observed in heart rate and heart variability, suggesting that activity of the autonomic nervous activity was little changed by coffee flavor. Reaction time for pushing button to target signal during CNV was shorten compared to the control. Psychological state of deactivation-sleepiness in GACL was reduced by coffee flavor. These results suggested that smelling coffee flavor stimulated arousal level of psychological state measured by psychological tests and the central nervous activity measured by CNV, and also activated responsibility to sensory-motor reflex measured by reaction time to target sound stimuli.

#### 1PK-175

##### Neural activities to frequency-modulated sounds in the left and right primary auditory cortex of guinea pigs observed by optical recording

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Neural activities to frequency-modulated (FM) sounds with different FM sweep rates in the primary auditory field (AI) of the left and right auditory cortices of the guinea pig were investigated using optical imaging with a voltage-sensitive dye (RH795). Eight guinea pigs were anesthetized with ketamine (80mg/kg) and xylazine (40mg/kg). Activity patterns to the FM sounds (upward and downward linearly swept frequency : 0.5-16.5 kHz in 16-400ms duration or FM sweep rate 0.04-1 kHz/ms) at 75 dB SPL were recorded from the AI on both sides. After the upward FM sound stimulation at the lower FM sweep rates (0.04-0.25 kHz/ms), the initial neural activity appeared at a dorsal region in the isofrequency band corresponding to the start frequency of the FM sound and then the secondary active spot appeared and moved crossing the isofrequency bands with the frequency sweep. At the higher FM sweep rates (0.5-1 kHz/ms), the initial neural activity appeared at the dorsal region of the higher isofrequency band. The activity patterns to the high-sweep FM sounds (accordingly short duration) were different from those to short-duration white noise and tones. These activity patterns were often observed in the left cortex which had the wider auditory field. We discuss the functional difference on the frequency processing between the left and right auditory cortices of guinea pigs.

#### 1PK-176

##### Human association cortex is composed of functionally relevant micro-modules : a high-resolution fMRI study using resting-state functional connectivity

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Recent fMRI studies have revealed that the human cerebral cortex can be parcellated based on resting-state functional connectivity (rs-fc). In order to know whether the parcellated areas are related to any particular brain function, we delineated boundaries in the pIFC using rs-fc, and compared them with brain activations associated with cognitive functions, which ought to reflect a neural processing supporting that function. High-resolution fMRI, which was used to discriminate signals from two banks of a sulcus, revealed a collection of micro-modules of the size of  $\sim 12$ mm, much smaller than Brodmann areas. The sizes of the activations were  $\sim 5$  mm and were smaller than that of the micro-module. The comparison of the spatial relationship between the boundaries and the activations revealed that the brain activations were less likely to be located on the boundaries. These results suggest that the micro-module in the human association cortex works as a functional unit that forms the neural network supporting a cognitive function.



## 1PK-177

### Emotional context modulates the fixation duration on the eye regions of facial stimuli

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Healthy human adults show strong tendency to fixate on the other's eye region for disproportionately longer duration than the other facial regions. However, the details about the underlying mechanism engendering this strong bias is yet to be clarified. In order to elucidate this point, the present study sought to clarify the factors influencing the fixation duration on the eye region in human adults. To this end, we recorded the scanning pattern of the several types of facial stimuli under different emotional contexts. After the facial stimulus appeared, a target stimulus, to which the participants were to make response, was presented at the periphery of the stimulus display. There were three types of target stimuli, namely, emotionally positive target (PT), aversive target (AT) and neutral target (NT). The type of the target stimuli set the emotional context of the task. The results have shown that the fixation duration on the eye region was strongly modulated by the type of the target stimuli. More specifically, PT and AT significantly increased the fixation duration on the eye region compared to NT, irrespective of the attributes of the facial stimuli themselves. This finding indicates the possibility that the bias to fixate on the eye region is engendered as an automatic response to emotionally-charged situations.

## 1PK-178

### Rationale of stereotaxic thalamotomy in view of brain activities related with parkinsonian tremor

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Thalamotomy is effective to treat parkinsonian tremor, but the rationale has not been established on its invasive means. In examining 170 cases of the electrode-guided thalamotomy for Parkinson's disease, we tested several relevant lines of working hypothesis as follows. (1) The so-called tremor neurons are localized in the thalamus, and the lesion there stops tremor. (2) Many nearby thalamic neurons fire in a highly rated tonic pattern, and some of them become contingently synchronized with tremor. (3) Tremor is generated centrally in the thalamus. (4) Tremor is maintained peripherally as well as centrally elsewhere beyond the thalamus. (5) The surgery gives no influence on progression of the disease. We were given direct evidences by observation for (1) and (2) as critical for the rationale, and indirect partial evidences for (3), (4) and (5). For (3) we examined conditions of the target stimulation and injury via the electrode to change tremor. For (4) we conducted passive and active muscle manipulations to see that peripheral kinesthetic afferents and central motor initiatives both contributed to maintenance of tremor. For (5) we found a quantitatively linear relationship between tremor neuron activities and alleviated tremor, implying their direct causality with no time consumption for any plastic processes building up tremor as an on-going symptom. Suppression of tremor is combined direct and indirect effect of the thalamic lesion. Integrative explanations for each parkinsonian motor symptoms should be given as the rationale for the surgical indication.

## 1PK-179

### Involvement of Rat Basolateral Amygdala and Medial Prefrontal Cortex in Expression of Fear-related Behavior in Response to Predator Odor

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Our previous study finds that the medial prefrontal cortex (mPFC) is involved in olfactory perception and emotional behavior; however, it is still unknown how olfactory information is processed in the mPFC and limbic area, such as amygdala, to control emotional behavior. In the present study, we investigated the extracellular multi-unit activity in the anterior cingulate cortex (ACC), prelimbic cortex (PL), infralimbic cortex (IL) and basolateral amygdala (BLA), using the freely moving rats subjected to the odor exposure test. In the test, animals were exposed to a mixture of cis-3-hexenol and trans-2-hexenal (green odor), 2,5-dihydro-2,4,5-trimethylthiazoline (TMT) and cat odor. We found that cat odor, but not TMT, induced prolonged activations in neurons in the PL and BLA with immobility. Simultaneous exposure of cat odor and green odor induced transient activations in neurons in the ACC, and attenuated the neuronal activity in the PL, while prolonged action in the BLA remained unchanged. We discuss the interaction between the subregions of mPFC and BLA to control fear-related behavior according to the features of odors.

## 1PK-180

### Timing representations in cue and delay activity of monkey striatal neurons during duration discrimination

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To clarify neural mechanisms of time perception in the basal ganglia, we have been studying neuronal activity during duration discrimination in the monkey striatum. Two Japanese monkeys were presented two visual cues, blue and red squares, successively for different duration (the first cue, C1 and the second cue, C2). Cue duration ranged from 0.2 to 1.6 s. Each cue was followed by a 1.0 s delay period (the first delay, D1 and the second delay, D2). After the D2 period blue and red squares were presented simultaneously. Subjects were required to choose the longer-presented cue. We recorded 498 phasically active neurons and found that 385 neurons showed task-related activity. Only 14 neurons responded to C1, and 97 neurons did to C2. Some of the C2 response neurons increased or decreased the activity depending on C1 duration. Activity of 49 neurons changed during D1 period and that of 157 neurons varied during D2 period. Some of the D1 response neurons increased or decreased the activity depending on C1 duration. These C1-dependent C2 or D1 activities might represent C1 durations for later comparison. Some of the D2 response neurons showed order-based temporal representation of the two cues. The D2 activity of these neurons differed between long-short and short-long trials. A few neurons exhibited two phasic responses following C2 onset. Intervals between the two responses changed depending on C2 duration. These results suggest that striatal activity during cue and delay periods have different functions in duration discrimination.

## 1PK-181

### Traveling back to the past with emotional slow breathing with amygdala and hippocampus activities

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Respiration activity has an important role for olfactory perception. Every breath including odor molecules activates the olfactory-related limbic areas associated with emotional experience and memory retrieval with respiratory changes. It is well known that some odors enable individuals to mentally travel back into their personal past and elicit special emotion or memory retrieval. This is called autobiographical memories (AM). In this study, we investigated how brain areas during stimulation of odor associated with personal memory differ from those activated by control odors. During presentation of odors related to AM and control odors, electroencephalograms (EEG) and respiration were simultaneously recorded. We found that odor-induced AM retrieval was associated with increasing tidal volume and decreasing respiratory frequency more than during presentation of control odors. EEG dipole analysis was used to determine the generators of the odor-induced inspiration related potentials. We found that odors associated with AM activated the entorhinal cortex, hippocampus, and amygdala to a greater extent than did control odors. These activations were observed from 50 ms to 100 ms after the onset of inspiration. We discussed assumptions how deep and slow breathing is related to pleasantness and memory retrieval associating with the limbic activations.

## 1PK-182

### Role of the hippocampal functional connectivity in gender-related character : A resting-state fMRI study

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Hippocampus is known to be related not only to memory but also various cognitive functions and emotional control. Recent study shows that the hippocampal volume is affected by the prenatal androgen exposure as measured by the second to fourth digit ratio (2D : 4D) in female. Thus, the hippocampus may be an important region to construct gender-related character. In this study, we measured global functional connectivity in the hippocampus with other regions ("regional global connectivity," rGC), and examined its correlation with 2D : 4D. A 3-Tesla MRI (Philips) was used to obtain structural and resting state functional (BOLD) images of healthy subjects (68 males and 53 females, 18-30 years old) with their eyes closed. After preprocessing of BOLD signals, cross-correlation coefficients of each voxel (6x6x6 mm) with all other voxels were calculated and averaged to determine rGC in each voxel. Pearson's correlation between rGC and 2D : 4D revealed to be significant ( $p < 0.05$ , corrected for multi-comparison with Monte Carlo simulation) at bilateral hippocampus, with negative correlation in males and positive in females. The results suggest that the low functional connectivity of the hippocampus in female is related to the aggressive behavior. In contrast, high functional connectivity in male may be related to the risk-taking decision making, since 2D : 4D is known to be inversely related to the day traders' ability.

## 1PK-183

### Task-dependent modulation of saccade-related activity in primate thalamic mediodorsal neurons

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To examine whether or not task-dependent modulation was observed in thalamic mediodorsal neurons, we compared saccade-related activity while monkeys performed two oculomotor delayed-response (ODR) tasks. In the ordinary ODR task, monkeys were required to make a memory-guided saccade to the location where a visual cue had been presented 3 s before, whereas in the rotatory ODR task, they were required to make a memory-guided saccade 90° clockwise from the cue direction. We compared firing rates of saccade-related activity while monkeys performed the same direction of the saccades under these two task conditions. We found that firing rates of saccade-related activity was greater during the rotatory ODR performance compared to the ordinary ODR performance. The magnitude of saccade-related activity observed in the thalamic mediodorsal nucleus was task-dependent although the saccade with the same parameters was performed in both conditions. The present result indicates that, when the task becomes more difficult and complex, task-related activity such as saccade-related activity can be enhanced.

## Poster Presentations Nutrition, Metabolism, Thermoregulation(1)

## 1PK-184

### Capillary endothelial Fatty Acid Binding Protein 4 and 5 play a critical role in fatty acid uptake in heart and skeletal muscle

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Capillary endothelial cells (ECs) are functional interface between the plasma and tissue fluids. Fatty acids (FA) are major energy substrates in various organs including heart, skeletal muscle and adipose tissue, but it is uncertain how they can reach interstitial space through endothelial layer of muscle-type continuous capillary. Fatty acid binding proteins (FABPs) are cytoplasmic FA chaperones that regulate the cellular and systemic metabolism of lipids. FABP4 (aP2) is believed to be exclusively expressed in adipocytes and macrophages while FABP5 (mall) is detected more widely. FABP4/5 have been implied in the pathogenesis of metabolic syndrome in patients with over-nutrition. Here we provide evidence for a novel function of FABP4 and FABP5 as endothelial FA carriers between microvessels and interstitial fluid functioning in trans-endothelial FA transport. Both FABP4/5 were abundantly expressed in capillary ECs. FA uptake was markedly reduced while glucose uptake was reciprocally and dramatically elevated in the heart (20 folds) and the red skeletal muscle (4 folds) in mice doubly deficient for FABP4/5, as estimated *in vivo* by uptake of FA analogue, 125I-BMIPP, and glucose analogue, 18F-FDG. An increase in glucose utilization during fasting was independent of insulin signaling and attributed to allosteric and post-transcriptional regulation. Identification of tissue-specific trans-endothelial FA transport by FABP4/5 may lend a new approach to limit FA utilization for treatment of metabolic diseases such as obesity and diabetes.

## 1PK-185

### Agouti-related protein and sympathetic nerve regulate TNF- $\alpha$ mRNA abundance in white adipose tissue

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TNF- $\alpha$  contributes to insulin resistance in obese rodents and humans. The increased production of TNF- $\alpha$  associated with obesity has been thought to be due to macrophage infiltration into white adipose tissue (WAT). We now show that *i.c.v.* injection of Agouti-related protein (AgRP) increased the amount of TNF- $\alpha$  mRNA in epididymal (epi) WAT of mice without changing the abundance of mRNAs for macrophage chemoattractant protein-1 or macrophage markers, and this effect is mediated through inhibition of sympathetic nerve activity in epiWAT. Surgical denervation and  $\beta$ -adrenergic blockade each up-regulated TNF- $\alpha$  mRNA in epiWAT. Norepinephrine or isoproterenol down-regulated TNF- $\alpha$  mRNA in epiWAT explants or in the stromal vascular fraction (SVF) derived therefrom by acting at  $\beta_2$ -adrenergic receptor ( $\beta_2$ -AR) and activating protein kinase A. Tissue-resident macrophages in the SVF contained high levels of  $\beta_2$ -AR and TNF- $\alpha$  mRNAs. Mice deficient in all  $\beta$ -ARs manifested an increased plasma TNF- $\alpha$  concentration and an increased TNF- $\alpha$  mRNA abundance in WAT, with no change in macrophage marker mRNAs. Finally, sympathetic nerve activity was reduced and norepinephrine failed to down-regulate TNF- $\alpha$  mRNA in WAT of mice with diet-induced obesity. Sympathetic nerve activity and  $\beta_2$ -AR signaling thus play an important role in regulation of TNF- $\alpha$  expression in WAT.

## 1PK-186

### Role of G protein-coupled receptor 40, a free fatty acid receptor, in the proliferation and differentiation of cultured neural stem cells

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Docosahexaenoic acid (DHA) is essential for the growth and functional development of the brain. Polyunsaturated fatty acids (PUFAs), including DHA, are known to regulate neurogenesis; however, the exact mechanism of underlying PUFAs-related neurogenesis has not been described conclusively. The present study aimed to examine the role of free fatty acid-activated G protein-coupled receptor (GPR) 40, which is a free fatty acid receptor, in the proliferation and differentiation of cultured rat fetal neural stem cells (NSCs). GPR40 mRNA expression levels in NSCs were analyzed by quantitative RCR at 0, 1, 4, and 7 days after differentiation. The expression levels of GPR40 were lower in the undifferentiated state than in the differentiated state. GW1100, a GPR40 specific inhibitor, did not affect NSC proliferation; however, GW1100 treatment with DHA enhanced NSCs proliferation, suggesting that GPR40 inhibition can maintain NSCs in the undifferentiated state. GPR40 activation by GW9508 increased the levels of Tuj-1 (a neuronal marker) mRNA expression as well as the percentage of Tuj-1-positive cells; this suggests that GPR40 activation enhanced neuronal differentiation. Moreover, DHA decreased Hes1 and presenilin 1 mRNA expression in the differentiating NSCs. In contrast to DHA, GW9508 did not affect Hes1 and presenilin 1 mRNA expression. These results indicate that different mechanisms can be involved in DHA-induced neuronal differentiation of NSCs.

## 1PK-187

### Relationship between insulin response to glucose and insulin-induced lowered shift of core temperature threshold for thermoregulation

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Esophageal temperature ( $T_{es}$ ) threshold for thermoregulation is lowered during hyperinsulinaemia with oral glucose intake compared to fructose intake. It is unknown whether the response is associated with insulin sensitivity. METHODS: Seven healthy men performed two trials in a climate chamber (28°C of  $T_a$ , 40% of RH). After 10 min baseline (BL), subjects ingested 75 g of glucose (GLU) or fructose (FRU) with 300 ml of water. After 20 min, passive heating (lower legs immersion, 42°C) lasted for 60 min.  $T_{es}$ , sweat rate and cutaneous vascular conductance at forearm were measured continuously, and plasma insulin ( $[Ins]_p$ ) and glucose concentration ( $[Glu]_p$ ) were determined. Insulino-genic index (ISI) that is known to correlate inversely with insulin sensitivity was calculated as the changes of  $[Ins]_p$  from BL to 30 min after intake divided by those of  $[Glu]_p$  during GLU. RESULTS: Lowered shift of  $T_{es}$  threshold for sweating ( $-8.65 \pm 3.82^\circ\text{C}/\text{nIU}$ ) and cutaneous vasodilation ( $-11.48 \pm 3.85^\circ\text{C}/\text{nIU}$ ) by a given increase in  $[Ins]_p$  from FRU to GLU at 30 min after intake were negatively correlated with ISI ( $10.0 \pm 1.5 \text{ IU}/\text{mmol}$ ) ( $R = -0.76$  and  $-0.62$ ). CONCLUSIONS: The responses of insulin-induced lowered shift of  $T_{es}$  threshold for thermoregulation are higher in subjects with lower ISI, suggesting that the responses are associated with insulin sensitivity.

## 1PK-188

### Activation of TRPC6 channel by $\beta_3$ -adrenergic mitochondrial uncoupling in mouse brown adipocytes

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In brown adipocytes,  $\beta_3$ -adrenergic activation induces lipid hydrolysis, enhancing TCA cycle and electron transfer, and the free fatty acid thus produced activates an uncoupling protein, UCP1, generating heat. Under this condition, cytosolic  $Ca^{2+}$  in adipocytes rises di- or tri-phasicly via the initial mitochondrial  $Ca^{2+}$  release, then the endoplasmic reticulum (ER) and the subsequent plasmalemmal  $Ca^{2+}$  entry. On the other hand,  $\alpha_1$ -adrenoceptor elicits  $IP_3$ -induced  $Ca^{2+}$  release from the ER and associated store-operated  $Ca^{2+}$  entry. In this study, we have investigated the mechanism of  $Ca^{2+}$  entry by  $\beta_3$ -adrenergic mitochondrial uncoupling in cultured brown adipocytes. In  $Ca^{2+}$  imaging,  $Ca^{2+}$  entry evoked by thapsigargin or cyclopiazonic acid was inhibited by 2-APB and low pH, but not by Ruthenium Red. TRPC6 channel activator, 1-Oleoyl-2-acetyl-sn-glycerol (OAG), evoked  $Ca^{2+}$  entry. The expression of mRNA and protein of TRPC6 channel and SOC-related molecules, STIM and Orai, were assessed by RT-PCR and Western-blotting method.  $\beta_3$  adrenergic stimulation thus causes  $Ca^{2+}$  release from mitochondria, which further elicits CICR from the ER and activates plasmalemmal  $Ca^{2+}$  entry via TRPC6 channel activation.

## 1PK-189

### Feeding inhibits voluntary exercise in the rat

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Reducing caloric intake and/or increasing exercise are recommended to reduce body weight and prevent metabolic syndrome. It is generally thought that increasing exercise will stimulate appetite to compensate to recover the decreased body weight. However, obesity induced by hyperphagia does not necessarily increase voluntary exercise. Thus obesity is hard to prevent and cure. But the relationship between food intake and voluntary exercise is not so simple. We examined the effects of feeding on voluntary exercise in the rat. Wistar male rats were used under temperature and light controlled (light on 6:00-18:00) conditions. The rats were given ordinary rat chow (OC) or high fat diet (HFD, 60% fat). Voluntary exercise was measured by running wheel activity (RWA). Fasting induced an increase in RWA, after returning the food RWA was significantly depressed compared with animal fed ad lib at least for a week. The reduced body weight during the fasting was not recovered to the level of the control animals during this period. When food availability was restricted during 11:00-13:00, the rats increased RWA before the feeding time. This increased exercise is regarded as a kind of food exploring activity. After establishing the food anticipatory activity, the food was changed from OC to a cafeteria diet (the rat could eat both the HFD and OC), then the food anticipatory activity was completely inhibited in a few days, but the rats ate more the cafeteria diet than OC during the restricted period. These results indicate that voluntary RWA is inhibited by food intake even in the case of negative energy balance.

## 1PK-190

### Effects of estrogen replacement on high-fat diet-induced insulin resistance in ovariectomized rats

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Menopause is associated with insulin resistance. In this study, we examined in vivo effects of estrogen on insulin sensitivity and insulin signaling in ovariectomized (OVX) rats fed on a high-fat diet (HFD). Female Wistar rats at 9 wk of age were ovariectomized and treated with placebo (Pla) or 17 $\beta$ -estradiol (1.5 mg/60-day release, sc) (E2) pellets 4 wk after OVX. Simultaneously, rats in either group were divided into two groups and given either standard chow diet (SD) or HFD. Four wk after replacement and HFD, intravenous glucose tolerance test (IGTT) was performed for assessment of insulin sensitivity. Two days later, 10<sup>5</sup> mol/l insulin was injected into the portal vein in each group of rats, and the liver and hind-limb muscle were dissected for insulin signaling analysis. The Akt and phosphorylated Akt in these organs were assayed by Western blotting. After 4 wk of replacement and HFD, HFD accumulated intra-abdominal fats in Pla group, while estrogen replacements suppressed the fat accumulation in either diet group. IGTT revealed that insulin sensitivity was decreased in the Pla group than in the E2 group of rats fed on SD. Furthermore, HFD reduced insulin sensitivity in the Pla group, while estrogen replacement restored it in OVX rats fed on HFD. In addition, insulin injection increased Akt phosphorylation in the liver of the E2 group, but not in the Pla group. These results suggest that HFD induces insulin resistance in the OVX rat, which is ameliorated by estrogen replacement.

## 1PK-191

### Role of UCP1 and muscle AMPK in diet-induced thermogenesis

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Brown adipose tissue (BAT) and skeletal muscle are the principle tissues in heat production in mammals. However, the role of the tissues in diet-induced thermogenesis (DIT) remains elusive. UCP1 plays an important role in thermogenesis in BAT, while AMPK is involved in the regulation of fatty acid oxidation in skeletal muscle. Here, we examined the effect of high calorie diet (HFD; high fat diet) on DIT in UCP1-KO mice, skeletal muscle-specific dominant-negative-AMPK transgenic (dnAMPK-mTg) mice and double-UCP1-KO-dnAMPK-Tg [UCP-KO/DN-AMPK-mTg] mice. Whole body energy expenditure (EE) is composed of DIT, exercise induced thermogenesis (Ex), and resting metabolic rate (RMR). We found a functional equation between energy expenditure and spontaneous locomotor activity in fasted mice. This equation was used to estimate RMR and DIT as well as Ex in UCP1-KO, dnAMPK-mTg and UCP-KO/DN-AMPK-mTg mice. When HFD or standard diet was applied to mice during the dark period, EE, Ex, DIT and RMR were not different in UCP1-KO or dnAMPK-mTg mice compared with that in wild type (WT) mice. However, DIT and EE, but not Ex or RMR, were decreased in UCP-KO/DN-AMPK-mTg mice during HFD feeding. These data suggest that both UCP1 and muscle AMPK are necessary for DIT in mice. BAT or muscle thermogenesis may be activated in UCP-KO and DN-AMPK-mTg mice in a compensatory manner.

## 1PK-192

### Optogenetic stimulation of neurons in the preoptic area inhibits metabolic heat production

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The preoptic area (POA) is the mammalian brain center for body temperature regulation. We have shown that the POA contains GABAergic inhibitory neurons that receive the pyrogenic mediator prostaglandin E<sub>2</sub>. These neurons project to sympathoexcitatory hypothalamic and medullary regions whose activation drives metabolic heat production (thermogenesis). Therefore, we hypothesized that GABAergic projection neurons in the POA provide tonic descending inhibition to regulate the tone of the sympathoexcitatory drive for metabolic thermogenesis. To functionally test this hypothesis, we examined the effect of stimulation of POA neurons on metabolic thermogenesis in brown adipose tissue (BAT) using an *in vivo* optogenetic technique in anesthetized rats. Virus-mediated delivery of channelrhodopsin-2 (ChR2) gene into the POA resulted in expression of ChR2 in many neurons in the POA. In these animals, sympathetic nerve activity in BAT, which was evoked by skin cooling, was consistently inhibited only during illumination of the POA through an optical fiber inserted into the brain. This result supports the hypothesis that POA neurons provide tonic inhibitory signals to control the sympathoexcitatory outflow to metabolic heat production.

## 1PK-193

### Activity of corticospinal and other neurons in macaque M1 in relation to locomotor movements on the treadmill

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To understand cortical mechanisms related to bipedal gait in humans, we recorded neuronal activity from M1 trunk/hindlimb regions in a monkey exerting quadrupedal and bipedal locomotion (QL and BL) on a treadmill. Of 81 neurons analysed, activity of 21 cells was recorded together with trunk/hindlimb EMGs. Spike-triggered averaging revealed that 6 cells showed post-spike facilitation and suppression, and synchrony facilitation in the hindlimb muscles. Of these neurons, 5 discharged phasically or phasically/tonically per step during QL. The phasic activity was broadly tuned to the touch-down event or late stance (ST)-phase. Following the transition from QL to BL, these cells sharpened their phasic activity with enhancing the peak discharge frequency. Interestingly, the peak activity of each cell during BL occurred in the late or terminal ST phase, but not in the mid-ST phase where all the target muscles displayed their peak activity. Population histograms of discharge frequency constructed from all analysed cells showed the same trend wherein M1 activity for BL was higher in the period from late to terminal ST phase than that for QL. Our results suggest that, for BL in monkeys, the output from M1 contributes to getting over the physical instability indwelling in BL itself and modulates on-going locomotor activities in the spinal circuitries, probably to smoothly transfer the center of body mass from one limb to the other. Such M1 function could be more significant in bipedal gait in humans.

## 1PK-194

### Relationship between masticatory performance and masticatory behavior in humans

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Masticatory performance (MP) is concerned closely with the masticatory function such as the mechanical breaking of ingested food into smaller pieces during mastication. However, actual relationship between MP and masticatory process from ingestion to swallowing has not been fully elucidated. Therefore, we investigate the relationship between the MP and both the bite size (length and volume) at the beginning of mastication and the number of chewing strokes until last swallowing (NCS). Forty-four adult subjects (26 males ; 18 females ; mean age, 23.3 yrs.) participated. Fish sausage (FS) with different diameters (13mm, 21mm) was used for the test food. Individual MP was measured by the sieving test using peanuts (3g) according to Manly and Braley (1950). NCS was counted by the masticatory counter. The bite volume, bite length and NCS during mastication of the FS with 21 mm in diameter were significantly larger than those of the FS with 13 mm in diameter. In contrast, the bite length of the FS with 21mm in diameter was significantly smaller than that of the FS with 13mm in diameter. There was no significant correlation between MP and the bite length, the bite volume, and the NCS. These results suggest that the degree of MP has no significant effect on the bite size and the NCS during natural mastication of food.

## Poster Presentations Motor Function(1)

## 1PK-195

### Superior tactile sensibility in sports involving ball handling

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The human hand can discriminate complex patterns of stimuli based on differential tactile input. This capacity may be decremented when disturbances exist. In sports such as pitching in baseball and shooting in basketball, good tactile sensitivity is an attribute of excellent performance. However, the ability to perceive tactile stimuli has not been compared between ball handling sports players and non-players. In the present study, we measured the ability to discriminate two stimuli applied concurrently to a thumb and each of the fingers. Each digit rested on two rows of four 1.3 diameter pins. Stimulation involved upward movement of the pins. Participants were asked to quickly judge which digit of the two was stimulated with a greater number of pins, with or without disturbance stimuli (8 pins on both digits) that were applied before the judging task. Subjects were ball handling sports players who belonged to a baseball or basketball team (BPs), and non-players who had no history of sporting activity involving ball handling (NPs). For the thumb and index finger pair, the accuracy of judgment in the NPs significantly decreased in the presence of a disturbance ( $p < 0.01$ ), while that in the BPs did not change. For other digit pairs, except the thumb and small finger pair, accuracy rates changed similarly. The present results suggest that the tactile sensibility of BPs was improved with sports training.

## 1PK-196

### The developmental differences in spontaneous spinal activities between rat and mouse using brainstem-spinal cord preparation

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In fetal rat isolated brainstem-spinal cord preparation, two kinds of activities were recorded from 4th cervical nerve root (C4); one is respiratory activity which is rhythmic and corresponding to phrenic nerve activity. The other is non-respiratory activity (NRA) which is not rhythmic, with high amplitude and might be corresponding to fetal movement. Previous study showed the critical point of disappearance of NRA is between E19 and E20, and NRA was induced by strychnine application after E20. The result indicated that the disappearance of NRA depends on the maturation of glycinergic system. In contrast, we investigated the spinal activities in C57BL/6 mouse brainstem-spinal cord preparation. Two kinds of activities were observed in neonatal mice preparation; one was rhythmic activity corresponding to respiratory movement and the other was not rhythmic and with small amplitude. This small non-respiratory activity (sNRA) was decreased by 5,7-Dichlorokynurenic acid and was increased by D-serine which are antagonist and agonist of strychnine-insensitive NMDA receptor glycine binding site, respectively. The critical point of disappearance of sNRA is probably between P3 and P4 because sNRA was not detected in P4 mice. It seems that the development of glycine neural circuit in mouse is slower than that of rat. These results suggest that the NMDA receptor glycine binding site plays an important role in generation of both rat and mouse fetal movement.

## 1PK-197

### Directional specificity for voluntary control of lip-closing force with visual-feedback

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A newly developed apparatus for measuring lip-closing force (LCF) during pursing like lip-closing can assess 8 directional forces (upper, lower, right, left and the 4 directions in between) separately. This study aimed to investigate the difference of voluntary control ability for these directional LCF. The experimental system consisted of the multidirectional LCF measurement apparatus, and a display that showed the exerted LCF for each direction in real time, along with a target value. Ten male subjects were instructed to control the LCF to maintain the target value using visual-feedback. The target value was set at half of the LCF with maximum effort. We estimated the matched time when the LCF was kept in the range of  $\pm 8\%$  of the target value. The accuracy rate was calculated by dividing the matched time by 3 seconds, which was the time between 1 second and 4 seconds after the onset of the LCF. The accuracy rate of the directional LCF differed significantly depending on the direction ( $p < 0.05$ , one-way ANOVA). In the assessment of the accuracy rate on the directional LCF separately, the rates of upper and lower directional LCF were significantly higher than those of oblique directional LCF ( $p < 0.05$ , t-test). This result suggests that voluntary control of the LCF might be recognized more easily in a vertical than in an oblique direction.

## 1PK-198

### Induction of oligodendrocyte progenitors from mouse iPS cells for transplantation to neonatal white matter injury

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Developing diffuse white matter injury (DWMI) caused by hypoxia is associated with permanent neurodevelopmental disabilities in preterm infants, and previously referred to as periventricular leukomalacia. As selective loss of oligodendrocyte progenitor cells (OPC) is reported in the developing DWMI, OPC transplantation would be a promising treatment to make up for neurodevelopmental abilities. We are now challenging OPC transplantation using mouse induced pluripotent stem (iPS) cells. To obtain OPC lineage cells suitable for transplantation, we first try to induce iPS cell-derived OPCs effectively, followed by removal of immature stem cells to prevent teratoma formation, and then check cell viability against cell damage in vitro. Effective induction to ectoderm was shown in case of EB formation with serum-free medium. Following to selection and expansion of neural stem cells, cells were differentiated to glial progenitor cells (GPC) with FGF-2 and EGF and then induced to OPC (47.4% of total cells) with PDGF. As some stem cells are still detected at this stage, we removed SSEA-1 (+) cells with MACS, resulting in 97% reduction. To check appropriate developing cells for the graft, iPS cell-derived GPC and OPC were treated with H2O2 for 24 h. Although more A2B5 (+)-GPCs were dead compared to non-treated controls (3.4-fold), less PDGFR (+)-OPCs were damaged by the treatment (1.8-fold), indicating OPC might be resistant to the cell damage. Data suggest that OPC rather than GPC will be appropriate for the transplantation to DWMI model rats.

## 1PK-199

### Activities of biceps brachii muscle after intercostal-musculocutaneous nerve transfer

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Intercostal-musculocutaneous nerve transfer has been applied to regain elbow flexion in patients who suffered severe brachial plexus injury. Since intercostal nerves originally innervate thoracic respiratory muscles, intercostal nerve transferred biceps brachii muscle (IC-biceps) shows respiratory activities in the early stages after surgery. However, 1 or 2 years later, patients regain voluntary control over the injured elbow. The purpose of this study is to evaluate activities of IC-biceps in monkey as an intercostal-musculocutaneous nerve transfer experimental model. One adult monkey (*Macaca fascicularis*) was used. Animals was anaesthetized with Ketamine, i.m. and gaseous anaesthesia. T3 and T4 intercostal nerves were transferred to the musculocutaneous nerve. All surgery was done using aseptic techniques. Four month after nerve transfer surgery, wire EMG recording electrodes were implanted in IC-biceps, triceps, deltoideus and T5 in the operated side, and biceps, triceps, deltoideus in the normal side. EMG signals and video images were recorded. IC-biceps showed voluntary movements and activities were separated from T5 and deltoideus, when animals extended and flexed their arms to eat small pieces of fruits with fingers. These results indicate that the descending pathways from the cortex and brainstem to spinal cord, and local neural circuit in the thoracic segments would be involved in motor control of IC-biceps.

## 1PK-200

### Electrophysiological effect of orexin on subthalamic neurons in rats : an in vitro study

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Orexin-A (ORX-A) and orexin-B (ORX-B) are newly identified neuropeptides in the lateral hypothalamus. ORXergic nerve fibers project to the subthalamic nucleus (STh) that plays a key role in motor control executed by the basal ganglia. Whole cell patch clamp recording using rat brain slice preparations was carried out to examine the electrophysiological effect of ORXs on STh neurons and to clarify the ionic mechanisms involved. Application of ORX-A and ORX-B elicited depolarization in STh neurons and the depolarization persisted in the presence of tetrodotoxin-containing artificial cerebrospinal fluid (TTX ACSF). When ORX-A and ORX-B were applied to STh neurons, the dose-response curve for ORX-B laid to the left of that for ORX-A. The ORX-B-induced depolarization was reduced in ACSF with high extracellular  $K^+$  concentration (high- $K^+$  TTX ACSF) and abolished in high- $K^+$ /low- $Na^+$  TTX ACSF in which NaCl in high- $K^+$  TTX ACSF was replaced with equimolar NMDG-Cl. An inhibitor of  $Na^+$ / $Ca^{2+}$  exchanger failed to inhibit the ORX-B-induced depolarization. The reversal potentials of ORX-B-induced net currents was about -90 mV under low- $Na^+$  TTX ACSF and that was -40 mV under  $K^+$  channel blocking using  $Cs^+$ -containing pipette solution. These results suggest that ORXs depolarize STh neurons post-synaptically and dose-dependently via a dual ionic mechanism including a decrease of  $K^+$  conductance and an increase of nonselective cationic conductance. It seems that ORXs are involved in motor control that is executed by the basal ganglia through the action to STh neurons.

## Poster Presentations Others(1)

## 1PK-201

### Effects of a catechin-rich beverage on stress-loaded diabetic rats

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We examined whether the intake of catechin-rich beverage ameliorates the increased oxidative stress induced by diabetes (DM). Eight weeks old-Wistar rats were divided into two groups; DM group and non-DM group. Rat was rendered diabetic by streptozotocin. After two weeks, both groups rats were further divided into four groups, respectively; freely water-intake group (W), freely intake of catechin-rich beverage group (T), and stress-loaded groups (W+S and T+S) of their respective group (W and T) in both DM and non-DM groups. We used one week-electric shock (0.5mA, 10sec/min, 30times/day) as a stressor. During these periods, body weight, intake volume of water and food were observed. Three weeks after the stress-load, the levels of fasting blood glucose, nitric oxide, oxidative stress, corticosterone and anti-oxidant capacity were measured in the serum of these rats. In general, catechin suppressed the increase in body weight of both DM and non-DM groups, but enhanced the intake of food and water in only DM group. The level of oxidative stress was significantly reduced in only T of DM group. The anti-oxidant capacity in DM group was decreased. Further, the level of corticosterone was decreased by catechin intake in only DM group. These findings suggest that catechin may ameliorate the diabetic state, because the compound reduced the level of oxidative stress.

## 1PK-202

### Thr239 suppresses ROS production in yeast mitochondrial NADH dehydrogenase (NDH-2)

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As with the proton-pumping multisubunit NADH dehydrogenase (complex I), the single subunit NADH dehydrogenases (NDH-2s) found in the mitochondria of plants and fungi serve as the major entry point into the respiratory chain. The NDH-2 catalyzes the electron transfer from NADH to ubiquinone (UQ) without energy transduction. A number of studies have investigated the potential use of NDH-2 from *Saccharomyces cerevisiae* (Ndi1) as a therapeutic agent against complex I disorders, such as sporadic Parkinson's disease which have been linked to reactive oxygen species (ROS) generation by complex I.

We reported previously that the enzyme-bound UQ in Ndi1 plays a key role in preventing the generation of ROS. Our recent studies suggest the Ndi1 reaction proceeds through a ternary complex (Ndi1-UQ-NADH) mechanism, where the bound UQ keeps oxygen from the reduced FAD. To confirm the theory, we investigated biochemical properties of Ndi1 and its Thr239 mutant which have relatively high non-physiological NADH oxidase activity. In the WT enzyme, NADH (but not NADPH) binding induced a stable charge transfer (CT) complex, while T239A mutant did not form the CT complex. Furthermore, the Thr239 mutation had an effect on the mid-point potential of FAD as well as the affinity for oxygen. These results suggest that the ROS production in Ndi1 is suppressed by Thr239, which is responsible for the formation of the stable interaction between Ndi1 and NADH and the stabilization of the FAD redox state.

## 1PK-203

### Sex difference in the effect of alcoholic beverages on gastric emptying in healthy volunteers

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**Background:** Alcohol is thought to modify appetite and food intake. The rate of gastric emptying and satiety are closely related to each other; delayed gastric emptying reduces hunger sensations. Although it has been reported that alcohol ingestion delayed gastric emptying of a solid meal, we previously observed that this effect of alcohol was not as clear in female subjects as in males. In the present study, we examined the retention time of alcohol in the stomach, and compared the effect of alcohol preload on the gastric emptying of a pancake between male and female subjects. **Methods:** The retention times of 60 ml of red wine, vodka, congeners, and mineral water were measured using a carbon (13C)-labeled acetic acid breath test. The experiments on the effects of alcohol preload on the gastric emptying of a pancake were conducted using a 13C-labeled octanoic acid breath test. **Results:** In male subjects, the retention time of wine and vodka was significantly longer than that of congeners and mineral water, and preloading with wine or vodka also significantly delayed gastric emptying of the pancake compared to preloading with congeners and water. In female subjects, the retention time did not differ among the 4 drinks, although the vodka and wine preloading slightly delayed gastric emptying of the pancake.

## 1PK-204

### Spontaneous vasoconstriction and neural regulation of submucosal venule in rat colon

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**Objective:** Functional properties of the venule in the gastrointestinal tract have been less understood than the arteriole. Here we examined spontaneous rhythmic vasoconstrictions and neural regulation of submucosal venules in the rat distal colon. **Method:** Changes in the diameter of venules in submucosal preparations of the rat distal colon were measured with video microscopy and analyzed using an edge-tracking software. **Results:** Venules exhibited spontaneous constrictions at a frequency of 6 min<sup>-1</sup>. This spontaneous activity was abolished by nifedipine (1 μM), CPA (10 μM), 2-APB (100 μM), niflumic acid (100 μM) or DIDS (100 μM). Transmural nerve stimulation or noradrenaline (1 μM) evoked a sustained venular constriction that was inhibited by phentolamine (1 μM). Capsaicin (100 nM) or calcitonin gene-related peptide (CGRP, 10 nM) induced a venular dilation that was attenuated by CGRP8-37 (2 μM), a CGRP receptor antagonist. Venular circular muscles positive for α-smooth muscle actin and perivascular sympathetic and CGRP-containing nerves of the venule were detected by immunohistochemistry. **Conclusion:** The spontaneous constrictions of the venule in the submucosa of rat distal colon appear to depend on Ca<sup>2+</sup> release from sarcoplasmic reticulum that opens Ca<sup>2+</sup>-activated Cl<sup>-</sup> channels to trigger Ca<sup>2+</sup> influx through L-type Ca<sup>2+</sup> channels. The venule is under the control of sympathetic and primary afferent nerves. The spontaneous constrictions may contribute to active drainage even when faecal pellets strongly distend the wall of the distal colon.

## 1PK-205

### Can infants distinguish between texture of diapers?

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Studies have shown that infants adapt to the surrounding environment through different sensory and cognitive abilities. However, few such studies have described the infants' sense of touch. Here, we assessed the ability of infants to distinguish between and their response to different diaper materials.

The prefrontal cortex (PFC) activities of 72 healthy Japanese infants exposed to tactile stimuli on their palm and lower back were measured by near-infrared spectroscopy. We divided infants into 2 groups according to different developmental stage: 44 infants aged 2-6 months and 28 infants aged 9-14 months. Two types of diaper surface material (P and Q) were chosen for tactile stimuli; the infants were exposed to each material at each body part 3 times for 30 s. Primary hand properties were studied using the Kawabata Evaluation System for Fabric; the mean deviation of the surface coefficient of friction was 0.007 and 0.012 for materials P and Q, respectively. Infants aged 2-6 months showed more significant increases in PFC activities for material P than Q on the palm of the left hand and left lower back. Infants aged 9-14 months also showed more significant increases in PFC activities for material P than Q on the left lower back. These findings suggest that infants can distinguish among diaper materials not only by their palms but also the lower back. Moreover, we found that infants find material P more pleasant to touch than Q.

Thus, we conclude that infants have enhanced sense of touch even on skin that is covered by diapers daily.



## 1PK-206

### Identification of binding regions on *Xenopus* dicalcin for its target glycoprotein in the egg-coating envelope

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Fertilization comprises taxon-specific sperm-egg interactions including sperm-binding to an egg-coating envelope, penetration through this envelope and fusion on an egg plasma membrane. Despite identification of several regulatory proteins and sugar residues, the molecular mechanisms whereby the fertilization is controlled remain largely unknown. We recently discovered that *Xenopus* dicalcin, present in the extracellular egg-coating envelope, remarkably suppresses fertilization *in vitro* through binding to gp41, a glycoprotein of the egg envelope. To reveal the molecular mechanism of the action of dicalcin, we set out to identify peptide regions of dicalcin responsible for the binding to gp41. As the first step, we prepared a set of deletion mutants of dicalcin and examined the binding activity of each mutants. The results indicated that the binding region is located within the N-terminal half of dicalcin. To narrow the range of the binding region, we next synthesized peptides that flank the N-terminal half of dicalcin and investigated the binding to gp41. The results showed that two peptide regions that locates separately at a distance of forty amino acids, retain the maximum binding activity. We further found that these peptides essentially inhibited the efficiency of fertilization *in vitro*. Thus, our present results determined the binding regions on dicalcin for its target, gp41, and may lead to the development of potent bioactive peptides that are capable of controlling the efficiency of fertilization *in vitro*.

## 1PK-207

### Transferrin-Transferrin receptor 1 signaling is required for mouse erythroblast enucleation through the mechanism independent of iron uptake

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During late stage of mammalian erythropoiesis, the nucleus undergoes extrusion from the cytoplasm while being surrounded by a segment of plasma membrane. However, the signaling pathways involved in this step of erythropoiesis have remained obscure.

Here, we used erythroblasts from the spleens of phlebotomized adult mice and erythroblasts from E14.5 mouse fetal livers to unveil the mechanisms of erythroblast enucleation. Enucleation was assessed by using the cell-permeable DNA staining dye SYTO16 and flow cytometer.

As the result, we demonstrated that mouse erythroblasts are not enucleated in the medium which does not contain transferrin (Tf) but are enucleated in the medium containing holo-Tf or apo-Tf. Anti-Transferrin receptor 1 (TfR1) monoclonal antibody blocks enucleation of both types of erythroblasts. Furthermore, reduction of TfR1 protein by TfR1 siRNA suppresses enucleation of mouse fetal liver erythroblasts without affecting their differentiation. These results indicate that Tf-TfR1 signaling has a crucial role in enucleation of mouse erythroblasts.

## 1PK-208

### Study of a mechanism of protective effect with saponins from Panax Ginseng against oxidative stress in blood preservation

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We studied to evaluate protective effects against oxidative stress on stored blood with ginsenosides extracted from saponin of Panax ginseng at 4°C. Deformability of erythrocytes, which were stored with citrate-phosphate-dextrose (CPD) in one week, decreased. However, erythrocyte deformability of stored CPD blood with saponins lesser decreased than without them. Saponins did not inhibit the shape change of erythrocyte and the peroxidation of membrane lipids. However, they inhibited the oxidation-induced decrease of thiol-group of membrane proteins. Then, using the thiol-group of membrane proteins as an index, we have screened the components of Panax ginseng extract and identified ginsenoside-Rg2 and Rh1 as the active ingredients. Rg2 and Rh1 inhibited the storage-induced decrease of thiol-group in Band 3 (anion-exchanger-1). These two ginsenosides made lactate production to decrease, but glucose consumption not to decrease. So it is suggested the ginsenosides induced hexose monophosphate pathway in the erythrocyte, then reduced glutathione regeneration increased.

## 1PK-209

### The mechanisms underlying Ca<sup>2+</sup>-mediated Ca<sup>2+</sup> sensitization in contraction of human detrusor smooth muscle

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Introduction and Objective : Ca<sup>2+</sup> sensitization requires stimulation of G protein-coupled receptor (GPCR) that activates rho kinase (ROK) and protein kinase C (PKC) pathways in smooth muscle. However, the only increase of [Ca<sup>2+</sup>]<sub>i</sub> without the activation of GPCR unexpectedly induced Ca<sup>2+</sup> sensitization in some studies. Therefore, the present study was undertaken to reveal the Ca<sup>2+</sup>-mediated Ca<sup>2+</sup> sensitization using human detrusor smooth muscle. Materials and Methods : The small strips (3-4 mm in length and 300-400 μm in diameter) were dissected from human urinary bladder and permeabilized with α-toxin. Results : The tension at constant 1 μM [Ca<sup>2+</sup>]<sub>i</sub> was inhibited by specific ROK inhibitor Y-27632 or predominantly by specific PKC inhibitor GF-109203X. The involvement of calmodulin in Ca<sup>2+</sup>-dependent activation of ROK was examined by Y-27632 before and after incubation with calmodulin inhibitor W-7 (100 μM). The inhibitory effects of 10 μM Y-27632 in the presence and absence of W-7 were 23.4±2.5% and 14.1±2.1%, respectively (n=6; P=0.029). Simultaneously, the inhibitory effects of 10 μM GF-109203X in the presence and absence of W-7 were 45.7±1.3% and 33.8±1.6%, respectively (n=6; P=0.003). Conclusions : This study demonstrated Ca<sup>2+</sup>-mediated Ca<sup>2+</sup> sensitization in human detrusor smooth muscle. The increase of [Ca<sup>2+</sup>]<sub>i</sub> activates Ca<sup>2+</sup>-sensitization, through activation of ROK and PKC. Further, this Ca<sup>2+</sup>-mediated Ca<sup>2+</sup> sensitization was independent of calmodulin.

## 1PK-210

### Role of ClC-5 in the intracellular H<sup>+</sup> transport in perfused bullfrog proximal tubule cells

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In this study, we examined the H<sup>+</sup> transport across the acid vesicles by measuring membrane potential with cytosolic pH (pH<sub>c</sub>) in perfused bullfrog proximal tubule cells with ion-selective microelectrodes or acridine orange. Elevating basolateral CO<sub>2</sub> from 1.5% to 5% at constant HCO<sub>3</sub><sup>-</sup> concentration induced a slight increase in pH<sub>c</sub> with a hyperpolarization of basolateral membrane or an increase in fluorescent intensity of acridine orange in acid vesicles. Moreover, pH in proximal tubular fluid was increased by elevating basolateral CO<sub>2</sub>. In the presence of 10<sup>-6</sup> M bafilomycin or 10<sup>-8</sup> M concanamycin, elevating basolateral CO<sub>2</sub> produced the decrease in pH<sub>c</sub> with depolarization or no increase in fluorescent intensity of acridine orange. In the presence of 10<sup>-6</sup> M NPPB (an inhibitor of Cl<sup>-</sup> channel), elevating CO<sub>2</sub> produced a significant hyperpolarization followed by a slow depolarization. We have already reported that hyperpolarization of basolateral membrane is always associated with increase in pH<sub>c</sub> and vice versa. From these experimental results, we concluded that H<sup>+</sup> transport by H<sup>+</sup> pump but also ClC-5 in acid vesicles might affect the regulation of pH<sub>c</sub> in the bullfrog proximal tubule cells.

## 1PK-211

### Diethylstilbestrol disturbs the non-genomic regulation of hyperactivation by progesterone and 17β-estradiol

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Mammalian spermatozoa have to be capacitated before fertilization. Generally, capacitated spermatozoa exhibit hyperactivation in flagellum. Sperm hyperactivation is a specialized movement of the sperm flagellum that creates the propulsive force for penetration of the zona pellucida. Hyperactivated spermatozoa exhibit large bend amplitude, whiplash and frenzied flagellar movements. Recently, I have been investigated that sperm hyperactivation was enhanced by progesterone via a non-genomic regulation, and progesterone-enhanced hyperactivation was suppressed by 17β-estradiol via another non-genomic regulation in hamster. Moreover, I suggested that enhancement of sperm hyperactivation was regulated according to rate of concentration of progesterone and 17β-estradiol in the last annual meeting. In the present study, I examined whether suppression of progesterone-enhanced hyperactivation by 17β-estradiol was disturbed by diethylstilbestrol in hamster spermatozoa. Diethylstilbestrol significantly but weakly suppressed progesterone-enhanced hyperactivation. When spermatozoa were simultaneously exposed to non-effective concentrations of 17β-estradiol and diethylstilbestrol, diethylstilbestrol accelerated a suppressive effect of 17β-estradiol, and strongly suppressed progesterone-enhanced hyperactivation together with 17β-estradiol. Those results suggest that diethylstilbestrol disturbs the non-genomic regulation of sperm function by progesterone and 17β-estradiol playing as accelerator of 17β-estradiol.

## 1PK-212

### Study of correlation between the quality and membrane potential using bovine ovum

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Ovums are selected for artificial fertilization based on morphology. However, some ovums and embryos can not continue to maintain development after artificial fertilization. To raise reproductive ratio, a new method for ovum selection from new point of view is needed. The membrane potential reflects situation of ion channels and completeness of cell membrane. On the other hand, it is possible to scratch ovums during separation from granulosa cells. Though it may effect quality of ovums, morphological analysis may not be able to distinguish them. Measurement of membrane potential may be applied for stage- and quality-selection of ovums. So we measured the membrane potential of unfertilized ovums and 2- to 8-cell period using bovine cells in this study. And we found wide dispersion of membrane potential in ovums after removing granulosa cells. It implies some "naked" ovums do not have ability that undergo development.

## 1PK-213

### Automated multiple intracellular recording in vivo

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Although multiple in vivo intracellular recording is a useful technique for understanding how neurons and neural circuits function, this is formidable tasks for researchers. In order to overcome this difficulty, we developed an automated intracellular recording (AIR) system. This AIR system can automatically move an electrode in the brain, find a neuron, activate a brief high frequency current to penetrate the neuron and inject the optimal negative current to recovery from the penetration damage. We evaluated the performance of the AIR system in anesthetized head-restrained mice. The success rate for one electrode was 63% (n=11 electrodes). The average stable recording time was 56 min, and a maximum time was 193 min. After stable intracellular recording from one neuron was finished, this system could continuously find another neuron and achieve the intracellular recording from it without changing the electrode. We could record from up to 4 neurons using 1 electrode. For multiple in vivo intracellular recording, we run 6 AIR systems in parallel and succeeded in simultaneous recording from 4 neurons; 2 neurons from the primary somatosensory and 2 neurons from the secondary motor area. This system will enable us to record from neurons that are synaptically connected and help us to understand the mechanism of the synaptic transmission in vivo.

## 1PK-214

### Comparison of self-study of the basic level of “step-by-step studies of human life sciences” at two different health care colleges

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We compared the effects of self-study of the basic level of “step-by-step studies of human life sciences” at two different health care colleges. For the 2012 school year, an e-learning account and a booklet of this innovative material were provided to the new students after entry registration for the 2-year course school A (n=146) and the 4-year course school B (n=326). In an anonymous survey on acquired knowledge from life science courses they had completed prior to entry registration, school A students answered significantly less ( $p < 0.05$ ). Before self-study, score of the evaluation test was  $63.4 \pm 1.2\%$  vs  $71.3 \pm 0.8\%$  (mean  $\pm$  SE) and 75% score could not be reached by 79.5% vs 54.6% of the students for schools A and B, respectively (for both,  $p < 0.01$ ). After about 3 months of self-study, the score of the evaluation test was  $91.4 \pm 0.7\%$  vs  $92.1 \pm 0.5\%$ , which for both A and B, were significantly higher compared to the scores before self-study ( $p < 0.01$ ), but were not significantly different between schools. In the anonymous survey asking about the change in confidence that ‘I can study more life sciences’ by working on ‘step-by-step’, the distribution of the answers in school A was significantly better ( $p < 0.05$ ).

## 2PK-001

### Effects of dexmedetomidine, alpha 2 agonist, on cardiac function, coronary vasoactivity, and ventricular electrophysiology in guinea-pig

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Both dexmedetomidine and clonidine are selective and specific alpha 2 agonists. We evaluated the inhibitory effects of dexmedetomidine on cardiac function with electrical field stimulation (EFS) and the direct effects on coronary artery or ventricular myocytes in guinea pig hearts, and compared these effects with those of clonidine. The heart was mounted on a Langendorff apparatus to measure coronary perfusion pressure (CPP). A saline-filled balloon was inserted into the left ventricle to measure systolic left ventricular pressure (sLVP). The EFS was applied to stimulate sympathetic nerve terminal. Action potential duration (APD) was investigated by the patch clamp methods. Dexmedetomidine almost completely inhibits the increase of LVP induced by EFS at concentrations  $> 10$  nM, with little affecting the basal LVP. In aged  $> 6$  weeks guinea pig, dexmedetomidine decreased CPP at concentrations  $< 10$  nM, but increased it at concentrations  $> 10$  nM in a concentration-dependent manner. On the other hand, dexmedetomidine decreased CPP at all concentrations in aged  $< 3$  weeks, and maximal inhibitory effect was found at 1 nM. Clonidine had minimal effects on CPP. Both dexmedetomidine and clonidine had little direct effects on ventricular dP/dt and/or APD. The present findings demonstrated that dexmedetomidine inhibits the increase of cardiac function activated by sympathetic stimulation significantly more than clonidine. In addition, the response of coronary artery resistance to dexmedetomidine alters during postnatal development.

## 2PK-002

### The Role of Plasma Hypoosmolarity in Closure of the Ductus Arteriosus

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The closure of the ductus arteriosus (DA) is regulated by environmental changes such as rising oxygen tension and withdrawal of PGE<sub>2</sub>. As we found that plasma osmolarity was decreased early after birth in rats, and that a hypoosmolarity sensor Transient Receptor Potential Melastatin (TRPM) 3 was highly expressed in rat DA, we hypothesized that plasma hypoosmolarity regulated closure of the DA via TRPM3. We found that hypoosmolarity (270 and 250 mOsm/kg) increased the tension of the rat DA rings (17.4% and 29.8% of 120 mM of KCl-induced contraction, respectively,  $P < 0.01$ ,  $n = 8$ ). In rat DA smooth muscle cells (SMCs),  $[Ca^{2+}]_i$  was increased by hypoosmolarity (270 mOsm/kg), which was attenuated in DA SMCs transfected with TRPM3-targeted siRNA. The TRPM3 activator pregnenolone sulfate (200  $\mu$ M) induced contraction of the rat DA (28.0% of KCl  $P < 0.001$ ,  $n = 6$ ). Conversely, DA closure was inhibited by 35% when plasma osmolarity was kept higher using 7.2% NaCl solution in vivo ( $n = 5$ ,  $p < 0.001$ ). Furthermore, we observed a similar decrease of plasma osmolarity in human preterm infants (3.2% decrease of cord blood). This decrease was blunted in extremely preterm infants including patent DA (PDA) patients. These results suggest that plasma hypoosmolarity early after birth contributes to closure of the DA via TRPM3.

## Poster Presentations Heart, Circulation(2)

## 2PK-003

### Altered neurotrophin expression profile in the NTS of SHR, a clue in the development of hypertension?

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Since the nucleus tractus solitarii (NTS) is a pivotal region for regulating the set-point of arterial pressure, we proposed a role for it in the development of neurogenic hypertension. Our previous findings suggest that the NTS of pre-hypertensive and hypertensive spontaneously hypertensive rats (SHRs) exhibits abnormal inflammatory condition with elevated anti-apoptotic factors, compared to the normotensive rats (Gouraud et al. *J Hypertens.* 2011, Gouraud et al. *Auton Neurosci.* 2011). Whether this chronic condition affects the neuronal plasticity and activity in the NTS remains unknown. To unveil the characteristics of the neuronal system in the NTS of SHRs, we investigated the expression of neurotrophin transcripts in both young and adult SHRs. The specific RT2 Profiler PCR Array for rat neurotrophins and receptors followed by data validation revealed that the expression of Gfra3 (Glial cell line-derived neurotrophic factor family receptor alpha-3), known to be involved in neurons survival and migration, and Tnfrsf 6 (Tumor necrosis factor receptor superfamily, member 6, Fas), known to be involved in neuronal death and neurons branching, are altered in the NTS of both adult and young SHRs. These profiles may be involved in the impairment of the neuronal circuitry regulating cardiovascular autonomic activity during the development of SHR. Supported by KAKENHI (19599022, 19-07458, 21300253).

## 2PK-004

### Impairment of E-C coupling in cardiac papillary muscle with fibrosis

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In pathophysiological conditions, such as cardiac hypertrophy, cardiac fibrosis is recognized as a maladaptive response. We made a rat model of cardiac hypertrophy by pulmonary artery banding (PAB). Four to six weeks after operation, the PAB rats (n=25) were divided into the interstitial fibrosis group [FIB; 17.12±1.47% of fibrosis area (n=6)] and the non-fibrosis with hypertrophy group [HYP; 3.04±0.26% (n=19)] judged by Masson Trichrome stain. They were compared with the sham-operated control rats [Sham; 2.53±0.24% (n=12)]. Papillary muscle diameter and muscle cell size in the right ventricle of FIB were almost identical to those in HYP. For measurement of tension with intracellular Ca<sup>2+</sup> transients, we used the aequorin method. The peaks of the Ca<sup>2+</sup> signals in FIB and HYP were significantly higher than that in the Sham. However, peak tension in FIB was significantly smaller than that in HYP and Sham. In connexin43 (Cx43) stain with IHC, we found less Cx43 accumulation in cell to cell long axis connective area in FIB compared with that in HYP and Sham. Cardiac hypertrophy with fibrosis showed less contractility with larger Ca<sup>2+</sup> transients. Impairment of tension development of the cardiac muscle with interstitial fibrosis is considered to be due to 1) a decrease in Ca<sup>2+</sup> sensitivity of the myofilaments, 2) the disturbance of Ca<sup>2+</sup> release mechanism and 3) asynchronous activation of each cardiac myocytes in the fibrotic area with less cell to cell communication.

## 2PK-005

### Simulation analysis of short-term desensitization of cardiac muscarinic K<sup>+</sup> current

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The activated cardiac K<sub>ACh</sub> current (I<sub>K<sub>ACh</sub></sub>) by high concentrations of ACh gradually decreases to a quasi-steady-state level within 1 min. This reduction of I<sub>K<sub>ACh</sub></sub> in the continuous presence of ACh is called short-term desensitization. Although this phenomenon has been known for a long time, its mechanism is not elucidated. By incorporating a concept of two distinct muscarinic receptors (m<sub>2</sub>Rs) and K<sub>ACh</sub> channels with different affinities for ACh and the G-protein β subunits (G<sub>β</sub>), respectively, we constructed a model of muscarinic modulation of I<sub>K<sub>ACh</sub></sub> and checked if the constructed model reproduces short-term desensitization. The model is composed of two parts: an allosteric model of the interaction between G<sub>βγ</sub> subunits and the K<sub>ACh</sub> channel, and a G-protein cycle model describing the interaction between m<sub>2</sub>Rs and G<sub>i/o</sub> proteins. The incorporated m<sub>2</sub>Rs conferred a I<sub>K<sub>ACh</sub></sub> response over a wide range of [ACh]<sub>s</sub>. The K<sub>ACh</sub> channels contributed to reproduction of apparent response of the peak and quasi-steady-state components in short-term desensitization, and also accounted for the effects of ACh preperfusion and recovery from short-term desensitization observed in atrial myocytes. Incorporation of the present I<sub>K<sub>ACh</sub></sub> model into the action potential models of nodal cells conferred recurring spontaneous firing after a short break in the presence of excess ACh. Therefore, two qualitatively different populations of K<sub>ACh</sub> channels and m<sub>2</sub>Rs may participate in the short-term desensitization of I<sub>K<sub>ACh</sub></sub> in native myocytes and vagal escape at nodal cells.

## 2PK-006

### Histamine H<sub>1</sub> receptors in the nucleus tractus solitarius attenuate cardiac baroreflex in rats

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Axons of histamine-containing neurons are known to project from the posterior hypothalamus to many areas of the brain, including the nucleus tractus solitarii (NTS), a central brain structure that plays an important role in regulating cardiovascular system. We have previously shown that histamine H<sub>1</sub> receptors are expressed in NTS neurons and regulate the set-point of arterial pressure and heart rate (HR). In the present study, we examined whether H<sub>1</sub> receptors in the NTS can modulate the cardiac baroreflex. After 2-Pyridylthylamine (10-25 nmol), a histamine receptor H<sub>1</sub> agonist, was microinjected into the NTS of urethane-anaesthetized Wistar rats, (i) both arterial pressure and HR were significantly increased, (ii) the cardiac baroreflex in response to phenylephrine induced hypertension was significantly attenuated, and (iii) bradycardia induced by L-glutamate microinjections into the NTS was significantly inhibited. These findings suggest that histamine H<sub>1</sub> receptors may regulate the cardiac baroreflex via acting postsynaptically to inhibit NTS neurons controlling HR.

## 2PK-007

### Hyperoxic air insufflation aggravates the systemic inflammatory response during cardiopulmonary bypass

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Cardiopulmonary bypass (CPB) is indispensable for cardiac surgery. Generally, the blood of patient is exposed to hyperoxic air via oxygenator and maintained in hyperoxic condition during CPB. The purpose of this study is to investigate whether hyperoxic condition aggravates the systemic inflammatory response during CPB.

Rats were divided into the Sham group, the normoxia CPB (PaO<sub>2</sub>: 100-150 mmHg) group and hyperoxia CPB (PaO<sub>2</sub>: 400-500 mmHg) group. CPB pump flow was initiated and maintained at 60 ml/kg/min. Blood samples were collected before (baseline), and 60 min and 120 min after initiation of CPB. During CPB, blood pressure and Hb were maintained around 70 mmHg and 10g/dl, respectively.

Pro-inflammatory markers (TNF- $\alpha$ , IL-6) and biochemical makers (LDH, ALT, AST) were significantly elevated in the hyperoxia CPB group compared with other groups. On the other hand, anti-inflammatory marker (IL-10) was significantly elevated in the normoxia CPB group compared with other groups. From these data, it is highly possible that hyperoxic condition aggravates the systemic inflammatory response and the organ damage during CPB.

## 2PK-008

### Voluntary Wheel-running Improves Blood Pressure and Endurance Capacity in Rat Heart Failure Model

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The aim of this study was to examine whether voluntary wheel-running exercise improve blood pressure and endurance capacity in a rat heart failure model. One-month-old Wistar male rats underwent an aortic-banding operation. In aortic-banding (Band) and sham-operation (Sham) groups, voluntary wheel-running distance for 10 days was gradually decreased as the rats aged. There was no difference in this running distance between both groups 2, 4, 6, 9, 12 and 18 months after the operation. In "Band" group, systolic/diastolic blood pressures, which were measured by a tail-cuff method, were gradually decreased 6 months later, and were significantly lower than those in "Sham" group 6-18 months after the operation. Eighteen months later, endurance capacity, which was evaluated by increment in blood lactate just after treadmill exercise, was significantly lower in "Band" group than in "Sham" group. In "Band" group, however, systolic/diastolic blood pressures and endurance capacity could be improved by voluntary wheel-running exercise for 3 weeks. These results suggest that daily light-exercise such as walking is a potential strategy for improving both systolic/diastolic blood pressures and endurance capacity in patients with heart failure.

## 2PK-009

### Ameliorating effect of foot bath on peripheral hemodynamics assessed by augmentation index of radial pressure waves

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One feels the warmth spread through one's body after the feet soaking in warm water, which is in a subjective way. We investigated ameliorating effect of foot bath on peripheral hemodynamics using an augmentation index (AI) of radial pressure waves as an indicator of reflected waves. The present study has been approved by the Ethics Committee of Fukushima Medical University. Twenty-seven healthy women aged 21 to 32 years soaked their feet in hot spring water (Nametsu Hot Spa, Nihonmatsu City, Fukushima, Japan) at 40-42°C for 15 min in a room temperature at about 19°C. Pressure waves measured noninvasively in the left radial artery using an applanation tonometry (Omron Healthcare, HEM9000-AI) and calibrated by blood pressure measured in the right brachial artery by means of oscillometric method. Pressure waves, blood pressure, body temperature (leg, arm and neck) were measured before and immediately and 15 min after the soaking feet in hot spring water. Systolic and diastolic pressures decreased significantly immediately after the bathing. The value of AI declined significantly immediately and 15 min after the bathing. Skin temperature at the neck showed a slight but significant increase after the bathing. In conclusion, foot bath is beneficial for remedying peripheral hemodynamics.

## 2PK-010

### Comparison of repolarization heterogeneity during and after exercise in healthy males

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#### (Background)

It has recently suggested that an increase of transmural dispersion of repolarization (TDR) is associate with arrhythmogenesis. But, there are many unresolved questions about the universality of the evaluation method for the arrhythmogenesis using the Tp-e/QT ratio.

#### (Purpose)

The aim of this study was to investigate changes in Tp-e interval, to compare the characteristic ratio (RR,QTc,Tp-e/QT) at the same heart rate before and after exercise.

#### (Subjects and Methods)

The subjects were 19 healthy volunteers, underwent electrocardiogram and measurements of blood pressure (BP) at rest, and ergometric exercise test (target HR is 200% of control). ECG intervals and repolarization characteristics (RR,QTc,Tp-e/QT) were calculated at rest, at peak of exercise and full recovery.

#### (Results)

Tp-e/QT ratios were significantly elevated after exercise compared with before exercise at same heart rate, in all subjects. A significant correlation ( $r=0.538$ ,  $p=0.015$ ) was observed between delta Tp-e/QT (peak exercise-before exercise) and delta BP (peak value-control value).

#### (Discussion)

It is considered that the elevation of systolic blood pressure increase the value of Tp-e/QT due to the action potential prolongation of endocardial in physiological manner.

#### (Conclusion)

Repolarization characteristics, the Tp-e/QT ratio is modulated by the autonomic nervous system and baroreflex mechanism.

## 2PK-011

### Effect of Hyperpolarization-Activated Current $I_f$ on Robustness of Sinoatrial Node Pacemaking : theoretical study in connection with intracellular $Na^+$ concentration changes

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To elucidate the effects of hyperpolarization-activated current  $I_f$  on robustness of sinoatrial node (SAN) pacemaking in connection with intracellular  $Na^+$  concentration ( $Na_i$ ) changes, we investigated 1) the impacts of  $I_f$  on dynamical properties of SAN model cells during inhibition of L-type  $Ca^{2+}$  channel currents ( $I_{CaL}$ ) or hyperpolarizing loads, and 2)  $I_f$ -dependent changes in  $Na_i$  and their effects on dynamical properties of model cells. Bifurcation analyses were performed for  $Na_i$ -variable and  $Na_i$ -fixed versions of Maltsev-Lakatta model for rabbit SAN cells; equilibrium points (EP), limit cycles (LC) and their stability were determined as functions of parameters. In the  $Na_i$ -variable system, increasing  $I_f$  conductance ( $g_f$ ) shrank  $I_{CaL}$  conductance ( $g_{CaL}$ ) region of unstable EPs and rhythmic firings. Increased  $g_f$  yielded an elevation in  $Na_i$  at EPs and during spontaneous firings, which caused EP stabilization and shrinkage of parameter regions of unstable EPs and rhythmic firings. As  $g_f$  increased, the unstable EP and stable LC regions determined with applications of hyperpolarizing bias currents ( $I_{bias}$ ) shrank in the  $Na_i$ -variable system, but enlarged in the  $Na_i$ -fixed system. These results suggest that 1)  $I_f$  attenuates robustness of SAN cells via facilitating EP stabilization, and 2) the enhancing effect of  $I_f$  on pacemaker robustness is reversed by concomitant increases in  $Na_i$ .

## 2PK-012

### Depressed length-dependent activation in left ventricular muscle of the knock-in mouse model of dilated cardiomyopathy with troponin T deletion mutation K 210

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We investigated how length-dependent activation is altered in a DCM mouse model with a deletion mutation K210 in the cTnT gene. Confocal imaging in isolated living hearts revealed that the cardiomyocytes were significantly enlarged in K210 hearts, with striation patterns similar to those in wild type (WT) hearts. Skinned muscle preparations were isolated from left ventricular papillary muscles of WT and K210 mice. An increase in sarcomere length shifted the mid-point ( $pCa_{50}$ ) of the force- $pCa$  curve leftward by  $\sim 0.21$   $pCa$  units in WT. In K210,  $Ca^{2+}$  sensitivity was lower, and the length-dependent shift of  $pCa_{50}$  ( $\delta pCa_{50}$ ) was less pronounced with a mean value of  $\sim 0.11$   $pCa$  units. The rate of active force redevelopment ( $ktr$ ) was significantly slower in K210 at half-maximal activation.  $pCa_{50}$ ,  $\delta pCa_{50}$  and  $ktr$  became similar in WT and K210 preparations following thin filament reconstitution with the identical troponin complex. An increase in thin filament cooperative activation upon an increase in the fraction of strongly bound cross-bridges by MgADP increased  $\delta pCa_{50}$  to  $\sim 0.21$   $pCa$  units in K210 preparations. These findings are consistent with the notion that the depressed Frank-Starling mechanism in K210 hearts is the result of a reduction in thin filament cooperative activation.

## 2PK-013 (SPK-6)

### Blockade of GABAergic inputs into the RVLM neurons enhances respiratory modulation of the cardiovascular sympathetic nerve in the *in situ* arterially-perfused preparation of rats

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It has been known that neurons in the rostral ventrolateral medulla (RVLM neurons) generate the activity of the cardiovascular sympathetic nerve (SNA), and receive the respiratory modulation from the respiratory center. Recently, we have reported that respiratory-related inhibitory inputs into the RVLM neurons in hypertensive rats are attenuated than that in normotensive rats. However, it is still unclear what kinds of inhibitory inputs are related with the respiratory-related inhibitory inputs. In this study, we evaluated effects of blockade of GABAergic inputs into the RVLM neurons on respiratory modulation of the SNA in the *in situ* arterially perfused preparation of rats. We injected a GABA<sub>A</sub> receptor antagonist, bicuculline (5 mM, 50 nL), into the RVLM bilaterally, and analyzed the effect on respiratory modulation of the SNA by the phrenic nerve activity-triggered average of the SNA. As a result, blockade of GABAergic inputs into the RVLM neurons elevated the basal SNA and enhanced the respiratory related SNA. The respiratory-phase relation of SNA in the presence of bicuculline in normotensive rats was similar with that in the absence of bicuculline in hypertensive rats. These data may indicate that enhancement of respiratory related modulation of cardiovascular sympathetic nerve in hypertensive rats is caused by attenuation of GABAergic inputs into the RVLM neurons.

## 2PK-014

### Properties of the mechanisms of blood vessels in Guinea pig liver

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The liver has a role as a reservoir of blood in the body and provides the blood for the circulation when it needs, for example, at the acute hemorrhage. For the function, the blood vessels in the liver seem to evoke the vasoconstriction. But the contraction/relaxation mechanisms of the blood vessels have been less understood. In the present study, blood vessels lying on the outlet of the liver, were isolated to observe the contraction directly *in vivo*. The isometric tension of the blood vessel was recorded in 37°C Krebs solution. When the vessel was depolarized with 40mM  $K^+$ , it significantly evoked vasoconstriction. 3-10  $\mu M$  phenylephrine evoked vasoconstriction. 1-10  $\mu$  Acetylcholine (ACh) evoked small but significant vasoconstriction. The 1  $\mu$  ACh evoked vasoconstriction on the tension evoked by the 10  $\mu$  phenylephrine. While the vasoconstriction was evoked in presence of 10  $\mu$  phenylephrine, sodium nitroprusside, a nitric oxide donor, did not evoke any vasorelaxation significantly. These results suggest that the blood vessel lying on the outlet has  $\alpha$  receptor to evoke vasoconstriction, but the mechanism for the vasorelaxation through cGMP is not developed.

## 2PK-015

### Analysis of nuclear Ca<sup>2+</sup> transient in mouse cardiomyocytes with GECO, a recently developed genetically encoded Ca<sup>2+</sup> indicator

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Nuclear calcium (Ca<sup>2+</sup>) signaling plays pivotal roles in various cellular functions including cell growth, differentiation, and cell death. In cardiomyocytes, rise in nuclear Ca<sup>2+</sup> is generally thought to result from cytosolic Ca<sup>2+</sup> transients with passive diffusion through nuclear pores. However, several studies suggest the independent regulation of nuclear Ca<sup>2+</sup> mediated by inositol 1,4,5-trisphosphate receptor (IP<sub>3</sub>R)-dependent signaling, which may be more important for local nuclear functions such as gene transcription, leading to hypertrophy. To clarify nuclear Ca<sup>2+</sup> signaling in living cells, we focused on a recently developed genetically encoded Ca<sup>2+</sup> indicator GECO. GECOs can simultaneously visualize multichromatic Ca<sup>2+</sup> images of different organelles including nucleus and mitochondria in a single cell. To distinctively analyze nuclear and cytosolic Ca<sup>2+</sup> transients in cardiomyocytes, we made adenoviruses encoding nucleus- and cytosol-targeted GECOs. Fluorescence images were analyzed in the neonatal mouse cardiomyocytes infected with adeno-GECOs or loaded with a synthetic Ca<sup>2+</sup> indicator Fluo-4/AM. With both indicators, electrical stimulation-elicited nuclear Ca<sup>2+</sup> oscillations were synchronized to cytosolic Ca<sup>2+</sup> transients, but with slower decline kinetics. ATP, an IP<sub>3</sub>R agonist, elevated the baseline of nuclear Ca<sup>2+</sup> signal, supporting the evidence for IP<sub>3</sub>R-dependent nuclear Ca<sup>2+</sup> signaling. We are now studying which molecules are involved in regulating the nuclear Ca<sup>2+</sup> signaling in cardiomyocytes.

## 2PK-016

### Effects of obstructive sleep apnea on sympathetic nerve activity and systemic arterial pressure in conscious rats

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Responses of sympathetic nerve activity to obstructive sleep apnea were studied. Wistar male rats were chronically instrumented with electrodes for measurements of renal (RSNA) and lumbar (LSNA) sympathetic nerve activity, and electroencephalogram, electromyogram, and electrocardiogram and with catheter for measurement of systemic arterial pressure, and with a tracheal balloon for induction of apnea. At least 3 days after recovery period, the tracheal balloon was inflated for 40 seconds during non-rapid eye movement (NREM) sleep. RSNA and LSNA increased immediately after onset of the tracheal balloon inflation and by approximately 3~4-fold compared with the pre-inflation level. Heart rate decreased in biphasic manner; it began to fall gradually during the initial several seconds after the onset of the inflation, and then it fell abruptly by approximately 200 beats/min. Systemic arterial pressure increased due to the tracheal balloon inflation. It is therefore concluded that the sympathetic nerve activity play a critical role in increasing systemic arterial pressure during obstructive sleep apnea in rats.

## 2PK-017

### Pharmacological inhibition of STAT3 protect heart from lipopolysaccharide-induced cardiac dysfunction through the inhibition of Jak2/STAT3/iNOS signaling

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Cardiac damage caused by cytokines/endotoxin has been reported to occur through the activation of pro-inflammatory cytokine-mediated Jak2/STAT3 signaling, leading to upregulation of iNOS. We thus hypothesized that pharmacological inhibition of STAT3 with WP1066, an analogue of the selective STAT3 inhibitor AG480 for clinical use, may protect heart from lipopolysaccharide (LPS)-induced cardiac dysfunction through the inhibition of Jak2/STAT3/iNOS signaling. LPS (5 mg/kg) elicited a significant and robust decrease of left ventricular ejection fraction (LVEF) in mice (C57BL/6) with or without the treatment of WP1066 (10 mg/kg i.p. for 1 hour), but the magnitude of the decrease was much less in mice pretreated with WP1066 after LPS injection (WP1066+LPS vs LPS: 15±1.9 vs 26±2.0%, n=5-6, P<0.01). We also examined the activation of JAK/STAT pathway. The tyrosine phosphorylation of STAT3 (Tyr705) in left ventricle (LV) was reduced more by 39±3% (n=4 to 5, P<0.01) in mice treated with WP1066, while it did not alter the phosphorylation of STAT1 (Tyr701). WP1066 also inhibited the production of iNOS in LV after LPS injection (LPS vs WP1066+LPS 100±11.4 vs 51±6.8%, n=6, P<0.01) with the decrease of the indexes for liver and renal dysfunction. Together, STAT3 inhibition with WP1066 may protect multiple organs other than heart from damage caused by cytokines/endotoxin.

## 2PK-018

### Time course of changes in sympathetic nerve activity and heart rate during development of hypertension in spontaneously hypertensive rats

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Potential contribution of sympathetic nerve activity to the development of hypertension were studied in spontaneously hypertensive rats (SHR). Male SHR and Stroke-Prone SHR (SHRSP) were chronically instrumented with bipolar electrode for measurements of renal (RSNA) and lumbar sympathetic nerve activity (LSNA) and a telemeter for measurement of arterial pressure (AP). Responses of AP, heart rate (HR), RSNA and LSNA were measured continuously and simultaneously from 8 to 12 weeks of age in SHR and SHRSP. At 8 weeks, the mean level of AP in SHRSP was significantly higher than that in SHR. AP increased progressively during 8-11 weeks in both SHR and SHRSP. The mean level of HR in SHRSP was not different to that in SHR at 8 weeks and it decreased gradually during 8-11 weeks in both SHRSP and SHR. We succeeded in measuring RSNA and LSNA over 4 weeks in several SHRSP. RSNA and LSNA apparently did not increase while development of hypertension occurred. It is therefore likely that possible contribution of sympathetic nerve activity to the development of hypertension in SHRSP remains uncertain.

## 2PK-019

### Epac1 pathway plays an important role for the development of heart failure through the activation of PKA

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Prolonged activation of sympathetic nerve and thus cAMP-mediated activation of PKA induces harmful effects on cardiac myocytes. Recent studies have shown that cAMP can activate Epac1, exchange protein directly activated by cAMP, as well. However, its role in the pathogenesis of heart failure remains unknown. In order to examine the role of Epac1 in the heart, we have generated the cardiac-specific type 5 adenylyl cyclase, a major cardiac AC isoform, transgenic mice (AC5TG) and Epac1-deficient AC5TG (AC5TG-EpacKO). Chronic isoproterenol (ISO) infusion (5mg/kg/day for 7 days) induced a similar degree of cardiac hypertrophy between AC5TG and AC5TG-Epac1KO. However, subsequent cardiac dysfunction was prevented in AC5TG-Epac1KO (AC5TG vs. AC5TG-Epac1KO : from 73.15±1.82 to 59.92±4.08 vs. from 75.5±2.44 to 69.72±2.62%, n=5-6). Cardiac myocyte apoptosis and fibrosis, hallmark of cardiac remodeling, were also examined by TUNEL staining and Masson-trichrome staining. Cardiac myocyte apoptosis was prevented in AC5TG-Epac1KO (AC5TG vs. AC5TG-Epac1KO : from 0.326±0.111 vs. 0.188±0.069%, n=7) and cardiac fibrosis was also prevented in AC5TG-Epac1KO (AC5TG vs. AC5TG-Epac1KO : from 0.078±0.041 vs. 0.037±0.029%, n=7) after chronic ISO infusion. Taken together, cAMP-Epac1 pathway plays an important role for the development of heart failure through the activation of cAMP-PKA pathway.

## 2PK-020

### $\alpha_{2A}$ Adrenergic Agonist, Guanfacine, Can Activate Cardiac Vagal Nerve Without Sympathetic Over-suppression

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**Background :** Vagal activation has recently become a therapeutic option for heart failure (HF). We have reported that  $\alpha_2$  adrenergic agonist, medetomidine, enhances vagal acetylcholine (ACh) release to the heart. However, medetomidine sometimes causes hypotension because of sympathetic over-suppression. This may prevent its clinical use. Since  $\alpha_{2A}$  adrenergic agonist, guanfacine, is reported to correct autonomic imbalance in HF patients, guanfacine may become another choice for HF treatment. **Methods :** In anesthetized rabbits, a microdialysis probe was implanted into the right atrial myocardium. 10 and 100  $\mu\text{g}/\text{kg}$  of intravenous guanfacine were tested and 20-min dialysate samples were collected. Another dialysate sample was collected after bilateral cervical vagotomy. Dialysate norepinephrine (NE) and ACh concentrations were measured by high-performance liquid chromatography. **Results :** 10  $\mu\text{g}/\text{kg}$  of guanfacine scarcely affected heart rate (HR) and mean blood pressure (MBP). Then dialysate NE and ACh concentrations did not change. 100  $\mu\text{g}/\text{kg}$  of guanfacine significantly decreased HR from 264±8 bpm at baseline to 128±18 bpm ( $P<0.01$ ), but did not affect MBP. This dose of guanfacine significantly increased dialysate ACh concentration from 6.7±1.2 nM to 41.7±8.4 nM ( $P<0.01$ ), but did not affect NE level. Vagotomy suppressed this increase in ACh concentration. **Conclusions :** Guanfacine enhanced vagal ACh release to the heart without sympathetic over-suppression. Guanfacine may be more favorable than medetomidine in HF treatment.

## 2PK-021

### Circulatory dynamics during sinusoidal work rate forcing of bilateral knee extension exercise

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The purpose of this study was to clarify the control of peripheral circulation during sinusoidal (sine) work rate (WR) forcing of bilateral knee extension (KE) exercise, especially focusing on the relationship of blood flows (BF) to lower limb with active muscle (femoral artery; FA) and to upper limb with non-active skeletal muscle (brachial artery; BA). Ten healthy male subjects performed the bilateral KE exercise with a constant WR (mean of sine WR) for a 30-min; for the next 16 min of sine WR with a 4-min period between 20 W and 60% of peak  $\text{VO}_2$ . During protocol, we measured the pulmonary gas exchange, heart rate (HR), mean arterial blood pressure (MAP), stroke volume, the blood velocity and cross sectional area of FA and BA, and skin BF (SBF) and sweating rate (SR) of forearm, respectively. The variables :  $y(t)$  were fitted as,  $y(t) = M + A \cdot \sin((2\pi/T) \cdot t - \theta)$ , where  $t$  : time,  $M$  : mean level,  $A$  : amplitude,  $T$  : period (of sine WR, 240 s),  $\theta$  : phase shift. All variables traced the sine wave adequately ( $r^2 > 0.50$ ). The phase shifts of variables regarding to  $\text{O}_2$  delivery to active muscles (such as pulmonary  $\text{VO}_2$ , HR, BF in FA) to WR forcing were similar; 50-70°. On the other, the response of BF in BA had the anti-phase (180°) while the forearm SBF and SR were delayed similar to variables of  $\text{O}_2$ -delivery. In conclusion, the circulatory control to non-active limb seems to be differential to the closed linkage along with  $\text{O}_2$ -delivery to active muscle.

## 2PK-022

### Differential Effects of Spinal Cord Injury on Flow Autoregulation in Celiac and Mesenteric Beds

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The aims of this study were to investigate the effects of spinal cord section on vascular tone and flow autoregulation in celiac and superior mesenteric beds in order to understand the influences of spinal cord injury on regional vascular functions. Celiac flow (CeF) and mesenteric flow (MF) in conscious rats were observed using an implanted electromagnetic flowmeter. Arterial pressure (AP) was measured in the terminal aorta. The spinal cord was transected at Th1 under ether anesthesia. One hour after spinal transection, celiac resistance (CeR) decreased significantly in spontaneously hypertensive rats (SHR) but not significantly in control Wistar rats (NCR), while mesenteric resistance (MR) increased significantly in both rats. CeR was not further decreased significantly by ganglionic blockade with hexamethonium (C6 : 25 mg/kg iv) in either spinal NCR or SHR but was significantly decreased in both neuraxis-intact rats, while MR in both spinal rats was significantly decreased but was not significantly altered in either neuraxis-intact NCR or SHR. MR in both rats was suppressed reflexively in a neuraxis intact state. Flow autoregulation disappeared in the celiac bed following spinal transection but was maintained at a lower flow level in the superior mesenteric bed. Vasopressin sensitivity increased in both spinal rats in mesenteric bed but only in spinal SHR in celiac bed. These results suggest that spinal cord injury impairs regional vascular functions by decreasing cardiac output due to dilation of capacitance vessels and depressing central nerve control in the both beds.



## 2PK-023

### Prostaglandin E<sub>2</sub>-EP4 signaling increased expression of atherogenic genes in human coronary smooth muscle cells

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Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) production is increased in atherosclerosis and its actions are mediated through EP receptors. However, distribution and roles of PGE<sub>2</sub> receptors (EPs) in coronary smooth muscle cells (CASCs) remain unknown. First, we examined mRNA expression of EP subtypes in human CASCs (hCASCs) by quantitative RT-PCR. We found that expression level of EP4 mRNA was much higher than that of EP1, EP2 and EP3, suggesting that EP4 is a primary EP subtype in hCASCs (EP1 : EP2 : EP3 : EP4=15 : 33 : 1 : 2140, p<0.0001, EP 4 vs. EP1, EP2 or EP3, n=4). To examine the role of EP4 signaling in hCASCs, we then performed expression analysis using quantitative RT-PCR and LC/MS/MS in hCASCs stimulated with EP4 agonist ONO-AE1-329 for 24 h (n=4) and 48 h (n=2), respectively. Quantitative RT-PCR analysis revealed that EP4 stimulation increased mRNA expressions of atherogenic genes, such as cyclooxygenase-2 (1.4-fold, p<0.01) and biglycan (1.3-fold, p<0.05), and decreased that of fibulin-2 (0.8-fold, p<0.05). Similarly, LC/MS/MS analysis showed that EP4 stimulation increased secretion of atherogenic proteins, such as IL-6 (1.6-fold), VEGF-A (1.4-fold), fibronectin (1.3-fold) and MMP-2 (1.3-fold). On the other hand, secretion of TIMP-3, anti-atherogenic protein, was decreased by EP4 stimulation (0.7-fold). These data suggest that EP4 signaling in hCASCs contributes to progression of atherosclerosis.

## 2PK-024

### Effect of work rate on oscillations of respiratory sinus arrhythmia during submaximal graded exercise

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We previously demonstrated that in humans mental stress exerts an influence on the oscillations of respiratory sinus arrhythmia (RSA), inducing incoherent phase lag with respect to breathing in addition to a decrease in the amplitude of RSA (A<sub>RSA</sub>). However, whether such incoherent oscillations of RSA could occur under physical stress has not been shown and was investigated in this study. After 4-min of resting control, eight healthy subjects performed a graded cycle ergometer exercise started with unloaded pedaling. The workload was increased by 20% of ventilatory threshold (T<sub>vent</sub>) every 4 min until 100% of T<sub>vent</sub>. Breathing pattern, beat-to-beat R-R intervals and gas exchange were recorded. Analytic signals of breathing and RSA were obtained by Hilbert transform and the degree of phase synchronization ( $\lambda$ ) and A<sub>RSA</sub> were quantified. By using spectral analysis, heart rate variability was estimated for the low-frequency (LF) and high-frequency (HF) bands. The A<sub>RSA</sub> decreased during 60, 80, and 100% T<sub>vent</sub> compared to that observed at rest and during 0% and 20% T<sub>vent</sub>. The  $\lambda$  decreased only at 100% T<sub>vent</sub> (P<0.01). The  $\lambda$  was correlated neither with the normalized HF nor with the A<sub>RSA</sub>, but was correlated with the normalized LF/HF ratio (P<0.01). These results indicate that physical stress exerts an influence on RSA differently from mental stress. We assume that exercise intensity at T<sub>vent</sub> elicits sympathetic nerve activation, which modulates dynamic transfer of vagal-cardiac nerve traffic, leading to incoherent oscillations of RSA during exercise.

## 2PK-025

### The Characterization of Changes in Cardiac Functions in Cardiovascular-Specific overexpression of Prostaglandin E Receptor EP4 mice

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Prostaglandin E receptor EP4 is abundantly expressed in the heart and vessels and is known to be up-regulated in cardiovascular diseases, such as atherosclerosis. However, the roles of up-regulated EP4 in the cardiovascular system are not fully understood. To examine the roles of EP4, we created transgenic mice overexpressing EP4 in the cardiovascular system. A Cre-loxP system was utilized under SM22 promoter. EP4loxP/loxP or EP4loxP/- mice were intercrossed with SM22-Cre transgenic mice to obtain EP4loxP<sup>+/+</sup>/SM22-Cre mice (transgenic) and EP4<sup>-/-</sup>/SM22-Cre mice (control) (10-13 weeks old, n=4). Transgenic mice were born in Mendelian ratios with the expected distribution of genotypes and gender, and had normal development up-to 2 months of age. RT-PCR and immunohistochemistry showed that human EP4 was abundantly expressed in the aorta and heart in transgenic mice but not in control mice. Expression level of human EP4 mRNA was higher in the left ventricle than in the right ventricle (2.31±1.36 fold, p<0.05). Body weight and heart weight were not different between in transgenic and control mice. Echocardiographic and catheterization studies showed that systolic and diastolic cardiac functions were not different between in transgenic and control mice. These results suggest that overexpression of EP4 in the cardiovascular system did not affect cardiac function at least under basal conditions. Further studies are required to examine the roles of EP4 in pathological conditions in which PGE<sub>2</sub> is abundantly produced.

## 2PK-026

### Left ventricular dysfunction induced by mild ischemia-reperfusion in *in situ* rat hearts is attenuated by a new calpain inhibitor

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We have previously indicated that a new soluble calpain inhibitor, SNJ-1945 (SNJ) attenuates cardiac dysfunction after cardioplegia arrest-reperfusion by inhibiting the proteolysis of  $\alpha$ -fodrin in *in vitro* study. In order to apply to clinical use, however, *in vivo* study is required as the approach to realistic medical treatment. The aim of the present *in situ* study was to investigate whether SNJ attenuated LV dysfunction (stunning) after mild ischemic-reperfusion (mI-R) in rat hearts. SNJ (60  $\mu$ mol/L, 5 ml i.p.) was injected 30 min before 30 min-gradual and partial coronary occlusion at proximal left anterior descending artery. To investigate LV function, we obtained curvilinear end-systolic pressure-volume relation by increasing afterload 60 min after reperfusion. In mI-R group, specific LV functional indices at midrange LV volume (mLVV), end-systolic pressure (ESP<sub>mLVV</sub>) and pressure-volume area (PVA<sub>mLVV</sub>: a total mechanical energy per beat, linearly related to oxygen consumption) significantly decreased, but SNJ reversed these decreases to time control level. Furthermore, SNJ completely inhibited the proteolysis of  $\alpha$ -fodrin after mI-R, although SNJ partially inhibited the decrease of LTCC and SERCA2a proteins. Our results indicate that improvements of LV function following mI-R injury are associated with inhibition of the proteolysis of  $\alpha$ -fodrin in *in situ* rat hearts. In conclusion, SNJ would be a promising tool to protect the heart from the stunning.

## 2PK-027

### The role of NCS-1 in the development of right ventricular hypertrophy with chronic hypoxia in mice

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Chronic hypoxia causes right ventricular (RV) hypertrophy associated with pulmonary arterial hypertension (PAH). A recent study reported that neuronal calcium sensor-1 (NCS-1) is an important regulator of immature heart function and (pathological) hypertrophy induced by hormonal stimulation (Nakamura et al. *Circ Res.* 2011). However, it is uncertain whether NCS-1 is involved in the development of the RV hypertrophy associated with PAH. To solve this problem, male NCS-1 knockout mice (7 wk) and their corresponding wild-type mice (C57 BL/6-NCR, 7wk) were subjected to chronic hypoxia (CH, 8% O<sub>2</sub>) for 3 weeks. Right ventricular systolic pressure (RVSP), mean arterial blood pressure (MAP) and heart rate (HR) were measured under anesthesia. RV, left ventricle plus septum (LV+S) and body weights were weighed. The CH-induced RV hypertrophy was attenuated in NCS-1 knockout mice compared to wild-type (RV/BW: 1.2 ± 0.1 vs 1.6 ± 0.1 mg/g, p=0.005, RV/LV+S: 0.44 ± 0.03 vs 0.52 ± 0.05, p=0.065), while the CH-induced RVSP elevation was not different between the two types of mice (36 ± 1 and 37 ± 5 mmHg, p>0.05). There were no differences in MAP and HR between these mice (p>0.05). These results suggest that NCS-1 plays an important role in the development of RV hypertrophy caused by PAH during chronic hypoxia.

## 2PK-028

### In vivo assessment of norepinephrine kinetics at cardiac sympathetic nerve ending in the anesthetized mice

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**Purpose:** To assess NE kinetics at cardiac sympathetic nerve endings by microdialysis technique in the anesthetized mice. **Methods:** Dialysis probe was implanted in the left ventricular myocardium of anesthetized mice (C57BL/6J) and perfused with Ringer solution at 2µl/min. A sampling period of 15 min was chosen (sample volume=30µl). Dialysate NE (norepinephrine) and an intraneuronal metabolite, DHPG (dihydroxyphenylglycol) concentration was measured using HPLC. We investigated the effects of locally administered pharmacological agents on dialysate NE and DHPG levels. **Results:** High K<sup>+</sup> (100 mM) increased dialysate NE from 49±17 to 143±28 pM and DHPG concentration from 1498±261 to 1770±414 pM (n=5). Desipramine (100µM), a reuptake inhibitor increased dialysate NE concentration from 96±29 to 459±115 pM, but did not change dialysate DHPG concentration (from 1697±98 to 1348±133 pM). In the presence of desipramine, high K<sup>+</sup> increased dialysate NE concentration from 459±115 to 1132±348 pM, but did not change dialysate DHPG concentration (from 1348±133 to 1333±114 pM). Pargyline (100mM), a monoamine oxidase inhibitor increased dialysate NE concentration from 54±9 to 111±19 pM, but decreased dialysate DHPG concentration from 1662±225 to 837±70 pM. **Conclusion:** Simultaneous monitoring of myocardial interstitial NE and DHPG levels provides information about NE kinetics at cardiac sympathetic nerve ending in the anesthetized mice.

## 2PK-029

### Abnormal cross-bridge dynamics in the in situ beating rat heart in early diabetes

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Diabetes is independently associated with a specific cardiomyopathy. Using synchrotron radiation small-angle X-ray scattering this study aimed to investigate in the *in situ* heart and in real time whether changes in cross-bridge dynamics and myosin interfilament spacing underlie the early development of this myopathy. Experiments were conducted using anesthetised Sprague-Dawley rats three weeks after treatment with either vehicle (control) or streptozotocin (diabetic). Left ventricular diffraction patterns were recorded during baseline and dobutamine infusions. Myosin mass transfer to actin filaments was assessed as the change in intensity ratio (I<sub>10</sub>/I<sub>11</sub>). In diabetic hearts cross-bridge dynamics were most notably abnormal in the diastolic phase (P<0.05) and to a lesser extent the systolic phase (P<0.05). A transmural gradient of contractile depression was observed. Elevated diabetic end-diastolic intensity ratios were correlated with the suppression in diastolic function (P<0.05). In addition the expected increase in systolic and diastolic myosin head transfer by dobutamine was significantly blunted in diabetic animals (P<0.05). Interfilament spacing did not differ between groups. The data demonstrate for the first time that myosin heads are displaced from actin filaments in the fibres of beating hearts, which is directly related to impaired ventricular function.

## 2PK-030

### EAD induced by the late inactivation component of Na<sup>+</sup> channel in human ventricular cell model

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After the rapid inactivation of Na<sup>+</sup> channel, a long-lasting inactivation has been observed over several hundred milliseconds. Recently, Undrovinas et al (1999) found that this slow component was further retarded in human ventricular myocytes dissected from failing hearts, and suggested arrhythmogenic influences of the delayed component. We examined this view by developing a comprehensive human ventricular cell model including a new model of the Na<sup>+</sup> channel inactivation kinetics. Indeed, we found that early afterdepolarization was induced by retarding the rate of slow inactivation. To get a quantitative insight, we applied the bifurcation analysis by taking the extent of slow inactivation as the bifurcation parameter. The analysis indicated that an unstable equilibrium point and limit cycle exist when the extent of inactivation is lower than 75%. In the time base simulation, four different patterns of spontaneous low membrane oscillations were identified for each segment of varying bifurcation parameter, separated by the bifurcation points. With the normal kinetics of the delayed inactivation, the inactivation proceeds more than 75% within the initial 150 ms of the plateau phase of the action potential, and thereby no EAD occurs. The time interval for the 75% inactivation is defined as a time-window susceptible for EAD. Under the pathophysiological condition, this time-window will be prolonged over a normal action potential duration.

## 2PK-031

### Aspartic acid promotes closure of the rat ductus arteriosus

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Recently, an immediate commencement of amino acid supplementation in very preterm infants (VPI) is recommended. However, the effect of amino acid on closure of the ductus arteriosus (DA) remains unknown. We hypothesized that the difference of amino acid composition influenced the prevalence of PDA in preterm infants. The serum amino acids of human VPI were analyzed. Fetal rats on the 21st day of gestation were intraperitoneally administered with aspartic acid (0.4%) or saline. Thirty minutes after injection, internal diameters of the DA and pulmonary artery (PA) were measured. The level of serum aspartic acid was lower in human VPI on the day of birth than in cord blood ( $4.70 \pm 1.99 \mu\text{M}$  vs  $15.6 \pm 10.6 \mu\text{M}$ ,  $p < 0.05$ ,  $n = 4$ ). The ratio of diameter of the DA to that of the PA was smaller than in aspartic acid group than in saline group ( $0.51 \pm 0.14$  vs  $0.64 \pm 0.17$ ,  $n = 11$ ,  $p < 0.05$ ). These data suggest that aspartic acid promotes closure of the DA. Supplementation of aspartic acid may be beneficial for VPI.

## 2PK-032

### Effects of ghrelin on survival and cardiac remodeling of dilated cardiomyopathy mice with troponin mutation

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Ghrelin is a growth hormone-releasing peptide, originally isolated from the stomach wall. In addition to its anabolic effects through the release of growth hormone, therapeutic effects of ghrelin have been reported in animal models of acute myocardial infarction. Recently, we created a knock-in mouse model of dilated cardiomyopathy (DCM) using a deletion mutation  $\delta\text{K210}$  in the *TNNT2* gene. The knock-in mice developed markedly enlarged hearts with left ventricular (LV) systolic dysfunction and frequent sudden cardiac death, closely recapitulating the clinical phenotypes of human patients. In this study, we attempted to examine whether ghrelin is beneficial for the treatment of DCM using this knock-in mouse model. Comparing to the saline treatment group, administration of ghrelin ( $150 \mu\text{g}/\text{kg}/\text{day}$  s.c.) prolonged the life span of DCM mice. On the echocardiographic study, ghrelin reduced LV end-diastolic dimension and increased % ejection fraction of LV. On the histological study, ghrelin decreased the heart-to-body weight ratio, prevented the cardiac remodeling and fibrosis, and markedly decreased the expression of brain natriuretic peptide. The expression of brain natriuretic peptide (BNP) also markedly decreased in ghrelin treatment group. These results suggested that ghrelin has a therapeutic benefit for the treatment of DCM.

## Poster Presentations Neuron, Synapse(2)

## 2PK-033

### Live cell imaging of CAPS protein – associated catecholamine release from large dense-core vesicles in PC12 cells using VMAT2-pHluorin

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$\text{Ca}^{2+}$ -dependent activator protein for secretion (CAPS) family protein is known as the proteins that is associated with exocytosis of large dense-core vesicle (LDCV) which contains neuropeptide and biogenic amine. CAPS family proteins play an important role in the priming step prior to vesicle membrane and the plasma membrane. However, the detail kinetics of CAPS regulated LDCV release is unclear. In the present study, we analyze the release kinetics of LDCV in PC12 cell by using fluorescent imaging technique. We constructed plasmid which is SE-pHluorin (pH sensitive GFP) in the luminal loop region of vesicular monoamine transporter 2 (VMAT2), and performed fluorescent imaging of LDCV release in PC12 by KCl and electrical stimulations. The release kinetics of LDCV seems different between soma and dendrites. Now we study the detail of the different release kinetics of LDCV in soma and dendrite, and their mechanisms associated with CAPS family proteins and calcium dependency.

## 2PK-034

### Expression of CAPS family proteins and their involvement in large dense-core vesicle release in mouse primary dopamine neurons

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The calcium-dependent activator protein for secretion (CAPS) family consists of two members, CAPS1 and CAPS2, and is known to be involved in regulation of large dense core vesicle (LDCV) release. It has been experimentally shown that CAPS1 regulates release of catecholamines such as dopamine (DA) and norepinephrine from neuroendocrine cells, whereas CAPS2 promotes release of two neurotrophins BDNF and NT-3. DA neurons play an important role in brain function, such as voluntary movement and reward predict. DA is synthesized in the ventral tegmental area (VTA) and substantia nigra (SN). VTA neurons project to the cortex and striatum, whereas SN neurons projects to the striatum. Interestingly, CAPS2 was shown to be highly expressed in DA neurons from rat and mouse brains. In the present study, to analyze a role of CAPS protein in DA release, we established a method to culture primary DA neurons by co-culturing cells from the midbrain (including VTA and SN) and striatum of mice and verified immunocytochemical distribution of CAPS proteins in cultured DA neurons. The result shows that CAPS1 and CAPS2 are expressed in both VTA and SN DA neurons of mice. By cellular imaging mouse primary DA neuron cultures expressed the vesicle monoamine transporter 2 fused with pH sensitive GFP (VMAT2-pHluorin), we will also discuss kinetics of CAPS1- and CAPS2-mediated DA release from LDCVs.

## 2PK-035

### Regulation of secretion dynamics of brain-derived neurotrophic factor (BDNF) by calcium-dependent activator protein for secretion 2 (CAPS2)

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Brain-derived neurotrophic factor (BDNF) has a pivotal role in neuronal survival and development. Calcium-dependent activator protein for secretion 2 (CAPS2) is a dense-core vesicle associated protein that promotes secretion of BDNF (Shinoda et al, PNAS, 2011). It is controversial whether BDNF secretion in cultured hippocampal neurons differs between somato-dendritic and axonal compartments, or occurs from axons but not dendrites. It also remains to be clarified whether CAPS2 is similarly involved in spatio-temporal kinetics of BDNF secretion from different compartments such as soma, dendrites vs. axons, and synaptic sites vs. extra-synaptic sites. In the present study, we tried to clarify the promoting activity of CAPS2 in local BDNF release from the subcellular compartments and its calcium sensitivity. We visualized secretion of BDNF-4xSEpH, which consists of four copies of super ecliptic pHluorin fused to the C-terminus of BDNF with a short linker sequence, from cultured mouse hippocampal neurons electrically stimulated to induce depolarization. We analyzed effects of CAPS 2 on local BDNF release (somato-dendritic vs. axonal and synaptic vs. extrasynaptic) in CAPS2-KO hippocampal cultures with or without overexpression of exogenous CAPS2 and simultaneous calcium imaging with Fura2-AM.

## 2PK-036

### Comparison of BDNF release kinetics due to differences in the stimulus and analysis of the Ca<sup>2+</sup> channels involved in the activity CAPS2

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Brain-derived neurotrophic factor (BDNF) is known to be involved in synaptic plasticity, development of neural circuits and induction of activity-dependent gene expression. BDNF is thought to be packed in the large dense-core vesicle (LDCV). BDNF containing LDCV is secretion vesicle via exocytosis, which is facilitated by Ca<sup>2+</sup>-dependent activator protein for secretion 2 (CAPS2) protein. It has remained unclear, however, about mechanisms and kinetics of CAPS2 promote secretion of BDNF. In this study, We transfected BDNF-pHluorin (pH sensitive GFP fused with BDNF) into hippocampal primary cultured neurons and measure the spatio-temporal BDNF release by time-lapse imaging technique. Furthermore, we have begun to identify the Ca<sup>2+</sup> channels involved in CAPS2 associated BDNF secretion.

## 2PK-037

### Synaptic transmission in hippocampus of CAPS1 conditional KO mice

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Calcium dependent activator protein for secretion 1 (CAPS1) was discovered as a soluble protein which is necessary to restore Ca<sup>2+</sup>-dependent norepinephrine secretion in PC12 cells. Now CAPS1 is known as a homolog protein of UNC31, whose mutations cause a defect of coordinated movement in *C. elegance*. Moreover, it is revealed that CAPS1 has MUN domain (Munc13 homologous domain) which is important in vesicle priming activity of Munc13 during synaptic vesicle exocytosis in mice. Taking together, it was suggested that CAPS1 is involved in the process of exocytosis, especially in the priming step. Although CAPS1 was initially shown to be required for exocytosis of dense-core vesicles (DCVs), whether CAPS1 is also involved in that of synaptic vesicles (SVs) is still controversial. Since CAPS1 KO mice are postnatal lethal, there are little information about biological significance of CAPS1 at the level of tissue or individual. To investigate whether CAPS1 is involved in SVs release process, we have recently generated CAPS1 conditional knockout (cKO) mice (Sadakata et al, submitted). Supposing that CAPS1 cKO mice are defected in basic synaptic transmission and/or synaptic plasticity, we studied electrophysiological properties of hippocampal synapses in CAPS1 cKO mice. We will show the results on basic transmission, PPF and LTP in CA3-CA1 synapses.

## 2PK-038

### Ca<sup>2+</sup>-dependent activator protein for secretion (CAPS) proteins are expressed in hypothalamic oxytocin neurons and colocalized to oxytocin in posterior pituitary

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The Ca<sup>2+</sup>-dependent activator protein for secretion (CAPS) protein family consists of two distinct members CAPS1 and CAPS2 and is involved in the exocytosis pathway of dense core vesicles (DCVs). Neuropeptide oxytocin (OXT), which is important for maternal and social behavior, is thought to be secreted by DCV exocytosis but the underlying molecular mechanism is largely unknown. It is noteworthy that CAPS2 knockout mice showed impairments in social interaction, maternal nurturing behavior and feeding behavior. To clarify whether CAPS family proteins are associated with OXT release from DCVs, we analyzed the localization of CAPS1 and CAPS2 in mouse hypothalamus and pituitary by immunohistochemistry and immunocytochemistry. We showed that CAPS1 was predominantly co-localized with OXT in the posterior pituitary, whereas CAPS2 observed in the intermediate lobe and the posterior pituitary at intermediate and low levels, respectively. In addition, we revealed that CAPS1 is co-localized with OXT in the paraventricular nucleus and supraoptic nucleus. Taken together, our results suggest that CAPS1 is the major member of the CAPS family to regulate secretion of OXT-containing DCVs. We will also show a role of CAPS family proteins in OXT secretion by analyzing the knockout and knockdown effects of either CAPS1 or CAPS 2.

## 2PK-039

### The proteome analysis to reveal the mechanism of excitatory synaptic vesicle release enhanced by BDNF

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Brain derived neurotrophic factor (BDNF) is secreted in the brain and plays critical role in neuronal survival and development and in synaptic development and plasticity. BDNF enhances both Ca<sup>2+</sup>-dependent and independent synaptic vesicle release, although the mechanisms of this process are not fully understood. In the present study, we carried out the proteome analysis to reveal the mechanism of synaptic vesicle release enhanced by BDNF. We found that the synaptic vesicle release enhanced by BDNF was observed at the excitatory terminal but not the inhibitory terminal on hippocampal cultured neurons treated with exogenous BDNF. Furthermore, our study suggests that BDNF affects the release probability of the excitatory synaptic vesicle by likely changing the subcellular distribution of synaptic vesicle-associated proteins or their compositions in vesicles. In order to clarify the mechanism of excitatory synaptic vesicle release enhanced by BDNF, we fractionated the excitatory synaptic vesicle from the hippocampal neurons which were treated with or without exogenous BDNF, and analyzed all the proteins present in the excitatory synaptic vesicle by mass spectrometry. We will show protein compositions in excitatory synaptic vesicles from neurons with or without BDNF stimulation.

## 2PK-040

### Switching of exo- and endocytosis : The role of post-synaptic SYT3

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The synaptotagmin (SYT) family consists of multiple isoforms which regulate a variety of membrane-trafficking in cells. One of the well characterized functions is to act as a Ca<sup>2+</sup> sensor for synaptic vesicle fusion at presynaptic sites. SYT1 and SYT2 play a major role in the molecular mechanism of exocytosis and also exhibit vesicle recycling via endocytosis in axons. By contrast, SYT3 has been suggested to undergo recycling in dendrites but less studied than SYT1 and SYT2. Here, we show that SYT3 localizes to postsynaptic membranes in hippocampus and may regulate exo and endocytosis in a Ca<sup>2+</sup> or neural activity-dependent manner. We expressed a SYT3 fused to a pH-sensitive derivative of GFP (pHluorin) in cultured hippocampal neurons and monitored its fluorescent dynamics in response to high KCl or electrical stimulation to induce depolarization. The results showed that fluorescence intensity of SYT3-pHluorin significantly decreased after the induction of depolarization, suggesting a possibility that SYT3-pHluorin is internalized inside the lumen of acidified organelles via endocytosis event. We also found that the endocytosis of SYT3-pHluorin needed adequate levels of Ca<sup>2+</sup> influx. However, at lower levels of Ca<sup>2+</sup> influx exocytosis rather than endocytosis of SYT3-pHluorin was elicited. These results suggest that SYT3 undergoes both exocytosis and endocytosis depending on the intensity of neural activity. We will also show candidate proteins that likely interact with SYT3 in rat hippocampus.

## 2PK-041

### Expression and function of brain-specific BAD-LAMP

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The brain and dendritic cell-associated lysosome-associated membrane protein-like molecule (BAD-LAMP) has been shown to localize in a subset of early endocytic organelles in subpopulations of cortical projection neurons in mice. UNC-46, a homolog of BAD-LAMP, is required for trafficking of the vesicular GABA transporter in *C. elegans*, and its knockout animals cause defects in GABA-mediated behavior. We independently identified BAD-LAMP (CD ID 06343) as a developmentally-regulated gene during mouse cerebellar development by *in silico* cloning using the cerebellar development transcriptome database (CDT-DB). BAD-LAMP shows high expression levels in the brain and is down-regulated in the cerebellum but up-regulated in the other brain region such as the cerebrum during the postnatal stage. We generated several expression plasmid encoding BAD-LAMP-fluorescent protein in which the full-length cDNA of BAD-LAMP was C-terminally tagged and subcloned into pEF-BOS. Exogenously-expressed BAD-LAMP in COS7 cells was predominantly localized in the cytoplasm. In this presentation, we will also show immunohistochemical localization of BAD-LAMP protein during brain development and effect of BAD-LAMP siRNA knockdown on GABA transporter trafficking.

## 2PK-042

### In vivo corticocortical interactions in mice

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Little is known about how corticocortical interactions affect cortical processing. Here, we studied functional and anatomical connections between the primary somatosensory cortex (S1) and the secondary motor cortex (M2). We performed cortical voltage-sensitive dye imaging to monitor the S1 and the M2 activities, somatic patch-clamp recording to measure membrane potential changes and dendritic calcium (Ca) imaging with a two-photon microscope from the L5 pyramidal neurons to investigate how the single cell integrates synaptic inputs. Local application of TTX into S1 decreased the M2 activity, and vice versa. Anatomical experiments demonstrate corticocortical connections between the two regions. M2 electrical stimulation evoked dendritic Ca increase in S1 L5 pyramidal neurons, which were decreased by application of CNQX to the brain surface, suggesting that the synaptic inputs evoked the dendritic Ca activity. The synaptic inputs from M2 evoked dendritic Ca activity without back propagating action potentials showing that distal dendrites receive the synaptic inputs. We also showed that M2 inputs evoked somatic hyperpolarizations in the S1 neurons through activation of interneurons. These results indicate that the M2 feedback inputs modulate the S1 activity, suggesting that the circuit regulates the processing of somatosensory information.

## 2PK-043

### Dendritic spine dynamics in the long-lasting synaptic suppression after repetitive LTD induction

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Behavioral memory is fixed through repeated task performance or exercise. However, the cellular mechanisms underlying this repetition-dependent memory consolidation are unrevealed. We previously showed in the organotypic slice cultures of the rodent hippocampus that repeated inductions of LTP lead to a slowly-developing long-lasting synaptic enhancement coupled with new synapse formation (RISE; Repetitive LTP-Induced Synaptic Enhancement), while that repeated inductions of LTD result in a long-lasting synaptic suppression accompanied by synapse elimination (LOSS; LTD-repetition-Operated Synaptic Suppression). In this study we pursued the process of synapse elimination using time-intermittent confocal microscopy. Spines on the CA1 pyramidal cell apical dendrites gradually decreased in the number over 3 weeks after 3 exposures to DHPG, a mGluR agonist and an LTD-inducing reagent. The largest decrease occurred within the first 1 week. Chasing individual spines revealed that the spines were in a dynamic equilibrium between generation and retraction and that the rate of retraction was increased while the rate of generation unaffected. This dynamics is not symmetric to that during the establishment of RISE reported by us previously, where both rates were transiently increased and the rate of retraction returned to a pre-stimulus level earlier resulting in a net increase in the spines' number.

## 2PK-044

### Activity-dependent endogenous taurine release facilitates the excitatory neurotransmission in the neocortical marginal zone of neonatal rats

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Marginal zone (MZ) including Cajal-Retzius (CR) cells is vital for lamination of developing neocortex. Besides excitatory GABAergic neurotransmission in the MZ, CR cells show glycine receptor (Gly-R)-mediated depolarization. However, its endogenous agonists and physiological roles are not yet known. Here, we performed voltage-sensitive dye imaging on tangential slices prepared from neonatal rats. Electrical stimulations evoked optical signals, which spread out radially over MZ. They were inhibited by antagonists of GABA<sub>A</sub>-R or Gly-R, but not by glutamate-R. Inhibition of NKCC1 also reduced the spread of excitation, suggesting that Cl<sup>-</sup>uptake mediated by NKCC1 promotes the GABA<sub>A</sub>- and Gly-R-mediated excitatory actions. Furthermore, microdialysis analysis using the tangential slices revealed that GABA and taurine but not Gly or glutamate were released in MZ in response to the electrical stimulation. Tetrodotoxin, voltage-sensitive Na<sup>+</sup> channel blocker, completely abolished Taurine release. Immuno-electron microscopic analysis revealed taurine was not localized in presynaptic structures. These results suggest that activation of Gly-Rs by activity-dependent release of endogenous taurine facilitates the excitatory neurotransmission in the MZ that is considered to be mediated by GABA.

## 2PK-045

### Periodic Synaptic Currents and Their Integration in Neurons in The Pontine Nuclei

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Principal neurons in the pontine nuclei receive excitatory synaptic inputs from the cerebral cortex and integrate those inputs to trigger action potentials, which are delivered to the cerebellar cortex via mossy fibres. It has been reported that the in vivo cerebellar mossy fibres fire low-frequency spontaneous action potentials in the resting state and high-frequency bursting action potentials upon sensory stimulation. It has not been known, however, how those firings are generated and modulated in the pontine principal neurons. In this study we made whole-cell path-clamp recordings from the pontine neurons in rats anaesthetized by ketamine/xylazine to characterize the synaptic integration in these neurons. Spontaneous excitatory postsynaptic currents (sEPSCs) recorded in the voltage-clamp mode had a large variation in the amplitude, with an overall average amplitude being 127±171 pA (mean±s.d., n=5 cells). Interestingly, the sEPSCs occurred in a form of short bursts that take place in slow oscillatory cycle (4.2±0.3 Hz, n=5). Those oscillatory synaptic inputs may reflect periodic activity in the cerebral cortex. Spontaneous inhibitory postsynaptic currents were detected only in a subset of cells (2/6 cells). In the current clamp mode, we observed that each burst generates one or a few action potentials. These findings suggest that the pontine principal neurons require integration of multiple synaptic inputs to transmit signals to the cerebellum.

## 2PK-046

### Residual $\text{Ca}^{2+}$ controls readily releasable pool in sympathetic neuron

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At the presynaptic terminal, an action potential (AP) increases transiently  $\text{Ca}^{2+}$  up to 200  $\mu\text{M}$  at mouths of  $\text{Ca}^{2+}$  channels, which link synaptic vesicles (SVs) through SNAREs and a  $\text{Ca}^{2+}$ -binding protein that initiates SVs exocytosis. Immediately after SVs exocytosis,  $\text{Ca}^{2+}$  concentration declines to the resting level. Special and temporal change in residual  $\text{Ca}^{2+}$ , which controls synaptic efficacy (Mochida et al., 2008), appears to depend on synaptic activities. Here we examined the residual  $\text{Ca}^{2+}$  on presynaptic plasticity applying paired-pulse protocol in the presence of high or low speed  $\text{Ca}^{2+}$  chelators, BAPTA- and EGTA-AM, respectively. We recorded paired excitatory post synaptic potential (EPSP) with various inter-stimulus-interval (ISI) of two consecutive APs from synapses formed between rat superior cervical ganglion neurons in long culture. 50  $\mu\text{M}$  BAPTA and 1  $\mu\text{M}$  EGTA reduced the first EPSP amplitude by 40% and 70% with the ISI of all range. In control, paired-pulse depression (PPD) was observed with the ISI of <100 ms. BAPTA reduced the PPD. In contrast, EGTA did not reduce the PPD but increased the paired-pulse ratio with the ISI of all range. These results suggest that rapid recovery of readily releasable pool (RRP) is regulated by a low affinity  $\text{Ca}^{2+}$ -sensor. To confirm this possibility, we measured recovery rate of the RRP from depletion of SVs with APs train. The recovery has two phases, fast (~10 s) and slow. BAPTA delayed the fast and slow phases, while EGTA delayed only the slow phase. These results suggest that special and temporal change in residual  $\text{Ca}^{2+}$  controls the RRP replenishment.

## 2PK-047

### Information tuning via synapse elimination in the whisker sensory thalamus of developing mice

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Lemniscal fibers in the thalamus undergo two kinds of developmental processes: somatosensory map segregation and subsequent synapse elimination. However, whether the synapse elimination further tunes somatosensory map has been unknown. We here provide a new insight using transgenic mice, *Krox20-Cre::Ai14*, where whisker-related lemniscal fibers are specifically labeled with tdTomato. In the mice, whisker-related lemniscal boutons are characterized as tdTomato-positive / VGlut2-immunopositive puncta, whereas non-whisker-related boutons be as tdTomato-negative / VGlut2-immunopositive ones. Surprisingly, the whisker sensory thalamus had even proportion of both types of boutons just after the barreloid segregation. In the subsequent synapse elimination period, the densities of both types of boutons dramatically decreased, while the proportion of whisker-related boutons gradually increased up to over 80%, indicating selective elimination depending on the information on fibers. The number of lemniscal fibers onto each relay neuron during the map segregation did not vary so much. These results suggest that the functional significance of synapse elimination in the thalamus is to reject inappropriate innervations within a roughly segregated map.

## 2PK-048

### Contribution of the lateral diffusion to the of AMPA receptor trafficking at the parallel fiber-Purkinje cell synapse

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Lateral diffusion of AMPA receptor (AMPA-R) is considered to play important roles in receptor trafficking at the synaptic membrane. However, at the synapse in the cerebellar Purkinje cell (PC), to what extent lateral diffusion contribution to AMPA-R trafficking was not examined. Here, we investigated extent of contribution of lateral diffusion to AMPA-R trafficking at the parallel fiber (PF)-PC synapse. We examined effect of tetanus toxin (TeTx), a blocker of SNARE-dependent exocytosis. Intracellularly applied TeTx caused gradual reduction of PF-EPSC amplitude and attained quasi-steady state. This reduction of EPSC-amplitude was conventionally attributed to the constitutive endocytic elimination of AMPA-R. Other possible reason of this reduction would be escape of AMPA-Rs from the synaptic membrane via lateral diffusion. This mechanism should not be affected by block of endocytosis. Thus, whether TeTx-induced reduction of EPSC-amplitude was affected by dynasore, a membrane permeable dynamin blocker, was examined. Results clearly showed that TeTx-induced reduction of EPSC amplitude was completely blocked by dynasore. This observation strongly suggested that contribution of lateral diffusion to AMPA-R trafficking at PF-PC synapse was minimal. Image-analysis of GFP-labeled GluA2, expressed in cultured PC, also supported this conclusion.

## 2PK-049

### Contrary changes of phasic- and tonic-inhibition onto thalamic relay neurons in mice with infraorbital nerve cut

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The rodent ventral posterior medial (VPM) nucleus, which is a subdivision of somatosensory thalamus, receives an excitatory input through the medial lemniscal synapse, which transports peripheral whisker information from the trigeminal nucleus to the VPM. In addition, the VPM also receives nearly all of the inhibitory inputs from the thalamic reticular nucleus (TRN). We previously reported that the complete transection of the infraorbital nerve (ION) resulted in multiple innervation of medial lemniscal fibers onto VPM neurons around 5 days after operation (POD5). However, it remains still unclear the postoperative change of inhibitory inputs onto VPM neurons after ION cut (IONC). We report here that IONC rapidly reduced the amplitude of evoked-inhibitory postsynaptic currents (eIPSCs) by minimum stimulation of TRN in the mouse VPM neuron from POD1. Similarly, the amplitude of miniature IPSCs (mIPSCs) by asynchronous release and spontaneous IPSCs (sIPSCs) were also reduced by IONC from POD1. In contrast, tonic GABA currents onto VPM neurons dramatically increased by IONC at POD7, tonic GABA currents also increased in VPM neurons, which had multiple lemniscal fibers by IONC. Therefore, these data indicate that phasic inhibition onto VPM neurons is decreased by IONC, whereas tonic inhibition is increased. In addition, synaptic- and extrasynaptic-inhibition onto VPM neurons after IONC rapidly change in advance of rewiring of lemniscal synapses by IONC.

## 2PK-050

### Synaptic vesicle protein-2A(SV2A)regulates vesicular pool at sympathetic neuron synapses

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Three isoforms of synaptic vesicle glycoprotein-2 (SV2A, B and C), trans-membrane proteins of the synaptic vesicle, are expressed in synapses of rat superior cervical ganglion (SCG) neurons in culture (Schivell et al., 2005). Among the isoforms, SV2A is known to be critical for regulated exocytosis in central and SCG neurons. To explore the functional mechanisms of SV2A in synaptic vesicle (SV) exocytosis, we employed a RNA-interference strategy for target-specific and solely presynaptic SV2A knockdown at SCG neuronal synapses. SV2A-siRNA decreased SV2A fluorescence intensity of the soma by approximately 60% and also excitatory postsynaptic response (EPSP) amplitude by 33%. SV2A loss-function impaired synaptic transmission during a short train of repetitive action potential firing at 20 Hz. The readily releasable pool (RRP) size estimated from EPSPs responded to the train was attenuated by 50%. SV2A loss-function decreases readily releasable synaptic vesicles after an exocytosis and delayed the RRP recovery after full depletion. Moreover, SV2A loss-function reduced 25% of Ca<sup>2+</sup> current amplitude measured from the soma without changing I-V relation ship. These data suggest that SV2A functions in the RRP formation with SV recruitment and that SV2A-deficits could lead to aberrant presynaptic Ca<sup>2+</sup> channel function that regulates synaptic transmission.

## 2PK-051

### Study on the hypoosmolarity sensing mechanism in arginine-vasopressin neurons : Reexamination on the taurine hypothesis

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Arginine-vasopressin (AVP), an antidiuretic hormone, is essential for homeostasis of body fluid osmolarity. When the plasma osmolarity decreases, AVP secretion from axon terminal of AVP neurons is suppressed. The hypoosmolarity sensing mechanism in osmosensor neurons is currently explained by the hypothesis that reduced osmolarity subsides the neuronal excitability due to hyperpolarization caused by glycine receptor anion channel activation by taurine released from osmotically swollen glial cells. However, Haam et al. (2012 J Neurosci) recently reported that GABA is excitatory in AVP neurons. Prompted by the paradoxical result, the present study reexamined the taurine hypothesis with using supraoptic glial cells and AVP neurons isolated from AVP-enhanced GFP transgenic rats. By nano-UPLC Q-TOF MS analysis, glial cells were consistently found to release taurine in response to hypoosmotic stress. In AVP neurons under perforated patch-clamp, however, taurine was found to induce depolarization, but not hyperpolarization, in a manner sensitive to strychnine. Thus, it appears that further investigation is needed before the accurate mechanism is determined.

## 2PK-052

### An experimental platform to study physiology, morphology and development of the vertebrate presynaptic terminal with genetic manipulations

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The calyx-type synapse of chick ciliary ganglion has been intensively studied for decade as a model system to study synaptic development, morphology and physiology. Here, we established a method to manipulate the molecular and functional organization of the presynaptic neurons of this model synapses. We introduced pCAGGS plasmid vectors containing EGFP gene into the midbrain neuroblast by in ovo electroporation method at embryonic day 2 (E2). The ciliary ganglion with oculomotor nerve was isolated from each E8-14 embryo. We found dozens of the calyx-type presynaptic terminals and axons labeled with EGFP fluorescence. Wide range of transgenes of interest could be introduced with this method. When the Brainbow I.I construct was introduced with NCre plasmid, the color coding of each presynaptic axon facilitated the dissociation of intertangled projections. With the expression of one of chimeric variants of channelrhodopsins, the presynaptic axon terminal was directly photostimulated. The vesicular dynamics was directly visualized with pH-sensitive exo/endocytosis probe. The presynaptic calcium influx triggered by a single action potential was detected by genetically-encoded calcium indicator. It is suggested that the above system would provide an experimental platform to unveil the molecular mechanisms underlying the morphology, physiology and development of synapses.

## 2PK-053

### Difference in synaptic strengths among competing inputs and absolute synaptic strengths contribute to distinct phase of climbing fiber synapse development in cerebellum

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In developing cerebellum, a single climbing fiber (CF) input is strengthened among multiple CF inputs in each Purkinje cell (PC) from P3 to P7, and only the strengthened CF extends its innervation along growing PC dendrites from P9. In parallel, surplus CF synapses on the PC soma are eliminated depending on two distinct mechanisms from P7 to P11 (early phase) and P12 to P16 (late phase). In this study, we examined development of CF-PC synapses in mice with PC-selective deletion of stargazin (TARPy2), a major AMPA receptor auxiliary subunit in PC (PC- $\gamma$ 2 KO). Whereas absolute amplitudes of CF-EPSCs of PC- $\gamma$ 2 KO mice were about half of those of wild-type mice during cerebellar development, difference in synaptic strength between the strongest CF and the weaker CFs in each PC developed normally in PC- $\gamma$ 2 KO mice. Besides, the early phase of CF elimination occurred normally in PC- $\gamma$ 2 KO mice. By contrast, extension of CFs along PC dendrites was reduced and higher percentage of PCs remained innervated by multiple CFs at P12 to P16 in PC- $\gamma$ 2 KO mice. These results suggest that difference in synaptic strengths among multiple CFs and absolute synaptic strengths contribute to distinct phases of CF synapse development.



## 2PK-054

### Does a long term HFS on STN induce the plasticity of a synaptic transmission onto SNr GABA neurons in rat slice preparations?

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As an effective therapeutic, deep brain stimulation on subthalamic nucleus (STN-DBS) has been applying to the patients of advanced Parkinson's disease. It was suggested that DBS contributed to recover to the normal activity from the abnormal hypersynchronous rhythmic burst firing of the parkinsonian basal ganglia. In fact, reports indicating that the reduction of hypersynchronous rhythmic activity through STN-DBS is associated with symptomatic relief are increasing in the patients and the parkinsonian model rat. However, the precise mechanism under this therapeutic effect of STN-DBS is still to be elucidated. I have applied a high frequency stimulation on STN (STN-HFS) under the same condition as STN-DBS for the human patient (constant current pulse, amplitude : 100-500  $\mu$ A, frequency : 125 Hz, duration : 100  $\mu$ s, for 20 min) to observe effects on the IPSC which was evoked by an electrical stimulation on internal capsule, including a putative direct pathway onto substantia nigra pars reticulata (SNr) GABA neurons in the rat brain slice preparations. At 80 minutes after STN-HFS under current-clamp mode, the amplitudes of IPSC were increased in four (normalized amplitude :  $1.398 \pm 0.102$ ;  $\text{mean} \pm \text{S.E.M.}$ ), decreased in one (0.64) and not changed in four neurons ( $0.933 \pm 0.036$ ). After STN-HFS under voltage-clamp mode ( $V_h = -70$  mV), the IPSC amplitude were increased in one, decreased in two and not changed in two neurons. These prolonged effects on synaptic transmissions might be one reason why DBS has the beneficial effects on the neuropsychiatric disorders.

## 2PK-055

### Optogenetic manipulation of presynaptic activity in chick ciliary ganglion during activity-dependent synapse elimination

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**[Introduction]** In nervous systems, a presynaptic neuron once branches extensively and projects its axons to many postsynaptic neurons in early developmental stages. The number of branches is reduced with development and the connections between presynaptic and postsynaptic neurons become refined. In the chick ciliary ganglion a postsynaptic ciliary neuron is multiply innervated by the presynaptic axons of a midbrain neuron in the Edinger-Westphal nucleus in the early stage. However it receives only one calyx-type presynaptic terminal before embryonic day 14 (E14). It has been hypothesized that the refinement of neuronal connections is dependent on the activity of either presynaptic terminal, postsynaptic cell or both. Here the involvement of presynaptic activity is investigated using optogenetics.

**[Method]** Plasmid containing channelrhodopsin was introduced in E2 embryo using *in ovo* electroporation. The synaptic current was measured from the postsynaptic ciliary neuron at E14 under conventional whole-cell patch clamp.

**[Results & Discussion]** In calyx-type presynaptic terminals channelrhodopsins was expressed and the light-dependent synaptic currents were evoked in the postsynaptic neurons. Therefore, the activity of a presynaptic axon/terminal is directly manipulated to evoke action potentials by light. Our optogenetic approach would facilitate to reveal the relationship between the presynaptic activity and the synaptogenesis.

## 2PK-056

### Dynamics of oscillating squid giant axons in response to periodically modulating impulses and modeling analysis

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Pulse patterns encode information processed in digital circuits. To achieve this, the generation of each pulse must be independent of previous device activity, and for this to happen, the rise and fall times of each pulse corresponding to the duration of its transient regime should be sufficiently short. Similarly, when the nervous system uses spike patterns to transmit information, spike generation should be independent of previous neuronal activity. Thus, it is necessary to understand the transient dynamics of spike generation in order to understand how information is transmitted. In this study, we examined the responses of oscillating squid giant axons to pulses with a sinusoidally modulating instantaneous frequency that was the inverse of the inter-pulse interval. We found that the axon response showed the following three properties : 1) the responses to increasing and decreasing instantaneous frequencies were different, i.e., the responses depended on input history ; 2) the same input could lead to different membrane potentials ; and 3) there existed some transient synchronizations of input pulses and spikes. We evaluated these responses using a mathematical model of membrane potential dynamics. To explain the above response properties, we used a stochastic phase transition operator that depicts the global dynamics of the given model in response to a single impulse. Our findings show that spike generation depends on the past activity of the membrane.

## 2PK-057

### Properties of dendritic filtering in auditory coincidence detector neurons of birds

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Difference of sound arrival time between two ears (interaural time difference, ITD) is a major cue for sound source localization. In birds, neurons in nucleus laminaris (NL) are the coincidence detector of bilateral synaptic inputs and involved in processing of ITDs. Importantly, NL neurons are tuned to a specific frequency of sound (characteristic frequency, CF) and have several morphological and functional specializations depending on CF. In particular, the length of dendrites varies along the frequency axis and the low-CF neurons have dendrites 7-20 times longer than the other CF neurons have. However, the long dendrites could deteriorate the precision of coincidence detection due to its filtering effects on synaptic potentials, and the functional roles of long dendrites for the ITD processing still remain elusive. Previously, we reported that the expression level of HCN1 channel is robust at the dendrites of low-CF neurons. The time courses of spontaneous EPSCs those recorded at the cell body of low-CF neurons exhibited relatively uniform distribution, and this distribution expanded through the blockage of HCN channels. This indicates that the HCN1 channel might contribute to compensate the filtering effects of dendrites in low-CF neurons. In this study, we analyzed the filtering properties of NL dendrites directly with the focal uncaging of MNI-glutamate. We will further examine the synaptic integration at the focal dendrites to access the functional roles of dendrites in the NL neurons.

## 2PK-058

### Facilitation of the swallowing reflex following administration of cannabinoids in rats

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This study was designed to elucidate whether cannabinoids facilitate the swallowing reflex. Successive swallowing reflexes were induced by applying continuous electrical stimuli to the superior laryngeal nerve (SLN) in anesthetized rats. Onset latency of the first swallowing reflex and the time interval between each swallow was measured with/without CB1 receptor agonist WIN 55-212-2 (WIN) intravenous (i. v.) administration.

Onset latency and time interval were dose-dependently decreased following WIN i.v. administration. CB1 antagonist (AM251) eliminated the effect of WIN, otherwise no obvious change was occurred with preadministration of CB2 antagonist (AM630) to WIN i.v. administration. Premicroinjection of AM251 into the nucleus tractus solitarius (NTS) where the swallowing center exists also blocked the effect of WIN i.v. administration.

Immunohistochemical study revealed that there are more GABAergic neurons than glutamatergic neurons with CB1 receptors. These findings suggest that WIN bind to CB1 receptors and cause suppression of neurotransmitter release. This phenomenon may result in facilitation of the swallowing reflex due to predominate effect of glutamatergic neurons, in comparison with that of GABAergic neurons, in the NTS.

## 2PK-059

### Glia-driven neuronal activity and behavior

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Dynamic activity of glia has repeatedly been demonstrated, but if such activity is independent from neuronal activity, glia would not have any role in the information processing in the brain or in the generation of animal behavior. Evidence for neurons communicating with glia is solid but signaling pathway leading back from glial-to-neuronal activity was often difficult to study. Here, we introduced a transgenic mouse line in which channelrhodopsin-2 was expressed in astrocytes. Selective photostimulation of these astrocytes *in vivo* triggered neuronal activation. Using slice preparations, we show that glial photostimulation lead to release of glutamate which was sufficient to activate AMPA receptors on Purkinje cells and to induce long-term depression of parallel fiber to Purkinje cell synapses through activation of metabotropic glutamate receptors. Finally, we show that neuronal activation by glial stimulation can lead to perturbation of cerebellar modulated motor behavior. These findings demonstrate that glia can modulate the tone of neuronal activity and behavior. This animal model is expected to be a potentially powerful approach to study the role of glia in brain function.

## Poster Presentations Ionic Channel, Receptor(2)

## 2PK-060

### Binding of the Gq protein stabilizes the active conformation of the M1R and M3R, but not of P2Y1R

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We have recently reported that binding of the Gq protein stabilizes the active conformation of the muscarinic acetylcholine receptor type 1 (M<sub>1</sub>R). Analyses of fluorescent resonance energy transfer (FRET) between the fluorescent proteins tethered at the third intracellular loop (i3) and the C-tail revealed that co-expression of Gq protein enhanced the agonist-induced FRET decrease and decelerated the recovery of the decreased FRET upon wash-out of the ligand. Then we aimed at examining whether or not these effects are observed in Gq coupled receptors other than the M<sub>1</sub>R, such as the muscarinic receptor type 3 (M<sub>3</sub>R) and purinergic receptor type 1 (P2Y<sub>1</sub>R). We first confirmed that the receptors fused with the fluorescent proteins interacted with the Gq protein and then monitored the intra-subunit FRET in the absence and presence of the Gq protein. In the absence of the Gq protein, application of agonists induced the decreases in FRET between YFP at the i3 and CFP at the C-tail in both constructs, reflecting the agonist-induced conformational changes. The agonist-induced FRET decrease was enhanced by co-expression of the Gq protein in the M<sub>3</sub>R FRET construct but not in the P2Y<sub>1</sub>R one. Similarly, the Gq-induced deceleration of the FRET recovery upon wash-out of ligands was observed in the M<sub>3</sub>R but not in the P2Y<sub>1</sub>R. These results suggested that the effects of the Gq coupling on the activated conformation of the receptor differ depending on the type of receptors.

## 2PK-061

### Analysis of the Transient Outward and Ultra-rapid Delayed Rectifier Potassium Current in Isolated Right Atrial Myocytes from Atrial Fibrillation Patients

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**OBJECT :** We aim to investigate changes of the transient outward ( $I_{to}$ ) and the ultra-rapid delayed rectifier ( $I_{kur}$ ) potassium current in human atrial myocytes associated with chronic atrial fibrillation (AF) and the effects of AF on the gene expression of the Kv4.3 and Kv1.5 potassium channel subunit. **METHODS :** We obtained forty-four fresh cardiac specimens isolated from thirty-three patients. Total mRNA was isolated and quantitative real-time PCR was performed to evaluate mRNA expression. Potassium currents were studied with the patch clamp technique after the atrial myocytes isolated from the right atrial appendages. **RESULTS :** The presence of AF was associated with a reduction in Kv4.3 expression ( $P < 0.05$ ), which was paralleled by a reduction in  $I_{to}$  current densities in this group of patients ( $P < 0.05$ ). AF also down-regulated the current densities of  $I_{kur}$  ( $P < 0.05$ ), but there was no significant change of Kv1.5 expression between two groups. **CONCLUSION :** Chronic AF in humans reduces  $I_{to}$  current and  $I_{kur}$  current by transcriptional down-regulation of the Kv 4.3 potassium channel subunit. Altered gene expression is an important component of the electrical remodeling process and may contribute to repolarization abnormalities in AF.

## 2PK-062

### Inhibition of Kir2.x inward rectifier $K^+$ channels by local anesthetics, lidocaine and bupivacaine

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The members of the Kir2 family of the inward rectifier  $K^+$  channels play important roles in the control of resting membrane potential and cellular excitability, and they are shown to be expressed in neurons of the central nervous system including the spinal cord. Local anesthetics (LAs) block nerve conduction by inhibiting voltage-gated  $Na^+$  channels, and high concentrations of LAs are applied in the spinal anesthesia, which have the potentials for neurotoxic effects. In this study, we examined the effects of lidocaine and bupivacaine on the whole-cell currents of the human Kir2.1, 2.2 and 2.3 homomultimeric channels stably expressed in HEK293 cells. Both lidocaine and bupivacaine at the concentrations of 0.1-10 mM suppressed the Kir2 currents in a dose-dependent manner, but the inhibitory effects by lidocaine were immediately observed and completely reversed by its removal, while the effects by bupivacaine were slowly progressing and hardly reversed. Lowering of the external pH from 7.4 to 6.6, which decreases the concentrations of their neutral forms to nearly one-fifth, attenuated the inhibitory effects caused by both LAs, suggesting that the sites of the actions on the channels are located within the membrane or intracellularly. Because a decrease in the resting  $K^+$  conductance increases the membrane excitability of neuronal cells, our results suggest that the suppression of the Kir2 currents may contribute to the mechanism of the neurotoxic side effects caused by administrations of lidocaine and bupivacaine.

## 2PK-063

### Functional linkage between TRPV4 and ANO1

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A non-selective cation channel TRPV4 ( $P_{Ca} : P_{Na} = 6 : 1$ ), which is activated by warm temperature ( $33^\circ C <$ ) or cell membrane extension, is expressed in various tissues. We reported the physiological significance in several cell types including skin and esophageal keratinocytes, and hippocampal neurons. Although the TRPV4 expression level is the highest in choroid plexus epithelial cells (CPEC) among brain regions, the TRPV4 function is still unknown. Since secretion of cerebrospinal fluid (CSF) from CPEC is very important to maintain the brain environment, we hypothesized that TRPV4 activity affects CSF secretion. To investigate this possibility, we focused on anoctamins (ANOs) activated by intracellular free  $Ca^{2+}$ . In this study, expression of ANO1, 4 and 6 in choroid plexus was found by RT-PCR. Furthermore, expression of ANO1 was confirmed by immunostaining in CPEC. Additionally,  $Ca^{2+}$ -activated  $Cl^-$  channel currents were observed for the first time in CPEC. Next, we recorded ANO1-mediated currents in HEK293T cells expressing TRPV4 and ANO1. We found that ANO1-mediated currents increased dramatically with TRPV4 activation by a low concentration of GSK1016790A (100 nM), a selective TRPV4 agonist. In addition, ANO1-mediated currents were inhibited completely in the absence of extracellular  $Ca^{2+}$ . These results indicate that  $Cl^-$  efflux through ANO1 depends on TRPV4 activity. Thus, we propose the concept that functional linkage between TRPV4 and ANO1 enhances water transport from CPEC to ventricle.

## 2PK-064

### The speed of recovery from inactivation of Kv4.2-KChIP4 complex changes depending on the stoichiometry

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Kv4.2 is a member of voltage-gated  $K^+$  channel family and its function is regulated by various auxiliary subunits including  $K^+$  Channel Interacting Protein 4 (KChIP4). It is known that coexpression of KChIP4 increases Kv4.2 current amplitude, decelerates inactivation and accelerates recovery from inactivation of Kv4.2. We aimed at elucidation of the regulation mechanism which remains unknown, focusing on the stoichiometry of the molecular complex. First, mixtures of cRNA of Kv 4.2 and KChIP4 at various ratios were injected to *Xenopus* oocytes and the expressed channel properties were analyzed under two electrode voltage clamp. We observed that recovery from inactivation of Kv4.2 changed depending on the ratio of injected cRNA (Kv4.2 : KChIP4). The time constant of recovery from inactivation at -80mV was  $291.4 \pm 21.2$  (10 : 0),  $72.0 \pm 5.8$  (10 : 1), and  $43.5 \pm 2.7$  ms (10 : 10). As a next step, we constructed two concatemers, KChIP4-Kv4.2 and KChIP4-Kv4.2-Kv4.2, whose stoichiometry of the molecular complex (Kv4.2 : KChIP4) are expected to be 4 : 4 or 4 : 2. The time constant of recovery from inactivation at -80mV of KChIP4-Kv4.2 was  $45.3 \pm 0.01$  ms, close to that of Kv4.2 with high expressed KChIP4. That of KChIP4-Kv4.2-Kv4.2 was  $88.4 \pm 3.1$  ms, close to that of Kv4.2 with low expressed KChIP4. These results show that the speed of recovery from inactivation of Kv 4.2 changes depending on the stoichiometry of Kv4.2 and KChIP4 complex.

## 2PK-065

### Substrate specificity of the voltage-sensing phosphatase(VSP)changes with membrane potential

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Voltage-sensing phosphatase (VSP) consists of the two domains: voltage-sensor domain and the cytoplasmic region with phosphoinositide-phosphatase activities. VSPs dephosphorylate the 5' phosphate from phosphatidylinositol 3,4,5-trisphosphate [PI (3,4,5) P<sub>3</sub>] and phosphatidylinositol 4,5-bisphosphate [PI (4,5) P<sub>2</sub>] upon voltage depolarization. However, it remains unknown whether VSPs dephosphorylate the 3' phosphate from PI (3,4,5) P<sub>3</sub>. To address this issue, the phosphatase region was reacted with radiolabeled phosphates. TLC assay showed that VSPs dephosphorylate the 3' phosphate of phosphatidylinositol 3,4-bisphosphate [PI (3,4) P<sub>2</sub>] but not that of PI (3,4,5) P<sub>3</sub>. To test whether voltage-dependent dephosphorylation of PI (3,4) P<sub>2</sub> in live cells, the PI (3,4) P<sub>2</sub> level was monitored in *Xenopus* oocytes by using the pleckstrin homology (PH) domain from tandem PH domain containing protein (TAPP1) fused with GFP. The fluorescence intensity increased during depolarization to 0 mV, consistent with 5' phosphatase activity of VSP toward PI (3,4,5) P<sub>3</sub>. However, depolarization to 60 mV showed a decrease of fluorescence intensity, indicating that PI (3,4) P<sub>2</sub> is dephosphorylated at the 3' position. These results suggest that VSPs have the 3' phosphatase activity toward PI (3,4) P<sub>2</sub>, and the level of PI (3,4) P<sub>2</sub> changes depending on the membrane potential.

## 2PK-066

### Characterization of type 1 ryanodine receptor channels carrying C-terminal malignant hyperthermia-associated mutations

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Type 1 ryanodine receptor (RyR1) is a Ca<sup>2+</sup> release channel in the sarcoplasmic reticulum and plays a pivotal role in excitation-contraction coupling in skeletal muscle. RyR1 is the major target for malignant hyperthermia (MH), an autosomal-dominant pharmacogenetic disorder triggered by volatile anesthetics such as halothane. It is widely believed that MH mutations cause hyperactivation of the Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release (CICR), resulting in abnormal Ca<sup>2+</sup> homeostasis in skeletal muscle. To date, over 150 mutations have been identified in the RyR1 gene of MH patients, and many of them are located in one of three 'hot spots', i.e., the N-terminal, the central, and the C-terminal regions. Whereas mutations in the N-terminal and the central regions have been extensively investigated, it remains unclear how the C-terminal mutations affect the CICR activity. In this study, we expressed several RyR1 channels carrying the C-terminal MH mutations in HEK cells and examined their CICR activity by live-cell Ca<sup>2+</sup> imaging and [<sup>3</sup>H] ryanodine binding. Our results suggest that the C-terminal MH mutations may cause hyperactivation of the CICR, primarily by sensitization to the activating Ca<sup>2+</sup>.

## 2PK-067

### PKA modulates the Ca<sup>2+</sup> channel activity induced by calmodulin in guinea-pig ventricular myocytes

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Our previous studies show that calmodulin (CaM) reverses rundown of the Ca<sup>2+</sup> channel in excised patches, however, the reversal effect of CaM is time-dependent, suggesting that additional cytoplasmic factors are required for CaM interaction with the Ca<sup>2+</sup> channel. In the present study, we examined the role of PKA-mediated phosphorylation in regulation of the Ca<sup>2+</sup> channel activity induced by CaM in the inside-out patches. The Ca<sup>2+</sup> channel activity disappeared and CaM hardly induced the channel activity after the patch excised into internal solution for 10 minutes. PKA catalytic subunit (PKAc) together with ATP maintained a small channel activity in the inside-out patch and subsequent application of CaM induced a high channel activity, suggesting that PKA-mediated phosphorylation modulates the interaction of CaM and the Ca<sup>2+</sup> channel. PKA also increased the Ca<sup>2+</sup> channel activity induced by CaM in the inside-out patches. In the presence of PKAc, CaM completely prevented rundown of Ca<sup>2+</sup> channel. Interestingly, cAMP mimicked the effect of PKAc, implying that the inactive PKA still anchored on or near the Ca<sup>2+</sup> channel in the inside-out patches. Similar to PKAc effect, phosphatase inhibitor, okadaic acid (OA), maintained the channel availability and attenuated the time dependence of CaM effect. These results suggest that PKA-mediated phosphorylation regulates the Ca<sup>2+</sup> channel activity by modulating the interaction of CaM with the Ca<sup>2+</sup> channel.

## 2PK-068

### Autoimmune disorder phenotypes in HVCN1 gene deficient mice

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Voltage-gated proton channel (Hv channel) is expressed in blood cells, microglia and epithelial cells. It has been shown that Hv channel plays key role in regulating activities of NADPH oxidase in phagocytes through regulation of membrane potential and intracellular pH. However, biological functions of Hv channel at the whole animal body level have not been fully understood. Here we demonstrate that knockout mice of HVCN1 gene, encoding Hv channel, show splenomegaly, autoantibodies and nephritis, which are reminiscent of phenotypes of autoimmune disorders. The number of activated T cells was larger in HVCN1-deficient mice than in wild-type mice, and this was remarkably enhanced upon viral infection. The production of superoxide anion in T cells upon stimulation with PMA showed two phases and only the second phase was significantly attenuated in the HVCN1-deficient mice. These results suggest a potential role of Hv channel in regulating T cell homeostasis.

## 2PK-069

### Development of planar patch clamp system with stretchable resin

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Every cell responds to mechanical stimuli. So far it is supposed that several ion channels have mechanosensitive properties by electrophysiological experiments, but its mechanism for mechanosensitivity is still not clear. In the conventional patch clamp recording, a membrane suction was applied to record the mechanosensitive response of ion channels. However, it is difficult to obtain the exact stretch ratio of the plasma membrane by the suction. Our purpose is to develop a novel planar patch clamp system which is capable of giving a direct stretch stimulus to the membrane to record the mechanosensitive response of ion channels. We used HEK 293 cells transfected with SAKCA (stretch-activated  $K_{Ca}$ ) channels. Planar electrode was fabricated with Polydimethylsiloxane. We achieved a Gigaohm seal rate of 37% and current recording of single ion channels using the electrode. Observation by scanning electron microscope confirmed that the longest diameter of the aperture increased up to 37.7% by stretching the planar electrode. In this chamber, gigaohm seal was achieved with a maximum seal resistance of 13 G $\Omega$ . Furthermore, we obtained results that open probability of the mechanosensitive SAKCA channel was increased in response to stretching of the planar electrode. We will continue developing this system to achieve high efficiency and high accuracy recording of mechanosensitive response of ion channels.

## 2PK-070

### Biochemical and histological identification of MaxiK channel orientation in the peri-nuclear ER membrane of HEK293 cells

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Our goal is to decide the MaxiK channel orientation in the peri-nuclear ER membrane. The MaxiK channel currents expressed in the nuclear envelope (NE) of HEK293 cells were enhanced by an increase in luminal  $Ca^{2+}$  and activated by the depolarization of the ER membrane. The immunofluorescent staining for Calnexin, an ER resident membrane protein showed that the orientation of the NE membrane was intact in our HEK293 NE preparation. The results suggest that NE MaxiK channels have unconventional orientation in the ER membrane. Based on the above, we carried out biochemical and histological studies for our initial issue. First, we prepared a couple of EGFP-fused MaxiK channels, one fused EGFP to C-terminus of MaxiK and the other to N-terminus, where we inserted a specific recognition site of TEV protease enzyme into the junction of MaxiK and EGFP. Subsequently the protease and the mutant channels were co-expressed in HEK293 cells. We examined accessibility of the enzyme to its recognition site by detecting the mutant protein digestion with the electrophoretic mobility shift assay. Second, we examined the MaxiK channel's C-terminus orientation using an immunogold labeling electron microscopy with an anti-C-terminus antibody in the NE preparations of HEK293 cell.

## 2PK-071

### Aroma-oil compounds inhibit the compound action potentials of frog sciatic nerves

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We have previously revealed that transient receptor potential (TRP) channel agonists such as capsaicin, menthol and allyl isothiocyanate, inhibit nerve action potential (AP) conduction without TRP activation. The present study examined whether many kinds of aroma-oil compound, some of which are TRP agonists, have a similar action and if so which structures of them are important for nerve conduction inhibition. The experiments were performed by applying the air-gap method to the frog sciatic nerve. A chemical, tetrahydrolavandulol, where the six-membered ring of menthol is opened, was found to inhibit compound APs (CAPs) with an efficacy comparable to that of menthol. Therefore, we focused on aroma-oil compounds that do not have six-membered rings. Aroma-oil compounds having-CHO group (aldehydes), citral and citronellal, reduced the peak amplitude of the CAP with the  $IC_{50}$  values of 0.48 mM and 0.50 mM, respectively. Aroma-oil compounds having-OH group (alcohols), geraniol and citronellol, also inhibited CAPs; their  $IC_{50}$  values were 0.53 mM and 0.38 mM, respectively. Taking into consideration previously-reported data, an efficacy sequence of aroma-oil compounds for CAP inhibition was phenols (thymol, carvacrol and eugenol) > alcohols > aldehydes > ketones ((+)-carbone, (-)-carbone, (+)-menthone, (-)-menthone and pulegone) > oxides (1,8-cineole and 1,4-cineole) >> carbon hydrates (limonene). It is suggested that aroma-oil compounds inhibit nerve conduction in a manner specific to their chemical structures.

## 2PK-072

### The optimization of the electrophysiological characterization of the voltage-gated proton channel on the artificial membrane

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The gating of the voltage-gated proton channel protein (VSOP/Hv1) is known to be controlled by membrane voltage and pH. VSOP/Hv1 lies with a number of fundamental questions, voltage gating, sensing the pH difference across membrane and proton selective permeation. Until now, the protein activity has been studied by electrophysiological technique exclusively in native cell or heterologous expression systems. Although the proton conductance of VSOP has been reported to be accelerated by the addition of unsaturated fatty acid on native cells, the mechanism of the effect is totally unresolved. The reconstitution system is ideally suited for answering these questions. We therefore set out to construct the system of the expressed VSOP on the artificial membrane.

The purified mouse-VSOP from heterologously expressed cells was reconstituted into the liposome consisting of POPE/POPC/POPS. The activities of the voltage-gated proton channel upon liposomal pH change in the external compartment were reported last year. We report here our trial of recording single channel of VSOP/Hv1 in the reconstituted proteoliposome. We also examined the effect of fatty acids on the mouse VSOP channel currents.

## 2PK-073

### Inhibition by daikenchuto of the compound action potential of frog sciatic nerve

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Various kinds of traditional Japanese medicine inhibit nerve action potential (AP) conduction. Daikenchuto (DKT), rikkosan, kikyoto and rikunshito at a concentration of 2 mg/ml reduced the peak amplitude of compound AP (CAP) in the frog sciatic nerve by 70, 30, 25 and 15%, respectively. DKT, which was the most effective, had a half-maximal inhibitory concentration (IC<sub>50</sub>) value of 1.1 mg/ml in reducing CAP peak amplitude. On the other hand, a TRPV1 agonist capsaicin and a TRPM8 agonist menthol reduced frog sciatic nerve CAP amplitudes without TRPV1 and TRPM8 activation. A TRPA1 agonist allyl isothiocyanate or cinnamaldehyde also reduced CAP peak amplitude. As a result, it was suggested that the DKT activity is partly attributed to TRP agonists contained in DKT which inhibit CAPs without TRP activation. The present study was undertaken to confirm our idea that TRP agonists contribute to the DKT-induced CAP inhibition; the air-gap method was applied to the frog sciatic nerve. Another Kampo medicine kakkonto at 2 mg/ml reduced CAP peak amplitude by only 12%, a value much less than that of DKT. Although DKT is composed of three kinds of crude medicine, ginseng, Japanese pepper and processed ginger, hydroxy- $\alpha$ -sanshol (TRPV1 and TRPA1 agonist) contained in DKT reduced CAP peak amplitude in a dose-dependent manner. A ginger component zingerone (TRPV1 agonist) reduced CAP peak amplitude (IC<sub>50</sub>=8.3 mM). These results give further evidence for an involvement of TRP agonists in the DKT-induced CAP inhibition.

## 2PK-074

### HCN channels regulate spontaneous firing rate of olfactory receptor neurons for axonal targeting to the olfactory bulb

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The olfactory receptor neurons (ORNs) utilize spontaneous firing activity for establishing and maintaining the olfactory neural network; however (1) how the spontaneous activity is generated and (2) the role of spontaneous activity in glomerular formation in the olfactory bulb are not established. By applying extracellular recording in the sliced mouse olfactory epithelium, we found that HCN channels depolarized the membrane of ORNs and boosted the spontaneous firing activity by sensing the cAMP levels that was largely maintained by the basal activation of Gs-coupled  $\beta$ 2-adrenoceptors (ADRB2). Exogenous co-expression of odorant receptors and ADRB2 in HEK293 cells implied that the activation levels of HCN channels might be differentially set in an odorant-receptor specific manner. Furthermore, we generated mice with HCN4 channel over-expressed by Tet-system, in which the spontaneous firing rate of ORNs was much higher than in non-transgenic littermates and the number of the olfactory glomeruli was drastically reduced in the dorsal olfactory bulb. Thus the rate of the spontaneous ORN firing would be intrinsically regulated by HCN channels via the basal activation of a unique set of GPCRs within an optimal range to maximize the olfactory network diversity.

## 2PK-075

### Involvement of TRPV2 in oral persistent pain following mucosal injury

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Some previous studies have reported that TRPV1 is involved in oral persistent pain following mucosal injury. On the other hand, the involvement of TRPV2 in oral persistent pain remains unclear. In this study, we determined if TRPV2 was involved in altered mechanical and heat sensitivity in the buccal mucosa (intra-oral tissue) or whisker pad skin (extra-oral tissue) following each tissue incision. Male Sprague-Dawley rats underwent a buccal mucosa or whisker pad skin incision, and the head-withdrawal latency (HWT) to mechanical or heat stimulation of the buccal mucosa or whisker pad skin was analyzed. Moreover, the expression of TRPV2 in trigeminal ganglion (TG) neurons innervating the buccal mucosa or whisker pad skin was examined. The HWT to heat stimulation of the buccal mucosa significantly decreased after buccal mucosal incision, although the HWT of whisker pad skin showed no change after whisker pad skin incision. The HWT to mechanical stimulation of the buccal mucosa or whisker pad skin significantly decreased after each tissue incision. The number of TRPV2-immunoreactive (IR) TG neurons innervating the buccal mucosa was significantly larger than that of whisker pad skin. The number of TRPV2-positive TG neurons innervating the buccal mucosa or whisker pad skin significantly increased on day 3 after each tissue incision. These findings suggest that TRPV2 plays a pivotal role in mechanical and heat hyperalgesia in oral structures following mucosal injury.

## 2PK-076

### Phosphatase activity of VSP is graded in a reflection of the sequential motion of the voltage sensor

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Voltage-sensing phosphatase (VSP) is composed of the voltage sensor domain (VSD) and the cytoplasmic region which has an enzymatic activity to dephosphorylate phosphoinositides. We have shown that the phosphatase activity of VSP is coupled to the movement of the VSD over all membrane potential where VSD moves (Sakata et al., 2011). However, little is known about mechanisms how the phosphatase activity is coupled to the movement of VSD. One of the possibilities is that the phosphatase activities are exhibited only when VSD is in a fully activated state, and the number of molecules in the active state increases as the membrane potential becomes more positive. The other is that the enzymatic activity of single VSP proteins can be graded upon distinct activated states of VSD. In this case, partial activation of VSD is coupled to certain level of the phosphatase activity. To distinguish between two possibilities, we studied VSD mutant of Dr-VSP, Dr-VSP (T156R/I165R), whose Q-V plot was fitted by the sum of two Boltzmann equations. Analysis of 'gating' current and voltage clamp fluorometry showed that VSD of this mutant moves in two steps. Measurements of the phosphatase activity demonstrated that both transitions of VSD trigger the phosphatase activity of Dr-VSP (T156R/I165R). These imply that partial activation of VSD is coupled to the phosphatase activity, and the phosphatase activity at the single protein level could be graded dependent upon the magnitude of the movement of the VSD.

## 2PK-077

### Involvement of the store operated calcium channels in the spontaneous calcium transients in striatal GABAergic neuron

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We previously reported that the long-lasting spontaneous intracellular  $Ca^{2+}$  transients (spontaneous  $[Ca^{2+}]_i$  transients), which lasted up to about 300 s, were observed in striatal cells and were caused by the  $Ca^{2+}$  release from the intracellular  $Ca^{2+}$  store (ER) via  $IP_3$  receptor. But, it is unclear what mechanism contributes to maintaining  $[Ca^{2+}]_i$  in a high concentration for such a long time during a single event of the spontaneous  $[Ca^{2+}]_i$  transient. The store operated  $Ca^{2+}$  channel (SOCC) is thought to be activated by the depletion of  $Ca^{2+}$  in ER. We hypothesized that the depletion of the  $Ca^{2+}$  in ER opens SOCC results in maintenance of the  $[Ca^{2+}]_i$  in a high concentration where the spontaneous  $[Ca^{2+}]_i$  transients exhibited. To test this hypothesis, we demonstrate that functional SOCCs existed in striatal GABAergic neurons using  $Ca^{2+}$  imaging, and compared the amount of the  $Ca^{2+}$  influx from the extracellular space under administration of TTX between where cells exhibited the  $[Ca^{2+}]_i$  transients and not by means of the  $Mn^{2+}$  quenching of the fluorescent  $Ca^{2+}$  indicator. The rate of the  $Mn^{2+}$ -quenching in the cells exhibiting the spontaneous  $[Ca^{2+}]_i$  transients was faster than in those exhibiting no spontaneous  $[Ca^{2+}]_i$  transients. These results suggested that the spontaneous  $[Ca^{2+}]_i$  transients led to opening SOCCs in the striatal cells, and that the SOCC might concern the maintenance of  $[Ca^{2+}]_i$  during the spontaneous  $[Ca^{2+}]_i$  transients.

## 2PK-078

### Pre-exposed temperature affects temperature thresholds for TRPM8 activation through binding of phosphatidylinositol 4,5-bisphosphate

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Cold sensation is one of the important senses for living things to survive. This fundamental sense is known to be well affected by ambient temperature. Transient Receptor Potential Melastatin 8 (TRPM8), a nonselective cation channel expressed in a subset of peripheral afferent fibers, is known to act as a cold sensor having a threshold for activation of about 27°C. Although the cold temperature threshold of TRPM8 is affected by menthol or pH, ambient temperature is not reported as a factor changing the cold temperature threshold. Since the cold temperature threshold was thought to be unchanged by changes in ambient temperature, the relativity of temperature sensing in different ambient temperatures could not be understood at molecular levels with thermosensitive TRP channels. Here we showed pre-exposed temperature changed cold temperature threshold of mammalian TRPM8 in the heterologous expression system. Moreover, reduction in phosphatidylinositol 4,5-bisphosphate ( $PIP_2$ ) induced attenuation of changes in cold temperature threshold by changes in pre-exposed temperature. Changes in the cold temperature threshold by pre-exposed temperature was also attenuated in a single mutation at R1008 in C-ter region. These findings suggest that the ambient temperature affects the cold temperature threshold of TRPM8 through binding of  $PIP_2$ .

## 2PK-079

### TRPC6-mediated membrane depolarization may regulate cell cycle progression of bone marrow stromal cells

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Resting membrane potential (RMP) is controlled by ion channel, transporter and pump activities in the cell membrane and also affected by electrical spread between neighboring cells via gap junctions. RMP varies depending on cellular conditions such as proliferation, differentiation, and apoptosis, and shows cell-cycle dependent changes in both normal and cancer cells. However, in most types of non-excitabile cells, the exact roles of RMP in their physiology and pathophysiology remains poorly understood. In this study, we explored the role of RMP in undifferentiated bone marrow stromal cells (BMSC) function by the patch clamp technique. In BSMCs in S-phase which was determined by a fluorescent cell cycle by indicator Fucci, RMP was found deeper than in the other phases. Most of BSMCs with deeper RMP (less than -60mV) showed a prominent inward rectifying IV-relationship. Synchronization of BSMCs at G<sub>1</sub>, S, G<sub>2</sub> or M phase revealed that RMP evaluated by a voltage-sensitive dye DiBAC<sub>4</sub>(3) was the deepest in the S-phase. As TRPC6 channel expression was found significantly decreased in the S-phase<sup>1</sup>, we next knocked down its expression by siRNA strategy. This procedure not only deepened RMP, but altered the cell-cycle distribution of BSMCs with more preference at G<sub>2</sub>/M phase. These results suggest that TRPC6 may play an important role for controlling the cell cycle progression of BMSC through regulating resting membrane potential. Reference 1) Ichikawa and Inoue, *J. Physiol. Sci.* 61 (Suppl.1): S121, 2011.

## 2PK-080

### ATP directly binds to cardiac $Ca^{2+}$ (Cav1.2)channels

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Although it is known that activity of Cav1.2  $Ca^{2+}$  channel depends on the intracellular ATP level, its underlying mechanism is still unclear. In cell-free patches, ATP, in the presence of calmodulin (CaM), maintains activity of the  $Ca^{2+}$  channels, implying direct binding of ATP to the channel. In this study, we tested the possible ATP binding to the channel by using photo-affinity labeling method. First, we examined two ATP analogues, i.e., EDA-ATP-biotin (biotin conjugated to ribose; ATP-rB) and 6AH-ATP-biotin (biotin conjugated to  $\gamma$ -phosphate; ATP-pB), for their potency to affect channel activity. In the inside-out patches of guinea-pig myocytes, 1 mM ATP-rB (+1  $\mu$ M CaM) but not ATP-pB supported channel activity, indicating that ATP-rB could be substituted for ATP. Then we used azido-ATP-rB to photo-affinity label the channel, and found that the reagent bound to the  $Ca^{2+}$  channel purified from guinea-pig heart with an apparent K<sub>d</sub> of about 0.5 mM. Finally, we examined the binding of azido-ATP-rB to fragments of the channel, N-terminal tail (NT) and three regions of C-terminal tail (CT1, CT2 and CT3 from proximal to distal), and found that the reagent bound to NT and CT1, but not to CT2 or CT3. These results support the hypothesis that ATP directly binds to Cav1.2  $Ca^{2+}$  channel and thereby regulates activity of the channel.

## 2PK-081

### Spatio-temporal regulation of intracellular $\text{Ca}^{2+}$ and NO signaling by the TRPC5 channel complex

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Cation channels encoded by the transient receptor potential (*trp*) gene superfamily are characterized by a wide variety of activation triggers that act from outside and inside the cell. We have previously reported that a class of TRP channels is sensitive to changes in redox status via modifications of its cysteine residues, such as electrophilic reaction and S-nitrosylation (*Nature Chem. Biol.* 2006 & 2011; *Channels* 2008). However, the roles of the  $\text{Ca}^{2+}$  influx via redox-sensitive TRP channels in regulating  $\text{Ca}^{2+}$  signaling pathways remain elusive. Here we show that nitric oxide (NO)-activated TRPC5 forms a "channelsome", a molecular assembly centered upon a channel, to regulate  $\text{Ca}^{2+}$  and NO signaling in endothelial cells. Upon vasodilator receptor stimulation, TRPC5 is activated via the PLC $\beta$  cascade. This activation of TRPC5 elevates the intracellular  $\text{Ca}^{2+}$  concentration and stimulates the formation of  $\text{Ca}^{2+}$ -calmodulin (CaM) complex. Subsequently,  $\text{Ca}^{2+}$ -CaM binds to endothelial NO synthase (eNOS) which disrupts the inhibitory association between the eNOS and the scaffolding protein caveolin-1. Once eNOS is released from caveolin-1, eNOS relocate to interact directly with TRPC5. This enables eNOS to produce NO in the vicinity of TRPC5, leading to an efficient secondary activation of TRPC5 and an amplification of  $\text{Ca}^{2+}$  influx and NO production. We report a novel pathway in which  $\text{Ca}^{2+}$  and NO signaling are spatio-temporally regulated by TRPC5, eNOS and caveolin-1 in endothelial cells.

## 2PK-082

### TRPC6 is involved in the wound healing of HaCaT cells via ATP- $\text{Ca}^{2+}$ signaling

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We have previously reported that stretch-induced ATP- $\text{Ca}^{2+}$  signaling plays a crucial role in the wound healing of HaCaT keratinocytes. The present study examined the potential involvement of TRPC6 in this signaling by using hyperforin, a TRPC6 activator/up-regulator. A narrow scar was made in the confluent monolayer of HaCaT cells cultured on a silicone chamber coated with collagen, and the ATP release and the intracellular  $\text{Ca}^{2+}$  increase in response to a transient uniaxial stretch (20%, 1 sec) were live-imaged. Besides the facilitation of wound healing, hyperforin amplified the stretch-induced ATP release from the frontier cells facing the scar and also the consequent intercellular  $\text{Ca}^{2+}$  wave mediated by metabotropic ATP receptors (P2Y2 and P2Y4). The  $\text{Ca}^{2+}$  response and the wound healing were significantly inhibited by apyrase (ATP-hydrolyzing enzyme), suramin (P2-receptor antagonist) and diC8-PIP<sub>2</sub> (the water-soluble PIP<sub>2</sub> analogue), which can down-regulate TRPC6 via the competitive inhibition of DAG. Thus, hyperforin may enhance the stretch-induced  $\text{Ca}^{2+}$  response by augmenting ATP release and TRPC6 activity, though we do not know yet how hyperforin enhances ATP release. It is suggested that TRPC6 contributes to the facilitation of wound healing by amplifying ATP- $\text{Ca}^{2+}$  signaling, the process of which may be activated by forces generated in frontier migrating cells and dragging forces exerted directly and indirectly on the rear cells connected to the frontier cells.

## 2PK-083

### Modulation of TRPP3 channel function by increasing temperature

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Transient receptor potential (TRP) ion channels are sensitive to multiple stimuli such as temperature, mechanical and osmotic stress. We have previously demonstrated that murine TRP polycystin 3 (TRPP3) forms a voltage-dependent cation channel that exhibits large tail current induced by repolarization after depolarization. In the present study, we investigated heating effects on TRPP3 channels. Single-channel events of the TRPP3 channel were recorded at hyperpolarized potentials under whole-cell configurations to analyze the channel properties. Heating to 40°C increased the single channel conductance accompanied by a decrease in the open probability. Dwell time analysis showed that both open and closed dwell times at 40°C are shorter than that at room temperature. Interestingly, lowering temperature (from 40°C to room temperature) markedly enhanced the TRPP3 channel activity. This rebound activation was due to an increase in the open probability but not in the single channel conductance. Tail currents of TRPP3 channels were increased by heating from 20°C to 32°C, but decreased at more than 36°C. Heating caused bimodally shifts of voltage-dependency of the TRPP3 channel; heating from 20°C to 32°C and to 36°C directed activation curves toward left and right, respectively. In addition, rebound activation of tail currents by cooling from 40°C was observed. These results suggest that increase in temperature has two disparate effects on TRPP3 channels.

## 2PK-084

### The properties of $\text{Mg}^{2+}$ -inhibited TRPM7-like channel in mouse osteoclasts

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We found previously that stimulation of osteoclast precursor cells with receptor activator of NF- $\kappa$ B, an osteoclast differentiation-inducing factor, up-regulates expression of transient receptor potential subfamily melastatin 7 (TRPM7). Although TRPM7 has been suggested to contribute  $\text{Mg}^{2+}$  uptake in mammalian cells, its role in osteoclasts is still unknown. Therefore, we examined the properties of cation currents in osteoclasts to clarify the functional role of TRPM7. Under whole-cell recording conditions in intracellular and extracellular  $\text{Mg}^{2+}$ -free solutions, outwardly rectifying cation currents were induced in mouse osteoclasts. Intracellular or extracellular application of  $\text{Mg}^{2+}$  inhibited the outwardly rectifying cation currents, called  $\text{Mg}^{2+}$ -inhibited cation current ( $I_{\text{MIC}}$ ). Extracellular 2-aminoethoxyphenylborate or intracellular spermine inhibited the  $I_{\text{MIC}}$ . On the other hand,  $I_{\text{MIC}}$  activity was prolonged by intracellular application of PIP<sub>2</sub>, and potentiated by extracellular divalent cation-free or acidification. The inward component of  $I_{\text{MIC}}$  evoked by extracellular acidification remained unchanged even in the presence of physiological concentration of  $\text{Mg}^{2+}$ . These properties of the  $I_{\text{MIC}}$  in osteoclasts are similar to those reported for TRPM7. Osteoclasts extrude  $\text{H}^+$  and  $\text{Cl}^-$  and form an acidic compartment to dissolve inorganic component of bone matrix. Abrogation of  $\text{Mg}^{2+}$ -dependent block of inward currents at extracellular acidic milieu is suitable for uptake of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  eluted from bone matrix during bone resorption.



## Poster Presentations Cell Physiology, Molecular Physiology (2)

### 2PK-085

#### A role of PI3 kinase in insulin-induced enhancement of cAMP-stimulated Cl<sup>-</sup> secretion in renal epithelia A6 cells

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Insulin is known to increase ENaC-mediated Na<sup>+</sup> reabsorption in the kidney that is related to hypertension in the diabetics. Although the role of insulin on ENaC-mediated Na<sup>+</sup> reabsorption is well investigated in renal epithelial cells, it is poorly understood how insulin acts on the Cl<sup>-</sup> secretion, which plays an important role in regulation of the body fluid content. In this study, to clarify how insulin acts on the Cl<sup>-</sup> secretion, we pretreated A6 cells with 1 μM insulin and measured forskolin (FK)-stimulated Cl<sup>-</sup> secretion and apical Cl<sup>-</sup> channel activity detected as NPPB (a Cl<sup>-</sup> channel blocker)-sensitive I<sub>sc</sub> and G<sub>t</sub>, respectively. Insulin pretreatment had no effect on basal Cl<sup>-</sup> secretion. Interestingly, insulin pretreatment enhanced FK-stimulated Cl<sup>-</sup> secretion with a further increase in NPPB-sensitive G<sub>t</sub>. The insulin action enhancing the cAMP-stimulated Cl<sup>-</sup> secretion required at least 4 hours, suggesting the possibility that insulin enhances the cAMP-stimulated Cl<sup>-</sup> secretion via gene expression. Indeed, insulin increased mRNA expression of CFTR Cl<sup>-</sup> channel and NKCC1. Further, PI3 kinase is known as a major signal in the insulin action. Pretreatment with LY 294002 (a PI3 kinase inhibitor) reduced the insulin action on both the Cl<sup>-</sup> secretion and mRNA expression of CFTR Cl<sup>-</sup> channel and NKCC1. These observations suggest that insulin potentiates the cAMP action stimulating Cl<sup>-</sup> secretion by upregulating mRNA expression of CFTR Cl<sup>-</sup> channel and NKCC1 via PI3 kinase-dependent pathways.

### 2PK-086 (SPK-7)

#### Structural and functional analysis of membrane microdomain as a platform for cell signaling pathway of Ca<sup>2+</sup>-sensitization of vascular smooth muscle contraction

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Abnormal vascular smooth muscle (VSM) contractions such as vaso-spasm are caused by a Rho-kinase (ROK)-mediated Ca<sup>2+</sup>-sensitization of VSM contraction. As an upstream mediator of the Ca<sup>2+</sup>-sensitization, we previously identified sphingosylphosphorylcholine (SPC). The degrees of SPC-induced Ca<sup>2+</sup>-sensitization correlated well with serum total and LDL-cholesterol (Chol) levels, and inversely with HDL-Chol levels. Furthermore, depletion of VSM Chol destroyed Chol-enriched membrane microdomains such as caveolae and lipid rafts, and abolished the SPC-induced Ca<sup>2+</sup>-sensitization. However, mechanisms by which SPC transduces the Ca<sup>2+</sup>-sensitizing signals exclusively through membrane microdomains are unknown. In this study, we tested if SPC preferably interacts with membrane microdomains and affects their structural homeostasis. Firstly, we examined the interaction of human VSM cells with SPC using the surface plasmon resonance measurement. We obtained the first direct evidence that VSM cells have very high affinity for *d*-SPC, but not *l*-SPC, indicating highly structural specificity of SPC. Secondly, we examined the effects of SPC on the surface structure of the VSM cells using scanning and transmission electron microscope. SPC altered dramatically surface structural characteristics of the membrane microdomain. These results support the important role of membrane microdomains such as caveolae and lipid rafts in SPC-induced Ca<sup>2+</sup>-sensitization of VSM contraction.

### 2PK-087

#### Ca<sup>2+</sup>-regulated exocytosis enhanced by PPAR α in guinea pig antral mucous cells : NO and cGMP accumulation

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Acetylcholine (ACh) stimulates Ca<sup>2+</sup>-regulated exocytosis, which consists of an initial phase followed by a sustained phase. We have reported that the initial phase is enhanced by PPARα in antral mucous cells. However, the signals following the activation of PPARα still remain uncertain. In this study, we examined the signals following PPARα activation on the Ca<sup>2+</sup>-regulated exocytosis in antral mucous cells.

Guinea pigs were anesthetized by pentobarbital-Na (70 mg/kg, ip). Antral mucous cells were isolated by a collagenase digestion. The exocytotic events were observed by video-microscopy.

A PPARα blocker (GW6471) abolished the enhancement of the initial phase induced by a PPARα agonist (GW7647), but increased the sustained phase. The similar increase has been reported by a PKG inhibitor. We examined the effects of a PKG inhibitor on the Ca<sup>2+</sup>-regulated exocytosis enhanced by GW7647. A PKG inhibitor abolished the enhancement of the initial phase and increased the sustained phase. Similar responses were observed by a NOS1 inhibitor. GW6471 did not affect the Ca<sup>2+</sup>-regulated exocytosis enhanced by 8BrcGMP or an NO donor (NOC12). Analyses of Western blotting and immunohistochemistry demonstrated that NOS1 exists in antral mucous cells.

Thus, PPARα stimulates NOS1 leading to NO synthesis and then NO accumulates cGMP resulting in the enhancement of Ca<sup>2+</sup>-regulated exocytosis in antral mucous cells.

## 2PK-088

### [Cl<sup>-</sup>]<sub>i</sub> regulation of ciliary bend angle, not beat frequency, in mouse bronchiolar ciliary cells : effects of a mucolytic, carbocistein

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The ciliary beating of the bronchiole plays a key role in the mucociliary transport. Bronchiolar ciliary cells were isolated from lungs of mice (C57Bl/6J) by an elastase treatment and the beating cilia were observed using a high-speed camera (500Hz). A mucolytic, carbocistein (CCys), used to relieve the symptoms of respiratory disease reported to stimulate the ciliary beating. The present study demonstrated that CCys induced cell shrinkage, suggesting a decrease in [Cl<sup>-</sup>]<sub>i</sub>, and that it increases the ciliary bend angle, not beat frequency, by 30%. The effects of CCys on the ciliary beating were not affected by bumetanide, HCO<sub>3</sub><sup>-</sup> free solution, and inhibited by NPPB and glibenclamide, suggesting that CCys stimulates Cl<sup>-</sup> channels. These suggests that a decrease in [Cl<sup>-</sup>]<sub>i</sub> stimulated by CCys increases ciliary bend angle. Previous studies revealed that the outer dyneins control the frequency and the inner dyneins control bend angle. In conclusion, in bronchiolar cilia, CCys decreases [Cl<sup>-</sup>]<sub>i</sub>, which stimulates activities of the inner dyneins in the axoneme.

## 2PK-089

### Ca<sup>2+</sup>-regulated exocytosis enhanced by a PKG inhibitor in guinea pig antral mucous cells : cAMP accumulation by inhibition of PDE2A

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In antral mucous cells, Ca<sup>2+</sup>-regulated exocytosis activated by acetylcholine (ACh) consists of an initial phase followed by a sustained phase. The addition of 8BrcGMP enhanced the initial phase, which was inhibited by a PKG inhibitor (Rp8BrPETcGMPS). But it induced a transient increase in the sustained phase, which was abolished by a PKA inhibitor (PKI), suggesting that the enhancement is caused by cAMP accumulation. Since the phosphodiesterase 2 (PDE2) is a cGMP-dependent enzyme, the inhibition of PKG may stimulate cAMP accumulation. We hypothesized that the enhancement of the sustained phase induced by Rp8BrPETcGMPS was caused by an inhibition of PDE2.

Guinea pigs were anesthetized by pentobarbital-Na (70 mg/kg, ip). Antral mucous cells were isolated by a collagenase digestion. The exocytotic events were observed by video-microscopy. The enhancement of the sustained phase induced by Rp8BrPETcGMPS was mimicked by a PDE2 inhibitor (BAY-60-7550). Analyses of Western blotting and immunohistochemistry demonstrated that PDE2A exists in antral mucous cells. ACh increased cGMP contents and Rp8BrPETcGMPS and BAY-60-7550 increased cAMP contents in antral mucosa during ACh stimulation.

Thus, the cGMP accumulation during ACh stimulation enhances the Ca<sup>2+</sup>-regulated exocytosis but suppresses cAMP actions. This may prevent excessive mucin exocytosis in antral mucous cells to maintain a continuous mucin release.

## 2PK-090

### Intracellular pH-dependent regulation of the pool size of readily-available H<sup>+</sup> channels

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Voltage-gated proton channels (H<sup>+</sup> channels) are expressed in many cell types. Once activated, H<sup>+</sup> channels could dissipate pH imbalance across the membrane. The throughput must depend on the number of readily-available channels, however. In this study, we have examined whether the pool size of the readily-available H<sup>+</sup> channels could be modulated by the intracellular pH (pHi), in microglia and osteoclast-like cells. Under whole-cell clamp recordings, pHi was elevated immediately after applying NH<sub>4</sub>Cl (2-10 mM) into the extracellular medium. The pHi in the vicinity of the channels, monitored with the reversal potentials, returned to the control level by washing NH<sub>4</sub>Cl. Then the electrophysiological properties of whole-cell H<sup>+</sup> currents in control and after the washout of NH<sub>4</sub>Cl were compared. The properties did not alter when the exposure was brief (2 min). More prolonged exposure (>5 min) decreased the whole-cell H<sup>+</sup> conductance, although the gating kinetics and the voltage-dependence for activation remained constant. The reduction of the H<sup>+</sup> conductance depended on the pHi elevated during the exposure to NH<sub>4</sub>Cl (>pH 6.8) and also accompanied by decreases in the cell capacitance. Dynasore, a dynamin blocker, inhibited the decreases in the H<sup>+</sup> conductance partially. Similar results were observed in both microglia and osteoclast-like cells. These data suggest that the pool size of readily-available H<sup>+</sup> channels could change dependently on the pHi and that membrane dynamics is involved in its regulation.

## 2PK-091

### The role of Kv7.1 K<sup>+</sup> channels in thromboxane A<sub>2</sub>-induced cell proliferation of human colonic cancer cells

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Thromboxane A<sub>2</sub> (TXA<sub>2</sub>) is an eicosanoid produced from prostaglandin H<sub>2</sub> via thromboxane synthase (TXS). It is well known that TXA<sub>2</sub> is a potent inducer of platelet aggregation and vasoconstriction. We have previously found that TXS is up-regulated in tissues of human colorectal carcinoma and that 9,11-epithio-11,12-methanothromboxane A<sub>2</sub> (STA<sub>2</sub>), which is a stable analogue of TXA<sub>2</sub>, triggers human colon cancer cell proliferation in a concentration-dependent manner. However, the mechanism is poorly understood. In the present study, we found that Kv7.1 K<sup>+</sup> channels (also called KVLQT1 and KCNQ1) are highly expressed in tissues of human colorectal carcinoma compared to in normal human colorectal tissues. Next, we investigated whether TXA<sub>2</sub> affects expression of the Kv7.1 K<sup>+</sup> channel in human colon cancer KM12-L4 cells. In cells treated with STA<sub>2</sub> for two days, expression of the Kv7.1 K<sup>+</sup> channel was significantly increased. In contrast, TXB<sub>2</sub>, which is an inactive metabolite of TXA<sub>2</sub>, failed to enhance the expression. Whole-cell patch-clamp recordings showed that voltage-independent K<sup>+</sup> currents in TXA<sub>2</sub>-treated cells were greater than that in non-treated or TXB<sub>2</sub>-treated cells. The currents were sensitive to chromanol 293B, which is a specific inhibitor of Kv7.1 K<sup>+</sup> channel. In addition, chromanol 293B concentration-dependently inhibited STA<sub>2</sub>-triggered cancer cell proliferation. These results suggest that Kv7.1 K<sup>+</sup> channel is functionally involved in the mechanism of TXA<sub>2</sub>-induced cell proliferation in colonic cancer.

## 2PK-092

### Epac1 promotes smooth muscle cell migration via calcium/calcieneurin-dependent cell polarization

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Vascular remodeling largely depends on migration of vascular smooth muscle cell (VSMC). We have reported that activation of exchange protein activated by cAMP 1 (Epac1) promotes VSMC migration. However, the intracellular mechanisms of Epac1-mediated migration remain unknown.

Aortic VSMCs in primary culture were obtained from Epac1<sup>-/-</sup> and wild-type (WT) mice. Migration of VSMCs was examined under stimulation with platelet-derived growth factor (PDGF)-BB using the time-lapse microscope. Intracellular Ca<sup>2+</sup> concentrations were monitored by using Fura2-AM.

PDGF-BB-induced directional migration of Epac1<sup>-/-</sup> VSMCs was significantly decreased (0.50-fold vs WT, p<0.001, total cell count 124). PDGF-BB-mediated intracellular Ca<sup>2+</sup> elevation of Epac1<sup>-/-</sup> VSMCs was significantly reduced (0.86-fold vs WT, p<0.01 n=10). To assess the establishment of cell polarity under stimulation with PDGF-BB, expression of phosphorylated cofilin in VSMCs was evaluated by immunocytochemistry. Dephosphorylation of cofilin in Epac1<sup>-/-</sup> VSMCs was significantly decreased (0.39-fold vs WT, p<0.001, total cell count 487). Dephosphorylation of cofilin in WT VSMCs was suppressed by cyclosporine A, a calcineurin inhibitor (10 μM, 60 min), (0.23-fold vs control, p<0.001, total cell count 223).

These results suggest that Epac1 promotes directional migration of VSMC via calcium/calcieneurin-mediated dephosphorylation of cofilin.

## 2PK-093

### Blockade of Kv2.1 channels potentiates glucose-induced insulin release in mouse islet β-cells

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Voltage-dependent potassium channels are involved in repolarization of excitable cells. In pancreatic β-cells, activation of delayed rectifier K<sup>+</sup> (Kv) channels possibly repolarize cells and attenuate glucose-stimulated action potentials to suppress insulin secretion. Inhibition of the β-cell Kv current would be expected to prolong action potentials and enhance glucose-induced insulin secretion. Among Kv channel families, Kv2.1 is reportedly expressed in islet β-cells as the major component of Kv currents in rodents. This study aimed to determine the effects of pharmacological or genetic blockade of Kv2.1 channels on the glucose-induced insulin release in islet β-cells of mice. In islets isolated by collagenase digestion, guangxitoxin-1E (GxTx), a Kv2.1 channel blocker, significantly increased glucose-induced insulin release without altering basal insulin release at 2.8 mM glucose. Glucose-induced insulin release from isolated islets of Kv2.1<sup>(+/+)</sup> mice was significantly greater than that of wild-type mice. Blockade of Kv2.1 channels by GxTx potentiated glucose (8.3 mM)-induced [Ca<sup>2+</sup>]<sub>i</sub> increases in β-cells as monitored by fura-2 microfluorometry, without altering basal [Ca<sup>2+</sup>]<sub>i</sub> levels and KCl (25 mM)-induced [Ca<sup>2+</sup>]<sub>i</sub> increases at 2.8 mM glucose. These results suggest that Kv2.1 channels physiologically restrict glucose-induced Ca<sup>2+</sup> influx and thereby attenuate insulin secretion in β-cells. Blockade of this channel can promote glucose-induced insulin release, providing a potential therapeutic tool to treat type 2 diabetes.

## 2PK-094

### Effect of tungstate on HeLa cells proliferation

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Sodium tungstate exists an oxo-anion as well as vanadate, and that acts as a weak oxidizing agent in aqueous solution. Oral administration of tungstate to diabetic rats normalizes glycemia, increases amount of GLUT4 mRNA and expression of GLUT4 on the plasma membrane. Isolated human peripheral blood lymphocyte treated with sodium tungstate reduced cell progression and increased in tungstate-dependent early apoptosis. Several studies have been reported about the effect of sodium tungstate on whole-body level in rat, but studies are few about the effect of that on molecular and cellular level. In this study, we investigated that the effect of sodium tungstate on HeLa cells. The addition of sodium tungstate to cell cultures resulted in significant reductions in the quantity of HeLa cells. The half maximal effective concentration of tungstate for cell growth was 0.8±0.1 mM. The cell viability was measured by using flow cytometry, and the cells treated with 1 mM sodium tungstate were almost viable. The results suggested that the effect of sodium tungstate on HeLa cell proliferation was not damage to cell viability but inhibition of proliferation. Furthermore, the inhibition of cell proliferation by sodium tungstate was improved by treatment with DIDS, which was an anion channel blocker, and bumetanide, which was a potassium sodium chloride channel blocker. Sodium tungstate might be related to chloride transport in the cell.

## 2PK-095

### Involvement of the extracellular pH in skeletal muscle insulin resistance

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Insulin resistance in the skeletal muscle is manifested by diminished insulin-stimulated glucose uptake and is a core factor in the pathogenesis of type 2 diabetes mellitus, but the mechanism causing insulin resistance is still unknown. The type 2 diabetes mellitus is associated with extracellular acidification, which would affect various cellular physiological functions. Therefore, we hypothesized that the extracellular pH in the skeletal muscle was lowered under pathophysiological conditions and the lowered pH affected insulin sensitivity of skeletal muscle cells. In the present study, we confirmed effects of the extracellular pH on the insulin signaling pathway in a rat skeletal muscle-derived cell line, L6 cell. The phosphorylation level of the insulin receptor was significantly diminished in low pH media. The phosphorylation level of Akt, which is a downstream target of the insulin signaling pathway, was also declined in low pH media. Moreover, the binding affinity of insulin to insulin receptor was reduced by lowering extracellular pH. We also tried to clarify the effect of lower pH conditions on the glucose uptake in L6 cells. Our present study suggests that lowered extracellular pH conditions may produce the pathogenesis of the insulin resistance in skeletal muscle cells.

## 2PK-096

### Identification of functional mutations of ryanodine receptor in malignant hyperthermia

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Ryanodine receptors (RyRs), located in the sarcoplasmic reticulum membrane, are required for intracellular  $\text{Ca}^{2+}$  release that is involved in a wide range of cellular functions. Malignant hyperthermia (MH) is a pharmacogenetic complication of general anesthesia resulting from abnormal  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (CICR) via the type 1 RyR (RyR1) in skeletal muscles. More than 100 mutations in the RyR1 gene have been reported in MH patients. However, there were only a few experimental results confirming those mutations being responsible for the increment of the CICR sensitivities, since such a long cDNA of RyR1 not only required much complicated procedures for making desired mutations but also caused its low transfection efficiency of the mutant DNAs. We improved the method for making MH mutants in the cDNA of RyR1. We characterized the functional mutations on RyR1 in HEK293 cells with Tet-regulated RyR1 expression for functional assay. Rabbit RyR1 channels carrying corresponding mutations (L13R, Q155K, R163C, D166G, R533H) were expressed. It was found that R163C and Q155K mutations of the RyR1 resulted in enhanced  $\text{Ca}^{2+}$  release activity, therefore these mutations would be responsible for the MH incidence. These results suggest that exploration of the functional mutations of RyR1 is probably effective in preventive diagnosis of patients associated with MH disease.

## 2PK-097

### Neurotrophin prevents lidocaine-induced inhibition of axonal transport in cultured mouse dorsal root ganglion

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Lidocaine is widely used antiarrhythmic and local anesthetic agent, while it is known to have neurotoxicity and inhibit axonal transport. Neurotrophin, an analgesic agent, is ordinarily used in Japan for the treatment of various chronic pain in the fields of orthopedics, neurology, and anesthesia. The ingredient of this drug is a non-protein extract from inflamed skin of rabbits inoculated with vaccinia virus. In this study, we investigated the effect of the combination of neurotrophin and lidocaine on axonal transport in cultured mouse dorsal root ganglion (DRG) neurons. Mouse DRG neurons were cultured for 48 hours and used for experiments. Movement of organelles in neurites was observed in real-time using video-enhanced microscopy. Simultaneous addition of neurotrophin blocked the lidocaine-induced inhibition of axonal transport. This result suggests that neurotrophin appear to prevent neurotoxicity, it may lead to relieve neuropathic pain by lidocaine.

## 2PK-098

### Regulation of cardiac Na/Ca exchanger by local Ca signaling mediated by PMCA

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Heart cells have two different sarcolemmal Ca transporters: the plasma membrane Ca-ATPase (PMCA) and the Na/Ca exchanger (NCX). In mouse ventricular cells, the expression of *Ncx1*, *Pmca1*, and *Pmca4* was confirmed by RT-PCR. Although PMCA has long been believed to make negligible contribution to the  $[\text{Ca}]_i$  homeostasis in heart cells, whole-cell clamp experiments in mouse ventricular myocytes showed that it was not true. Inhibition of PMCA by vanadate and carboxyeosin enhanced the amplitude of the NCX current in a dose-dependent manner, and the enhancement of the NCX current involved a shift of its  $[\text{Ca}]_i$ -dependence to the left. This NCX current enhancement by PMCA inhibition was not observed when the  $[\text{Ca}]_i$  was strongly buffered with 10 mM-BAPTA. A computer simulation of the Ca concentration profile around a PMCA molecule, based on Neher's diffusible chelator model (1986), predicted 1) that the operation of a PMCA molecule developed a  $[\text{Ca}]_i$  well of around 50 nm diameter in its vicinity, and 2) that the  $[\text{Ca}]_i$  decline in the well could well explain the NCX current enhancement by PMCA inhibition. Similar enhancement of the NCX current was also observed when ATP hydrolysis was inhibited by AMP-PNP. These results indicate a potential role of the PMCA as a signaling molecule that regulates the NCX according to metabolic states of the cell, by changing the local  $[\text{Ca}]_i$ .

## 2PK-099

### Oscillatory changes in the activity of $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter control cell cycle progression via changes in the intracellular concentration of $\text{Cl}^-$ in MKN28 human gastric cancer cells

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We previously demonstrated that the reduction of intracellular chloride concentration ( $[\text{Cl}]_i$ ) inhibits the cell proliferation of gastric cancer MKN28 cells by diminishing the transition rate from G1 to S cell cycle phase. If there are oscillatory changes of activity of  $\text{Cl}^-$  transporters during the cell cycle progression, oscillatory changes of the  $[\text{Cl}]_i$  would also occur. However, mechanisms involved in modulation of cell cycle progression by  $\text{Cl}^-$  transporters are still poorly understood. To clarify the underlying mechanisms, we examined the relationships between the expression of  $\text{Na}^+/\text{K}^+/\text{2Cl}^-$  cotransporter (NKCC) and the change of  $[\text{Cl}]_i$  during the cell cycle progression in gastric cancer MKN28 cells. We measured the  $[\text{Cl}]_i$  of the cells in each cell cycle stage (G1, S and G2/M) released from synchronization by using a double thymidine block method. The  $[\text{Cl}]_i$  was reduced in the M phase followed by  $[\text{Cl}]_i$  in the G1 and S phase. Protein expression of NKCC was high in the S and G2 phases and low in the M and early G1 phases. These results strongly suggest that cell cycle-dependent expression of NKCC leading to oscillatory changes of  $[\text{Cl}]_i$  plays important roles in the cell cycle progression in MKN28 cells.

## 2PK-100

### Release of granzyme B from NK92 cells which was crosslinking-stimulated by CD2

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Natural killer (NK) cells play a key role in the innate immune system through the rapid secretion of cytokines and the ability to lyse virally infected cells or tumor cells. CD2 are surface glycoprotein receptors and important for NK cell activation. Nevertheless, activation of NK92 cells through CD2 crosslinking-stimulation has not been completely understood. Here we analyzed the effects of CD2 crosslinking-stimulation on NK92 cell activation. We confirmed the expression of CD2 on the NK92 cell surface. The majority of NK92 cells expressed CD2 on their cell surfaces. We found that CD2 crosslinking-stimulation enhanced release of granzyme B from NK92 cells. CD2 crosslinking-stimulation markedly induced phosphorylation of extracellular signal-regulated kinase (ERK) 1/2 compared with non-stimulated NK92 cells. Furthermore, we discovered that tyrosine phosphorylation of ERK1/2 was quickly induced within 1 min in an antibody concentration dependent manner. Mitogen-activated protein kinase extracellular signal-regulated kinase (MEK) inhibitor U0126 is a chemically synthesized organic compound that inhibits activation of ERK1/2. U0126 treatment inhibited the release of granzyme B, compared with CD2 crosslinking-stimulated NK92 cells, in a concentration-dependent manner. These results suggest that CD2 crosslinking-stimulation enhances the release of granzyme B and phosphorylation of ERK1/2, which are critical events during NK cell-mediated cytotoxicity.

## 2PK-101

### Effect of cesium on HeLa cells proliferation

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Cesium (Cs), as well as potassium (K), is a member of alkaline metal elements. In the study of transporter, Cs has been used as a K channel blocker on cells. Doses of CsCl by the intraperitoneal route in mice indicated that a rapid transfer of Cs from blood to tissue compartments (Pinsky and Bose, 1984). Effects of Cs on the whole bodies had been investigated. However, it is not clear that how the Cs is transported through which kinds of way at the molecular levels, and that the effect of Cs on the cell metabolisms. We examined the proliferation of HeLa cells cultured with different types of alkali metals such as Li, Na, K, Rb, and Cs. Among them, only Cs inhibited the proliferation of the cells. The proliferation decrease is dependent on Cs concentration. Microscopic examination of the Cs-treated cells, cell membrane was not smoothly. Two different methods of live and dead assay were performed (i.e., LDH assay and Calcein-PI stain). In the concentration indicated cell proliferation inhibition, the HeLa cells membrane was not damaged by Cs. Cs uptake into the cell as intracellular cation was confirmed by capillary electrophoresis. These results suggested that Cs added to extracellular media uptake into the cell and influence cell proliferation.

## 2PK-102

### Regulation of thioredoxin interacting protein (TXNIP) expression by p44/p42 MAPK-p90RSK pathway

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Thioredoxin interacting protein (TXNIP) is an anti-tumor protein, down-regulated in cancer cells. Molecular analysis concerning the regulation of TXNIP amount could lead to develop novel cancer therapies. We have reported that a monosaccharide D-allose inhibits the cell cycle progression of cancer cell lines through the induction of TXNIP expression. Here we elucidated the signal transduction pathway regulating TXNIP amount in hepatocellular carcinoma cell line HuH-7.

D-allose transiently activated p44/p42 MAPK and p90RSK, a substrate of p44/p42 MAPK, and then up-regulated TXNIP. Inhibition of p44/p42 MAPK phosphorylation by PD98059 reduced the expression level of TXNIP. These results suggest that transient activation of p44/p42 MAPK-p90RSK pathway caused by D-allose could lead to TXNIP up-regulation.

We also examined the mechanism of TXNIP down-regulation. Serum stimulation caused a sustained activation of p44/p42 MAPK and p90 RSK, and rapid degradation of TXNIP. Inhibition of p44/p42 MAPK phosphorylation by PD98059 weakened this effect on TXNIP level. These results indicate that sustained activation of p44/p42 MAPK-p90 RSK pathway could lead to the TXNIP reduction. Taken together, the p44/p42 MAPK-p90RSK pathway could take part in both up-regulation and down-regulation processes of TXNIP. Further molecular analysis and in vivo administration analysis of D-allose would lead us to have better understanding of the new cancer therapies using this monosaccharide.

## 2PK-103

### Oct-3/4 induces CpG demethylation in the MGMT promoter to induce temozolomide resistance in glioblastoma cells

Takahashi, Hisaaki; Kobayashi, Yukako; Sugimoto, Kana; Yano, Hajime; Tanaka, Junya (*Molecular and Cellular Physiology, Grad School of Med, Ehime Univ, Japan*)

Alkylating agents, such as temozolomide, are the most effective cytotoxic agents used to treat malignant gliomas. A cellular DNA repair enzyme, O<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT), reverses alkylation at the O<sup>6</sup> position of guanine. Thus, the expression level of MGMT is closely related to the sensitivity of brain tumors to alkylating agents. MGMT expression is controlled by methylation/demethylation of cytosine phosphate guanosine (CpG) islands in the promoter region of the MGMT gene. Oct-3/4, a self-renewal regulator in stem cells, is expressed in various kinds of solid tumors including glioblastoma, and is involved in tumor progression and malignancy in glioblastomas. However, little is known regarding MGMT expression in glioblastomas. Therefore, in the present study, we investigated whether Oct-3/4 is involved in the sensitivity of glioblastomas to temozolomide, through the expression of MGMT. Oct-3/4 over-expression resulted in decreased susceptibility to temozolomide compared with control cells, as assessed by LDH assay. In Oct-3/4 over-expressing cells, the expression of MGMT mRNA was up-regulated, as shown by qPCR analysis. The methylation status of 27 CpG sites within the MGMT promoter was analyzed by genomic sequencing of bisulfite-modified DNA. Oct-3/4 over-expressing cells showed enhanced demethylation of CpG islands. These results suggest that Oct-3/4 promotes the temozolomide resistance in glioblastoma cells by up-regulating MGMT expression through epigenetic changes in the MGMT promoter region.

## 2PK-104

### Oct-3/4 contributes to acquire anti-cancer drug resistance of glioblastoma cells

Hosokawa, Yuki; Takahashi, Hisaaki; Kawabe, Yuya; Sugimoto, Kana; Yano, Hajime; Tanaka, Junya (*Molecular and Cellular Physiology, Grad Sch of Med, Ehime Univ, Japan.*)

Drug-resistance presents a major obstacle to the efficacy of chemotherapeutic treatment of cancers. Oct-3/4, a self-renewal regulator in stem cells, is expressed by various kinds of solid tumors including glioblastoma. Although Oct-3/4 is involved in tumor progression, malignancy and prognosis in glioblastomas, little is known regarding drug resistance in glioblastoma. In the present study, we investigated whether Oct-3/4 contributes to drug resistance in glioblastoma cells by using established Oct-3/4-overexpressing glioma cells. Compared with control cells, Oct-3/4 overexpression resulted in decreased susceptibility to anti-cancer drugs, carboplatin, VP-16, and doxorubicin, as assessed by LDH assay and inhibited apoptotic PARP cleavage. In Oct-3/4-expressing cells, the expression of drug efflux pump genes, ABC transporters, was upregulated as assessed by qPCR analysis. Oct-3/4-expressing glioma cells also showed enhanced-efflux of doxorubicin (Dox) by detecting intracellular Dox fluorescence using flow cytometry. Furthermore, established drug-resistant U251 glioma cells (U251/Dox cells) in long-term culture with growth medium containing Dox, demonstrated upregulated Oct-3/4 and ABC transporter expression. Inhibition of Oct-3/4 expression in U251/Dox cells by shRNA downregulated the expression of ABC transporter mRNAs. These results suggest that Oct-3/4 promotes the acquisition of a drug-resistant phenotype in glioblastoma cells. Therefore, suppression of Oct-3/4 might be a potential therapeutic target for treatment of glioblastomas.

## 2PK-105

### Oct-3/4 promotes angiogenesis in glioblastoma through VEGF production

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Accumulating evidence shows that the expression level of Oct-3/4, a self-renewal regulator in stem cells, positively correlates with various solid tumors involved in glioma. However, little is known regarding the influence of Oct-3/4 in the angiogenesis of glioblastomas. In the present study, we examined the potential angiogenic functions of Oct-3/4 in glioblastomas. We established Oct-3/4-overexpressing human glioblastoma cells (U251/Oct-3/4), prepared from human glioblastoma cells (U251), and transplanted U-251/Oct-3/4 and U251/enhanced green fluorescent protein (EGFP) cells into the right thigh of nude mice. Compared with U251/EGFP control cell-derived subcutaneous tumors, U251/Oct-3/4 cells developed 10-fold larger tumors with aberrant blood vessel formation 8 weeks after transplantation. By the aberrant blood vessel formation, the necrotic area in Oct-3/4 cell-derived tumors was smaller than that of control cells. The expression levels of angiogenesis-related genes, such as hypoxia-inducible factor (HIF) and vascular endothelial growth factor (VEGF), were upregulated in U251/Oct-3/4 cells. Furthermore, ELISA analysis revealed that U251/Oct-3/4 cells secreted higher amounts of VEGF protein than control cells in vitro. Indeed, the conditioned media of U251/Oct-3/4 expressing cells possessed the ability to induce blood vessel formation in a three-dimensional collagen culture of rat abdominal aorta. These results suggest that Oct-3/4 promotes tumor development of glioblastoma by angiogenesis through VEGF production.

## 2PK-106

### Cell fate analysis of macrophages/microglia using CD11b-Cre transgenic mice

Holmes, Ayano; Takahashi, Hisaaki; Sugimoto, Kana; Yano, Hajime; Tanaka, Junya (*Molecular and Cellular Physiology, Grad Sch of Med, Ehime Univ, Japan.*)

Microglia are activated during brain pathology and release a variety of bioactive substances that may modify neuropathological events. Although microglial cells are considered the only cell population of mesodermal origin in the brain, we have previously shown that rat primary microglial cells can transdifferentiate into neuroectodermal cells such as neurons, oligodendrocytes and astrocytes, through two-step culture using a highly concentrated serum-supplemented media. Our study suggests that microglial cells have similar properties to stem cells in that they can differentiate to neurons and may contribute to the restoration or modification of injured brain. To clarify this hypothesis in vivo, we developed cell fate analysis using a transgenic mouse expressing green fluorescent protein (GFP) driven by the microglia/macrophage-specific promoter. Transgenic mice are currently generated by injection of exogenous genes into mouse embryos. Recently, another approach to transgenesis has been developed using lentiviruses to deliver genes into mouse embryos. Although careful handling and training are required for lentivirus use, a technically demanding and expensive microscopic system are not required. We attempted to develop a transgenic mouse expressing Cre recombinase driven by CD11b, the microglia/macrophage-specific promoter, and report on the progress of cell fate analysis in this meeting.

## Poster Presentations Sensory Function(2)

## 2PK-107

### Effects of 1,8- and 1,4-cineole on spontaneous excitatory synaptic transmission in adult rat spinal substantia gelatinosa neurons

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Various plant-derived chemicals activate TRP channels expressed in the central terminal of primary-afferent neuron, resulting in a presynaptic enhancement of spontaneous glutamatergic transmission in spinal substantia gelatinosa (SG) neurons. Many of the properties of the TRP channels have been examined in the cell body of primary-afferent neuron. We have suggested that there may be a difference in property between TRP channels in the central terminal and cell body of primary-afferent neuron. In order to further address this issue, we examined the effects of plant-derived 1,8- and 1,4-cineole on spontaneous glutamatergic transmission in the SG neurons. Bath-applied 1,8-cineole reversibly enhanced the frequency of spontaneous EPSC without a change in its amplitude with an  $EC_{50}$  value of 2.5 mM. 1,4-Cineole increased spontaneous EPSC frequency about 20-fold effectively more than 1,8-cineole. These cineole activities were unaffected by TRPV1 antagonist capsazepine while being inhibited by TRPA1 antagonist HC-030031. The spontaneous EPSC frequency increase produced by 1,8-cineole was much greater than that expected from TRPM8 activation by 1,8-cineole compared to (-)-menthol. It is concluded that cineole enhances the spontaneous release of L-glutamate onto SG neurons, possibly through TRPA1 activation; this action of 1,4-cineole is much greater than that of 1,8-cineole. These results could serve to know the property of the central TRP channels.

## 2PK-108

### Cooling – Gustation interaction : Conditioning cold stimuli enhance taste sensitivity

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It is well known that the gustatory nerve fibers are not only sensitive to taste stimulation but also respond to changes in temperature. Furthermore it has been reported that heating or cooling in the small areas of the tongue could cause sensations of taste. We have recently shown that transient cooling of the mouth improved dysgeusia. In this study, we tested modulatory effects of cold stimulation on for taste sensitivity in Japanese dental students. Sensory evaluation for the four basic tastes (sweetness, saltiness, sourness, bitterness) were examined by the instillation method, in which taste stimuli were applied to the entire oral cavity. The estimation was done by a blind test. The taste stimuli consisted of five concentrations of the four basic taste solutions. We estimated the recognition threshold (the concentration at which the taste could be identified as one of four basic tastes). Conditioning cold stimulation was applied by keeping an ice cube in the oral cavity for 1 min. We confirmed that the tongue temperature returned to the body temperature 3 min after its removing from the mouth. Afterwards, the test for the four basic tastes was performed. We found that cooling of the tongue enhanced the sensitivity of all basic tastes. These results suggest that cold-sensitive neurons may play an important role in the human gustatory system.

## 2PK-109

### Oxytocin facilitates inhibitory transmission without a change in excitatory transmission in adult rat spinal substantia gelatinosa neurons

Jiang, Chang-Yu; Fujita, Tsugumi; Xu, Nian-Xiang; Luo, Qing-Tian; Kang, Qin; Matsushita, Akitomo; Ohtsubo, Sena; Kumamoto, Eiichi (*Dept Physiol, Saga Med Sch, Saga, Japan*)

Oxytocin is thought to be involved in various physiological actions including antinociception. We have previously reported that oxytocin produces a membrane depolarization by activating oxytocin receptors without a change in spontaneous glutamatergic excitatory transmission in spinal substantia gelatinosa (SG) neurons which play a pivotal role in regulating nociceptive transmission from the periphery. The present study further examined the effect of oxytocin on synaptic transmission by applying the whole-cell patch-clamp technique to the SG neurons of adult rat spinal cord slices. Oxytocin (0.5  $\mu$ M) did not affect the peak amplitude of monosynaptically-evoked primary-afferent A $\delta$ -fiber EPSC recorded at a holding potential of -70 mV. Spontaneous inhibitory transmission was increased by oxytocin in a dose-dependent and repeated manner.  $EC_{50}$  values for oxytocin in increasing GABAergic and glycinergic spontaneous IPSC frequencies were 0.024  $\mu$ M and 0.038  $\mu$ M, respectively. These inhibitory transmission enhancements were mimicked by [Thr<sup>4</sup>,Gly<sup>7</sup>]-oxytocin, and were inhibited by [d(CH<sub>2</sub>)<sub>5</sub><sup>1</sup>,Tyr(Me)<sup>2</sup>,Thr<sup>4</sup>,Orn<sup>8</sup>,des-Gly-NH<sub>2</sub><sup>9</sup>]-vasotocin and voltage-gated Na<sup>+</sup>-channel blocker tetrodotoxin. It is concluded that oxytocin enhances spontaneous inhibitory transmission, the action of which is due to an increase in the neuronal activities of inhibitory SG neurons. Such inhibitory transmission enhancements produced by oxytocin could contribute to its antinociceptive effect.

## 2PK-110

### Developing a method for recording gustatory neuron responses in the nucleus of the solitary tract of awake mice

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In this study, we develop a method for recording gustatory neuron responses in the NST of awake mice (C57BL/6). For chronic recording, a cranioplastic cap was fixed to the skull. An intraoral cannula was implanted in the face to deliver taste stimuli. After recovery from the surgery (ca. 1 week), the mouse was mounted in the stereotaxic device with a sling which restrained the body movement, and trained to obtain 0.25M sucrose and water through the cannula. In the recording sessions, a glass-coated tungsten microelectrode was inserted in the gustatory area of the NST localized in advance. After single- or multi-unit activity was recorded, each of the five standard taste stimuli (0.1 M NaCl, 0.5 M sucrose, 0.01 M citric acid, 0.0001 M quinine HCl, and 0.01 M disodium 5'-inosin monophosphate) was delivered via the intraoral cannula. We obtained two taste-responsive multiple-units in the NST of two awake mice. These units responded differently to the standard taste stimuli; both responded best to sucrose. The activity of these taste responsive units was not affected by mouth movements (licking). Water rinse tended to reduce baseline activity of these responsive units. These results indicate that the present experimental model is useful for the investigation of gustatory information processing in the NST.

## 2PK-111

### Studies on the visibility of “color map” and “monochrome map” : Evaluation by the search time and search distance

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Recently, an opportunity for us to see a map is increasing by the spread of mobile information terminals. Generally about the map, anyone needs to see easily and to search the target quickly. About intelligible map, Ninomiya et al. (2008) studied an abbreviation map generation algorithm for mobile information terminals. About exploring the target on map, Ichimura et al. (2009) studied information searching behavior based on trace of eye movement. However, the difference in visibility in the search for “color map” and “monochrome map” has not been investigated quantitatively. In the present study, we evaluated “color map” and “monochrome map” of visibility quantitatively with search time and distance. In the experiment, we showed “color map” and “monochrome map”, then the subjects explored a search target. The map was placed 25cm in front of the right eye position. Pupil at the same distance was taken through the half mirror by Infrared CCD camera. We investigated in the case of one or two search target. As a result, it was found that both of the search time and distance were shorten in “color map”. In addition, there was a clear difference in both of the search time and distance in two search targets. It is considered that we can easily search “color map” than “monochrome map” due to the hue and saturation.

## 2PK-112

### Studies on pupil reaction during gazing ambiguous figure

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The first study of the optical illusion has been done in the 1890's, but recently studied again by the development of the computer. However, most studies have been done in a psychological field. In addition, among optical illusion, there is the ambiguous figure that one figure looks like two, but is not investigated in detail. We examined and focused on a Rubin vase in the ambiguous figure in this study and how see it by measuring a pupil area. We showed ambiguous figure with a PC for five seconds. A pupil of right eye was taken by infrared CCD Camera when we watched an ambiguous figure. We recorded this pupil image to a HDD and then transferred to a PC through a video capture board. The pupil area per frame was analyzed by software. Because color figure is based on visibility, we examined not only the gray scale but also color scale of figure as the pupil reaction. We examined the change of the pupil area when we watched a figure of high visibility and low visibility under the condition that the viewpoint of the figure was instructed or not. When a viewpoint was instructed, the pupil area decreased depending on the brightness of the figure at the high visibility and increased depending on the area of the figure at the low visibility. When the viewpoint was not instructed, the pupil area did not change depending on the brightness and the area regardless of high visibility, low visibility. It was found that ambiguous figure how the visible, is according to whether or not to gaze it.

## 2PK-113

### 2,4,6-Trichloroanisole is a potent blocker of olfactory signal transduction

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Off-flavor substances that are generated naturally in foods/beverages cause large damages on products. One of the most powerful off-flavors is 2,4,6-trichloroanisole (TCA) that is known for inducing the corktaint of wines. Since TCA and other off-flavor substances cause damages on wide varieties of products also, understanding of mechanisms of such off-flavors is one of the most urgent issues. Generally, such off-flavors have been thought to produce unpleasant smells. Here, however, we show with human psychophysical tests that TCA and related substances actually reduce flavors of wines with ppt level contaminations. Furthermore, it was shown in single olfactory cells that TCA blocked transduction channels without causing excitatory responses. It was much more potent (100-1000 times) than by olfactory masking agents that have been widely used for perfumery compounds, and even than by a well-known pharmacological blocking agent, l-cis-diltiazem. To explain such super-efficiency, the TCA effect showed the time-integration and slow recovery from the blockage. Surprisingly, this blockage was observed when 1 aM TCA was applied to the cell. The sequence of block matched perfectly the human recognition for off-flavors, TCA >>> 2,4,6-trichlorophenol. We further confirmed that the potent block was caused by a haloarene structure having a side chain that increases LogD. The present findings thus not only show the mechanism of flavor loss, but also provide possible molecular architectures that invent olfactory masking agents and powerful channel blockers.

## 2PK-114

### The role of *Poxn*-expressing neurons in different processes of ingestion behavior of *Drosophila*

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The *Pox neuro* (*Poxn*) gene of *Drosophila* plays an important role in the development of peripheral and central nervous system (CNS); those functions might be involved in the reception and processing of taste signals. The peripheral *Poxn*-expressing neurons function as taste sensors. The CNS *Poxn*-expressing neurons project to several brain regions including the central body and antennal lobes, but their function in ingestion behavior are still unclear. To determine the contribution of peripheral or CNS *Poxn*-expressing neurons to ingestion behavior of *Drosophila*, we performed the proboscis extension reflex (PER) test and two choice test to several mutant fly strains with dysfunction of peripheral taste neurons or both peripheral and CNS *Poxn*-expressing neurons. Although the responsibility to sugar solutions was significantly reduced in fly strains with dysfunction of peripheral taste neurons in PER test, these strains were able to choose sucrose solutions with higher concentration from two kinds of solution (40 mM and 30 mM) normally. Fly strains with dysfunction of both peripheral and CNS *Poxn*-expressing neurons shown declined selectivity for these sucrose solutions. These results suggested that the *Poxn*-expressing peripheral neurons might contribute mainly to triggering PER, and CNS *Poxn*-expressing neurons might be involved in later processes like food choices based on the difference of taste intensity.



## 2PK-115

### A new efficient measurement of contrast sensitivity function in the behaving Long-Evans rat

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As an animal model, there is a growing need for rodents in visual neuroscience because of their high applicability to genetic manipulation and optical control of a specific neuron, which enables activation/inhibition of a specific network or neuron-type at a specific time. This has also been accompanied by another need for a measurement system of fundamental visual ability of various visual aspects. Although two laboratories (Busse et al. 2011; Histed et al. 2012) recently developed contrast-sensitivity assessment systems of mice, both required a long-term training period for animal's learning of the task (3-4 weeks for Busse; 8-10 weeks for Histed). To resolve this problem, we elaborated a new assessment system, in which rats were trained to detect a grating patch presented in right or left visual field in a two-alternative forced-choice task by operant conditioning, with completed learning the task within 2 weeks. The specification of our system and its advantages are as follows: 1) a freely moving condition to motivate the rat's action and to minimize stress from restraint; 2) a spout-lever for rapid learning of the task-related lever-pulling manipulation (Kimura et al. 2012); and 3) a staircase method for rapid determination of contrast threshold. Using this system, we examined which grating parameters affect visual performance, finding that size influenced contrast-sensitivity but temporal frequency and direction did not. Thus, our system provides a promising visual behavior model with rapid task learning and efficient assessment of visual performance.

## 2PK-116

### Nociceptive behavioral responses to mechanical, thermal and chemical stimulation in the adulthood of rats with neonatal dopamine depletion

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Previous data provide evidence that dopamine system is involved in not only motor processes but also modulation and processing of somatosensory information. Although rat with dopamine depletion using 6-hydroxydopamine (6-OHDA) in the adulthood exhibits akinetic motor activity, rat with dopamine depletion in the neonate exhibits a spontaneous motor hyperactivity, suggesting the effect of dopamine depletion depends on the lesioned developmental period. Moreover, the hyperactivity of neonatally 6-OHDA treated rats is ameliorated with psychostimulant such as methamphetamine. It has been reported that dopamine depletion in the adulthood results in a hyperalgesic response to nociceptive stimulation, whereas the effect of neonatal dopamine depletion on nociceptive response in the adulthood is still unclear. To clarify behavioral characteristics of nociceptive response in the adulthood of neonatally 6-OHDA treated rats, we performed the behavioral analyses with von-Frey test, tail flick test and Formalin test. We also examined whether methamphetamine attenuates the formalin-induced nociceptive response. The neonatally 6-OHDA treated rats showed a hyperalgesic response in Formalin test, but not in von-Frey test and tail flick test. Although the formalin-induced nociceptive response of control rats was dose-dependently decreased by methamphetamine, the hyperalgesic response of the neonatally 6-OHDA treated rats was not affected, suggesting a crucial role of dopamine system for tonic pain and the complex mechanism.

## 2PK-117

### Asymmetric glycinergic inputs between ON and OFF pathway in the mouse retina

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The formation of direction selectivity is one manifest feature of the dendritic computation in the retina and cholinergic amacrine cells plays a pivotal role for this dendritic computation. However, the input-output relationship of the cholinergic amacrine cells at the synaptic level has not been elucidated. In this study, we characterized the responses of the cholinergic amacrine cells to glutamate, acetylcholine and glycine in the mouse retina. Responses to glutamate were driven by AMPA/KA type receptors and accompanied an increase in the frequency of GABAergic IPSCs. Responses to acetylcholine were mediated by nicotinic receptor but not by muscarinic receptor. Although an amplitude of acetylcholine response was very small, acetylcholine-induced currents accompanied a vigorous increase in the frequency of GABAergic IPSCs. There was no significant difference of response amplitude to glutamate or acetylcholine between ON- and OFF-cholinergic amacrine cells. On the other hand, the glycinergic inputs in the ON-cholinergic amacrine cells were significantly stronger than those in the OFF-cholinergic amacrine cells. Glycine responses were inhibited by an application of strychnine. The result suggests that ON-pathway-specific glycinergic responses make a sharp contrast of the OFF-pathway-specific purinergic responses. These pathway-specific responses in cholinergic amacrine cells might be involved in the pathway specific adjustment of dynamic range to achieve the best visual acuity in the mouse retina.

## 2PK-118

### New subarea in the rostradorsal part of the primary auditory cortex in mice

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Transcranial flavoprotein fluorescence imaging reveals fine structures of cortical activity in mice. Using this method, we reexamined tonotopic maps of the primary auditory cortex (AI) in C57BL/6 mice. Tonal stimuli produced cortical responses in AI, and the response peaks shifted from the caudal to the rostroventral parts of AI, when the frequency was changed from 5 kHz to 40 kHz. However, previous studies have reported that the tonotopic axis of AI is present between the caudal and rostradorsal parts of AI in mice. The discrepancy between the present finding and the previous studies suggests that the rostradorsal part of AI may have another tonotopic map, which is independent from the map in other parts of AI. To test this possibility, we investigated whether any heterogeneity in cytoarchitecture was found within AI. We stained AI with a monoclonal antibody SMI-32 to non-phosphorylated neurofilaments, since SMI-32 staining has been used in many previous studies to identify various auditory subareas in the cortex and thalamus. SMI-32 staining revealed that the rostradorsal part of AI has enriched nonphosphorylated neurofilaments while other parts of AI were not. Using two-photon calcium imaging, we confirmed that the rostradorsal part of AI had a tonotopic map that was clearly different from the map in other parts of AI. These physiological and anatomical findings indicate that rostradorsal part of AI can be parcellated from AI.

## 2PK-119

### The retinal intrinsic response of suprachoroidal –transretinal stimulation(STS)for retinal prosthesis : relationship between threshold current and features of implantation surgery

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As retinal prosthesis we proposed suprachoroidal-transretinal stimulation (STS), which uses electrode array implanted into sclera. The intrinsic retinal response evoked by STS was recorded, and the relationships between its threshold current and some features of implant operation were investigated.

Seven eyes of seven cats were studied under general anesthesia. The electrode array for STS was inserted into the pocket made in sclera. Each array had two platinum electrodes (0.5 mm in diameter and 0.3 mm in height). The reflectance of 800-880nm was recorded via fundus camera, and biphasic pulses (0.03-2.0 mA, 0.5 ms/phase, 20 Hz frequency) were applied to each electrode. The impedance of each electrode was measured with LCR meter, and the depth of retinal indentation by the electrode head with optical coherent tomography. The residual scleral thickness between the electrode and the retina was histologically examined.

The threshold current was positively correlated with the scleral thickness, and negatively correlated with the electrode impedance and the retinal indentation by the electrode. These results showed that implantation surgery influences the threshold current of retinal prosthesis by STS.

## 2PK-120

### Involvement of rostral ventromedial medulla cells in motor cortex induced anti-nociceptive effects in normal and chronic constriction injury rats

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Motor cortex stimulation provides anti-nociceptive effects in chronic constriction injury (CCI) rats by spinal cord inhibition. It has also shown that even in normal rats spinal cord neurons reduces responses to nociceptive stimuli during motor cortex stimulation, but the precise mechanisms of this spinal cord inhibition remains to be unknown. In this study, we tested the rostral ventromedial medulla (RVM) involvement in this motor cortex stimulation-elicited spinal cord inhibition in normal rats and CCI rats made by sciatic nerve ligation. Single unit activities of the RVM cells were recorded with tungsten microelectrodes under pentobarbital anesthesia. Prior to cortical stimuli, the RVM cells were classified into three groups, ON-, OFF-, and Neutral cells, based on their responses to nociceptive pinch stimuli applied at the hind paw. Cortical stimulus current intensity was ranged 30-110 $\mu$ A. We found that OFF cells facilitated, OFF cells inhibited, ON cells facilitated, and ON cells inhibited by motor cortex stimulation in normal rats, and OFF cells facilitated in CCI rats. These results suggest that motor cortex can drive the spinal cord in the two opposite directions, facilitatory or inhibitory, via the RVM, however the balance between facilitation and inhibition may change in CCI rats.

## 2PK-121

### A HDAC inhibitor, trichostatin A persists and enhances synaptic plasticity in the olfactory bulb

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Epigenetic mechanisms play a critical role in transcriptional regulation, synaptic plasticity, and memory formation. Specifically, histone-associated heterochromatin undergoes changes in structure during the early stages of long-term memory formation. Young rats can learn their dam's odor and approach her without visual information. In order to establish olfactory learning, the pairing of odor and somatosensory stimulation is crucial. We have shown that synaptic plasticity in the olfactory bulb (OB) underlies aversive olfactory learning. Epigenetic modifications are recognized to represent a principal interface between intracellular signaling pathways and gene expression. Chromatin is known to be post-translationally regulated by acetylation of histones via histone acetyltransferases. This process causes chromatin structure to relax, leading to enhanced transcription, and can be reversed by histone deacetylases. We have reported that trichostatin A (TSA) infusion during odor-shock training enhanced a conditioned odor aversion. We hypothesized that elevating levels of histone acetylation might facilitate induction of long-term potentiation (LTP). Therefore, we measured field EPSPs derived from granule cells to investigate the effect of TSA on induction of LTP at the mitral-to-granule cell synapse in slice preparations of the main olfactory bulb. The results indicated that TSA could enhance the amount of potentiation triggered by high frequency stimulation and facilitate LTP-induction with subthreshold tetanus at mitral/granule dendrodendritic synapses in the OB.

## 2PK-122

### Effect of mGluR2 activation on granule cell activities at the reciprocal synapse in the mouse accessory olfactory bulb

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By measuring the reciprocal synaptic currents from mitral cells in the AOB, we have demonstrated that an agonist for group II metabotropic glutamate receptors (mGluR2/mGluR3), DCG-IV, suppressed dendrodendritic inhibition (DDI) in a reversible manner while the mGluR2/mGluR3 antagonist LY341495 enhanced it. The effects of these drugs were markedly impaired by genetic ablation of mGluR2, indicating that DCG-IV-mediated suppression of DDI is mediated by mGluR2. Miniature EPSCs (mEPSCs) recorded from granule cells were reduced in both their frequency and amplitudes by the extracellular application of DCG-IV, suggesting that mGluR2 can modulate the synaptic transmission from mitral to granule cells through both presynaptic and postsynaptic mechanisms.

In the present study, we have given attention to the effect of mGluR2 activation on postsynaptic properties (that is, granule cell activities). AOB slices were prepared from 23-to 35-day-old Balb/c mice. Using the patch-clamp technique in whole-cell configuration, the current responses of granule cells were recorded in the presence of an antagonist for GABAergic transmission, picrotoxin (50  $\mu$ M). An extracellular application of DCG-IV reduced the response of granule cells to glutamate, providing further evidence for mGluR2 to modulate the synaptic transmission from mitral to granule cells through a postsynaptic mechanism. Additionally, DCG-IV inhibited Ca<sup>2+</sup> currents in granule cells, suggesting that mGluR2 reduces the GABAergic transmission to some extent through the inhibition of Ca<sup>2+</sup> channels.

## 2PK-123

### Distribution of tyrosine hydroxylase immunoreactive amacrine cells in the developing gerbil retina

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The development of catecholaminergic neurons in mammalian retina has been examined with antibody against tyrosine hydroxylase (TH). We have examined shape localization and change in number of the TH-immunoreactive (TH-IR) amacrine cells in the retina from postnatal day (P) 1 to adult gerbils using immunocytochemical method. The gerbils were perfused intracardially with a fixative. After the removed eyeballs were frozen with a rapid jet of liquid carbon dioxide, they were cut with section of 16 $\mu$ m using a cryostat. The sections and the whole mounts were examined by ABC immunocytochemical staining method. We have found two kinds of TH-IR amacrine cells. One type (type A) of TH-IR cells have monostratified dendrites extending in the outermost layer of the IPL, while the other type (type B) cells have dendrites extending in the middle of IPL. The type A somata were larger and more densely stained than those of the type B cells. The type A somata showed no significant growth, while the type B somata showed significant growth between P7 and P14 days. After P 14 little change was seen in the sizes of the type B somata. After P28, the diameters of type A somata did not overlap with those of the type B somata. These results suggest that two kinds of dopaminergic amacrine cells have different developmental properties in the developing gerbil retina. Eye opening is an important period for the maturation of dopaminergic amacrine cells, for the maturation of the IPL.

## 2PK-124

### The neural representation of lightness in the cat primary visual cortex

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Lightness (perceived luminance) of surface is greatly influenced by the luminance of neighboring and more distant regions, which has been thought to reflect a part of perceptual lightness constancy. Recently, we found that about 30% of neurons in the lateral geniculate nucleus (LGN) of anesthetized cats show visual responses representing the lightness and its constancy. In that study, the late (50-100 ms after stimulus onset) but not early (0-50 ms) responses to stationary presentation (500 ms) of a uniform large disk with diameter of 20° covering neuron's receptive field (center stimulus, CS) is not only changed by the luminance of the CS, but also modulated by simultaneous luminance change of the background area surrounding the CS (background stimulus, BS). However, it remains unknown how the BS modulates the CS response. To examine the possibility that the integrated information of CS and BS comes from primary visual cortex (V1), we conducted the same experiments in V1 as the LGN, where the multi-point extracellular recording was performed. Sixty percent of V1 neurons significantly responded to the CS, and those neurons were distinguished by BS-induced modulation: one increased the visual responses as the BS luminance dissociates from the CS luminance, and the other did as the BS luminance verges towards the CS luminance. It suggests that the former would be the source of the late response of LGN neurons. To clarify this point, we are analyzing the time course of the neuronal responses.

## 2PK-125

### Response-based surround suppression of the cat primary visual cortex

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Neurons in the primary visual cortex (V1) show suppressive phenomenon known as surround suppression (SS). Visual stimuli located outside a neuron's classical receptive field (CRF) don't elicit spiking response, but frequently suppress the CRF response. Since the strength of SS changes in proportion to the similarity of stimulus features such as orientation between CRF and its surround, SS has been thought to play a role in figure-ground segregation and in gain control of responses. Here, it remains an open question by which way SS adjusts the response gain, according to overall network-response or individual neuron-response. To answer this question, we performed multi-points extracellular recording from anesthetized cats and examined the effects of stimulus phase on SS. The CRF was stimulated with stationary sinusoidal grating patch with various sizes and phases. We isolated 34 simple and 46 complex cells. The simple cells showed different shapes of the size-tuning curves depending on the grating phases. Interestingly, the strength of SS and spatial summation properties seem to be associated with the response magnitude for each grating phase. This suggests that the activity levels of simple cells are individually adjusted by SS. In contrast, complex cells responded almost equally to various phases. The complex cells seem to form such features by gathering the response-gain-adjusted inputs from simple cells. Thus, phase-dependency of SS seems to be explained well by hierarchical feedforward information processing.

## Poster Presentations

### Oral Physiology

#### 2PK-126

##### Modulation of two types of jaw-opening reflexes by stimulation of the red nucleus

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The red nucleus (RN) is cytoarchitecturally divided into two parts; the parvicellular part (RNP) and the magnocellular part (RNM). The present study aimed to (1) compare the effects of RN stimulation between low- and high-threshold afferent-evoked jaw-opening reflexes (JORs), and (2) compare the largeness of the effects between the RNP and the RNM. The experiments were performed on rats anesthetized with urethane and  $\alpha$ -chloralose. The test stimulation was applied to the inferior alveolar nerve (a single pulse, 0.1 ms in duration, 1 Hz) to evoke the JOR. The stimulus intensity was either 1.2 (low threshold) or 4.0 (high threshold) times the threshold. The electromyograms were recorded from the anterior belly of the digastric muscle. The conditioning stimulation (1 pulse, 0.2 ms in duration, 1 Hz, 100  $\mu$ A) was applied to the RN. The interval between the conditioning and test stimuli was varied. Conditioning electrical stimulation of the RN significantly facilitated the JOR evoked by innocuous stimulus. Significant facilitation was observed at a conditioning-test interval of 5-12 ms. On the other hand, stimulation of the RN significantly suppressed the JOR evoked by noxious stimulus. Significant suppression was observed at a conditioning-test interval of 30 ms. The facilitatory effect was not different between the RNM and the RNP. The suppressive effect of the RNM was significantly larger than that of the RNP. These results suggest that the RN has different functional role on the control of the JOR.

#### 2PK-127

##### Neural activity in the somatosensory cortex following oral stimulation applied to preferred chewing side : An MEG study

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Little is known whether evoked cortical response following oral stimulation depends on preferred chewing side (PCS). Somatosensory evoked fields (SEFs) were measured in 12 healthy volunteers following tongue and hard palate stimulation. Evident PCS determined by subjective and objective methods was right in six participants and left in six participants. Clear responses were detected over the both hemispheres for either tongue or palate stimulation. We evaluated the cortical activity via activated root-mean-square (aRMS), which is the time-averaged activity between 10 and 150 ms from the 18-channel RMS over the each hemisphere. For tongue stimulation, aRMS of the contralateral- and ipsilateral-hemisphere was  $8.23 \pm 5.39$  and  $4.67 \pm 3.06$  fT/cm for PCS, respectively, and  $5.11 \pm 3.81$  and  $4.03 \pm 2.85$  fT/cm for non-PCS. For palate stimulation, aRMS of the contralateral- and ipsilateral-hemisphere was  $5.35 \pm 2.00$  and  $4.62 \pm 2.33$  fT/cm for PCS, respectively, and  $4.63 \pm 1.93$  and  $4.14 \pm 2.07$  fT/cm for non-PCS. Although there was no significant difference of aRMS of the contralateral hemisphere between PCS and non-PCS in the hard palate, aRMS of the contralateral hemisphere for PCS was significantly greater than that for non-PCS in the tongue. The present finding suggests that the discrepancy in the effect of PCS on SEFs between tongue and hard palate may depend on the presence or absence of fine motor activity.

#### 2PK-128

##### Relationship between Morphogenesis of Circumvallate Papillae and Distribution of $\alpha$ -Gustducin and Neural Cell Adhesion Molecule (NCAM) in the Epithelium and the Taste Bud

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In the present study, we used DIC images and images obtained in transmission mode by LSM to examine the histology of the same specimens that we had monitored the fluorescence of AlexaFluor 488 and 633. By combining images, we were able to define clearly the histological locations of keratins 13 and 14 (K13 and K14), type II and type III collagens (CII and CIII),  $\alpha$ -gustducin and neural cell adhesion molecule (NCAM) during the morphogenesis of circumvallate papillae and taste buds. K14 immunoreactivity was first detected in the basal layer of the epithelium of the circumvallate papillae on postnatal day 0 (P0) and K13 immunoreactivity was detected on P7. The respective pattern of K13 and K14 immunoreactivity differed during the development of the circumvallate papillae. Both CII and CIII appeared in conjunction with the morphogenesis of the circumvallate papillae, and in the connective tissue (CT) that surrounded the lingual muscle during myogenesis of the tongue. However, CIII was more distinct in these areas than CII.  $\alpha$ -Gustducin appeared in the cytoplasm of taste cells during their formation after birth, while NCAM appeared in the epithelium of circumvallate papilla-forming area. However, these two markers of taste cells were distributed in similar proportion within mature taste cells.

## 2PK-129

### Assessment of paracellular transport upon onset of secretory stimulation in the isolated perfused submandibular salivary gland

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Primary saliva is a mixture of fluid secreted through paracellular route and transcellular route. These components were estimated as 60 and 25  $\mu\text{l/g-min}$ , respectively. The paracellular fluid secretion could include a pressure-independent and a pressure-dependent components: 25 and 35  $\mu\text{l/g-min}$ , respectively. To assess whether the pressure-dependent paracellular transport corresponds to the wider paracellular route (wider than 5 angstrom in radius; Murakami 2001), The submandibular salivary gland was surgically isolated from rat (Wistar male) and perfused arterially on the stage of confocal microscope with ultrafast z-axis drive (5-Live). Using sulfo-rhodamine B in the perfusate, the appearance of the fluorescent signal in the intercellular canaliculi was observed upon secretory stimulation with carbachol (1  $\mu\text{M}$ ) and other secretagogues. The system allowed us to obtain 64 images every 2 s and gave 3D reconstructed image every 2 s. Isoproterenol (1  $\mu\text{M}$ ) decreased the fluorescence intensity in the canaliculi, suggesting lowering fluid secretion through the pressure dependent paracellular route.

## 2PK-130

### Appetite for vitamin C in vitamin C-deficient rats

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Vitamins are essential micronutrients that contribute to a healthy life. However, the behavioral mechanism for ingesting vitamin C (VC) is unclear. Therefore, we conducted the behavioral and neural experiments in Osteogenic Disorder Shionogi (ODS)/Shi Jcl-*od/od* (*od/od*), which are unable to synthesize VC. In the behavioral experiment, the preferences for VC solution were measured before and after deprivation of VC solution in *od/od* and their wild type (+/+) rats by using two-bottle preference test (vs. distilled water; 48hours). In the neural experiment, integrated responses of the chorda tympani nerve in both rats were recorded. The results are as follows: After deprived VC solution for 25 days, the preferences for VC solution in *od/od* rats were significantly greater than those in +/+ rats. These results did not depend on increase of consumption of VC but on decrease of consumption of distilled water. The chorda tympani nerve responses to VC solution were observed in *od/od* rats even if they were deprived VC solution. These results suggest that VC-deficient rats may recognize VC as a tastant and reject distilled water.

## 2PK-131

### Involvement of the endocytosis pathway in secretory granule formation as revealed by fluorescent dye-uptake into rat parotid acinar cells

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Parotid acinar cells produce many secretory granules (SGs), one type of intracellular organelle. The shape of SGs is spherical and their diameter is about 1  $\mu\text{m}$ , and therefore they can be recognized under light microscopy by immunostaining. That is an advantage to using SGs as a model for organelle investigation. It has been considered that the formation of SGs occurs from the Golgi complex. In this study, we reveal a novel process of SG formation by endocytosis after secretion using fluorescent dye-uptake. Isoproterenol was injected into rats to induce the secretion of SGs by their parotid glands, which were excised 2 hours after the injection. The dispersed acinar cells, which had no SGs, were then incubated with Lucifer Yellow (LY) dye as a tracer for 3 hours. The uptake of LY into newly-formed SGs was estimated by the following methods: 1) the co-localization of LY with amylase was observed by confocal laser microscopy, 2) The intracellular localization of LY was confirmed by electron microscopy, 3) The fluorescence of LY was detected in purified SGs, and 4) the secretion of LY upon stimulation was examined using perfused acinar cells. In addition, acinar cells were cultured in the presence of LY for 2 days to allow the SGs to mature and enlarge, after which they could be observed clearly by confocal laser microscopy. These results demonstrate that SGs contain LY, revealing the contribution of endocytosis to the process of SG formation in parotid acinar cells.

## 2PK-132

### Development of secretory granule-specific pH indicator in rat parotid acinar cells

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Aim of this study is the development of organelle-specific pH sensor. In parotid gland, secretory granules store salivary amylase and releases contents by exocytosis. Amylase is sorted at the trans-Golgi network into forming immature secretory granules (ISGs) and kept in ISGs that become mature secretory granules (MSGs). During maturation, maintenance of intra-granular pH has been considered to be important factor for protein retention and storage. Difference of intra-granular pH might be a good marker to distinguish ISGs from MSGs. However, since secretory granules of parotid gland rapidly eliminate known fluorescence pH sensors such as LysoTracker, it was difficult to observe pH-related events. To keep pH sensor in parotid secretory granules stably, we developed a parotid granule-specific pH indicator. By using expression system of reporter Halo Tag protein that has N-terminal 25 amino acids of amylase as signal peptide (SS25H), whole secretory granules were visible in primary cultured parotid acinar cells. Next, a pH sensor that can bind to Halo Tag protein was synthesized from a fluorescence pH indicator, SNARF-1 succinimidyl ester, and Halo Tag-specific amine ligand. Synthesized fluorescence pH indicator, SNARF-O2, was clearly bound to SS25H in cell lysate covalently. Also, in confocal microscope, SNARF-O2 was observed in spherical compartment similar to amylase granule in primary cells. In conclusion, we succeeded to synthesize granule-specific pH indicator that applicable to detect internal pH of secretory granules.

## 2PK-133

### Leptin suppresses sweet taste responses of mouse fungiform taste cells

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Leptin, an anorexigenic mediator that reduces food intake by acting on hypothalamic receptors, selectively suppresses sweet taste responses. This effect would be mediated by leptin receptor Ob-Rb on sweet taste receptor cells. However, suppressive effect of leptin on taste responses of sweet sensitive taste cells still has not been elucidated. In this study, we examined the effect of leptin on sweet taste responses of taste cells in mouse fungiform taste buds. In about half of sweet sensitive taste cells, bath application of 20 ng/ml leptin suppressed responses to sweeteners (<80% of control response). The effect of leptin was concentration dependent and reached maximal level at 10-20 ng/ml. On the other hand, responses of sour sensitive taste cells to acids were not affected by bath application of leptin. When the cells were adapted to several concentrations (1-5 ng/ml) of leptin, increases in 10 ng/ml leptin still affected sweet responses of taste cells. These results indicate that leptin actually suppresses taste responses of sweet sensitive taste cells. Supported by JSPS KAKENHI Grant Number 18077004, 18109013, 23249081 (YN) and 21791808, 23689076 (RY).

## 2PK-134

### Periodontitis leads to impairment of salivary functions

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This study was designed to clarify whether periodontal disease leads to hyposalivation. At first, we investigated salivary functions in the experimental periodontitis model, developed by a unilateral ligature around the second upper molar, pilocarpine-induced salivary secretion, gland weight, gland histology and muscarinic cellular responses were investigated. The periodontitis model showed a significant reduction in pilocarpine-induced salivary secretion, bilateral atrophy of salivary glands and bilateral vacuolization in acinar cells. According to Ca<sup>2+</sup>-imaging and Western blotting, there were no differences in the muscarine-induced intracellular Ca<sup>2+</sup> mobilization in acinar cells and the M3 receptor expression level in the salivary gland between the sham and the periodontitis model. Second, we clinically investigated improvement in salivary secretion in 15 periodontitis patients following periodontal therapy. In the periodontitis patients, the salivary flow rate was recovered to healthy levels during periodontal therapy. These results from humans and animals suggest that periodontitis leads to hyposalivation.

## 2PK-135

### Effects of anti-cancer using a novel magnetic nano-particle

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Background Radical surgery for patients with oral cancer causes dysfunctions such as dysphagia, dysarthria, and mastication disorder, resulting in losing their quality of life. Here, we developed a novel magnetic anti-cancer drug (=EI236). We evaluated the anti-cancer effects of EI236 using oral cancer animal model. Methods First, we performed STEM (Scanning Transmission Electron Microscopy)-EDX (Energy-dispersive X-ray) to prove that EI236 was taken into the VX2 cancer cells (Squamous cell carcinoma cell), resulting in being cytotoxic to the cells. Second we established rabbit's oral cancer model to examine the anti-cancer effect in vivo. We transplanted VX2 cancer cells into the tongues of rabbits. Rabbits were divided into 4 groups: 1) control group, 2) intravenous EI236 injection (5mg/kg×7days) (iv group), 3) intravenous EI236+electromagnet (DDS (Drug Delivery System) group), 4) intravenous EI236+electromagnet+alternating magnetic field (HT (Hyperthermia) group). The size of tumors was measured daily for 7 days. After 7 days, anti-cancer effects were evaluated by histopathological analysis. Results We confirmed by STEM-EDX that EI236 acts anti-cancer effect by being taken into cancer cells. DDS group and HT group showed significant decreases in tumor volume. In particular, HT group showed anti-cancer effect by histopathological analysis. Conclusion EI236 shows a potency of new drug for the treatment of oral cancer.

## 2PK-136

### Magnetic resonance imaging of the temporomandibular joint in the mouse

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High magnetic field magnetic resonance imaging (MRI) was applied to the temporomandibular joint (TMJ) in the mouse. The purpose of this study was visualization of the internal structure of the TMJ, including the articular cartilage, and the upper and lower joint cavities. Temporomandibular joints from 35 male C57BL6 mice (5-10 weeks old) were examined. Using scout images, slice position were set according to the previous report (K. Satoh *et al.*, Arch Oral Biol 2011, 56: 1382-9). 2D T<sub>1</sub>-weighted MR images with a spatial resolution of 65 μm were obtained by 5 min. The temporal bone and mandibular condyle were depicted as low signal intensity, and the articular disk was depicted as an intermediate signal intensity. After intravenous injection of Gd-DTPA, the articular disk, and the upper and lower joint cavities could be assigned clearly using T<sub>2</sub>-weighted MR images. After MRI examination, all mice were sacrificed for the histological examinations. Paraffin-embedded TMJ blocks were cut in the sections at a 8 μm thickness. Sections were stained with hematoxylin and eosin. Histological structures observed with MRI were closely agreed with those observed with hematoxylin-eosin staining under light microscopy, suggesting that MRI is a useful method for analyzing the complex structure of the TMJ in the mouse.

## 2PK-137

### Can the rats discriminate the components of the mixed taste solutions?

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We conducted behavioral studies to investigate whether Wistar/ST rats can discriminate the components of mixed taste solutions. In this study, we compared the numbers of licks for 10 sec in conditioned group with those in unconditioned group. Rats in conditioned group were acquired conditioned taste aversion to mixture solutions of 5mM saccharin Na (Sacc) and 0.03M NaCl (preferable mixture), or that of 5 mM Sacc and 1.0M NaCl (unpreferable mixture) by intraperitoneal administration of 0.15M LiCl. Rats in unconditioned group were injected saline instead of LiCl. As results, when the numbers of licks in rats conditioned to preferable mixture and those in unconditioned group were compared, the numbers of licks for preferable mixture, 5mM Sacc, 0.03 M NaCl and 0.1M NaCl in conditioned group were significantly smaller than those in unconditioned group. On the other hand, when the numbers of licks in rats conditioned to unpreferable mixture and those in unconditioned group were compared, the numbers of licks for 5mM Sacc, 0.03M NaCl and 0.1M NaCl in conditioned group were significantly smaller than those in unconditioned group. These results suggest that the rats can discriminate the components of the mixed taste solutions, and that palatability of taste solution may affect the acquisition of the conditioned taste aversion.

## 2PK-138

### Effect of Danshen component, salvianolic acid B on intracellular Ca<sup>2+</sup> level of rat submandibular gland cells

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Objective : Danshen (DS) has been shown to induce salivary fluid secretion in vitro, which starts after a latent time and then increases slowly. In addition, it is proved that the secretion is not induced by activation of muscarinic or  $\alpha$  adrenergic receptors. However, when Ca<sup>2+</sup> was removed from the perfusate, DS failed to induce salivary secretion. Our purpose was to ascertain whether DS causes an increase of intracellular calcium in rat submandibular gland (SMG) cells. To reduce the masking effect by dark colour of DS granule, DS component salvianolic acid B (SAB) was used in this study. The effect of SAB on intracellular Ca<sup>2+</sup> level in rat SMG cells was studied. Methods : Double-wave-length fluorospectrophotometer was used to measure [Ca<sup>2+</sup>]<sub>i</sub> of the isolated SMG cells. Calcium indicator Indo-1-AM was used instead of Fura-2-AM to avoid autofluorescence from SAB. Three doses of SAB : 150, 200, and 250  $\mu$ g/ml were added to the SMG cell suspension separately. Intracellular Ca<sup>2+</sup> mobilization was measured for 20 min after SAB administration. Result : Each concentration of SAB could not evoke [Ca<sup>2+</sup>]<sub>i</sub> mobilization. Conclusion : Ca<sup>2+</sup> dependence has been shown in DS induced fluid secretion, whereas intracellular Ca<sup>2+</sup> mobilization was not observed.

## 2PK-139

### Extracellular signal-regulated kinase phosphorylation in trigeminal spinal nucleus and upper cervical spinal cord neurons induced by experimental tooth movement

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Continuous mechanical pressure on the teeth during orthodontic treatment produces discomfort or pain to patients. Though the discomfort and pain induced by teeth movement usually declined within a few days, some patients complain to the long-lasting discomfort and pain. Thus, understanding of the molecular and cellular mechanisms of the discomfort and pain associated with teeth movement is important to develop the appropriate treatment for orthodontic patients.

Here, we showed that experimental teeth movement by orthodontic appliance resulted in a rapid and transient phosphorylation of extracellular signal-regulated protein kinase (ERK) in the trigeminal spinal subnucleus interpolaris and caudalis transition zone (Vi/Vc), Vc and upper cervical spinal cord (C1-C2) in rats. The phosphorylated ERK (pERK) was observed in neurons but not in astroglia and microglia. Single-plane scanning analysis indicated that the pERK was localized in the neuronal nuclei. In addition, mechanical allodynia in the periodontal tissue was produced by teeth movement on day 1 after placement of the orthodontic appliance.

These findings suggest that ERK phosphorylation in Vi/Vc, Vc and C1-C2 neurons play a pivotal role in the tooth pain during orthodontic treatment.

## 2PK-140

### Estrogen acts through mGluR 1 to modulate TMJ-evoked activity of trigeminal subnucleus caudalis neurons

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Sex hormone status is a risk factor in painful temporomandibular disorders (TMD). Previously we reported that estrogen (E2) enhanced nociceptive processing of TMJ input by laminae I-II, but not laminae V units. The mechanisms for this enhancement are not known. To determine if estrogen interacts with metabotropic glutamate receptor 1 (mGluR1) to modulate nociceptive input from the TMJ region, single units were recorded in laminae I-II at the spinomedullary (Vc/C2) junction from ovariectomized female rats (OvX) treated with high E2 (40ug/d, HE2), low E2 (4ug/d, LE2) and male rats. Under isoflurane anesthesia TMJ units were activated by ATP (1 mM, 20ul) injected into the joint space. The mGluR1 antagonist, CPCCOEt (50-500uM) was applied topically to Vc/C2 surface 10 min before test injections of ATP. ATP-evoked responses of TMJ units in HE2 were enhanced versus LE2 (p<0.05). Topical 50uM CPCCOEt did not affect ATP-evoked responses in LE2. By contrast, the enhanced ATP-evoked response normally seen in HE2 rats was blocked by topical 50uM CPCCOEt (p<0.05). This suggested that mGluR1 activation contributed to the E2-mediated enhanced response in HE2 rats. It is concluded that estrogen acts in part through a mGluR1 dependent mechanism to enhance TMJ nociceptive processing by laminae I-II neurons in the medullary dorsal horn.

## 2PK-141

### Characteristics of Brain Functions during Jaw Reaction Movements in Man

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Comparing the values of reaction time (RT) in finger and jaw movements, it was found that jaw RT was slightly longer than that presumed by its neural pathway. To examine this prolongation of jaw RT, contingent negative variation (CNV) reflecting the brain cognitive condition was recorded at Cz of the international 10-20 method for 15 healthy adults. Each task consisted of repeated single trials in which a warning signal (S1) of click tone was followed 1.5 seconds later by an imperative visual signal (S2) to which a quick motor response were required. The finger or jaw RT to S2 was also measured by analyzing motion of a LED reference point fixed to the subject's chin or finger tip with a photo sensor. A whole mean CNV amplitude over the S1-S2 interval was obtained for each task. The CNV amplitude recorded during the jaw task was lower than that during the finger task in all subjects. A statistically significant negative correlation was found between the differences of CNVs amplitude and those of RTs. These results suggest that the skill of jaw reactive movement was not developed compared with that of finger reactive movement.

## Poster Presentations

### Endocrinology

## 2PK-142

### Localization and function of V-ATPase in salivary glands of mouse

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Vacuolar H<sup>+</sup>-ATPase (V-ATPase) is localized in intracellular membranes of organelle such as the vacuole, lysosome, Golgi apparatus, synaptic vesicle, and is known to acidify intracellular compartments as well as pump H<sup>+</sup> across the plasma membrane. V-ATPase is composed of two large domains, a cytosolic (V<sub>1</sub>) domain and a transmembrane (V<sub>o</sub>) domain, and at least 13 distinct subunits. V<sub>1</sub> domain, consisted of A-H subunits complex, is responsible for hydrolyzing ATP. V<sub>o</sub> domain, consisted of a, c, c', c'', and d subunits complex, is involved in H<sup>+</sup> translocation. V<sub>o</sub> domain is united with V<sub>1</sub> domain and functions. The localization and function of the V-ATPase in the salivary glands remains to be studied. Here we examined the expression of V-ATPase by the RT-PCR method and localization of V-ATPase by the immunohistochemical analysis. In major salivary glands, a2, a3, d1, B2, C1, E2 subunit isoforms of V-ATPase were expressed commonly. The immunoreactivity of B2 subunit was found in the epithelial duct cells of salivary glands. We then examined phenotypes of knockout mice of the a3 subunit isoform (a3-KO mouse). The size of salivary glands of the a3-KO mouse is very small, and there is little saliva production. Intraoral salivary pH was also lower (i.e., acidified) than a control mouse. These results suggest that V-ATPases play a critical role for modifying pH in duct cells of salivary glands.

## 2PK-143

### Negligible role of neurosteroids in modulating forebrain GABA(A)receptor function on ADH release

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We reported previously that under basal conditions, the excitability of hypothalamic ADH neurons is tonically inhibited by GABA (A) receptor (-R) activity in the forebrain (AV3V), and that an osmotic or volemic stimulus may excite the neurons by preventing the AV3V GABAergic function. On the other hand, the GABA (A)-Rs involved in tonic inhibition of neural activity are known to include a subtype of which binding affinity to GABA is highly affected by neurosteroid (NS) formed in the brain. In addition, the AV3V contains NS sensitive neurons, as well as NS-Rs and its mRNA. Therefore, it seems possible that NS may participate in ADH release or other phenomena solely or in association with AV3V GABA (A)-R function. This study aimed to examine the validity of the view. Experiments were done in awake rats. Results were as follows. (1) AV3V infusion with a NS capable of inhibiting functions of GABA (A)-R by acting on its steroid-sensitive subunit (SSS) did not alter plasma AVP, osmolality, blood pressure or heart rate. (2) Rises in plasma ADH and other variables evoked by AV3V injections of a GABA (A)-R antagonist were not affected by the pretreatment with another NS capable of enhancing GABA (A)-R functions via binding to the SSS. (3) AV3V injections of a drug that blocks NS biosynthesis did not change ADH secretion triggered by an osmotic or a volemic stimulus. These facts suggest that roles of AV3V GABA (A)-Rs in ADH release and other phenomena are hard to be modulated by NS, in other words, the receptors are composed of subunits with negligible NS sensitivity.



## 2PK-144

### Aberrant cerebellar development of mice deficient in dual oxidase maturation factors(DUOXA)

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Thyroid hormone (TH) plays key roles in the brain development. The rodent cerebellum has been used as a model to study of the mechanisms of TH action. Dual oxidase maturation factor (DUOXA) is a specific maturation factor of dual oxidase, which is a rate-limiting enzyme for TH synthesis. Thus, mutation of DUOXA leads to congenital hypothyroidism both in humans and mice. In this study, we aim to clarify the role of TH on development of cerebellum using DUOXA knockout (KO) mice. As previously reported, the concentration of TH was extremely low in homozygote KO (HO) mice compared with those in heterozygous (HT) mice. Although HO mice appeared to be normal at birth, a significant reduction in body weight became apparent especially after postnatal day 14 (P14). In HO mice, the time on accelerating rotarod was significantly decreased compared with those in HT mice on P25, whereas total moving distance in the open field and grip strength test was not affected, indicating that the function of motor coordination was retarded in HO mice. Cresyl violet staining showed that proliferation and migration of granule cells were delayed after P15 in KO mice. But morphological changes of cerebellum on P25 were not evident in spite of dysfunction of rotarod on P25. Catch-up growth of cerebellum cannot normalize functional abnormality. These results indicate that this mice could be utilized to study the effect of hypothyroidism on brain development.

## 2PK-145

### Milk-ejection related afferent pathways from the ventral tegmentum to the supraoptic nucleus relayed by the dorsomedial nucleus

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We have previously suggested that neurons in the dorsomedial hypothalamic nucleus (DMH) projected to oxytocin (OT) secreting neurons in both sides of hypothalamus and that these projections might contribute to the synchronized activation of OT neurons during milk-ejection reflex. In the present experiments, responses of OT neurons in the supraoptic nucleus (SON) to electrical stimulation of the mid-brain ventral tegmentum (VT) was examined, because this structure was suggested that it was included in the afferent of milk-ejection. To test a possibility that afferents from the VT to the SON are relayed by the DMH, effects of microinjection of lidocaine into the DMH on responses of OT or vasopressin (VAP) cells to electrical stimulation of the VT were also examined. Fifty and 53% of OT cells in ipsilateral and contralateral SON to the stimulated site, respectively, were orthodromically excited by electrical stimulation of the VT. Twenty-two % of vasopressin (VAP) cells in both side of the SON to the stimulated site were also excited orthodromically by electrical stimulation of the VT. Microinjection of lidocaine into the DMH reduced the excitatory response to the electrical stimulation of the VT in 2 out of 3 OT cells and in one VAP cell. Results of the present experiments suggest that OT cells receive excitatory input from both side of the midbrain VT and that these inputs are probably relayed by the DMH.

## 2PK-146

### The opposite effects of lipopolysaccharide to thyroxine on thyroid hormone-regulated functions in astrocyte

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Astrocyte plays essential roles for development and homeostasis of the brain. Thyroid hormone (TH, thyroxine; T<sub>4</sub>, triiodothyronine; T<sub>3</sub>) regulates development and various functions of astrocyte. Perinatal infection/inflammation is a major risk factor for developmental disorder of the brain. Lipopolysaccharide (LPS) is an endotoxin of gram-negative bacteria that induces a variety of reactions in many cells including cerebellar astrocyte. Some of such effects are similar to those seen in perinatal hypothyroidism. In the present study, we investigated the effect of LPS on TH-regulated astrocyte functions, such as actin polymerization and activity of iodothyronine deiodinase type 2 (D2) that converts T<sub>4</sub> to T<sub>3</sub> using C6, glioma-derived clonal cells and rat primary astrocytes. As low as 10 ng/ml LPS induced the expression of glial fibrillary acidic protein (GFAP) and D2 mRNAs. T<sub>4</sub> (10 nM) promoted actin polymerization, whereas 10 ng/ml LPS markedly induced actin depolymerization. Furthermore, T<sub>4</sub> partly rescued LPS-induced actin depolymerization in a dose dependent manner. LPS induced D2 activity in a dose- and time-dependent manner, whereas T<sub>4</sub> but not T<sub>3</sub> blocked it. T<sub>4</sub> restored LPS-induced D2 activity. LPS-induced actin depolymerization and D2 activity were blocked by p38 MAPK inhibitor, while T<sub>4</sub>-induced pathways were not affected by any inhibitors examined. In conclusion, LPS action to astrocytes may be partly exerted through p38 MAPK signal transduction pathway that is independent from T<sub>4</sub>-regulated pathway.

## 2PK-147

### Role of SCGB3A2 in the mouse pituitary gland

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Secretoglobin (SCGB) 3A2 was originally identified as a downstream target in the lung for the homeodomain transcription factor Nkx2\_1, which is expressed in the thyroid, lung, and ventral forebrain. The Nkx2\_1-null mouse has no thyroid, severely hypoplastic lungs and no pituitary. In this study, we first determined the localization of Nkx2\_1 and SCGB3A2 in the mouse pituitary gland by immunohistochemical analysis and RT-PCR to understand the role of SCGB3A2 on pituitary hormone production. Additionally, we investigated the effect of SCGB3A2 in pituitary hormone expression using primary culture of rat pituitary cells. Nkx2\_1 was shown by immunohistochemistry to be localized in the posterior pituitary lobe, whereas SCGB3A2 was shown by immunohistochemistry and RT-PCR to be localized in both anterior and posterior lobes. Double staining for SCGB3A2 and pituitary hormones revealed that SCGB3A2 was localized mainly in gonadotrophs: in 47.9% of FSH-secreting cells and in 42.7% of LH-secreting cells. SCGB3A2 was expressed in anterior and posterior lobes from postnatal day 1 and postnatal day 5 respectively, and SCGB3A2 expression coincided with the region of FSH-secreting cells. Additionally, SCGB3A2 dramatically inhibited the expression of LH and FSH mRNA in rat pituitary primary cell culture. These results suggest that SCGB3A2 regulates FSH/LH production in the anterior pituitary lobe and that transcription factors other than Nkx2\_1 may regulate SCGB3A2 expression.

## 2PK-148

### Olfactory response to the abdominal gland-derived steroids in the newt, *Cynops pyrrhogaster*

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The abdominal gland of the sexually developed male newt, *Cynops pyrrhogaster* was found to excrete androstenedione and pregnenolone. Each steroid exhibited an attracting activity toward the sexually developed females. The preference of the females for both steroids was shown to be completely abolished by plugging the bilateral nostrils, indicating that these steroids act on the olfactory organ. In order to determine the sensitivity of the olfactory receptor cells in the ventral nasal epithelium to androstenedione and pregnenolone, electro-olfactograms (EOGs) were recorded. In the sexually developed females, the vomeronasal epithelium showed a greater response to the steroids than the main olfactory epithelium. The EOG response of sexually developed females and undeveloped females treated with prolactin and gonatotropin was significantly greater than those of the hormone-treated males or untreated females. It was concluded that the main site of action of these steroids resides in the vomeronasal epithelium and that sensitivity to the steroids in this region is sex- and hormone-dependent.

## 2PK-149

### Stimulation of insulin secretion by adenosine in mouse pancreatic islets via A<sub>2A</sub> receptors

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Adenosine receptors play a role in the modulation of various cell activities such as survival or apoptosis. Equilibrative nucleoside transporters (ENTs) bidirectionally transport nucleosides across the membrane and regulate the balance of intra- and extracellular nucleoside concentrations. However, their physiological or pharmacological functions such as hormone secretion in pancreatic  $\beta$ -cells are not fully clarified. We found that mRNAs of adenosine A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, A<sub>3</sub> receptors and ENT subtype 1 were expressed in mouse pancreatic islets and a  $\beta$ -cell line, namely Beta-TC6 cells. Extracellular adenosine markedly augmented insulin secretion in islets in a concentration-dependent manner and its effect was statistically significant at more than 100  $\mu$ M in the presence of high glucose (20 mM). The augmentation was clearly blocked by an A<sub>2A</sub> receptor antagonist SCH58261, but not by an A<sub>2B</sub> receptor antagonist MRS1754, an A<sub>3</sub> receptor antagonist VUF5574 and the ENT inhibitors, dipyridamole and NBTI. On the other hand, the effect of adenosine was synergistically potentiated by an A<sub>1</sub> receptor antagonist DPCPX. A stable adenosine analogue NECA, which has high affinity for A<sub>2A</sub> receptors, also had the stimulatory effect at more than 100  $\mu$ M. These results suggested that A<sub>2A</sub> receptors play a major role in the modulation of insulin secretion by adenosine in mouse pancreatic islet  $\beta$ -cells.

## 2PK-150

### Effect of oral administration of thyroxine on cerebellar function in congenital hypothyroid (rdw) rat

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The development and the function of the central nervous system (CNS) largely depend on thyroid hormones. Perinatal hypothyroidism induces various abnormalities in CNS. The purpose of this study is to determine the influence of thyroid function on several behavioral aspects by using rdw rats. An rdw rat is a hereditary dwarf rat that has a mutation of the thyroglobulin gene and is a useful model of congenital hypothyroidism. To examine the morphological changes in developing rdw rat cerebellum, immunohistochemistry using the antibody for calbindin-D28k, which is specifically expressed in Purkinje cells, was performed. The Purkinje cell dendrite was poorly developed in homozygote rdw rat. To assess cerebellar motor function, we performed rotarod-test on postnatal days 60. We found that the cerebellar motor coordination of rdw rat is significantly low compared to that of euthyroid rats. These results indicate that hypothyroidism can affect cerebellar development and motor function. Furthermore, rdw rats are characterized by hypoactive behavior, as evidenced by the decreased locomotor activities. We are currently examining whether these phenotypes are rescued by the oral administration of thyroxine (T<sub>4</sub>).

## 2PK-151

### Effect of thyroid hormone for cerebellar development –analysis of transgenic mice that specifically express mutant thyroid hormone receptor in Purkinje cells

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Thyroid hormone (TH) plays a critical role for brain development. Deficiency of TH during perinatal period causes abnormal brain development. However, the mechanism of TH for regulating brain development was not fully clarified. We generated a cerebellar Purkinje cell specific TR $\beta$ 1 (G345R) mutant expressing transgenic mice to study TH action during cerebellar development. Dominant negative effect of the mutant TR $\beta$ 1 was shown by reporter gene assay in CV-1 cells. We confirmed the TR $\beta$ 1 (G345R) localization specifically in Purkinje cells by immunohistochemistry (IHC). Body and cerebellar weights were no differences among wild type (Wt), heterozygote (He) and homozygote (Ho). However, Ho spent markedly shorter time on rotarod compared to Wt and He on P15 and P30 both in male and female. Cerebellar IHC results showed that granule cell migration was significantly delayed in Ho compared to those of Wt and He on P15. Primary culture of mouse cerebellum showed that Purkinje cell dendrite arborization was decreased in Ho and He groups. Realtime RT-PCR for brain-derived neurotrophic factor showed lower expression in Ho and He groups than that of Wt group. In summary, Purkinje cell-specific expression of mutant TR $\beta$ 1 (G345R) induces ataxia and delayed cerebellar development that is similar to hypothyroid mice. These data indicate that TH plays a crucial role through TR $\beta$ 1 in Purkinje cells to control normal cerebellar development.

## 2PK-152

### Possible Involvement of Brain-Derived Repressive Molecule(B-ReM)in Normal Cerebellar Development

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Thyroid hormone (TH) plays critical roles for normal brain development and its deficiency during the perinatal period known as cretinism in human. TH regulates brain development only during a limited period, which is called critical period of TH action. However, the molecular basis of TH action in developing brain has not yet been fully clarified. Interestingly, after the critical period, the activities of many genes that are altered by perinatal hypothyroidism return to the same levels as those of euthyroid animal despite morphological alterations. The mechanisms generating such critical period have not been understood. We recently cloned and characterized Brain-derived Repressive Molecule (B-ReM), a retinoid X receptor (RXR)-interacting protein, using a Sos-Ras yeast screening system and RXR-ligand binding domain as bait from human embryonic brain cDNA library. Functional studies using reporter gene assay showed the B-ReM as a transcriptional repressor in CV-1 cells. We investigated the expression of B-ReM protein in rat cerebellum at postnatal days (P) 1, 5, 10, 15, 20, 25, 30 by Western blotting. Immunohistochemistry showed specific expression of B-ReM within the nucleus of the cerebellar cortex cells except the external granule cell layer. The expression pattern of B-ReM was similar to that of steroid receptor coactivator (SRC)-1. These results suggest that the B-ReM may be related to the formation of critical period together with the other molecules.

## 2PK-153

### Mifepristone increases anti-inflammatory M2 macrophages in obese adipose tissues

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Mifepristone, a putative steroid receptor antagonist, clinically serves as an anti-cancer agent, eliciting both cytostatic and cytotoxic effects on malignant cells. However, the metabolic effects of long-term treatment with mifepristone have remained unclear. It is now widely appreciated that adiponectin is an anti-diabetic hormone that improves insulin resistance; M2 macrophages inhibit a chronic inflammation in obesity induced adipose tissues. We sought to explore *in vivo* metabolic actions of mifepristone by using a high-fat (HF)-diet model of C57BL/6NcrSlc mice. When these mice were fed with mifepristone along with HF diet, they exhibited marked improvement in insulin tolerance test, attenuated hepatic ballooning degeneration, and decreases in adipocyte size, compared with pair-fed mice that received HF diet alone without mifepristone. Intriguingly, mifepristone-treated mice exhibited significantly elevated plasma adiponectin levels and up-regulated gene expression of CD163 (marker of anti-inflammatory M2 macrophages). We did not observe differences in total body weight, visceral fat accumulation, and marker gene expression of inflammatory M1 macrophages with or without mifepristone. These results uncover a possibility that long term administration of mifepristone leads to anti-diabetic and anti-inflammatory effects, associated with promoted secretion of adiponectin from adipose tissues.

## 2PK-154

### Imaging analysis of of L-glutamine-induced GLP-1 secretion in enteroendocrine L cell line GLUTag cells

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Enteroendocrine L cells sense various nutrients (i.e. glucose, fatty acids, and amino acids) and secrete glucagon like peptide-1 (GLP-1). GLP-1 facilitates a glucose-dependent insulin secretion from pancreatic  $\beta$ -cells. Although it has been reported that amino acids induce GLP-1 secretion from the enteroendocrine L cells, the underlying mechanisms of amino acids-induced GLP-1 secretion have not been elucidated.

In the present study, we used a green fluorescent protein (GFP)-tagged tissue-type plasminogen activator (tPA-GFP) to label GLP-1-containing vesicles in enteroendocrine L cell line GLUTag cells and observed a GLP-1 release from the cells by using total internal reflection fluorescent microscopy.

We found that application of L-glutamine to GLUTag cells induces an intense and continuous release of tPA-GFP. However, depletion of extracellular  $\text{Na}^+$  ions, which block the L-glutamine uptake to the cells, had no effect on the L-glutamine-induced tPA-GFP secretion. We next observed the effect of ATP-sensitive  $\text{K}^+$  channel opener diazoxide on the L-glutamine-induced GLP-1 secretion. However, GLP-1 secretion was not inhibited by diazoxide completely, suggesting that L-glutamine-induced mitochondrial ATP synthesis would be only partially involved with GLP-1 secretion. Taken together, these results suggest that an alternative signaling pathway would be contributed to L-glutamine-induced GLP-1 secretion from GLUTag cells.

## 2PK-155

### Effects of ovarian sympathetic nerve stimulation on ovarian testosterone secretion in rats

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Our previous study demonstrated that ovarian estradiol secretion was decreased by electrical stimulation of the ovarian sympathetic nerve (the superior ovarian nerve: SON). The present study examined the effects of electrical stimulation of SON on the secretion of testosterone, which is precursor substance of estradiol, from the ovary in rats. Rats were anesthetized on the day of oestrous, and ovarian venous blood samples were collected through the catheter inserted into the ovarian vein. Testosterone concentration of ovarian venous plasma was measured using EIA. The secretion rate of testosterone from the ovary was calculated from the testosterone concentration and the rate of ovarian venous plasma. SON was electrically stimulated at the supra-maximal intensity for C-fibers. Under resting condition, testosterone concentration in ovarian venous plasma were approximately 3.5 times higher than in systemic arterial plasma. Electrical stimulation of the SON produced a decrease in the ovarian testosterone secretion rate, reaching about 74% of the prestimulus values during stimulation. The SON stimulation-induced decreases in testosterone secretion rate were blocked completely by alpha1-adrenoceptor antagonist (prazosin), but not by alpha2-adrenoceptor antagonist (yohimbine). We conclude that sympathetic nerves, which reach the ovary via the SON, have an inhibitory role on ovarian testosterone secretion via activation of alpha1-adrenoceptors.

## 2PK-156

### Developmental expression of estrogen receptor conducted sexual dimorphism in the BNST and SDN-POA

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In the adults of the estrogen receptor (ER)  $\alpha$  gene promoter 0/B transgenic rat, enhanced green fluorescent protein (EGFP) was observed in the sexually dimorphic brain regions including the bed nucleus of the stria terminalis (BNST) and the sexually dimorphic nucleus of the preoptic area (SDN-POA). In the BNST and SDN-POA, estrogen-ER $\alpha$  signaling causes the sexual dimorphism by acting in perinatal periods and 95% of EGFP-labeled cells were ER $\alpha$  immunoreactive in the adults, suggesting the usefulness of the transgenic rats for developmental studies in the sexual differentiation. Here we report the developmental expression of EGFP in the BNST-POA region during fetal and neonatal life. EGFP fluorescence was detected on embryonic day 18 and EGFP expressing cells were concentrated in the BNST but diffused in the POA. On embryonic day 21, EGFP cells in the POA aggregated to form the SDN-POA. The adult patterns of BNST and SDN-POA were established in early postnatal period. Although many ER $\alpha$  immunoreactive cells were distributed in the BNST-POA region, ER $\alpha$ /EGFP double-labeled cells were few in the perinatal brain. Since estrogen-ER $\alpha$  signaling causes the sexual dimorphism, C-terminally truncated ER $\alpha$  variants, undetectable forms in our antibody, may mediate estrogen action, or ER $\alpha$  expressing cells which surround the BNST and SDN-POA may play a key role for the sexual differentiation.

## 2PK-157

### Masculinization of the rat brain by neonatal dihydrotestosterone combined with pubertal androgen treatment

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Sex differentiation of mammalian brain is accomplished by androgenic action in early development. It has also well established that aromatization of testosterone converting to estrogen plays a substantial role in masculinization of the rodent brain, i.e. brain masculinization is due to estrogenic action via estrogen receptor rather than that of androgen. Here, we reevaluated the role of androgen receptor in sexual differentiation of rat brain by neonatal treatment of nonaromatizable androgen, dihydrotestosterone (DHT). DHT (100 mg/0.05 mL sesame oil) or oil was administrated for 3 consecutive days from the day of birth in Long-Evans female rats. Untreated males also served as controls. At 28 days old, all offspring were gonadectomized under pentobarbital anesthesia (45 mg/kg, ip). Half of offspring in each group were simultaneously subjected to subcutaneous implantation of a Silastic capsule containing testosterone (puberty androgenization, PA), and the other half were implanted with the same capsules at 50 days old (adult androgenization, AA). Sexual behavior with receptive females was weekly tested 3 times in those rats started from 60 (PA group) or 65 days old (AA group). Both of PA and AA males showed vigorous mount activity, whereas DHT-treated females with AA expressed a low level of mounts with estrous females. In contrast, DHT-treated females with PA showed mount activity comparable to that of male controls. The results suggest that neonatal androgen receptors latently masculinize rat brain, and pubertal androgen discloses the effect.

## Poster Presentations Membrane Transport

## 2PK-158

### K<sub>Ca</sub>3.1 channel is important for secretion in pancreatic duct cells

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**Introduction :** Potassium channels play a vital role in maintaining the membrane potential and the driving force for anion secretion in epithelia. In pancreatic ducts, however, identity of K<sup>+</sup> channels has not been extensively investigated. **Objectives :** The aim of our study was to functionally identify K<sup>+</sup> channels that contribute to secretion in pancreatic ducts. **Methods :** The molecular basis of K<sup>+</sup> channels is revealed using RT-PCR analysis and immunostaining. We measured equivalent short-circuit current (I<sub>sc</sub>), membrane potential, and single-channel currents using electrophysiological techniques. **Results :** Pancreatic duct cells expressed *KCNN4* coding for an intermediate-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channel (K<sub>Ca</sub>3.1). K<sub>Ca</sub>3.1 protein localized in luminal and basolateral membranes of duct cells. DC-EBIO (5,6-dichloro-1-ethyl-1,3-dihydro-2H-benzimidazole-2-one), an activator of K<sub>Ca</sub>3.1 channel, increased I<sub>sc</sub> in Capan-1 monolayer, consistent with a secretory response. Clotrimazole, a blocker of K<sub>Ca</sub>3.1 channel, inhibited I<sub>sc</sub>. K<sub>Ca</sub>3.1 channel blockers depolarized the membrane potential of cells in microperfused ducts dissected from rodent pancreas. Cell-attached patch clamp single-channel recordings revealed K<sub>Ca</sub>3.1 channels with an average conductance of 80 pS in freshly isolated duct cells. **Conclusion :** These results indicated that the K<sub>Ca</sub>3.1 channels may, at least in part, be involved in setting the resting membrane potential. Furthermore, the K<sub>Ca</sub>3.1 channels are involved in anion transport in stimulated pancreatic ducts.

## 2PK-159

### Adenosine receptors regulate Cl<sup>-</sup> channels in pancreatic duct cells

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**Introduction :** Pancreatic acini secrete ATP and nucleotide-modifying enzymes that include CD39 and CD73. Adenosine, the end product, elicited Ca<sup>2+</sup> signals in pancreatic duct cells that express adenosine receptors. However, effect of adenosine receptors on anion secretion has not been extensively investigated. **Objectives :** The present study aimed to determine whether adenosine receptors regulate anion channels in pancreatic duct cells. **Methods :** The molecular basis of adenosine receptors was revealed by RT-PCR analysis and immunostaining. We measured equivalent short-circuit current (I<sub>sc</sub>) in human adenocarcinoma cell line (Capan-1) monolayer. **Results :** Pancreas expressed adenosine A<sub>2A</sub> receptor (*Adora2a*). Adenosine A<sub>2A</sub> receptors localized in luminal membrane of duct cells. The luminal addition of adenosine (100 μM) significantly increased I<sub>sc</sub> in Capan-1 monolayer, consistent with a secretory response. This increase was inhibited by 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB), niflumic acid, or CFTRinh-172. **Conclusion :** These results indicated that the adenosine A<sub>2A</sub> receptors regulate both Ca<sup>2+</sup>-activated Cl<sup>-</sup> channels and CFTR Cl<sup>-</sup> channels in Capan-1 cells. Furthermore, the adenosine A<sub>2A</sub> receptors may be involved in anion transport in pancreatic ducts.

## 2PK-160

### Computational simulation of intracellular pH induced by NH<sub>4</sub><sup>+</sup> pulse in pancreatic duct cell

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The mechanisms for changes in intracellular pH (pH<sub>c</sub>) induced by NH<sub>4</sub><sup>+</sup>-pulse were analyzed in a mathematical model of pancreatic duct cell constructed by MATLAB/Simulink. Basolateral membrane contained Na<sup>+</sup>-HCO<sub>3</sub><sup>-</sup> cotransporter (NBC), K<sup>+</sup> channel, Na<sup>+</sup>-K<sup>+</sup> pump, and Na<sup>+</sup>-K<sup>+</sup>-Cl<sup>-</sup> cotransporter (NKCC). NKCC carried NH<sub>4</sub><sup>+</sup> in place of K<sup>+</sup>. Apical membrane contained CFTR anion channel and Cl<sup>-</sup>-HCO<sub>3</sub><sup>-</sup> exchanger (SLC26A6). Cell stimulation was mimicked by increasing the permeability of NBC, K<sup>+</sup> channel, CFTR, and SLC26A6. Addition of 20 mM NH<sub>4</sub><sup>+</sup> to the bath caused rapid cellular alkalinization to pH ~7.6 followed by slow recovery. Removal of NH<sub>4</sub><sup>+</sup> caused rapid acidification to pH ~7.1 followed by slow recovery. The alkalinization/acidification upon addition/removal of NH<sub>4</sub><sup>+</sup> was caused by membrane diffusion of NH<sub>3</sub> and consumption/production of H<sup>+</sup> by NH<sub>4</sub><sup>+</sup>-NH<sub>3</sub> buffering system. Most of the consumed/produced H<sup>+</sup> was buffered by intracellular HCO<sub>3</sub><sup>-</sup>-CO<sub>2</sub> and intrinsic buffering systems and only ~0.0003% of the consumed/produced H<sup>+</sup> caused the observed alkalinization/acidification. Recovery of pH<sub>c</sub> from alkalinization/acidification was due to HCO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> transport. When HCO<sub>3</sub><sup>-</sup> transport was activated, the rate of pH<sub>c</sub> recovery from alkalinization and acidification was increased by ~1.4 and ~1.1 times respectively. The data suggest that NH<sub>4</sub><sup>+</sup>-pulse-induced pH<sub>c</sub> changes are weakly affected by the rate of HCO<sub>3</sub><sup>-</sup> transport.

## 2PK-161

### Time-dependent mathematical model of dynamics in ENaC translocation : analysis of Na<sup>+</sup> transport in epithelial cells

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Epithelial Na<sup>+</sup> channels (ENaC) are translocated to the apical cell membrane from the intracellular store sites. Experiments measuring Na<sup>+</sup> transport indicate that at least 4 steps are involved in ENaC translocation : i.e., steps of insertion, endocytosis, recycling and degradation ; 1) the insertion step of ENaC translocated from the intracellular to the apical membrane ; 2) the endocytosis step of ENaC from the apical membrane to the intracellular space ; 3) the recycling step of ENaC insertion to the apical membrane after endocytosis ; 4) the degradation step of ENaC. Electrophysiological measurements provide us with only the information on currents through ENaCs located in the apical membrane. To understand more details about regulation of Na<sup>+</sup> transport in epithelial cells and which state is the rate-limiting one, we tried a mathematical model with electrophysiological data. This could lead us to prediction about the step involved in the intracellular trafficking suggesting that the amount of recycled ENaCs depends on quality control of ENaC in the intracellular store site. Thus, we established this mathematical model including steps of recycling and degradation. By analyzing experimental data with optimized/simplified mathematical model on functional expression of ENaCs, we could obtain more information from the experiment. The mathematical model leads us to novel understandings on ENaC-mediated Na<sup>+</sup> transport.

## 2PK-162

### Comparison of transport properties and expressions of L-type amino acid transporters in human pancreatic cancer-derived cell lines

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System L is a transport system responsible for the Na<sup>+</sup>-independent transport of large neutral amino acids including several essential amino acids. In several malignant tumors, a L-type amino acid transporter 1 (LAT1) is known to be up-regulated to support tumor cell growth. In this study, we compared the properties of [<sup>14</sup>C] L-leucine transport in human pancreatic cancer cells T3M-4, Panc-1, MIA PaCa-2 and BxPC-3. Every cell expressed heterodimeric system-L transporter LAT1 and 4F2hc with different profile. The uptakes of [<sup>14</sup>C] L-leucine by these cells are Na<sup>+</sup>-independent and inhibited by system L selective inhibitor BCH and LAT1-specific inhibitor JPH203 with different affinities. The profiles of the inhibition of [<sup>14</sup>C] L-leucine uptake by amino acids and amino acid-related compounds in these cells are comparable with those for the LAT1 expressed in *Xenopus* oocytes. The majority of [<sup>14</sup>C] L-leucine uptake is, therefore, mediated by LAT1 in these cells. These results suggest that the transport of neutral amino acids into human pancreatic-cancer cells mediated mainly by LAT1 and its specific inhibition in pancreatic cancer cells will be a new rationale for anti-cancer therapy.

## 2PK-163

### Conditional expression of KCC2 in GnRH neurons in vivo causes impairment of fertility

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GABA, the main inhibitory neurotransmitter in the adult brain, exerts an excitatory action in neonatal neurons and in neurons following various forms of neuronal injury. GABA acts excitatory when  $[Cl^-]$  is high, due to the high expression of the  $Na^+ - K^+ - 2Cl^-$  cotransporter (NKCC1), which mediates inward transport of  $Cl^-$ , and the low expression of the  $K^+ - Cl^-$  cotransporter (KCC2), which excludes  $Cl^-$  out of the cell. The decreased expression of NKCC1 and increased expression of KCC2 switches the GABA response from excitatory to inhibitory. To investigate the functional consequences of excitatory action of GABA in neuronal function *in vivo*, we generated transgenic mice with conditional overexpression of KCC2 or knockout of NKCC1 restricted in specific neuronal populations in a reversible fashion using tetracycline controlled gene expression system. We can control the temporal and regional expression of KCC2 or NKCC1. We investigated the role of excitatory action of GABA in gonadotropin-releasing hormone (GnRH) neurons using the mouse that the excitatory action of GABA can be modulated restricted in GnRH neurons. GnRH neurons form the final common pathway for the central regulation of reproduction. Female mice, which expressed KCC2 in GnRH neurons, show low rate of pregnancy and abnormal estrous cyclicity. These results suggest that excitatory action of GABA on GnRH neurons has an important role in female reproduction.

## 2PK-164

### Chloride-proton antiporter CIC-5 is functionally associated with gastric proton pump

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$Cl^-$ -secreting molecules for gastric acid (HCl) secretion have not been well established. In this study, we found that CIC-5, a  $Cl^-/H^+$  antiporter, was expressed in gastric parietal cells of rats, rabbits and humans. CIC-5 was colocalized with gastric proton pump ( $H^+$ ,  $K^+ - ATPase$ ) in the cells located at the luminal region of glands. It has been suggested that the luminal parietal cells more actively secrete acid than do the basal parietal cells, so CIC-5 may be involved in the mechanism of gastric acid secretion. In hog gastric mucosa, CIC-5 was highly expressed in tubulovesicles (TV) but not in apical canalicular membrane. In TV, CIC-5 was coimmunoprecipitated with  $H^+$ ,  $K^+ - ATPase$ . Notably, ATP-dependent  $^{36}Cl^-$  uptake in TV mediated by CIC-5 was abolished by SCH28080, a specific inhibitor of  $H^+$ ,  $K^+ - ATPase$ . To examine association between CIC-5 and  $H^+$ ,  $K^+ - ATPase$ , we constructed the tetracycline-regulated expression system of CIC-5 in HEK293 cells stably expressing  $H^+$ ,  $K^+ - ATPase$ . In CIC-5 expressing cells, CIC-5 was colocalized with  $H^+$ ,  $K^+ - ATPase$  at plasma membrane and coimmunoprecipitated with  $H^+$ ,  $K^+ - ATPase$ . SCH28080-sensitive  $^{36}Cl^-$  transport and  $Cl^-$  currents were clearly observed in CIC-5-expressing cells but not in control cells. In the cells expressing CIC-5 mutant (E211A) which lacks  $H^+$  transport activity, no significant  $^{36}Cl^-$  transport activity was observed. These results suggest that CIC-5 is functionally associated with  $H^+$ ,  $K^+ - ATPase$  in tubulovesicles of gastric parietal cells.

## 2PK-165

### The $Na^+ / H^+$ exchanger NHE1 directly binds to calcineurin A and amplifies the downstream NFAT pathway via 6-residues binding motif PVITID

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Cardiac hypertrophy is an adaptive response to various stresses, often resulting in heart failure. Calcium-dependent protein phosphatase calcineurin (CaN) is a key molecule to govern pathological cardiac hypertrophy. CaN dephosphorylates a downstream transcription factor NFAT, which in turn induces hypertrophy-related gene expression. Recently, we have found that the  $Na^+ / H^+$  exchanger NHE1, a pH-regulating transporter, activates the CaN-NFAT signaling, leading to cardiomyocyte hypertrophy via direct binding of CaN to the 6-residues motif (PVITID) in the cytosolic carboxy-terminal domain of NHE1. We suggested that localized higher pH produced by NHE1 enhanced the activity of CaN bound to NHE1 via sensitizing  $Ca^{2+}$ . Thus, we consider that CaN-binding site would serve as a platform to transmit the pH-signal to CaN-NFAT pathway. Since dephosphorylation of NFAT by CaN strongly depends on the interaction with each other, it is considered that CaN bound to NHE1 is required to be released from NHE1 before dephosphorylating NFAT. Therefore, we hypothesized that the affinity of CaN-binding site in NHE1 is just good enough for delivering the activated CaN from NHE1 to NFAT. We examined whether alteration of the binding affinity between CaN and NHE1 affects the CaN-NFAT signaling, and obtained the preliminary results consistent with our idea. We expect that ongoing detailed analysis may be a catalyst for therapeutic approach towards cardiac hypertrophy and heart failure.

## 2PK-166

### Heterogeneity in the uptake mechanism of glucose into insulinoma cells. A quantification by fluorescent D- and L-glucose derivatives

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D-glucose is a fundamental energy source for most organisms, but there are still many unknowns about mechanisms how individual cells take up D-glucose. 2-NBDG, a fluorescent D-glucose derivative, has been proved to be an effective tracer for monitoring D-glucose uptake into wide variety of cells including tumor cells. However, we questioned to what extent 2-NBDG uptake into tumor cells occurs through GLUTs in a stereoselective manner. Here we show that 2-NBDG, the L-form antipode of 2-NBDG, is taken up into insulinoma cells to a considerable amount. We discuss heterogeneity in the uptake mechanism of glucose into tumor cells based on pharmacological evaluation of the 2-NBDG and 2-NBDG uptake using a microplate reader.

## 2PK-167

### Secretory effects of luminal PGE<sub>2</sub> on human colorectal epithelia

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Prostaglandins (PGs) are known to have a variety of physiological and pathophysiological roles in the gastrointestinal (GI) tract. In the GI epithelia, PGs evoke transepithelial K<sup>+</sup> and Cl<sup>-</sup> secretion, and mucus secretion. Aim of the present study is to clarify the mechanism of the PG-evoked secretion. Methods : Mucosa-submucosal tissue preparations of human colon were made of the normal parts around the extracted colorectal cancer tissues. Short-circuit current (*I*<sub>sc</sub>) and tissue conductance (*G*<sub>t</sub>) were measured as indices of transepithelial electrogenic ion transport and permeability by utilizing the Ussing chamber. Piroxicam (10<sup>-5</sup>M) and tetrodotoxin (10<sup>-6</sup>M) were pretreated to remove the effects of endogenous PGs and neural activities. In addition, the expressions of EP<sub>1</sub>-EP<sub>4</sub> receptors in the human colon were analyzed by immunohistochemistry (IHC). Results : Luminal and serosal administrations of PGE<sub>2</sub> evoked an increase in *I*<sub>sc</sub>. The luminal PGE<sub>2</sub>-induced increase in *I*<sub>sc</sub> was weaker than the serosal PGE<sub>2</sub>-induced response, but the luminal administration of an EP<sub>4</sub> receptor agonist, ONO-AE1-329, increased *I*<sub>sc</sub> more potent than serosal PGE<sub>2</sub>. Luminal pretreatment of an EP<sub>4</sub> receptor antagonist, ONO-AE3-208, inhibited not only luminal, but also serosal PGE<sub>2</sub> and ONO-AE1-329. In IHC, EP<sub>4</sub> receptor-immunoreactivity was detected on the apical site of epithelial cells. Conclusion : The present results suggest that the luminal PGE<sub>2</sub> induces an electrogenic anion secretion via EP<sub>4</sub> receptors expressed on apical membrane in the human colonic epithelia.

## 2PK-168

### Characterization of a novel variant of the Na<sup>+</sup>/HCO<sub>3</sub><sup>-</sup> cotransporter 4 (NBC4) expressed in rat choroid plexus

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Secretion of HCO<sub>3</sub><sup>-</sup> through the apical membrane of the choroid plexus epithelial cells is an essential step to form the cerebrospinal fluid (CSF). Recently, it was found that Na<sup>+</sup>/HCO<sub>3</sub><sup>-</sup> cotransporter 4 (NBC4), an electrogenic member of the NBC family, was expressed in the choroid plexus. Boron WF et al. reviewed Na<sup>+</sup>-coupled HCO<sub>3</sub><sup>-</sup> transport activity of NBC4. They concluded that NBC4c is the only variant confirmed to have electrogenic Na<sup>+</sup>-coupled HCO<sub>3</sub><sup>-</sup> transport activity. Previously, we reported that a novel NBC4 isoform (NBC4g : truncated form of general isoform of NBC4c) expressed highly in rat choroid plexus and represented a DIDS-sensitive HCO<sub>3</sub><sup>-</sup> transport activity. Nevertheless, the tissue distribution and the transport properties of ions of NBC4g have not yet been characterized. In the present study, (1) RT-PCR analysis indicated that NBC4g isoform was clearly expressed, but NBC4c isoform was not detected in the rat choroid plexus. (2) Immunostaining and electron microscopy studies revealed the apical localization of NBC4g in the same epithelial cell. (3) Electrophysiological studies showed that NBC4g has an electrogenic and cAMP-dependent HCO<sub>3</sub><sup>-</sup> transport activity. We conclude that NBC4g, not NBC4c, is the essential isoform of NBC4 in the rat choroid plexus to maintain the appropriate concentration of HCO<sub>3</sub><sup>-</sup> in CSF.

## Poster Presentations Respiration

## 2PK-169

### New concept of flow-mediated CO<sub>2</sub> gas excretion from human pulmonary arteriolar endothelial cells

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We studied the physiological role of flow through pulmonary arterioles in CO<sub>2</sub> gas exchange. We previously established human pulmonary arteriolar endothelial cells (HPAoEC). The cells demonstrated marked immunocytochemical staining of PECAM-1, VEGF R2, ACE-1, and CA type IV on their cell surface. Ten seconds shear stress stimulation caused the co-release of H<sup>+</sup> and ATP via the activation of F<sub>1</sub>/F<sub>0</sub> ATP synthase on the HPAoEC. F<sub>1</sub>/F<sub>0</sub> ATP synthase was immunocytochemically observed on the cell surface of non-permeabilized HPAoEC. In the shear stress-loaded HPAoEC culture media supernatant, ATPase activity increased in a time-dependent manner. Ten seconds shear stress stimulation also produced stress strength-dependent CO<sub>2</sub> gas excretion from the HPAoEC, which was significantly reduced by the inhibition of F<sub>1</sub>/F<sub>0</sub> ATP synthase or CA IV on the endothelial cell surface. In conclusion, we have proposed a new concept of CO<sub>2</sub> exchange in the human lung, flow-mediated F<sub>1</sub>/F<sub>0</sub> ATP synthase-dependent H<sup>+</sup> secretion, resulting in the facilitation of a dehydration reaction involving HCO<sub>3</sub><sup>-</sup> in plasma and the excretion of CO<sub>2</sub> gas from arteriolar endothelial cells.

## 2PK-170

### Plastic changes of the neural expression of c-Fos protein in the medullary respiratory neurons following respiratory muscle training with CO<sub>2</sub> breathing in the Wistar rats

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Respiratory muscle training (RMT) can improve respiratory muscle weakness in chronic respiratory diseases. However, it is unclear whether RMT induces adaptations in the respiratory center as well as respiratory muscles. We hypothesized the respiratory center in the medulla would also adapt to the RMT and less neurons became to recruit to achieve same minute ventilation under hypercapnic stimulation. To clarify this hypothesis, the number of c-Fos positive medullary neurons after hypercapnic stimulation (7% CO<sub>2</sub>, 50% O<sub>2</sub>, 90 min) in the rats with RMT was compared to that in the untrained control rats. For the respiratory muscle training, we used the hypercapnic stimulation that consisted of placing each rat in a hypercapnic chamber in order to increase the minute ventilation. The RMT rats (n=2) were placed in a chamber filled with 7% CO<sub>2</sub> and 50% O<sub>2</sub> for 30 min, 5 times a week, for 4 weeks, whereas control rats (n=2) were placed in the same chamber filled with room air. After 1.5 hours from the completion of the last trial of the 4 weeks program, axial sections from the medulla oblongata were processed for c-Fos immunohistochemistry. It was found that the number of the c-Fos positive neurons in the RMT rats were smaller than those in the control rats at ventral and dorsal region of the medulla oblongata. These results suggest that the RMT would induce the plastic changes in the respiratory center to minimize the recruited number of the respiratory neurons and this might enlarge the dynamic range of ventilation.

## 2PK-171

### Long-lasting shortening of inspiratory burst duration by treatment with eugenol

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It is not well understood whether chemicals that are known as transient receptor potential (TRP) channel agonists or antagonists exert any effects on the medullary respiratory center. Previously, we have reported that capsaicin (a heat-sensitive TRPV1 channel agonist) or menthol (a cold-sensitive TRPM8 channel agonist) caused excitatory or inhibitory effects on respiratory rhythm generation in the brainstem-spinal cord preparation from newborn rat. In the present study, we examined effects of eugenol or carvacrol (that are TRP channel modulators) on respiratory rhythm generation in the brainstem-spinal cord preparation from newborn rat (P0-P3). Eugenol is contained in several plants including clove and is used as an analgesic drug. The preparations were superfused by modified Krebs solution at 25-26°C, and inspiratory C4 ventral root (or phrenic nerve) activity was monitored. In the newborn rat in vitro preparation, eugenol (0.5-1 mM) induced inhibition respiratory rhythm accompanied with strong inhibition of burst activity of pre-inspiratory neurons. After washed out, respiratory rhythm gradually recovered but the duration of inspiratory burst was extremely shortened as characterized by inspiratory phase composed of a single spike activity in inspiratory neurons. This continued for more than 1 hr after washed out. Carvacrol exerted effects similar to that of eugenol. These results suggest that eugenol or carvacrol inhibited cellular (and/or network) mechanisms that are essential for maintenance of burst duration of respiratory neurons.

## 2PK-172

### Post-apneic cardiorespiratory responses in patients with obstructive sleep apnea

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We previously reported that the immediate onset of ventilatory airway responses to hypoxia is influenced by 5-HT<sub>2</sub> receptor activity in the dorsomedial medulla oblongata of unanesthetized mice, and that post-hypoxic ventilation is suppressed via 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> receptors in the central amygdaloid nucleus. In addition, suppression of post-hypoxic ventilation has been reported in patients with obstructive sleep apnea. The present study sought to clarify post-apneic cardiorespiratory responses in patients with obstructive sleep apnea. We examined two patients exhibiting obstructive sleep apnea of a similar level of severity (apnea/hypopnea index ~30), measuring airflow, electrocardiography, sleep-wakefulness and percutaneous oxygen saturation obtained from diagnostic polysomnography. Specifically, airflow and electrocardiography in the initial stage of sleep were analyzed in detail using wave-analysis software. The results revealed that there were two types of onset responses in post-apnea, heart-rate change and respiratory-rate change, depending on sleep-wakefulness. The level of percutaneous oxygen saturation was reduced to a greater extent when respiratory rate increased with wakefulness, and did not change when heart rate increased. Our findings are interpreted in terms of classification of post-apneic responses, and response-related factors in patients with obstructive sleep apnea are discussed.

## 2PK-173

### Hyperpnea independent of limb movements at exercise onset in mice

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The aim of the present study was to test the hypothesis that factors independent of limb movements play an important role in hyperpnea during the early phase of treadmill exercise in mice. We measured ventilation and pulmonary gas exchange during constant treadmill exercise, the level of which was below the lactate threshold. We then compared the ventilatory response to a startle stimulus with the response to treadmill exercise. The startle response was elicited by subjecting stationary mice to a sudden movement of the treadmill belt. We demonstrated the following novel findings in this study. (1) Video analysis showed that the mice increased their respiration nearly simultaneously with the start of treadmill belt movement, before they started locomotion. (2) Ventilation increased in response to a sudden movement of the treadmill belt in ambulatory restricted mice. (3) Incremental changes in treadmill speed did not induce a further increase in ventilation during the early phase of treadmill exercise. (4) Hyperpnea during the early phase of treadmill exercise was plastic. These findings suggest the importance of factors independent of limb movements in the control of hyperpnea during the early phase of treadmill exercise in mice.



## 2PK-174

### Long-lasting facilitation of respiratory rhythm by treatment with TRPA1 agonist, cinnamaldehyde

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It is not well understood whether chemicals that are known as transient receptor potential (TRP) channel agonists or antagonists exert any effects on the medullary respiratory center. In the present study, we examined effects of TRPA1 agonist (cinnamaldehyde or allyl isothiocyanate) on respiratory rhythm generation in the brainstem-spinal cord preparation from newborn rat (P0-P3) and in the in situ perfused-preparation from juvenile rat (P11-13). The preparations were superfused by modified Krebs solution at 25-26°C, and inspiratory C4 ventral root (or phrenic nerve) activity was monitored. In the newborn rat in vitro preparation, cinnamaldehyde (0.5 mM) induced typically biphasic responses in C4 rate; initial short increase (0.5-2 min) and subsequent decrease followed by gradual recovery of the rhythm during 15 min bath application. After washed out, the rate of respiratory rhythm further increased and has been kept higher than that of control (200% of control) for more than 2 hrs (a kind of long-term facilitation). Allyl isothiocyanate induced effects similar to cinnamaldehyde. The long-lasting facilitation of respiratory rhythm was partially antagonized by TRPA1 antagonist, HC-030031 (10 µM). We have obtained similar results in the in situ perfused-preparation from P11-13 rats. Our findings suggest that activation of TRPA1 channels could induce a long-lasting facilitation of the respiratory rhythm.

## 2PK-175

### Effects of vagotomy on the hypercapnic ventilatory response in the decerebrate neonatal rat

Furuie, Hiroki; Iizuka, Makito (Center for Med. Sci., Ibaraki Pref. Univ. of Health Sci., Ibaraki, Japan)

We studied the effects of vagotomy on the ventilatory response to hyperoxic hypercapnia in the decerebrate neonatal rat. The rats at postnatal days 0-3 were assigned into three groups: bilaterally vagotomized, sham-operated, and intact group. After the recovery from the surgery, the rats were successively exposed to 0, 3, 6, 9% CO<sub>2</sub> (mixed with 50% O<sub>2</sub>, balance N<sub>2</sub>) at least for 10 min each, and changes in the respiratory frequency and tidal volume were examined using direct plethysmograph. Body temperature was maintained ranging from 33 to 38°C. The rats in all three groups showed similar ventilatory pattern under the control condition. Progressive hypercapnia increased both respiratory frequency and tidal volume in the intact and sham-operated rats. In the vagotomized rat, however, the hypercapnic stimulation, especially 9% CO<sub>2</sub>, decreased the respiratory frequency and increased the tidal volume extraordinarily. In fact, the vagotomized rat showed inspiration with gasp-like mouth opening under these conditions. As a result, minute ventilation of the vagotomized rat under 9% CO<sub>2</sub> did not differ from that under the control condition. These results suggest that, in the neonatal rat, feedback from the pulmonary stretch receptor via vagus nerve is essential for control ventilation with proper respiratory frequency-tidal volume relation to hypercapnia.

## 2PK-176

### Breathing is affected via dopamine receptors in the basolateral amygdala

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[Introduction] It has been reported that breathing is closely correlated with emotion. Dopamine receptor antagonists have been used effectively in treating affective disorders. It has long been reported that the amygdala plays an important role in controlling emotion via neurochemical mechanisms. For example, a previous study revealed that when the basolateral amygdala (BLA) of rats was directly perfused with a dopamine receptor antagonist, the startle reflex was suppressed. In the current study, we hypothesized that breathing is regulated by dopamine in the BLA, and examined the effect of dopamine antagonist administration in the BLA on breathing. [Methods] Adult male mice (C57BL/6N) were exposed to sevoflurane, and a microdialysis probe was inserted into the BLA through a pre-inserted guide cannula. We directly perfused artificial cerebrospinal fluid (ACSF), a D1-like (D1) antagonist and a D2-like (D2) antagonist in the BLA, while concurrently monitoring respiration and body temperature. [Results] The respiratory rate was significantly decreased when a D2 antagonist was perfused into the BLA, compared with ACSF. [Discussion] The current results suggest that dopamine regulates breathing through D2 receptors in the BLA. These findings indicate that respiratory rate may be an important indicator when using dopamine-related medications in the treatment of affective disorders.

## 2PK-177

### Involvement of the medullary raphe nuclei in the control of respiration

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Previous studies suggest that the midline medullary raphe nuclei may be involved in the regulation of breathing. Stimulation of caudal raphe nuclei causes a change in respiration. Anatomical studies demonstrate that raphe neurons have projections to the phrenic motor nucleus, the ventral respiratory group, and the dorsal respiratory group. Recent years, it has been demonstrated that the medullary raphe nuclei form one of several brainstem regions of central chemoreception. However, the details of the medullary raphe nuclei in the control of respiration has not been clarified. We found that respiratory neurons existed in spontaneously breathing or ventilated rat medullary raphe nuclei, and some of these neurons were antidromically activated from the C4-C5 spinalcord. In addition, most of the respiratory neurons of the medullary raphe nuclei responded to hypercapnia, these neurons were antidromically activated by electrical stimulation of the retrotrapezoid nucleus (RTN). Local injection of kainic acid into the medullary midline induced increases in amplitude and frequency of diaphragm activities, followed by a decrease in respiratory frequency. Apnea was elicited 14-20 minutes after injection of kainic acid in the midline medulla. These studies suggested that medullary raphe nuclei can play a role in respiration, we will discuss possible involvement of medullary raphe nuclei in respiration.

## 2PK-178

### The correlation of variations between coughing capability and respiratory function in supine and sitting position

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**Background :** Effective coughing with the intent of secretion removal is important for respiratory tract clearance. Cough peak flow (CPF) is a good indicator for assessing coughing strength and is often used clinically. CPF indicates the correlation between respiratory muscle strength and vital capacity, and is reported to be influenced by posture.

**Purpose :** The aim of this study was to evaluate variations in CPF and respiratory muscle strength (maximum expiratory pressure ; PEmax, maximum inspiratory pressure ; PImax) as well as vital capacity (VC) with postural change, and to investigate their relationship.

**Participants and Methods :** Eighteen healthy subjects participated in this study. We randomly measured CPF, PEmax, PImax, and VC with the subjects in a supine position and a sitting position. Each measurement was obtained twice, and the highest value was used for further calculations. To evaluate the effect of postural change, the differences in CPF, VC, PEmax, and PImax were calculated in the supine and sitting positions, and defined as  $\delta$ CPF,  $\delta$ VC,  $\delta$ PEmax, and  $\delta$ PImax respectively.

**Results :** We observed a significant correlation between  $\delta$ CPF and  $\delta$ VC as well as  $\delta$ PImax ( $r=0.48$ ,  $r=0.55$ ). However, the correlation with  $\delta$ PEmax was not significant.

**Conclusions :** When posture is changed from a sitting position to a supine position, the decline in inhalation function but not exhalation function causes a decline in CPF.

## 2PK-179

### Dopamine D1 receptors contribute to hyperpnea during constant-load exercise via metabolic control in mice

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We previously revealed that dopamine D2 receptors participate in exercise hyperpnoea via behavioral control of ventilation with unchanged pulmonary gas exchange in mice. In the present study, we examined the hypothesis that D1 receptors are also involved in the exercise hyperpnoea using a D1 receptor antagonist SCH 23390 (SCH) with simultaneous recording of ventilation and pulmonary gas exchange. The respiratory responses of mice injected with saline or SCH (50  $\mu$ g/kg body weight) were compared during treadmill exercise at a speed of 6 m/min. Each mouse was set in an airtight treadmill chamber equipped with a differential pressure transducer and open-circuit system with a mass spectrometer. At rest, SCH mice had significantly reduced respiratory frequency, minute ventilation and pulmonary gas exchange compared with saline mice. Treadmill exercise produced an abrupt increase and a sequential decline to the steady-state level in both groups of mice. SCH lowered the increased levels of respiratory frequency, tidal volume and minute ventilation during the steady state, as well as reducing the O<sub>2</sub> uptake, CO<sub>2</sub> output and body temperature throughout treadmill exercise. Hypercapnic ventilatory response between groups was similar. Thus, the D1 receptors contribute to a resting ventilation level and exercise hyperpnoea during the steady state in parallel with metabolic changes. The metabolic control of D1 receptors is essential for steady state ventilation.

## 2PK-180

### Effects of DMSO on nociception and respiratory control

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Dimethyl sulfoxide (DMSO), an organosulfur compound, dissolves both polar and nonpolar substances, and is widely used in pharmacological experiments as a solvent. DMSO is also used in humans as a cryoprotectant in autologous stem cell transplantation for chemotherapy of malignancies. However, severe neurological and respiratory adverse effects accompanied by DMSO infusion have increasingly been reported. Thus we hypothesized that DMSO may affect the physiological functions of nociception and respiratory control. The dose-response relationship of DMSO (cumulative doses 0.5, 1.5, 3.5, 7.5 and 15.5 mg/g, intraperitoneally injected at 50 min intervals) in nociception and respiratory control was analyzed in adult mice. For nociception, latency to licking of the hind paw or jumping from the thermal stimulus (55°C) was measured in hot-plate test before and after each DMSO injection. Respiratory function was assessed from frequency and tidal volume as well as minute ventilation during room air and hypoxic (7% O<sub>2</sub>) conditions by whole body plethysmography. Low DMSO doses (0.5 and 1.5 mg/g) affected neither nociception nor respiration. Higher doses, however, prolonged the response latency, and the highest DMSO dose (15.5 mg/g) immobilized animals. On the other hand, resting and hypoxic ventilation was relatively little affected. We suggest that DMSO itself suppresses nociception. Caution should be exercised when DMSO is used as a solvent in pharmacological experiments of pain.

## Poster Presentations Drug Actions

## 2PK-181

### Suppressive Effects of Nonsteroidal Anti-inflammatory Drugs Diclofenac Sodium, Salicylate and Indomethacin on Delayed Rectifier $K^+$ -Channel Currents in Murine Thymocytes

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Lymphocytes predominantly express delayed rectifier  $K^+$ -channels (Kv1.3) in their plasma membranes, and the channels play crucial roles in the lymphocyte activation and the proliferation. Since nonsteroidal anti-inflammatory drugs (NSAIDs), the most commonly used analgesic and antipyretic drugs, exert immunomodulatory effects, they would affect the channel currents in lymphocytes. In the present study, employing the standard patch-clamp whole-cell recording technique, we examined the effects of diclofenac sodium, salicylate and indomethacin on the channel currents in murine thymocytes and the membrane capacitance. Diclofenac sodium and salicylate significantly suppressed the pulse-end currents of the channel. However, indomethacin suppressed both the peak and the pulse-end currents with a significant increase in the membrane capacitance. This study demonstrated for the first time that NSAIDs, such as diclofenac sodium, salicylate and indomethacin, exert inhibitory effects on thymocyte Kv1.3-channel currents. The slow inactivation pattern induced by indomethacin was thought to be associated with microscopic changes in the plasma membrane surface detected by the increase in the membrane capacitance.

## 2PK-182

### Delayed administration of the nucleic acid analog 2Cl-C.OXT-A attenuates brain damage and enhances functional recovery after ischemic stroke

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2Cl-C.OXT-A (COA-Cl) is a novel nucleic acid analog that enhances angiogenesis through extracellular signal-regulated kinase 1 or 2 (ERK 1/2) activation. We performed in vitro and in vivo experiments to investigate whether COA-Cl can attenuate neuronal damage and enhance recovery after brain ischemia. In primary cortical neuron cultures, COA-Cl prevented neuronal injury after 2 h of oxygen-glucose deprivation through ERK 1/2 and purinergic receptor mediated mechanism. The effect of COA-Cl was evaluated in vivo with 60 min of middle cerebral artery occlusion combined with bilateral common carotid artery occlusion. We observed significantly reduced infarct volume and improved neurological deficits upon injection of COA-Cl. We also evaluated the effect of delayed COA-Cl administration on recovery from brain ischemia by continuous administration of COA-Cl from 1 to 8 days after reperfusion. Delayed continuous COA-Cl administration also reduced infarct volume. Furthermore, COA-Cl enhanced perinfarct angiogenesis and synaptogenesis, resulting in improved motor function recovery.

## 2PK-183

### Triptolide suppresses the production of IL-1 $\beta$ in adjuvant-arthritis rats

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Various extracts of the Chinese herbal medicine *Tripterygium wilfordii* Hook. f. (TWHF) have been reported to be therapeutically effective for rheumatoid arthritis (RA) in China, but their action mechanism has not been understood well. Although triptolide, a diterpenoid triepoxide from TWHF, suppresses the inflammatory cytokine production in vitro, the influence of triptolide on immune system is not fully understood well. The present study, therefore, was designed to examine the effects of triptolide on cytokines production in vitro and in vivo by using adjuvant arthritis rats. The first part of these experiments was designed to examine the effects of triptolide on arthritis rats. Adjuvant arthritis was induced in male Wistar rats by a single subcutaneous injection of 0.1 mL complete Freund's adjuvant into the paw. Arthritic rats were injected intraperitoneally with various doses of triptolide once a day for 1 week, starting one week after adjuvant injection. The minimum concentration of triptolide, which caused significant suppression of paw swelling, was 0.1 mg/kg. The second part of these experiments was carried out to measure the ability of cytokine production by lymphocytes from the arthritis rats which were treated with triptolide. Triptolide caused suppression of IL-1 $\beta$  production in lymphocytes which was activated by Concanavalin A. These data suggest that the therapeutic effects of TWHF in RA are in part due to the novel chondroprotective effects of triptolide via suppression of IL-1 $\beta$  production.

## 2PK-184

### Suppressive effect of Juzentaihoto on vascularization induced by B16 melanoma cells in vitro and in vivo

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Juzentaihoto (JTT) is well known to be one of Japanese herbal medicines, and used for the supplemental therapy of cancer patients with remarkable success. However, the precise mechanisms by which JTT could favorably modify the clinical conditions of cancer patients, including tumor metastasis are not well defined. The present study, therefore, was undertaken to examine the possible therapeutic mechanisms of JTT on cancer using B16 melanoma cell (B16 cell)/experimental mouse system. JTT was well mixed with rodent chow at 3.0% concentrations, and was administered orally ad libitum. Oral administration of JTT was started one week before tumor cell injection and continued throughout the experiment. Oral administration of JTT into mice significantly inhibited tumor metastasis in lungs after intravenous injection of B16 cells. JTT also significantly suppressed enlargement of tumor size in hind footpad after the subcutaneous injection of B16 cells. In the second part of experiments, the chamber that containing B16 cells was buried in the murine back. This chamber was covered with a collagen membrane, which was able to pass only smaller material than cell component. In JTT administrated group, vascular endothelial growth factor (VEGF) of chamber internal fluid significantly decreased, and vascularization of chamber circumference was also inhibited. These results strongly suggest that oral administration of JTT caused decrease in the generation of VEGF, which is responsible for vascularization, and results in inhibition of B16 cell metastasis.

## 2PK-185

### Palmitate suppresses the osteoclast differentiation in ovariectomized mice

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Osteoclasts are the only cell type capable of resorbing mineralized bone, and they act under the control of numerous cytokines produced by supporting cells such as osteoblasts and stromal cells. Among cytokines, receptor activator of nuclear factor- $\kappa$ B ligand (RANKL) or osteoprotegerin (OPG) was found to be a key osteoclast relation molecule that regulates its cognate receptor, RANK, on osteoclast precursor cells. The present study was designed to examine an inhibitory effect of the palmitate, an isoquinoline alkaloid originally isolated from *Coptis chinensis*, on an osteoclast differentiation in vivo by using ovariectomized (OVX) mice. The first part of experiments were designed to examine by histological approach of shank-proximal obtained from OVX mice treated with palmitate. They were divided into four groups of 5 mice each: the sham-operated (Sham), OVX, OVX-palmitate intake groups (1mg/kg, 10mg/kg), randomly. OVX mice were served by sonde forcibly with various doses of palmitate once a day for 12 week, starting two-weeks after OVX-operation. Palmitate caused significant suppression of the osteoclast number on tissue of 10mg/kg intake group. The second part of experiments was examined the cytokine level of serum from OVX mice treated with palmitate. In the mice treated by palmitate, RANKL increased, and OPG decreased. These results suggest that palmitate attenuates osteoclast differentiation through inhibition of RANKL expression, and activation of OPG expression. Therefore, palmitate might be a candidate for an anti-resorptive agent of disorders such as osteoporosis.

## 2PK-186

### Effect of Hachimijogan on Parkinson's disease model mouse

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Parkinson's disease (hereinafter, PD) is a degenerative disease indicated by extrapyramidal signs, and characterized by degeneration of the substantia nigra dopamine nerve cells. In Kampo medicine, symptoms causing aging unsuitable for a particular age and a decline in body functions are believed to be symptoms of 'Jinkyo' (kidney deficiency). The purpose of this research was to investigate the efficacy of hachimijogan and rokumigan, the representative medicine prescribed for Jinkyo, towards PD. hachimijogan and rokumigan were administered to rotenone-induced PD model mice, and the efficacy thereof was evaluated from the motor activity and drop in substantia nigra dopamine nerve cells. Furthermore, in order to investigate the action mechanism thereof, the 8-OH-dG in urine, which is the oxidative stress marker, was measured. A declined motor activity, reduced substantia nigra dopamine nerve cells, and increased 8-OH-dG concentration in urine were observed in PD model mice; however, these were significantly suppressed due to the administration of hachimijogan. On the other hand, the significant effect did not observed by the administration of rokumigan. From the above results, it was suggested that hachimijogan restrains the increase in oxidation stress as one of its action mechanisms, and includes a suppressing effect against the onset and advancement of PD.

## 2PK-187

### Lapachol suppresses fibril formation of fibroblasts derived from hypertrophic scars

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The pathogenesis and therapy of hypertrophic scar (HS) have not yet been established. Our aim was to investigate the inhibitory effects of lapachol, isolated from the stem bark of *Avicennia rumphiana* Hall. f., on HS-fibroblasts. The effects of lapachol on HS-fibroblast proliferation were measured using the MTT assay, cell-cycle analyses and lactate dehydrogenase (LDH) assays. The type I collagen  $\alpha$ -chain (COL1A1), IL-6 and PAI-1 mRNA and/or protein levels of HS-fibroblasts were quantitated by real-time PCR and ELISA. Lapachol at 25 and 50  $\mu$ M significantly inhibited the *in vitro* proliferation of HS-fibroblasts, but not fibroblasts from non-lesional skin sites. In addition, lapachol had no apparent effect on cell cycle and LDH activity in conditioned medium from lapachol-treated HS-fibroblasts was nearly equal to that in medium from vehicle-treated cells. Lapachol treatment also inhibited COL1A1 and PAI-1 mRNA levels in hypertrophic scar fibroblasts, but did not affect IL-6 mRNA levels. The protein levels of IL-6 and PAI-1 in conditioned medium from HS-fibroblasts treated with 50  $\mu$ M lapachol were lower than those from vehicle-treated HS-fibroblasts. Lapachol decreased the proliferation rate of HS-fibroblasts. As IL-6 and PAI-1 secretion was also lowered in lapachol-treated HS-fibroblasts, our findings suggested that lapachol may have suppressed extracellular matrix hyperplasia in wound healing and possibly alleviated the formation of hypertrophic scar.

## 2PK-188

### Effects of melanogenesis in each fraction of Hyugatouki (Angelica Furujiuga) extract.—Development of gray hair improvement medicine—

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Purpose *Angelica furcijuga* (AF) is an endemic species and perennial herb of Japanese parsley department that grows wild in the South Kyushu island in Japan. It is faced with extinction but its usefulness is attracted attention by success of organic grow. We have ever reported that the AF extracts from lobe and stem stimulated melanogenesis in mouse B16 Melanoma Cell (B16 cell) and mouse hair. In the present study, on the each fraction of the AF's extracts from lobe and stem, the effects of tyrosinase activity were examined using the B16 cell. Methods The B16 cell were cultured with the each fraction of AF's extracts, and drug susceptibility test was conducted. The tyrosinase activity was measured in the B16 cell, the effect to the ability of melanin production was checked. Tyrosinase mRNA in the B16 cell was measured by the real-time RT-PCR assay. Results and Discussion We observed difference that each fraction had effect on tyrosinase activity. In addition, we found the different effect of the each fraction on the expression of tyrosinase mRNA. This study suggests the certain fraction of AF's extracts contains the melanin production promoting substances.

## 2PK-189

### Effects of flumazenil on the anti-stress actions of coffee volatiles in mice

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This study was performed to clarify the mechanisms underlying the anti-stress actions of coffee volatiles. We first investigated the effects of flumazenil, a benzodiazepine receptor antagonist, on stress-induced hyperthermia and pentobarbital-induced sleep time in combination with volatile exposure in mice. Fresh medium-dark roasted and moderately powdered Guatemalan coffee beans were used. We chose these beans because they had the greatest effects on relaxation in human studies. The coffee powder (400 or 1200 g) was placed into several plastic cases in a test room with the temperature maintained at  $25 \pm 1^\circ\text{C}$ . Male ICR mice weighing 35 to 45 g were exposed to coffee volatiles in the test room. Four hours after the beginning of the exposure period, stress-induced hyperthermia and pentobarbital-induced sleep tests were performed. Flumazenil (0.3-3.0 mg/kg, i.p.) was injected 30 min prior to the start of both tests. In the stress-induced hyperthermia test, exposure to coffee volatiles dose-dependently reduced the stress-induced elevation in body temperature, suggesting an anti-stress effect of the volatiles; pretreatment with flumazenil inhibited this anti-stress effect. Further, coffee volatiles prolonged the pentobarbital-induced sleep time, suggesting that the volatiles have a hypnotic effect. Flumazenil also blocked the prolongation of pentobarbital-induced sleep time. Based on these results, we suggest that benzodiazepine receptors are involved in the anti-stress actions of volatile compounds in roasted coffee beans.

## 2PK-190

### Chemotherapeutic and drug delivery system with a novel nano-magnetic particle, EI236

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**Purpose:** We have developed a novel magnetic anticancer drug which is designated as EI236. Its ferromagnetic property contributes to new features. 1) Anti-cancer effect 2) drug delivery system (DDS), which is delivered by a magnet to the tumor, resulting in reduced side effects 3) MRI-contrast effect, which is visualized by MRI, evaluating the drug localization and concentration. Thus, the purpose of this study is to examine these features. **Method:** The magnetic evaluation of EI236 was confirmed by Electro Spin Resonance (ESR). Anti-tumor effect of EI236 was evaluated by MTT and TUNEL assays in mouse melanoma cells. To examine the anti-cancer effect and magnetic controlled delivery in vivo, we used a mouse model of melanoma grafted on tail. Mice were divided into 3 groups; 1) control group 2) intravenous EI236 injection group 3) intravenous EI236 injection+electromagnet (DDS) group. Furthermore the tumor regression and MRI-contrast effect of EI236 were measured by Magnetic Resonance Imaging (MRI). **Result:** EI236 decreased the cell proliferation and increased apoptosis in MTT and TUNEL assays. EI236 showed the magnetism at room temperature by ESR. We confirmed that application of a magnet enhanced the anti-cancer effect of EI236 in vitro and in vivo. These findings demonstrated that EI236 exhibited anti-cancer effect and DDS. **Conclusion:** Our findings suggest that EI236 can serve as a novel magnetic anti-cancer drug and will assist us in developing a novel and simultaneous treatment strategy of chemotherapeutic and DDS.

## 2PK-191

### New strategy of simultaneous hyperthermo-chemotherapy using a novel nano-magnetic anti-cancer drug

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**Aim:** Hyperthermic therapy, which kills cancer cells by increasing the temperature, is attempted in the advanced or difficult cases. Despite of its effectiveness, it has not been widely used. One reason is that it is technically difficult to increase temperature only in a target cancer site. Therefore, we have developed a new therapy using a novel nano-magnetic particle, i.e., EI236. EI236 exhibits not only anti-cancer effect, but also magnetism. Because of its magnetism, this drug can generate heat by itself when it is exposed to an alternating current magnetic field (ACMF). **Method:** To examine the anti-cancer and hyperthermal effects of EI236, we used a rabbit model of rabbit osteosarcoma cells grafted on legs. Rabbits were divided into five groups; 1) no treatment 2) intra-venous (iv.) EI236 injection 3) intra-arterial (ia.) EI236 injection 4) ia. Methotrexate (MTX) injection 5) ia. EI236+ACMF. The volume of tumor was measured daily, and then the samples were evaluated histologically by HE, Ki67, and TUNEL staining. **Results:** When EI236 was injected and the tumor was exposed to ACMF, it showed the greatest regression of tumor among all the groups examined. These results indicated that the exposure of ACMF greatly enhanced the anti-cancer effect of EI236. EI236 exhibited anti-cancer and hyperthermal effects. **Conclusion:** These findings suggest that EI236 can assist us in developing a new strategy of simultaneous hyperthermo-chemotherapy in the future.

## Poster Presentations

### Physical Fitness, Sports Medicine

#### 2PK-192

##### Effects of aging on the regulatory mechanisms of O-linked-N-acetylglucosamine modification in murine naive peritoneal macrophages

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Proinflammatory cytokine productions by macrophages in response to lipopolysaccharide (LPS) stimulation are well accepted to be gradually suppressed along with aging. The signal transduction under Toll-like receptor 4 (TLR 4) is inhibited by increased O-linked-N-acetylglucosamine (O-GlcNAc) modification, because the posttranslational modification occurs at serine/threonine residues and this competes with phosphorylation. In this study, to clarify the roles of O-GlcNAc modification in the aging-dependent deterioration of proinflammatory cytokine productions, the mRNA expression levels of O-GlcNAc cycling enzymes were compared between naive peritoneal macrophages isolated from 8-week-old (young) and 12-month-old (middle-aged) male BALB/c mice. The amounts of tumor-necrosis factor- $\alpha$  and interleukin-6 productions by the cells stimulated with 100 ng/ml LPS for 6 hours were clearly lower in the middle-aged mice than the young mice. The levels of total inhibitor of  $\kappa$ B $\alpha$  (I $\kappa$ B $\alpha$ ) protein were not different between both mice, although aging-dependent suppression of LPS-stimulated I $\kappa$ B $\alpha$  phosphorylation was observed. The levels of O-GlcNAc modified proteins were relatively higher in the middle-aged mice than the young mice. The mRNA levels of O-GlcNAcase in the middle-aged mice were 0.7-fold lower than the young mice, although those of O-GlcNAc transferase were similar between both mice. These results suggest that aging-dependent inhibition of TLR signaling is partly mediated by increased O-GlcNAc modification.

#### 2PK-193

##### Influence of oral rehydration solution for blood fluidity in exercise model rats

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Perspiration occurs when sudoriferous water is supplied by the blood, caused by the heat radiation mechanism in high temperature. Blood flow is controlled by its blood fluidity, volume and the cardiovascular system. It was reported that severe-intensity exercise decreased blood fluidity, but the water supply in exercise improved blood fluidity via suppression of platelet aggregation. In this experiment, we investigated the relation between blood fluidity and supply of oral rehydration solution (ORS) in rats loaded with forced exercise in high temperature environment. SPF male Wistar rats weighing 250 g were used. All animals were put in high temperature environment (WBGT 28°C) through whole experimental period. The rats were divided into five groups randomly: Exercise-Non water intake (EN), Exercise-Water intake (EW), Exercise-1/2 concentration ORS intake (E1/2O), Exercise-ORS intake (EO) and Baseline (BL). In a group of water supply, each solution was served before, midst and later exercise by sonde forcibly. The blood was collected before or later of exercise and blood fluidity and P-selectin level of serum were measured. In the EN and EO, blood sodium and P-selectin, platelet activation markers, increased while blood fluidity decreased significantly compared with the BL. We speculate that these rat models caused hypertonic dehydration from results of electrolytic concentration. The ORS supply in hypertonic dehydration by exercise might deteriorate blood fluidity via enhancement of platelet aggregation.

#### 2PK-194

##### Factors of immediate leukocytosis after acute strenuous exercise

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[Objectives] The aim of this study was to evaluate factors of immediate neutrophilia and lymphocytosis after acute strenuous exercise by measuring sympathetic hormones, stress hormones, and cytokines as indicators.

[Methods] The subjects were trained 16 males (age, 21 $\pm$ 1.3, height, 172 $\pm$ 5.8 cm, weight, 74 $\pm$ 15.4 kg, mean $\pm$ SD). They ran the 12 minutes Cooper test, and blood samples were collected before and after exercise. From the blood samples, leukocyte counts, neutrophil counts, lymphocyte counts, adrenaline, noradrenaline, adrenocorticotropic hormone (ACTH), cortisol, interleukin-6 (IL-6), interleukin-8 (IL-8), and blood lactate concentration were measured.

[Results] Leukocyte counts, neutrophil counts, lymphocyte counts, adrenaline, noradrenaline, ACTH, cortisol, and IL-6, increased significantly ( $p < 0.01$ ), while, IL-8 decreased significantly ( $p < 0.01$ ) after exercise. Neutrophils showed a significant correlation with adrenaline ( $r = .402$ ), noradrenaline ( $r = .580$ ), ACTH ( $r = .599$ ), cortisol ( $r = .609$ ), and IL-6 ( $r = .724$ ). Lymphocytes showed a significant correlation with noradrenaline ( $r = .697$ ), ACTH ( $r = .781$ ), cortisol ( $r = .512$ ), and IL-6 ( $r = .467$ ).

[Conclusions] Conventionally, immediate leukocytosis after acute strenuous exercise has been ascribed mainly as a result of increased sympathetic hormone, while, stress hormones and cytokines have been considered as factors involved in delayed leukocytosis. However, in this study, it became clear that stress hormones and cytokines also contribute to immediate leukocytosis.

## 2PK-195

### Blood flow of the prefrontal cortex was increased by stepping on 3-D high repulsion cushion(3-D HRC)

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Exercise stimulates the brain and is useful for keeping healthy life. 3-D HRC (cushion made by tangled fine polyethylene fibers) is widely used for training of football players in J-league and little leagues. It enhances the strength of muscle power in lower limbs and hip and also enhances balancing ability of the trunk. We hypothesized that the cushion is useful not only for motor function (muscles and balancing) but also for stimulation of the brain. The blood flow of the brain measured by NIRS (near infrared spectroscopy) was compared between simple stepping on the floor and on the cushion. Stepping on the latter significantly increased the blood flow of the brain especially in prefrontal cortex. The precise mechanism is not well known yet but it may be due to enhanced afferent stimuli from lower limbs, hip or trunk to the brain, thus the brain was much activated and got arousal. The further mechanisms of increase of blood flow in prefrontal cortex will be discussed.

## 2PK-196

### Combined Long-Term Caffeine Intake and Exercise Improves Insulin Resistance

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Purpose: This study examined the effects of long-term caffeine intake and regular exercise, as well as their combination, on muscle triglyceride (TG) and glycogen (Gly) content in the liver and skeletal muscle, an indicator of insulin resistance. Other metabolic syndrome (MS)-related parameters, including body weight (BW); visceral fat mass (VFM); and levels of fasting blood glucose (BG), serum insulin, leptin, and lipids, were also measured. Methods: The sedentary (OLETF-Sed), exercise (OLETF-Ex), caffeine-intake (OLETF-Caf), or caffeine-intake and exercise (OLETF-Caf&Ex) group for 5 weeks of treatment and comparison to a group of control rats. The OLETF-Caf and OLETF-Caf&Ex groups were fed rat chow containing 0.25% caffeine, and the OLETF-Ex and OLETF-Caf&Ex groups were encouraged to exercise every day. The pre- and post-treatment levels of serum biochemical components were measured after overnight fasting and the post-treatment TG and Gly content in the liver and quadriceps femoris was measured. Results: The caffeine-only, exercise-only, and combined treatments all resulted in reductions in BW and VFM and improvement in a number of MS-related factors. However, the combined treatment resulted in significant decreases in fasting BG, insulin, FFA, and leptin levels and in VFM compared to the other treatments. Conclusion: Combined long-term caffeine intake and exercise expenditure more effectively improves insulin resistance in both liver and skeletal muscles and MS risk factors in OLETF rats compared to caffeine intake or exercise expenditure alone.

## 2PK-197

### Influence of passive hyperthermia on arm cranking endurance performance and isokinetic peak torque of exercised and non-exercised muscle groups

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This study examined the effect of passive hyperthermia on arm cranking endurance performance and isokinetic peak torque of exercised (elbow flexors) and non-exercised (knee extensors) muscle groups. Eight subjects were immersed for 30 min in water at 36 and 40°C, and then exercised on an arm cranking ergometer at 60% of maximal oxygen uptake until voluntary fatigue in a temperate environment. Pre- and post-immersion in water and immediately following an arm-crank exercise, subjects performed two series of isokinetic shortening and lengthening maximal voluntary contractions (MVCs) of the elbow flexors and knee extensors: one series of single MVCs and one series of 25 endurance MVCs. Rectal temperature at the end of immersion was 37.1±0.3°C (±SD) and 38.1±0.3°C in the 36 and 40°C trials, respectively. During arm-crank exercise, time to fatigue was significantly less in the 40°C trial (41±13 min) than in the 36°C trial (52±12 min). Integral electromyography and peak torque for shortening single MVCs of the elbow flexors at post-immersion in water were significantly lower in the 40°C trial than in the 36°C trial. Peak torque during 25 endurance MVCs of the elbow flexors and knee extensors were similar between trials. These results indicate that passive hyperthermia reduces arm cranking endurance performance and isokinetic peak torque for shortening brief MVCs of exercised muscle groups.

## 2PK-198

### Effect of daily average steps on lower limb muscle mass after one year in community-dwelling elderly Japanese

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The purpose of this study was to investigate the effect of daily average steps (DAS) on lower limb muscle mass (LLMM) in elderly, community-dwelling Japanese subjects. Subjects were 239 (75 men and 164 women), aged 65-84 years old. They were healthy and has lived in Takatsuki city. We conducted a 1-year follow-up study in 2005. We have measured two times for DAS, LLMM, Grip strength (GS) at May 2005 (start point: SP) or at May 2006 (end point: EP). DAS was calculated from step counts for 7 consecutive days measured by a pedometer/accelerometer (Lifecorder Ex). LLMM was determined from the sum of lower limb muscle mass measured by bioelectrical impedance analysis (BIA) using the Body Composition analyzer MC-190. LLMM was shown by LLMM/body weight (LLMM/Wt). GS was measured using a dynamometer. In men, measured values (SP or EP), DAS was 8589±3455 (mean±standard deviation) or 8469±3628 steps/day, LLMM was 14.3±1.5kg or 14.3±1.6kg, LLMM/Wt was 24.6±2.6 or 24.4±2.2, and GS was 33.1±6.1kg or 34.8±5.6kg, respectively. In women, DAS was 8627±3628 or 8589±3455 steps/day, LLMM was 14.3±1.5kg or 14.3±1.6kg, LLMM/Wt was 19.8±1.7 or 19.8±1.6, and GS was 20.0±4.1 or 22.6±3.6kg, respectively. A significant correlation (p<0.01) was observed between DAS at SP and LLMM/Wt at EP in men. These results suggest that DAS is effective for an increase in LLMM/Wt in men.

## 2PK-199

### Is muscle oxyhemoglobin saturation determined using near-infrared spectroscopy related to muscle oxidative capacity?

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**Introduction :** A shorter muscle oxyhemoglobin saturation half recovery time ( $\frac{1}{2}\text{SmO}_2\text{RT}$ ) is related to muscle oxidative capacity. However,  $\text{SmO}_2$  reflects the dynamic balance between  $\text{O}_2$  supply and  $\text{O}_2$  consumption in the investigated muscle volume. The difference in  $\frac{1}{2}\text{SmO}_2\text{RT}$  is not only related to the  $\text{O}_2$  supply but also to  $\text{O}_2$  consumption. Thus, it may be inaccurate to state that  $\frac{1}{2}\text{SmO}_2\text{RT}$  is related to muscle oxidative capacity. The purpose of this study was to examine whether  $\frac{1}{2}\text{SmO}_2\text{RT}$  reflected  $\text{O}_2$  supply during recovery. **Methods :** Five healthy men performed a 10-s sustained isometric contraction maximal intensity hand grip exercise. Oxygenation of the flexor digitorum superficialis was measured using near-infrared spectroscopy, and brachial artery mean blood velocity (MBV) was measured using Doppler ultrasonography. Brachial blood flow (BBF) was calculated over a 2-s duty cycle as follows:  $\text{BBF (mL/min)} = \text{MBV (cm/s)} \times \pi r^2 \text{ (cm}^2\text{)} \times 60$ , where  $r$  is the radius of the brachial artery. **Analysis :** The average BBF (BBFmean) data was used during  $\frac{1}{2}\text{SmO}_2\text{RT}$  and the maximum amplitude (BBFmax) was calculated as the difference between the maximum BBF during the recovery phase and baseline value. The relationship between with  $\frac{1}{2}\text{SmO}_2\text{RT}$ , BBFmean, and BBFmax was determined using Pearson's rank correlation. **Results :** There was no correlation between  $\frac{1}{2}\text{SmO}_2\text{RT}$  and BBFmean ( $r = -0.465$ ,  $p = 0.4764$ ), or BBFmax ( $r = -0.115$ ,  $p = -0.8705$ ). **Conclusions :** These results indicate that muscle oxyhemoglobin saturation recovery time may not be related to the  $\text{O}_2$  supply of the muscle.

## 2PK-200

### Myostatin physiologically regulates skeletal muscle metabolism with growth and exercise through the AMPK signaling pathway

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**Purpose :** Recent studies on myostatin knockout mice have suggested that myostatin, a growth suppressing factor of skeletal muscle, regulates carbohydrate and lipid metabolism in skeletal muscle cells. To validate this at physiological condition, the effects of postnatal up-regulation of myostatin in combination with the effects of exercise were examined focusing on the involvement of the AMPK signaling pathway. **Methods :** F344 female rats (6-week) were divided into three groups; pre-experimental control group ( $n=7$ ), post-experimental control group ( $n=8$ ), and (post-experimental) exercise group ( $n=7$ ). Rats of the exercise group voluntarily ran on a rotary wheel ergometer with a load of 30% of body mass for 8 weeks from 6 weeks of age. Rats were allowed to take diet and water freely during the experimental period. Plantaris muscles were analyzed. **Result :** Independent of exercise loading, myostatin expression increased with growth. In the post-experimental control group, the expressions of phosphorylated AMPK $\alpha$  and PGC-1 $\alpha$  protein decreased correspondingly, with concomitant down-regulation of carbohydrate and lipid metabolism. In the exercise group, these decreases were inhibited. GLUT4, glucose transporter protein, did not change with growth but increased by exercise. **Conclusion :** We confirmed that myostatin physiologically regulates carbohydrate and lipid metabolism in skeletal muscle cells via AMPK signaling pathway.

## 2PK-201

### Usability of a muscle hardness meter on the detection of the superficial and the deep muscle stiffness changed by stretching

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Muscle stiffness is one of the objective indication of symptoms for muscles. The muscle hardness meter is useful on the superficial muscles for detecting hardness changes through physical therapy. We quantified muscle stiffness on the rectus femoris muscle and iliopsoas muscle before and after stretching in 22 healthy young persons by using a muscle hardness meter (NEUTONE TDM-N1, TRY-ALL). Muscle stiffness were changed from  $20.0 \pm 7.1$  N (mean  $\pm$  SD) to  $25.5 \pm 5.9$  on the rectus femoris muscles after stretching and from  $27.5 \pm 5.9$  to  $33.6 \pm 7.6$  on the iliopsoas muscles, respectively. There was no significant difference between muscle stiffness changes. The values before and after stretching on each muscle correlated significantly ( $P < 0.01$ ). Between hardness of the two muscles the significant correlation was observed over 22 subjects ( $P < 0.05$ ). The suppleness of the rectus femoris muscles were improved by the stretching and the improvement values showed the significant correlation between the relative changes (% gain) of muscle hardness after stretching ( $P < 0.05$ ). Therefore, the muscle hardness meter may be useful for clinical study of the deep muscles as well as the superficial muscles.

## Poster Presentations Others(2)



## 2PK-202

### Report of equal opportunity survey 2012 in the Japanese Physiological Society

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The Japanese Physiological Society (JPS) conducted a survey of equal participation of men and women at the 89th annual meeting in Matsuyama in March, 2012. The purpose was to clarify the ratio and fact of female members and explore the ideal equal opportunity in JPS. There were 551 respondents, equivalent to 21% of 2640 total JPS members (year 2011). Twenty-seven percent of the respondents were female, 69% male and 4% unknown. Since 20% of JPS members are female, women responded better. The survey including 18 questions revealed an apparent gender inequality; i.e. female physiologists are smaller or lower than men in terms of number, position, research fund, and term of work. Yet, there is a sign of increasing number of young women, indicating a hope. Free answer section of the survey could be divided into three groups, including suggestions, facts, and complaints. In suggestions, both men and women depicted improving cases in progress. In facts, however, some males answered that gender equality is unnecessary. This gives impression that the level of awareness of equal opportunity is high in females, while low in males. This difference could regress the gender equality. It is necessary to find a way to resolve this difference in the levels of recognition of inequality between men and women physiologists.

## 2PK-203

### Measurements of EEG during recall periodic stimulation

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In recent years, Brain-Machine Interface (BMI) to operate the machine with the potential change of the brain has been actively developed. There are two main ways to control the BMI. One is passive way using evoked potential, the other is active way using motor imagery. Many studies of BMI have been done in order to support the function of the body in a variety of ways. Therefore, the many types of way are possible to expand the possibilities of research of the BMI. For people who can't be covered in a conventional method, we focused on whether it can be used to recall the evoked potentials. After it continues to receive periodic stimulation, it might be able to be recalled as if they still continue to receive stimulation. Therefore, we consider that it is possible to recall the somatosensory evoked potential (SEP). On the experience of recall, a timing to perform the recall is important. We use a different stimulation to match the timing, however, we can't measure only the EEG during recall. In this case, if there is linearity between the evoked potentials by timing stimulation and by recalling stimulation, it is possible to measure EEG of recalling stimulation by subtracting EEG of timing stimulation from the overall. In previous measurement, we have confirmed that there is nearly linear at Fp1. This time, we confirmed that there is linearity in almost all places with newly manufacturing the multi-channel EEG. In addition, we report the results of measuring the EEG during recall stimulation.

## 2PK-204

### Ca<sup>2+</sup> imaging of subserosal cells in the guinea-pig proximal colon

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In the subserosal layer of the guinea-pig proximal colon, PDGFR $\alpha$ -positive fibroblasts and c-Kit-positive interstitial cells of Cajal (ICC-SS) have been identified by immunohistochemistry. Since only sparse PGP 9.5-positive nerve filaments are observed and thus innervation of the subserosal layer appears to be poor, it is assumed that some endogenous factors other than neurotransmitters may contribute to intercellular communication amongst cells in the subserosal layer. In this study, the responsiveness of subserosal cells to acetylcholine (ACh) and ATP was investigated by in situ Ca<sup>2+</sup> imaging using fluo-4 loaded preparations.

A subset of subserosal cells exhibited Ca<sup>2+</sup> transients in response to ATP (10  $\mu$ M), while not responding to ACh (10  $\mu$ M). ATP induced Ca<sup>2+</sup> transients were not mimicked by  $\alpha$ ,  $\beta$ -methylene ATP (10  $\mu$ M), suggesting that ATP may act via P2Y but not P2X receptors. These results indicate that subserosal cells, which responded to ATP but not to ACh, may communicate with neighboring cells using ATP. Since these cells were not positive for c-Kit antibodies, they are unlikely to be ICC, and identifications of the cell type will be discussed together with the results of ongoing observations.

## 2PK-205

### Cholinergic and non-cholinergic regulatory system in the esophageal motility of *Suncus murinus* (a house musk shrew)

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*Suncus murinus* (a house musk shrew; suncus) is a species of insectivore that has an ability to vomit in response to mild shaking or ingestion of chemicals. Since rodents including rats and mice do not show the emetic reflex, the suncus has been extensively used to examine emetic mechanism. Although the movement of the esophagus would be related to the emetic response, regulatory mechanisms for the suncus esophageal motility are unclear. Therefore, the aim of the present study was to clarify cholinergic and non-cholinergic components that regulate esophageal motility in the suncus. An isolated segment of the suncus esophagus was placed in an organ bath and the mechanical responses were recorded using a force transducer. Electrical stimulation of the vagus nerve evoked two-phase contractile responses in the esophageal segment. First contraction was inhibited by a blocker of nicotinic acetylcholine receptors on the striated muscle, and second one was inhibited by a blocker of muscarinic acetylcholine receptors on the smooth muscle. Next, to investigate whether non-cholinergic components are involved in the esophageal motility of the suncus, we used some agonists. Exogenous application of serotonin and histamine induced the contractile response of the smooth muscle as well as acetylcholine. These findings suggest that motility of the suncus esophagus is regulated by cholinergic and non-cholinergic components.

## 2PK-206

### Comparison of inhibitory effects of menthol derivatives on contraction at different positions of isolated mouse intestine

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We have reported that menthol derivatives (MD) showed concentration-dependent inhibitory effects on contraction of isolated mouse intestine induced by acetylcholine (ACh). This inhibitory effect of MD seems to involve activation of TRPM8 because MD shows no effect at the low temperature (23°C) where TRPM8 has been already activated. Here we compared inhibitory effects of MD on contraction at different positions of isolated mouse intestine. We divided into 5 intestinal positions, that is, jejunum (J), upper part of ileum (UI), lower part of ileum (LI), colon (C), and rectum (R). When amplitude of ACh-induced contraction by DMSO was 100% (control), under 50 µM MD, amplitude of contractions by 0.5 µM ACh became 52.13% (J), 75.24% (UI), 75.68% (LI), 36.62% (C), 49.07% (R) at 37°C and 55.34% (J), 99.81% (UI), 94.06% (LI), 74.25% (C), 70.87% (R) at 23°C, respectively. At all intestinal positions except jejunum, inhibitory effects of MD on ACh-induced contractions became weak at low temperature, suggesting involvement of activated TRPM8 on inhibitory effects of MD on ACh-induced contraction. It is not yet clear why different responses according to intestinal positions occurred, but expression of TRPM8 may be different.

## 2PK-207

### Characteristics of voltage dependent inward current in the rat embryonic heart early after the initiation of heartbeat

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**Background:** We have recently reported that the cardiac crescent begins to contract at embryonic day 9.99 to 10.13 in the rats. Meanwhile, the knowledge about voltage dependent inward currents which contribute to both automaticity and excitability in this phase of developing heart is far less revealed than in the matured heart. **Method and Result:** We enzymatically isolated myocyte from the beating embryonic heart at embryonic day 11.0 of Wister rats for whole cell patch-clamp. Normal Tyrode's solution with 1.8mM Ca<sup>2+</sup> was used for external solution. Inward currents were elicited by test potentials from -90 mV to +30mV from a holding potential of -90mV. The cellular capacitances were 10.0±0.46 pF (mean±SEM). Current to voltage (I-V) relationship showed a hump at a low voltage range from -70mV to -50mV and peaked at -20mV (-5.46±0.20 pA/pF). Application of 0.2mM Cd<sup>2+</sup> to the external solution almost completely blocked the inward current, indicating that this current was carried by L-type Ca<sup>2+</sup> channel, and the peak of I-V relationship was shifted to more negative voltages approximately by 10mV than in I<sub>CaL</sub> observed in the adult rat myocytes. **Conclusion:** At least two kinds of voltage dependent inward current were observed in the developing heart at the embryonic day of 11.0. Considering previous reports, the negative shift of I-V relationship appeared to imply the contribution of Ca<sub>v</sub>1.3.

## 2PK-208

### Nuclear Factor kappa B Inhibition Promotes Closure of Rat Ductus Arteriosus

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**Background:** Prostaglandin E2 (PGE2) plays dual roles in vasodilation and structural remodeling such as intimal thickening of the DA through adenylyl cyclase-cAMP pathway. However, DA remodeling is not completely prevented by inhibition of cAMP pathway, suggesting that there is a cAMP-independent PGE2 signal pathway. **Methods and Results:** We performed differential DNA microarray analyses to find a distinct gene expression profile between PGE2-specific receptor (EP4) stimulation and direct activation of adenylyl cyclase using forskolin (FSK) in cultured rat DA smooth muscle cells (SMCs). Signal pathway analysis indicated that the nuclear factor kappa b (NFkB) pathway was activated more in EP4-stimulated SMCs than in FSK-treated SMCs. DA explants from 21st fetuses were cultured for 48 hours in the presence or absence of the NFkB inhibitor IMD-0354 at a concentration of 10-6M. IMD-0354 treatment facilitated closure of DA explants through vasoconstriction and promoted SMC migration into the subendothelial space. When IMD-0354 was injected into 21st rat fetuses, NFkB inhibition advanced DA closure even in fetal circulation. **Conclusion:** Although some previous reports have showed that NFkB pathway promotes vascular remodeling, the present data demonstrated that NFkB inhibition facilitated DA closure. NFkB may play a central role in a cAMP-independent PGE2 signal pathway of the rat DA.

## 2PK-209

### Preventive effect of lignan on redox status of erythrocyte membrane against oxidative stress

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Some kind of polyphenol from sesame-seed or flaxseed has a preventive effect over atherosclerosis or diabetes, under which oxidative stress is pathologically important. We are studying to evaluate the effects of iron-induced oxidative stress and protective effects of lignan, which is a kind of polyphenol from flaxseeds, against oxidative damage on redox status of erythrocyte membrane. Heparinized venous blood was obtained from healthy donors, and immediately centrifuged. After a careful removal of plasma and buffy coat, erythrocytes were purified by three cycles of resuspension and washing with isotonic HEPES-buffered saline (HBS) and resuspended at adjustable hematocrit in HBS. The erythrocytes were treated with 0.2mM FeSO<sub>4</sub> containing a lignan (Secoisolaricresinol (SECO) or Matairesinol (Mat), Laricresinol (Lar)) and incubated at 37°C for 1 hour. Using thiol group of membrane proteins of erythrocyte as an index, we have screened lignans. (-)-SECO inhibited the oxidation-induced decrease of thiol-group of erythrocyte membrane. If the viscoelasticity of erythrocyte membrane increases according to the membrane oxidative injury, the erythrocyte deformability will fall and the blood viscosity will increase. To further analyze the function of erythrocyte flow, we are now trying to evaluate a viscosity of erythrocyte suspensions by a cone-plate viscometer.

## 2PK-210

### The mechanism of enhancement on plasminogen activation and thrombolysis by synthetic nonadecapeptide

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A synthetic nonadecapeptide (SP ; GPYLMVNVTGVDGKGNELL) binds to plasminogen (Plg) and subsequently enhances the activation of Plg by tissue-type plasminogen activator (t-PA). To identify the binding site for SP in Plg and elucidate the effects of SP on thrombolysis in vivo, we investigated the interaction between SP and Glu-Plg or Glu-Plg fragments digested by cyanogen bromide (CNBr) using FITC-labeled SP. FITC-labeled SP interacted with Glu-Plg, B-chain of plasmin and CNBr-digested fragment 2 (His585-Val786 in Glu-Plg). SP bound to the synthetic peptide B11 (Phe747-Gly763 in B-region of Glu-Plg) in a concentration-dependent manner. Furthermore, B11 peptide inhibited the binding of SP to Glu-Plg and the SP-induced Glu-plg activation by t-PA. Since the mutant B11 peptide, D750A (substitution of Asp750 to Ala) and deletion of Asp750 did not bind to SP, Asp750 was thought to be a critical amino acid in the binding between SP and Glu-Plg. When SP was administered in mouse thrombosis model, earlier recanalization was observed than in mice with vehicle administration in dose-dependent manner. However, SP did not show the recanalization in t-PA gene deficient mice. In conclusion, it was suggested that SP binds to the B-region of Glu-Plg and enhances the activation of Glu-Plg by t-PA. Furthermore, this study indicated that SP induces effective thrombolysis in vivo.

## 2PK-211

### Effects of supraspinal and spinal $\alpha 1$ -adrenoceptor subtypes on bladder activity in conscious rats

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In the previous studies in anesthetized rats, it was proposed that bulbospinal noradrenergic inputs to the sacral parasympathetic nucleus played an important role in micturition function. However, these findings were not confirmed in conscious rats. In the present study, we tested the effects of  $\alpha 1$ -adrenoceptor antagonists on the micturition reflex induced by infusing fluid into the bladder of conscious rats at the supraspinal and spinal level. The mRNA expression of three  $\alpha 1$ -adrenoceptor subtypes was detected in rat brain and lumbosacral spinal cord. Intracerebroventricular (i. c. v.) injection of the  $\alpha 1$ -adrenoceptor antagonist tamsulosin, the selective  $\alpha 1A$ -adrenoceptor antagonist silodosin and the selective  $\alpha 1D$ -adrenoceptor antagonist BMY 7378 significantly prolonged the intercontraction interval (ICI) but did not alter the maximum voiding pressure (MVP). Although intrathecal (i.t.) injection of BMY 7378 did not affect the ICI, tamsulosin and silodosin prolonged the ICI in a dose-dependent manner. The MVP was significantly decreased by i.t. injection of tamsulosin but not by silodosin and BMY 7378. The present results indicate that supraspinal  $\alpha 1A$ - and  $\alpha 1D$ -adrenoceptors are important for the regulation of reflex-bladder activity in conscious rats. Noradrenergic projections from the brainstem to the spinal cord could promote the afferent limb rather than the efferent limb of the micturition reflex pathway, and the main  $\alpha 1$ -adrenoceptors in the afferent limb of this reflex pathway may be  $\alpha 1A$ -adrenoceptors.

## 2PK-212

### Comprehensive analysis on expression profiles of genes encoding GABA receptor subunits in the rat GnRH neurons and GT1-7 cells

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GnRH neurons function as central regulators of the reproductive axis, and GABA is one of the major players in the regulation of GnRH neurons. In the present study, we comprehensively analyzed the expression profiles of genes encoding GABA receptor (GABAR) subunits in the rat GnRH neurons and GT1-7 cells. Rat GnRH neurons expressed  $\alpha 1-4$ ,  $\beta 1-3$ ,  $\gamma 1$ ,  $\gamma 2$ ,  $\epsilon$ , and  $\theta$  subunit mRNAs for ionotropic GABARs, and Gabbr1 and Gabbr2 subunit mRNAs for metabotropic GABARs, while GT1-7 cells expressed  $\alpha 2-5$ ,  $\beta 1$ ,  $\beta 3$ ,  $\gamma 1$ ,  $\gamma 3$ ,  $\delta$ ,  $\epsilon$ ,  $\pi$ ,  $\theta$ ,  $\rho 1$ ,  $\rho 2$ , Gabbr1, and Gabbr2 subunit mRNAs. Subsequently, we examined the functionality of GABARs in GT1-7 cells using a perforated patch-clamp technique and enzyme immunoassays. We observed inward ionotropic GABAR currents and inhibition of forskolin-induced cAMP formation via metabotropic GABARs. GnRH release from GT1-7 cells was augmented by GABA in a manner sensitive to picrotoxin. Forskolin-induced GABA release was suppressed by baclofen. The expression patterns of GABAR subunit mRNAs in GT1-7 cells were different from those in native GnRH neurons. However, the cells retained the functional GABARs, and they provide a useful model system for investigating the pharmacological responses of GABARs and the molecular biological regulation of GABAR gene expression.

## 2PK-213

### Effects of an antiepileptic drug phenytoin on oocyte $Ca^{2+}$ oscillations and on sperm motility of the mouse

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**BACKGROUND :** Oocytes produced during fetal life stay in the ovary for a long period until ovulation. It is therefore important that ovarian oocytes are not damaged by any drugs or chemicals. The present work studies effects of phenytoin, one of the most frequently used antiepileptic drugs, on the function of the oocyte. Phenytoin was also applied to mouse sperm for comparison.

**METHODS :** Oocytes or sperm were obtained from ovaries or epididymides of 8-12-week-old ICR mice. Oocytes were loaded with Fura-2/AM and their fluorescence  $Ca^{2+}$  images were monitored by an image analyser (ARGUS-50, Hamamatsu Photonics). The movement of sperm was monitored using a high-speed digital camera (CASIO ; EX-LIM EX-FC100) at 210 fps (frames per second).

**RESULTS :** 1) Oocytes isolated from the ovary showed spontaneous  $Ca^{2+}$  oscillations at regular intervals. 2) The oscillations were not affected by phenytoin when its concentration was lower than 20  $\mu g/ml$  (80  $\mu M$ ). 3) When the concentration of phenytoin exceeded 20  $\mu g/ml$ , the frequency of  $Ca^{2+}$  oscillations was irreversibly lowered. As for mouse sperm, no effect of phenytoin on motility was observed when its concentration was less than 20  $\mu g/ml$ , and the beating frequency was reversibly lowered at higher drug levels.

**CONCLUSION :** The data indicate that phenytoin has no toxic effects on mouse gametes at concentrations useful for suppressing epilepsy in humans (10-20  $\mu g/ml$ ), and that oocytes are more sensitive to the drug than sperm when its concentration exceeded these therapeutic doses.

## 2PK-214

### A Preparative Separation of Basophils and Eosinophils by a Continuous Flow-Through Density Gradient Cell Separation Method

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We have developed a continuous flow method for gentle and preparative-scale separation of basophils and eosinophils from the human peripheral blood based on cell density. Obtained fractions are further used for the purification of basophils with the specific antibody. A 10ml volume of the peripheral blood was diluted with acid-citrate-dextrose solution to hematocrit value of 20%, and injected into a rotating separation disk. The method typically yielded the population of basophils at 65% in the density of 1.079 g/ml and that of eosinophils at 85% in the density of 1.102 g/ml from healthy volunteers. Separated cells preserved their natural functions: basophils released histamine and eosinophils released eosinophil-derived neurotoxin (EDN) by A23187-induced degranulation. The expression of CD203c antigen, the activation marker of basophils, was higher in an atopic dermatitis (AD) patient than in healthy volunteers. After the activation by anti-IgE antibody, an increase of CD203c level in healthy volunteers and the AD patient were found at 51% and 81% of basophils, respectively, similar to the native basophils before the separation. These results suggested that functionally viable basophils could be separated by the present method from AD patients as well as from healthy volunteers. We believe that the present method is useful for a gentle and preparative-scale separation of basophils and other functional cells.

## 3PK-001

### Prostaglandin E2 receptor EP4 in coronary smooth muscle cells may play a role in promoting intimal thickening that precedes atherosclerosis

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Physiological intimal thickening (IT) precedes atherosclerosis in coronary artery (CA). However molecular mechanisms in IT are largely unknown. We have previously reported that prostaglandin E<sub>2</sub>-EP4 signaling promoted IT in the ductus arteriosus and that EP4 was highly expressed in smooth muscle cells (SMCs) of human CA (hCASMCs), suggesting involvement of EP4 signaling in IT of CA. We further obtained proximal right CA and left main trunk of CA from patients who were not died from cardiac diseases ranging in age from 0 day to 71 years old (n=8). Elastica masson stain and immunohistochemistry revealed that IT and EP4 expression was present even in 0 day old patients. IT gradually became prominent during development and EP4 was constitutively expressed in SMCs of the tunica media and IT at any ages examined. LC/MS/MS analysis and quantitative RT-PCR using normal hCASMCs showed that the EP4 agonist ONO-AE1-329 increased Pyk2, proline-rich tyrosine kinase-2, which has been reported to be involved in cell migration (1.4-fold increase in Pyk2 mRNA, n=6, p<0.05). In conclusion, these data suggested that EP4 signaling in hCASMCs contributed to IT formation via Pyk2.

## 3PK-002

### Analysis of frequency-dependent acceleration of relaxation by simulation model

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The frequency-dependent acceleration of relaxation (FDAR) is crucial in filling the ventricular cavity during the diastolic period at a higher heart rate. It has been suggested that CaMKII may be involved in FDAR based on the fact that CaMKII regulates a variety of Ca<sup>2+</sup>-regulated proteins, which are involved in cardiac excitation-contraction coupling in the cardiac myocytes. The activity of SR Ca<sup>2+</sup>-pump (SERCA) is enhanced by PLB phosphorylation at Thr-17 by CaMKII, or at Ser-16 by PKA. SERCA is also directly phosphorylated by CaMKII to increase the V<sub>max</sub> of Ca<sup>2+</sup> uptake. RyR receptor is also phosphorylated by CaMKII or PKA to increase (or decrease) its open probability. However, it has been suggested that the time course of phosphorylation and de-phosphorylation of PLB and RyR are slower than that of FDAR. We performed a system biological approach to test involvement of various mechanisms in FDAR by using our human ventricular cell model. So far, our analysis supported the central role of SERCA modulation, but the time course of FDAR was generally slower in simulation. The decreasing peak time and accelerate relaxation at higher frequency in experiments were better simulated when the right-shift of myofilament Ca<sup>2+</sup> sensitivity was also assumed. This new assumption is based on the fact that the Ca<sup>2+</sup> sensitivity of myofilaments is modulated in a frequency-dependent phosphorylation of TnI by PKA, resulting in the right shift of the Ca<sup>2+</sup>-contraction force relationship. We will discuss a possibility of PKA activation via field electrical stimulation or by unknown pathway.

## Poster Presentations Heart, Circulation(3)

### 3PK-003

#### Caffeine induced bidirectional ventricular tachycardia in anesthetized rat

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**Background and Purpose :** In previous studies, we showed that Bidirectional ventricular tachycardia (BVT) has been elicited by continuous administration of the doxapram hydrochloride and epinephrine under halothane anesthesia in adult Wister rats. The purpose of present study is to investigate the effect of caffeine on the cardiac excitation, particularly occurrence of BVT and repolarization property.

**Methods :** Adult female Wister rats were anesthetized with halothane gas (1.2%). ECG, respiratory movements and peripheral blood flow were recorded with a Biopac system MP-150. Caffeine (0.5mg/kg/min) and epinephrine (10 $\mu$ g/kg/min) were simultaneously and continuously injected through the femoral vein. The effect of drugs was evaluated by measuring RR, PR, QTc, JTp/JT and Tp-e/QT of body surface ECG.

**Results :** The caffeine and epinephrine administration elicited shortening of RR and prolongation of PR. QTc and JTp/JT of repolarization property were significantly prolonged and Tp-e/QT was significantly shortened before appearance of BVT.

**Discussion :** QTc and JTp/JT on the ascending limb of the T wave show prolongation with the intracellular Ca<sup>2+</sup> overload or disorder of ionic channels. Therefore we conclude that the caffeine and epinephrine produce an increase in intracellular Ca<sup>2+</sup> concentration and elicit BVT due to early after depolarization in ventricular myocytes.

### 3PK-004

#### Aging deteriorates the mesenteric lymph pump activity

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Efficient propulsion of lymph is essential for maintaining tissue fluid and macromolecules homeostasis, fat absorption, and immunity. Knowledge of how aging influences the regulatory mechanisms of lymphatic contractility is limited. We investigated the aging-associated changes in rat mesenteric lymph vessels (MLV) obtained from 9-mo and 24-mo old Fischer-344 rats. Following techniques were implemented : isolated pressurized MLV preparation, MLV video monitoring *in vitro* and *in situ*, immunohistochemical labeling. We found weakening of the aged MLV including diminished tone, and predominant decrease in contraction frequency which leads to decrease in their minute productivity. Application of L-NAME is able to enhance contractility of aged MLV which referred to status of surrounding tissue microenvironment. These findings demonstrate that aging remarkably weakens MLV contractility, there is, an additional source of some yet unidentified metabolites in aged tissues surrounding the aged MLV that stimulates lymphatic contractions and the effect of which may be counterbalanced or blocked by excessive nitric oxide (NO) release.

### 3PK-005

#### Chewing prevents stress induced heart failure in rats

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It is now well established that stress is linked to cardiovascular diseases by causing autonomic imbalance. We investigated whether chewing during stress acts as an active coping strategy which prevents stress induced heart failure on the right ventricle (RV) hypertrophy rat model. Male SD rats were given a single dose (60 mg/kg) of monocrotaline (MCT) intraperitoneally to induce pulmonary hypertension (PH) and RV hypertrophy. The following 3 groups were studied : rats treated with MCT (M group), rats treated with MCT plus immobilization stress (MS group), and MCT plus allowed to chew a wooden stick during immobilization stress (MC group). MS and MC group rats were exposed 1hr immobilization stress at two times per week for 3 weeks after MCT injection for 3 weeks. At the 6 week after MCT injection, the survival rate, heart tissue and lung tissue were evaluated. The ratio between RV weight and the weight of the left ventricle plus interventricular septum (RV/LV+IS) was taken as the index of RV hypertrophy. Chewing significantly reduced that stress induced lung weight gain (M : 2598 $\pm$ 236 mg, n=10 ; MS : 3597 $\pm$ 373 mg, n=7 ; MC : 2568 $\pm$ 177 mg, n=8 ; p<0.05) and RV/LV+IS ratio (M : 0.35 $\pm$ 0.02%, MS : 0.37 $\pm$ 0.02%, MC : 0.36 $\pm$ 0.03%). It also increases the survival rate (M : 50%, MS : 35%, MC : 40%). The results suggest that chewing under stress condition might relieve stress-induced lung remodeling and progressive heart failure.

### 3PK-006

#### A method of evaluating the aortic baroreflex in human

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Arterial blood pressure is sensed by arterial baroreceptors in the carotid sinus and aortic arch regions. The carotid baroreflex function in human has been extensively investigated using the variable-pressure neck chamber, but studies investigating the aortic baroreflex function were limited due to the technical difficulties. We therefore attempted to develop a system to examine aortic baroreflex function in human. The system was consisted of the neck chamber, a pressure transducer connected to the neck chamber, blowers that apply pressure to the neck chamber, a beat-to-beat based blood pressure monitor, and the control unit. We designed the system so that the carotid sinus transmural pressure to be maintained but the aortic pressure to be altered when blood pressure changes. To accomplish this, chamber pressure, arterial blood pressure, and estimated carotid sinus pressure (blood pressure-chamber pressure, ECSP) were continuously monitored and air pressure was automatically applied to the neck chamber corresponding to the changes in ECSP. Using this system, we tested whether ECSP was maintained when blood pressure changed. Changes in blood pressure were evoked by unilateral thigh cuff deflation after three minutes of thigh cuff inflation. Without the developed system, blood pressure and ECSP decreased 20-40 mmHg after the thigh cuff release. When we applied the developed system, blood pressure decreased after the thigh cuff release but ECSP did not change. We thus consider that aortic baroreceptor could be stimulated due to the changes in blood pressure, without changing carotid sinus pressure.

### 3PK-007

#### Implementation of a Ventricular Cell Model to a Cardiovascular Model Regulated by the Autonomic Nervous System

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Cardiovascular system is finely tuned by autonomic nervous system (ANS) to avoid excess loading of the heart and to meet the metabolic demand of the body. A number of mathematical models have been developed to simulate hemodynamic responses to various changes in posture, lower body negative pressure, gravity, exercise and so on; however, most of them are lumped-parameter models which are not suitable for investigating detailed molecular mechanisms underlying the dynamics of the system. In the present study, the lumped parameters, especially for the left ventricle, are replaced by realistic parameters based on molecular entities. Integrating a detailed ventricular cell model (Kyoto model) into a circulation model allows in-silico assessment of cardiac function at the molecular level. Our model of cardiovascular system is essentially based on Heldt et al. model (2002), which consists of vascular system and baroreflex system. Cardiac output generated by left ventricular contraction is calculated in a spherical model based on Laplace's law. Contraction force is calculated in a cell model (Kuzumoto model 2008) equipped with Negróni-Lascano model (1996) and regulated by ANS. It is confirmed that hemodynamic parameters calculated in the system are within the physiological range in a steady state. Transient response to tilt and myocardial oxygen consumption at different hemodynamic states will be discussed using the system model.

### 3PK-008

#### Effects of block and facilitation of HERG channel by Class III antiarrhythmic agents on cardiac action potential: a simulation study

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HERG channel underlies the rapidly activating component of the delayed rectifying potassium current ( $I_{Kr}$ ) in heart and plays an important role in terminating the cardiac action potential (AP). We have experimentally demonstrated a dual effect of some antiarrhythmic agents, such as nifekalant, on HERG channel. Besides blocking HERG channel, these compounds can facilitate its activation. To assess the clinical relevance of HERG channel modulations by compounds, we conducted simulations of cardiac AP in a modified Luo-Rudy model with HERG channel block and facilitation block and facilitation that were based on the experimentally observed effects of nifekalant on hERG channel.  $I_{Kr}$  block prolonged action potential duration (APD) in the block conditions either with or without facilitation. In addition, the refractory period in both conditions equally increased by 55 msec from control condition so that the ectopic cell excitation was suppressed. Moreover, in our simulation, we could observe an early afterdepolarization (EAD) when both  $I_{Kr}$  and the slowly activating component of  $I_K$  ( $I_{Ks}$ ) were blocked. Importantly, facilitation mechanism prevents hazardous prolongation of APD and the induction of EAD by accelerating the repolarization rate via an increase in  $I_{Kr}$  during prolonged phase 3. Therefore, antiarrhythmic agents that have both block and facilitation effects on HERG channel may have a lower risk for inducing EADs and triggered activity and thus be more suitable for the treatment of arrhythmias than pure HERG blocker.

### 3PK-009

#### Magnesium Influx Pathway in Cardiomyocytes Under Hypoxic Condition

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During hypoxia, increase in extracellular Mg concentration inhibits the decrease in cardiac intracellular Mg concentration and protects hearts effectively. To elucidate the underlying mechanism, we examined the relationship between the cardiac function and intracellular Mg by using Langendorff perfused rat heart model and mag-fura 2/AM fluorometry with isolated rat ventricular myocytes. In Langendorff perfusion, after stabilization, the hearts were applied isoproterenol (iso), which decreases intracellular free  $Mg^{2+}$  concentration ( $[Mg^{2+}]_i$ ), and then wash out it. Washout of iso caused the depression of cardiac function and decrease in cardiac total Mg content. Application of iso with high extracellular Mg concentration improved the recovery of cardiac function and prevented from declining total cardiac Mg content. In isolated cardiac myocytes,  $[Mg^{2+}]_i$  was not affected by high extracellular Mg concentration under the aerobic condition. However, under the hypoxic condition, high extracellular Mg concentration increased  $[Mg^{2+}]_i$ , in addition, this increase in  $[Mg^{2+}]_i$  was suppressed by TRPM7 inhibitors. We showed that decrease in cardiac total Mg content by hypoxia or iso application induces depression of cardiac contractile function. The depression of cardiac function by decrease in cardiac total Mg content was improved by extracellular high Mg concentration. This mechanism was suggested the possibility of increase in  $[Mg^{2+}]_i$  by influx from extracellular high  $Mg^{2+}$  space via TRPM7.

### 3PK-010

#### Differential effects of one-legged cycling on blood flows in proximal and distal muscles of the non-contracting leg in humans

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We have recently reported that centrally-induced vasodilator signal is transmitted to the non-contracting vastus lateralis muscle at the start period of voluntary exercise and during motor imagery (Ishii et al. J Appl Physiol 2012). To examine whether central command also contributes to the blood flow responses in the distal muscles of the non-exercising leg during voluntary one-legged exercise, we measured the relative changes in concentrations of oxygenated-hemoglobin (Oxy-Hb) of the tibialis anterior and triceps surae muscles with near-infrared spectroscopy. The Oxy-Hb of both non-contracting tibialis anterior and triceps surae muscles increased at the start of voluntary one-legged cycling, although the magnitude of the increases in Oxy-Hb in the tibialis anterior and triceps surae muscles seemed less than that of the vastus lateralis muscle. Motor imagery of the voluntary one-legged cycling also increased the Oxy-Hb of the non-contracting tibialis anterior and triceps surae muscles, as similarly as the vastus lateralis muscle. These present findings suggest that central command contributes to increasing the blood flow not only in the proximal muscles but also in the distal muscles of the non-exercising leg during voluntary one-legged exercise and during motor imagery of the exercise in humans.

### 3PK-011

#### Effect of vestibular lesions on responses of arterial blood pressure to neck flexion in rabbits

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Our previous data suggest that vestibular organs play a role in a transient drop in arterial blood pressure (ABP) induced by 45-degree head-down postural rotation (HDR) through the suppression of sympathetic nerve outflows (Exp. Brain Res. 2009). HDR stimulates the vestibular receptors, neck-muscle receptors and/or baroreceptors activated by head-ward fluid shift. Since these receptors are not separately stimulated, it is difficult to discriminate between the different effects of the three stimulated receptors on the cardiovascular system. In order to elucidate the effects, the receptors were stimulated by neck flexion (NF) without head-ward fluid shift, using urethane-anesthetized vagotomized rabbits. The animal's head was tilted to 45-degree head-down in 5 s, and kept at the position for a minute. NF induced a decrease in ABP, and then the pressure recovered toward the pre-NF level within a minute. Renal sympathetic nerve activity (RSNA) decreased before the ABP drop. Pretreatment with hexamethonium bromide suppressed the NF-induced drop of ABP. The NF-induced decrease in ABP was not different from HDR-induced decrease. Next, changes in ABP during NF were examined using vestibular-lesioned (VL) rabbits. NF-induced decrease of ABP in these VL rabbits was smaller than that in the control rabbits. These results suggest that both vestibular and neck-receptors which are activated by HDR may result in the transient drop of ABP through the suppression of sympathetic nerve.

### 3PK-012

#### Stimulation of subthalamic locomotor region increases myocardial tissue blood flow in anesthetized rats

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Feedforward control of cardiac sympathetic nerve activity has been reported to play an important role for rapid cardioaccelerator response at the onset of exercise (Tsuchimochi et al, Am J Physiol 2002, 2009). It is uncertain, however, whether myocardial perfusion is controlled by such a feedforward mechanism during exercise. To address this question, the effect of electrical stimulation of subthalamic locomotor region (SLR), the electrical or chemical stimulation of which is known to elicit coordinated locomotion and concomitant cardiorespiratory responses, on the left ventricular (LV) myocardial perfusion was examined in anesthetized rats. Changes in LV myocardial perfusion and muscle blood flow to the right gastrocnemius muscle were measured by a Laser-Doppler flowmetry. Arterial pressure and heart rate (HR) were also measured. Stimulation of SLR rapidly increased both LV myocardial perfusion and mean arterial pressure (MAP) in a stimulus intensity-dependent manner. Stimulation of SLR also increased gastrocnemius muscle blood flow gradually throughout the stimulus period. Beta-adrenergic blockade attenuated the rapid increase in myocardial perfusion evoked by SLR stimulation. These results suggest that a feedforward control of the myocardial perfusion plays an important role in meeting myocardial demands for adequate blood supply.

### 3PK-013

#### Positive and negative inotropic effects of left ventricular mechanical work and energetics in hypothermic and hyperthermic rat hearts

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We investigated the effects of cardiac hypothermia or hyperthermia on LV myocardial mechanoenergetics using the excised, cross-circulated rat heart model. We analyzed the left ventricular (LV) end-systolic pressure-volume relation (ESPVR) and the linear relation between the myocardial oxygen consumption per beat ( $VO_2$ ) and systolic pressure-volume area (PVA; a total mechanical energy per beat) in isovolumically contracting rat hearts during hypothermia (30°C), normothermia (36°C), and hyperthermia (42°C) under 300-bpm pacing. LV ESPVR shifted downward in cardiac hyperthermia compared with that in cardiac normothermia. In contrast, it shifted upward in cardiac hypothermia. There was no significant difference in the slope of  $VO_2$ -PVA relation among them. In 6 of 9 hearts, cardiac hyperthermia decreased the  $VO_2$  intercept, which is composed of each  $VO_2$  fraction consumed in excitation-contraction coupling (mainly consumed for calcium handling) and consumed for basal metabolism. In contrast, in the remaining 3 hearts, cardiac hyperthermia did not change the  $VO_2$  intercept. The results indicated that cardiac temperature is an important factor for controlling cardiac mechanoenergetics. Especially, excessively high cardiac temperature may induce calcium handling-disorder in E-C coupling.

### 3PK-014

#### The transcriptional regulator, Id2, regulates the cardiac calcium channels expression

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Inhibitor of differentiation/DNA binding protein 2, Id2, is a transcriptional repressor playing a role the heart development. In-vivo studies suggested that Id2 could regulate the expression of genes controlling the electrophysiological activity of the heart. Therefore, we tested the hypothesis that Id2 could regulate the expression of cardiac ion channels expression. For this purpose, we altered the level of Id2 expression in isolated ventricular cardiomyocytes of newborn rats. Id2 overexpression reduced the frequency of spontaneous cardiomyocytes action potentials. In addition, we measured a decrease expression and activity of cardiac L- and T-type voltage-gated calcium channels. On the other hand, the silencing of Id2 by siRNA increased both expression and activity of cardiac calcium channels. Interestingly, the Id2 overexpression prevented the steroid-dependent increase of cardiac calcium channels expression and activity upon addition of steroids known to induce cardiac hypertrophy and arrhythmia, aldosterone and corticosterone. Concomitantly, Id2 expression prevented the steroid-dependent increase of the action potential frequency in cardiomyocytes. In conclusion, our results show that Id2 regulates the expression cardiac calcium channels and prevents the pathological increase in cardiac calcium channel expression.

### 3PK-015

#### Assessment of a piezoelectric transducer sensor for cardiovascular monitoring in anesthetized guinea-pigs

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Piezoelectric transducers (PZTs), which detect mechanical vibrations, have the potential to evaluate cardiac movements by simply putting it under the body of animals. In the present study, we have assessed the heart beat-related PZT signals (h-PZT) in anesthetized guinea-pigs by simultaneously monitoring h-PZT signal, ECG, heart sound, aortic pressure, and central venous pressure. The obtained h-PZT signals displayed characteristic features as the ventricle and atrium changed their shapes during cardiac cycles. A transient positive wave, which occurred approximately at the onset of R wave in ECG, was followed by profound negative wave during systole. The negative wave peaked early in systole and then gradually returned. A small notch which corresponded to the second heart sound was observed at the onset of isovolumetric relaxation. In response to phlebotomy, the size of the negative wave was decreased, accompanied by flattening of h-PZT signals during diastole, whereas saline transfusion increased the negative wave during systole and produced transient outward wave during diastole. On the other hand, administration of isoproterenol accelerated the transient positive wave at the onset of systole markedly. These findings suggest that h-PZT signals might be useful to identify cardiac cycles and to evaluate cardiovascular dynamics during changes in blood volume and cardiac contractility.

### 3PK-016

#### Oscillation model of Ca and cAMP in cardiac myocytes

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In cardiomyocytes,  $\beta$ -adrenergic signal transduction by the sympathetic nervous system is very important for regulating cardiac function. It is well known that intracellular calcium concentration oscillates during cardiac myocyte contraction and relaxation. Because cardiac adenylyl cyclase is directly inhibited by free calcium, such oscillation may also induce oscillation of cAMP concentration within cardiac myocytes. However, there exists adenylyl cyclase isoforms that cannot be inhibited by calcium. Thus, whether oscillation of cAMP occurs in vivo in intact myocytes remain undetermined. We have made a simulated computer model using Cell Designer to understand the relationship between calcium and cAMP as regulated by various molecules within cardiac myocytes, such as PKA or PDE. The amount of calcium-inhibitable adenylyl cyclase isoform was determined according to experimental data obtained from various genetically engineered animal models. We have found that the presence of negative feedback mechanism is important for continuing such oscillation. It is also important that cardiac myocytes contain a large amount of calcium inhibitable adenylyl cyclase isoform. Further, the presence of intermittent catecholamine stimulation is required for lasting oscillation. Our understanding of such oscillation mechanism will be helpful to develop a prediction system to understand the pathophysiology of cardiomyocytes, such as arrhythmia.

### 3PK-017

#### Stromal interaction molecule 1 (STIM1) governs cardiac hypertrophy and fibrosis in response to increased afterload

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**[Background]** Our previous data showed transient receptor potential (TRP) channels and stromal interaction molecule 1 (STIM1) had a crucial role in potentiating cardiac hypertrophy through the  $Ca^{2+}$  entry related pathway. However, role of STIM1 in cardiac fibrosis is still elusive. The present study was designed to examine whether STIM1 is involved in cardiac fibrosis in vivo. **[Methods and Results]** We used STIM1 hetero-knockout (*STIM1*<sup>+/-</sup>) mice subjected to transverse aortic constriction (TAC) operation for the stimulation of fibrotic response. The littermate mice of WT and (*STIM1*<sup>+/-</sup>) were operated, and divided into 4 groups of WT sham, WT TAC, (*STIM1*<sup>+/-</sup>) sham and (*STIM1*<sup>+/-</sup>) TAC. Four weeks later, TAC induced cardiac fibrosis was significantly inhibited in (*STIM1*<sup>+/-</sup>) mice, which showed longer survival rate. DNA micro-array, RTPCR and Western blot analysis in the left ventricles of (*STIM1*<sup>+/-</sup>) showed the suppressed BNP and ANF expressions and specific increase of tissue inhibitor of metalloproteinase 4 (TIMP4) and the resultant matrix metalloproteinase 2 (MMP2) inactivation. In addition, RT-PCR showed that new splice variant of STIM1, named STIM1L was up-regulated in the heart of WT TAC, although the up-regulation was inhibited in (*STIM1*<sup>+/-</sup>). **[Conclusion]** These results provide definitive evidence that STIM1 plays a crucial role for cardiac fibrosis associated with TIMP4/MMP2.

### 3PK-018

#### Reconstruction of the unidirectional conduction of the ventricular action potential ; a simulation study

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It has been suggested that an unidirectional conduction of the action potential is involved in the reentry of action potential (AP) propagation within the cardiac tissue under various pathophysiological conditions. We examined possible abnormal conditions of unidirectional conduction of AP by connecting multiple ventricular cell models simplified for the membrane excitation through gap junctional conductance. The number of ion channel species of the model are largely minimized using only the major currents responsible for generating the characteristic ventricular AP ; such as rapid  $Na^+$  channel for the propagation adjusted for the maximum rate of rise 200 V/sec of AP, the L-type  $Ca^{2+}$  current to maintain the plateau potential, and inward rectifier  $K^+$  current ( $I_{K1}$ ) adjusted to a repolarization rate of  $\sim 2V/sec$ , in addition to  $Na^+$  and  $K^+$  background membrane conductances. The gap junctional conductance was adjusted to give the AP propagation velocity of 0.5 m/s with a cell length of 200  $\mu m$  for a single cell. Preliminary simulation indicated that the unidirectional conduction of AP was achieved by connecting a cluster consisting of larger number of cells, having a uniform potential profile, to a smaller cluster of less number of cells via a limited gap junctional conductance. Namely, AP propagation occurred only from the larger cluster toward smaller cell cluster. We aim at determining the semi-quantitative relationship between the stoichiometry of cell coupling, the amplitude of the  $Na^+$  current and gap junctional conductance by using a one-dimensional array of multiple cells.



### 3PK-019

#### Parasympathetic reflex vasodilation evoked by trigeminal afferent inputs in the rat basal cerebral arteries

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Parasympathetic vasodilator fibers are thought to be important in regulating the hemodynamics in the orofacial area since the parasympathetic vasodilation evoked by trigeminal-mediated reflex significantly increases blood flow in the orofacial tissues. Although basal cerebral arteries and cerebral blood vessels regulating the cerebral hemodynamics are known to be innervated by parasympathetic fibers, it is unclear whether these nerves are involved in the regulation of blood flow to the brain. In the present study, we explored this question by investigating the effects of activation of the parasympathetic nervous system evoked by electrical stimulation of the central cut end of the lingual nerve (LN) on the blood flows in both the internal carotid artery (ICABF) and the cerebral vessels (CBF), and the neural mechanisms mediating these responses in urethane-anesthetized, artificially ventilated and cervically vago-sympathectomized rats. Electrical stimulation of the LN elicited increase of the ICBF accompanied by an increase in the systemic arterial blood pressure (SABP), but not that in the CBF. The ICBF increases were significantly reduced by the intravenous administration of both autonomic cholinergic ganglion blocker hexamethonium and atropine. These indicate that the ICBF increase evoked by activation of the parasympathetic vasodilator fibers occurs via the trigeminal-mediated reflex, suggesting that the parasympathetic reflex vasodilation evoked by trigeminal inputs may play an important role in the maintenance of the cerebral hemodynamics.

### 3PK-020

#### Purinergic signalling in the rostral ventro-lateral medulla controls sympathetic drive and contributes to the progression of heart failure in rats

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Heart failure may lead to hypoperfusion and hypooxygenation of tissues which triggers ATP release within CNS circuits controlling sympathetic outflow. Here, we studied whether: a) activated brainstem astroglia release ATP and via ATP release activate sympathoexcitatory neurones of the rostral ventrolateral medulla (RVLM); b) ATP actions in the RVLM contribute to sympathoexcitation, progression of left ventricular (LV) remodeling and development heart failure. In vitro, optogenetic activation of RVLM astrocytes expressing channelrhodopsin-2 activated RVLM neurones in ATP-dependent manner. In vivo, optogenetic activation of RVLM astrocytes increased sympathetic renal nerve activity and arterial blood pressure. To interfere with ATP-mediated signalling by promoting its extracellular breakdown we developed a lentiviral vector to express an ectonucleotidase-transmembrane prostatic acid phosphatase (TMPAP) on the cellular membranes. In rats with myocardial infarction-induced heart failure, bilateral expression of TMPAP in the RVLM reduced plasma noradrenaline concentration, maintained LV end diastolic pressure, attenuated decline in dP/dTmax and shifted the LV pressure-volume relationship curve to the left. Conclusions: Activated RVLM astrocytes are capable of increasing sympathetic activity via release of ATP while facilitated breakdown of ATP in the RVLM attenuates the progression of LV remodeling and heart failure.

### 3PK-021

#### Clinical Manifestations and Electrophysiological Characteristics of K Channel Mutations Responsible for Short QT Syndrome

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**Background:** Short QT syndrome (SQTS) is a rare and lethal hereditary arrhythmia characterized by short QT interval on ECG. Mutations have been identified in four ion channel genes including *KCNH2* and *KCNQ1*. **Purpose:** To determine the genetic basis of SQTS in Japan and evaluate the electrophysiological properties of the mutations. **Methods:** We genetically screened four SQTS families and analyzed the functional properties of mutant channels using whole-cell patch clamp. **Results:** In addition to the previously reported two K channel mutations (*KCNQ1*-V141M and *KCNH2*-T618I), we found a novel *KCNH2* mutation I560T in an asymptomatic patient associated with paroxysmal atrial fibrillation (AF) and family history of sudden death. Heterologously expressed I560T channel showed 4.1 fold increase of the IKr current density than wild-type. Voltage-dependence of inactivation of I560T showed a depolarizing shift of +17.6 mV, and that of activation exhibited a shift of -13.2 mV. These gating properties of IKr, similar to the ones of T618I, tend to shorten the action potential duration in both ventricle and atrium, which predisposes affected individuals to malignant ventricular arrhythmias and paroxysmal AF. **Conclusions:** We identified three mutations in *KCNH2* and *KCNQ1* in Japanese SQTS families. These mutations similarly result in gain-of-function properties, but the clinical manifestations are considerably variable.

## Poster Presentations

### Neuron, Synapse(3)

#### 3PK-022

##### An optogenetic characterization of synaptic responses from the medial prefrontal cortex to amygdala

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The amygdala plays a pivotal role in emotional processing in concert with several brain regions. In rodents, it has been shown that the medial prefrontal cortex (mPFC) modulates activity of the amygdala, and that dysfunction of a neural circuit composed of mPFC and amygdala was induced when animals were exposed to intense stressful events. However, precise synaptic connectivity between mPFC and amygdala, and its changes by stress remain unknown. In this study, we utilized optogenetic techniques in mice to investigate specific synaptic response at mPFC-amygdala synapses, and the effect of stress on those synapses. Mice were injected with AAV vectors encoding channelrhodopsin 2 and yellow fluorescent protein (AAV-ChR2-YFP) into their mPFC. Three weeks after the injection, whole-cell patch-clamp recordings were performed from pyramidal neurons in the basolateral nucleus of the amygdala (BLA). We found that photo-irradiation (465 nm) of ChR2 at the axon terminal of the mPFC afferents to the BLA pyramidal neuron could evoke monosynaptic response. The photo-activated responses showed a gradual decrease in amplitude in response to repetitive stimulation of BLA pyramidal neurons, but not the ChR2-expressing neurons in the mPFC. These findings provide basic information for pursuing the functional alteration of the mPFC-amygdala circuit by stress.

#### 3PK-023

##### Electrophysiological Properties of Neuronal Cells in Mouse Dorsal Raphe Nucleus

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The dorsal raphe nucleus (DRN) is the origin of central serotonin (5-HT) system and plays an important role in the regulation of many physiological functions such as sleep/arousal, food intake and mood. In order to understand the regulatory mechanisms of 5-HT system, it is necessary to characterize the types of neurons in the DRN. Then, we performed the electrophysiological recordings in acute slices of glutamate decarboxylase (GAD) 67-GFP knock-in mice (postnatal days 21-27). We utilized this mouse to discriminate visually between GABAergic (GAD+) and non-GABAergic (GAD-) cells. Among GAD-cells, serotonergic neurons could be distinguished by immunoreactivity for tryptophan hydroxylase (TPH). Putatively, remaining GAD-neurons might be glutamatergic. Therefore, in this study, properties of three types of neurons were explored. Compared with serotonergic neurons, both GFP+ and glutamatergic neurons displayed a narrower half-height width of the action potentials. Moreover, the input-output relationship curve of serotonergic neurons were slower than that of both GFP+ and glutamatergic neurons. Thus, the neurons containing amino acid transmitters in the DRN have some distinct cellular profiles among DRN neurons. Further exploration of their synaptic and pharmacological properties in the DRN would provide a better understanding of 5-HT system in the brain.

#### 3PK-024

##### Improvement of fluoro-patch-electrode for recording calcium fluorometry and electrical activity from a single neuron

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Recordings of electrical activity in parallel with molecular dynamics from single neurons should be essential to decipher the neural circuit operation. Recent development of high-resolution optical imaging devices, such as two-photon microscopy and calcium imaging, have promoted our understanding of molecular dynamics within the active neuron; however, most neural activities are conducted in the deep brain tissue and are out of the reach of high-resolution optical imaging. Now, we have established a quartz patch electrode recording method intending to monitor the fluorescence signal from a neuron in parallel with the electrical activity. Through a quartz patch electrode, we succeeded to deliver and collect light from the tip of the patch electrode, and to record the neural electrical activities. By labeling neurons with a genetically encoded eCFP-eYFP based FRET responsible Ca<sup>2+</sup> indicator, or a synthetic Ca<sup>2+</sup> indicator, Ca<sup>2+</sup> responses of neurons were recorded, which were evoked in response to high K<sup>+</sup> medium or electrical stimulation in slice preparations, or in response to white noise sound stimulation from the inferior colliculus in vivo of a chick. The optical recording from the tip of quartz patch electrode is reliable, and will be a powerful approach to understand the electrical and molecular dynamics of neurons located in the deep brain tissue.

### 3PK-025

#### Integration of sound evoked synaptic currents and action potential generation in nucleus magnocellularis of young chicken

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In avian auditory system, the sound time information is extracted from auditory nerve fibers (ANFs) in the nucleus magnocellularis (NM). NM neurons generate spikes in Hi-Fidelity manner for middle/high characteristic frequency (CF, 500~ Hz) sound, because of a large EPSC generated by a small number of end-bulb shaped terminals formed on the cell soma, while for low CF (~500 Hz) sound, spike timing jitter is reduced during transmission because of an integration of many small EPSCs generated by a large number of small bouton shaped terminals. Since there is no direct observation of synaptic integration and firing in NM neurons *in vivo*, we recorded sound evoked synaptic activities and resulting action potentials simultaneously in NM neurons of chicks by *in vivo* loose-patch voltage-clamp recordings. We investigate how the different form of ANFs-NM synapse between low and high CF NM neurons contributes to the transmission of the time information of sound.

### 3PK-026

#### A novel GABAergic action mediated by functional coupling between GABA<sub>B</sub> receptor and two different high conductance K<sup>+</sup> channels in insect central neurons

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In this study, we report a novel coupling of GABA<sub>B</sub>-like receptor with two different high conductance K<sup>+</sup> channels, Na<sup>+</sup>-activated K<sup>+</sup> (K<sub>Na</sub>) channel and Ca<sup>2+</sup>-activated K<sup>+</sup> (K<sub>Ca</sub>) channel in Kenyon cells isolated from the mushroom body of the cricket brain. Single-channel activities of K<sub>Na</sub> and K<sub>Ca</sub> channels in response to bath applications of GABA and GABA<sub>B</sub> specific agonist SKF97541 were recorded with cell-attached patch configuration. The open probability (Po) of both K<sub>Na</sub> and K<sub>Ca</sub> channel was found to be increased by bath application of GABA and this increase in Po was antagonized by co-application of GABA<sub>B</sub> antagonist CGP54626 suggesting that GABA<sub>B</sub>-like receptors mediate these actions. Similarly GABA<sub>B</sub> specific agonist SKF97541 increased the Po of both K<sub>Na</sub> and K<sub>Ca</sub> channels. Perforated patch recordings using β-escin further revealed that SKF97541 increased the amplitude of the outward currents elicited by step depolarizations. Under the current clamp condition, SKF97541 decreased the firing frequency of spontaneous action potential (AP) and changed the AP wave form. The amplitude and the duration of AP were decreased whereas the afterhyperpolarization of AP was increased. Resting membrane potential, however, was not significantly altered by SKF97541. Taken together, these results have suggested that GABA<sub>B</sub>-like receptor is functionally coupled with both K<sub>Na</sub> and K<sub>Ca</sub> channels and this coupling mechanism may serve to prevent the AP formation and limit excitatory synaptic input.

### 3PK-027

#### Acute effect of X-irradiation on adult mouse brain

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Brain irradiation is an effective tool in the therapy of brain tumor. Although X-irradiation on brain is known to generate sometimes serious damage on brain function, the underlying mechanism of irradiation effects is still uncertain. In this study we examined the effects of X-irradiation on neurons *in vivo*; focusing on drebrin, an actin-binding protein, as a post-synaptic marker and as an indicator of synaptic function. In addition, we used Synapsin I and doublecortin as markers of pre-synapses and newly-generated neurons, respectively. Left hemispheres of the mouse brains were irradiated with a single dose of 10 Gy. We analyzed irradiation effects by comparing the immunofluorescence intensity between the irradiated and non-irradiated side. Mice were sacrificed after 0.5, 2, 24, and 48 hours post irradiation. The intensities of drebrin and Synapsin I in the neuropil region were decreased at 0.5 and 2 hours in the irradiated side. Interestingly, however, the staining intensities of those proteins were recovered within 24 hours, indicating that a single dose of 10 Gy irradiation induced a transient effect on both pre- and post-synapses. On the other hand, the neuronal cell death of newly-generated neurons was observed at 24 hours. Together it is indicated that a single dose of 10 Gy X-irradiation causes the cell death of newly-generated neurons and the transient effect on both of the presynaptic and postsynaptic elements of mature neurons.

### 3PK-028

#### Optogenetic activation of ChR2-expressing astrocytes evokes neuronal firing *in vivo*

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Astrocytes are supposed to be involved in synaptic transmission. Cytosolic calcium (Ca<sup>2+</sup>) level of an astrocyte has been widely used to monitor its activity. Indeed, we reported that astrocytic Ca<sup>2+</sup> increase induced by the cholinergic nucleus in the basal forebrain (nucleus basalis of Meynert; NBM) is necessary for the induction of synaptic plasticity in the cortex *in vivo* (Takata et al., 2011 JNS). In addition to Ca<sup>2+</sup> surge, NBM induces a few mV depolarization of astrocytic membrane potential. While astrocytes express voltage dependent channels, physiological role of astrocytic membrane fluctuation is not clear, perhaps due to lack of a method to depolarize astrocytes without Ca<sup>2+</sup> surge. Recently, we succeeded in generating transgenic mice expressing a light-sensitive channelrhodopsin-2 (ChR2) in astrocytes (Tanaka et al., 2012 Cell Rep), whose membrane potential was depolarized for a few mV without Ca<sup>2+</sup> surge upon light illumination. Unexpectedly, *in situ* hybridization for c-fos revealed that light-induced activation of astrocytes in the parietal cortex induced neuronal activation in the entire ipsilateral cortex. To investigate time course of the neuronal activity-propagation, we're now performing multi-site local field potential recording using silicon probes. Our preliminary results show that light-induced astrocytic depolarization evoked neuronal firing nearby.

### 3PK-029

#### Motor skills training improved motor function and enhanced synaptic plasticity in the sensorimotor and striatum following intracerebral hemorrhage in rats

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We investigated the effects of motor skills training on some kinds of motor function and synaptic plasticity associated protein following intracerebral hemorrhage (ICH) in rats. Male Wistar rats were placed in a stereotaxic apparatus and injected with collagenase into the left striatum to induce ICH. They were randomly assigned to sham or ICH. Each group was divided into motor skills training and control (no exercise) group. Motor skills training group were performed acrobatic training from 4 to 28 days after surgery. Motor functions were assessed by motor deficit score, horizontal ladder test and beam walking test at some time points after ICH. The number of DeltaFosB positive cells was counted using immunohistochemistry and PSD-95 protein levels at bilateral sensorimotor cortices and striata were analyzed by Western blotting at 14 and 29 days after ICH. Motor skills training following ICH were significantly improved motor function. The number of DeltaFosB positive cells and PSD-95 protein level of acrobatic group were significantly increased more than those of control group in bilateral sensorimotor cortices and striata. We demonstrated that motor skills training improved motor function after ICH in rats, and enhanced neural activity and synaptic plasticity in the striatum and sensorimotor cortex.

### 3PK-030

#### Synaptic Potentiation in the Nociceptive Amygdala Following Fear Learning in Mice

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Pavlovian fear conditioning is a form of associative learning, which depends on associative synaptic plasticity in the amygdala. While the central amygdala is suggested to play an active role in fear learning, little is known about its synaptic regulation. The capsular part of the central amygdala (CeC) receives direct nociceptive information from the lateral parabrachial nucleus (IPB), as well as highly processed polymodal signals from the basolateral nucleus of the amygdala (BLA). Therefore, we focused on CeC as a convergence point for polymodal and nociceptive signals, and show that fear conditioning results in synaptic potentiation in both BLA-CeC and IPB-CeC synapses. This potentiation is dependent on fear learning rather than on nociceptive or sensory experience, or fear retrieval. We also found that the synaptic weight of the IPB-CeC and BLA-CeC pathways was correlated in fear-conditioned mice, suggesting that fear learning may induced activity-dependent heterosynaptic interactions. This synaptic potentiation is associated with both postsynaptic and presynaptic changes. These results indicate that the CeC may provide an important locus of Pavlovian association in addition to the well-established plasticity of the lateral amygdala, and suggest that the multi-step system of the association contributes to the highly orchestrated nature of fear learning.

### 3PK-031

#### Firing pattern and morphological analysis of substantia gelatinosa neurons receiving TRPA1-expressing afferents in rat spinal dorsal horn

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The TRPA1-channel has been proposed to be a molecular transducer of cold and inflammatory nociceptive signals. It is expressed on a subset of small primary afferent neurons both in the peripheral terminals, where it serves as a sensor, and on the central nerve endings in the dorsal horn. We examined how activation of TRPA1 channels affects synaptic transmission onto substantia gelatinosa (SG) neurons using whole-cell patch-clamp recordings in adult rat spinal cord slices. We have shown that cinnamaldehyde (TRPA1 agonist) elicited a barrage of EPSCs in a subset of the SG neurons. In this study, we examined the firing patterns of SG neurons receiving CA-sensitive afferent fibers. Most (78%) of the CA-sensitive SG neurons were the delayed and sustained repetitive firing types, although CA-insensitive cells were also found in these two types of SG neurons. A previous study explored the relationship between the morphological class of SG neurons and their firing pattern, has shown that vertical cells exhibit the delayed or sustained firing type, and radial cells exhibit the phasic firing type. Moreover, central cells are reported to exhibit the initial firing pattern of discharge. These results indicate that activation of spinal TRPA1 pre-synaptically facilitates miniature excitatory synaptic transmission from primary afferents onto mainly delayed firing and sustained repetitive firing type neurons (these firing type were exhibited most of vertical and radial cells).

### 3PK-032

#### Functional roles of monocarboxylate transporters in maintenance of membrane potential and synaptic transmission in the cerebellum

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Neurons consume high levels of ATP molecules to maintain their activities. In addition to extracellular glucose, lactate of astrocyte origin is utilized as substrate of ATP production in neurons. Monocarboxylate transporters (MCTs) are key molecules in lactate transfer from astrocytes to neurons. Of three subtypes expressed in the CNS, MCT1 and 4 are expressed in astrocytes and MCT2 are in neurons, in particular at excitatory postsynapses, as recently shown in parallel fiber-Purkinje synapses (Bergersen et al., 2005). This study was undertaken to identify the effects of pharmacological blockage of MCTs on neuronal activities. We recorded excitatory postsynaptic current (eEPSC) from Purkinje neurons evoked by parallel fiber stimulation in cerebellum slices of mice and analyzed effects of a MCT inhibitor, alpha-cyano-4-hydroxycinnamic acid (4-CIN), thereon in the presence of extracellular glucose and intracellular ATP. 4-CIN (1 mM; 15 min) significantly decreased eEPSC amplitude to  $17.4 \pm 3.4\%$  of pre-application value ( $n=5$ ) and increased paired-pulse ratio from  $1.71 \pm 0.06$  to  $2.51 \pm 0.09$ ;  $n=5$ ), suggesting that the presynaptic release probability is decreased by MCT inhibition. 4-CIN induced an outward shift of the holding currents ( $+201.4 \pm 88.7$  pA;  $n=5$ ) and hyperpolarized the membrane potential ( $-16.4 \pm 3.4$  mV;  $n=8$ ). Those results suggest that maintenance of pre- and postsynaptic functions in addition to that of membrane potential primarily depends on MCT-mediated energy transfer.

### 3PK-033

#### Detection of interaural time difference and computer simulation

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Sounds are coded as a spike train of auditory nerve fiber (ANF) and various features of sounds are extracted in the cochlear nuclei of the brainstem. In birds, ANF innervates two cochlear nuclei; nucleus magnocellularis (NM) and nucleus angularis. NM is specialized for the transmission of sound phase in formation bilaterally to the nucleus laminaris (NL), where the interaural time difference (ITD) is first processed. The axonal projection from the contralateral NM to the NL is longer than that from the ipsilateral NM. The best ITD of NL neurons are between 0-400  $\mu$ s, which corresponds to the sound source located in from the center to the contralateral side. We compared the characteristic frequency (CF) of ipsilateral NM neuron and contralateral NM neuron that project to the same NL neuron. The CF of contralateral NM inputs were consistently higher than that of ipsilateral NM inputs. Because of the cochlear delay, high CF NM units fire temporally faster than lower CF NM units. These results indicate the cochlear delay have important role in the ITD processing. Furthermore, by using computer simulation, we will discuss the detail and advantages of ITD detection using the different CFs.

### 3PK-034

#### PKG accelerates vesicle endocytosis via Rho-associated protein kinase at the calyx of Held synapse

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At synapses, neurotransmitter is stored in synaptic vesicles, and the vesicles are fused into presynaptic terminal membranes to release the neurotransmitter when an action potential reaches to the presynaptic terminal. Fused vesicle membranes are retrieved into presynaptic terminals by endocytosis, and recycled to be reused for synaptic transmission. Previously we showed that presynaptic protein kinase G (PKG), which activated by NO released from postsynaptic cells, accelerated the time course of endocytosis at the calyx of Held synapses of rats after hearing onset. Although the PKG activity accelerated vesicle endocytosis via elevating presynaptic PIP<sub>2</sub> level, it was unknown how PKG regulates presynaptic PIP<sub>2</sub>. In this study, we found that Rho-associated protein kinase (ROCK) inhibitor slowed vesicle endocytosis. The effect of ROCK inhibitor was mimicked and occluded by a PKG inhibitor. The inhibitory effect of ROCK inhibitor on endocytosis was rescued by a direct injection of PIP<sub>2</sub> into presynaptic terminals. Consistently, the ROCK inhibitor lowered the PIP<sub>2</sub> level in the rat brainstem tissue, assessed by both ELISA and Mass-spectrometric analyses. These results suggest that ROCK links the retrograde NO-PKG pathway to intraterminal PIP<sub>2</sub>, thereby upregulating endocytosis of synaptic vesicles.

### 3PK-035

#### The functional synaptic connection of the GABAergic neurons in the striatum was examined by the multicellular calcium imaging

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The striatum is the primary input region of the basal ganglia. The striatal GABAergic projection neurons (MSNs) receive excitatory inputs from the cortex and send inhibitory outputs to the other nuclei of the basal ganglia. The striatal interneurons also receive excitatory inputs from the cortex, and make inhibitory synaptic connections to the MSNs. However, the striatal neuronal dynamics had not been clear. Therefore, we recorded the multicellular spontaneous activities of striatal GABAergic neurons using the calcium imaging. The activities of the GABAergic neurons were increased in the condition of perfusing Mg<sup>2+</sup>-free, 5 mM K<sup>+</sup> artificial cerebrospinal fluid (ACSF) in both of the corticostriatal slice and the striatal slice. In the condition of perfusing Mg<sup>2+</sup>-free, 5 mM K<sup>+</sup> ACSF, the activities of the GABAergic neurons in the striatal slice were higher than that of the corticostriatal slice. This result suggested that a MSN and a striatal interneuron which made inhibitory synaptic connection to that MSN received excitatory inputs from the same cortical projection neuron. We also showed synchronized activities of the multi GABAergic neurons in the corticostriatal slice. This result suggested that multi-MSNs were innervated by the same cortical projection neuron.

### 3PK-036

#### Cholinergic neurons in the rat medial vestibular and prepositus hypoglossi nuclei show distinct current responses dependent on the expression of specific nicotinic acetylcholine receptor subtypes

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Cholinergic system in the medial vestibular nucleus (MVN) and prepositus hypoglossi nucleus (PHN) plays important roles in horizontal eye movements. In our previous study using whole-cell recordings in brainstem slices obtained from choline acetyltransferase (ChAT)-tdTomato transgenic rats, we found that the application of acetylcholine (ACh) induced inward currents in cholinergic neurons in the MVN and PHN. The inward currents were composed of both nicotine- and muscarine-sensitive currents, and nicotine-sensitive currents were larger than muscarine-sensitive currents. In the present study, we characterized nicotine-sensitive currents in MVN and PHN cholinergic neurons. Based on the activation and desensitization kinetics, nicotine-sensitive currents were separated into fast, slow and fast & slow mixed types. Pharmacological analyses revealed that the fast and slow types were mostly abolished by methyllycaconitine (MLA) and dihydro- $\beta$ -erythroidine (DH $\beta$ E), respectively, and the mixed type was abolished by MLA plus DH $\beta$ E. This finding indicates that the fast and slow types of nicotine-sensitive currents are mediated by  $\alpha$ 7 and  $\alpha$ 4 $\beta$ 2 subtype of nicotinic ACh receptors, respectively. All these results suggest that cholinergic neurons in the MVN and PHN show distinct cholinergic responses, dependent on the expressions of  $\alpha$ 7 and/or  $\alpha$ 4 $\beta$ 2 nicotinic ACh receptors.

### 3PK-037

#### Excitatory synaptic inputs to tuberoinfundibular dopaminergic neurons in mice under fed and fasted condition

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Dopamine neurons located in the arcuate nucleus and adjacent periventricular region of the mediobasal hypothalamus are called tuberoinfundibular dopaminergic (TIDA) neurons (A12). A part of their functions is to inhibit PRL release from the anterior pituitary as a PRL inhibitory factor. Sulpiride, a dopamine D2 receptor blocker, acts as an antidepressant and stimulates food intake, suggesting that TIDA neurons are involved in the controlling feeding behavior but its role on feeding states is not documented. We determined the excitatory synaptic inputs to TIDA neurons during fed and fasted condition. To this end, transgenic mice carrying green fluorescent protein under the control of the rat tyrosine hydroxylase gene (Matsushita et al, *J Neurochem* 2002) were used. In fed and fasted male mice at the age of 8 weeks, whole-cell voltage-clamp techniques in acute slice were applied. TIDA neurons were identified by fluorescence microscopy. The frequency of miniature excitatory post synaptic potential (mEPSP) in fasted mice was significantly decreased compared to that in fed mice. However, the mean amplitude of mEPSP in fasted rats was not different from that in fed mice. The present study revealed that excitatory inputs to TIDA neurons were decreased during fasted condition. We suggest that TIDA neurons are involved in the controlling feeding behavior.

### 3PK-038

#### NEURON AND SYNAPTIC FUNCTIONS

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Differential gene expression specifies the highly diverse cell types that constitute the nervous system. With its sequenced genome and simple, well-defined neuroanatomy, the nematode *C. elegans* is a useful model system in which to correlate gene expression with neuron identity. The UNC-4 transcription factor is expressed in thirteen embryonic motor neurons where it specifies axonal morphology and synaptic function. These cells can be marked with an *unc-4::GFP* reporter transgene. Here we describe a powerful strategy, Micro-Array Profiling of *C. elegans* cells (MAPCeL), and confirm that this approach provides a comprehensive gene expression profile of *unc-4::GFP* motor neurons in vivo. Fluorescence Activated Cell Sorting (FACS) was used to isolate *unc-4::GFP* neurons from primary cultures of *C. elegans* embryonic cells. Microarray experiments detected 6,217 unique transcripts of which ~1,000 are enriched in *unc-4::GFP* neurons relative to the average nematode embryonic cell. The reliability of these data was validated by the detection of known cell-specific transcripts and by expression in UNC-4 motor neurons of GFP reporters derived from the enriched data set. In addition to genes involved in neurotransmitter packaging and release, the microarray data include transcripts for receptors to a remarkably wide variety of signaling molecules. The added presence of a robust array of G-protein pathway components is indicative of complex and highly integrated mechanisms for modulating motor neuron activity. Over half of the enriched genes we have described a microarray-based method, MAPCeL, for profiling gene expression in specific *C. elegans* motor neurons.

### 3PK-039

#### Climbing fiber network refinement and desynchronization of Purkinje cell population activity during postnatal development

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The inferior olive of the medulla oblongata sends glutamatergic afferents to the cerebellum. In adult rodents, axons of inferior olivary neurons divide into several climbing fibers (CFs) that innervate cerebellar Purkinje cells (PCs) in a one-to-one manner. Activation of CFs generates bursts of action potentials known as complex spikes (CSs) in PCs. CSs in neighboring PCs are synchronized due to electrical coupling between inferior olivary neurons. During early postnatal period, however, the pattern of CS activity in a population of PCs remains unclear. CF-PC synapses undergo dynamic changes during the first two postnatal weeks including selective strengthening of a single CF and elimination of redundant CFs in each PC. In this study, we used in vivo two-photon calcium imaging to monitor the CF responses from population of PCs during the period of the CF network refinement. We found that the CS activity was highly correlated among neighboring PCs in newborn mice and was progressively desynchronized during the first postnatal week. The synchrony reached the adult level at postnatal day 8. We also analyzed PC-specific *Ca<sub>v</sub>2.1* knock out mouse, in which the CF synapse refinement is impaired. The desynchronization of CS activity observed in wild type mice was incomplete in the knockout mouse. These results suggest that the desynchronization of CS activity partly result from the CF network refinement.

## Poster Presentations

### Ionic Channel, Receptor(3)

### 3PK-040

#### Comparison among various types of anticonvulsant in the extent of the inhibition of compound action potentials in the frog sciatic nerve

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Although anticonvulsants used for treating neuropathic pain are known to have various actions such as voltage-gated Na<sup>+</sup> and Ca<sup>2+</sup> channel inhibition and GABA<sub>A</sub>-receptor channel activation, it has not been fully examined whether there is a difference among various types of anticonvulsant in inhibiting nerve conduction. The present study examined the effects of anticonvulsants on fast-conducting and a voltage-gated Na<sup>+</sup>-channel blocker tetrodotoxin-sensitive compound action potential (CAP) recorded from the frog sciatic nerve by using the air-gap method. Classic types of anticonvulsant, carbamazepine (iminostilbene derivative) and lamotrigine (phenyltriazine derivative), reduced the peak amplitude of the CAP with the IC<sub>50</sub> values of 0.56 mM and 0.67 mM, respectively. These values were similar to those of local anesthetics, leobupivacaine and lidocaine (IC<sub>50</sub>: 0.45 mM and 0.74 mM, respectively). On the other hand, new types of anticonvulsant, gabapentin, sodium valproate and topiramate, at a high concentration such as 10 mM had no effect on CAPs. These results indicate that there is a difference in the extent of nerve conduction inhibition among anticonvulsants and that some anticonvulsants have an ability to inhibit nerve conduction with an efficacy similar to those of local anesthetics. These findings may serve to know which kinds of anticonvulsant should be used to alleviate neuropathic pain with a minimal side effect.

### 3PK-041

#### Differences in kainic acid-induced inward currents between vasopressin-eGFP and oxytocin-mRFP1 neurons isolated from the supraoptic nucleus in the transgenic rats

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The supraoptic nucleus consists of two types of magnocellular neurosecretory cells, arginine vasopressin (AVP) and oxytocin (OXT). Recently, we have developed the generation and characterization of rats that express an AVP-enhanced green fluorescent protein (eGFP) and an OXT-monomeric red fluorescent protein 1 (mRFP1) fusion transgene. These transgenic rats enable the visualization of AVP or OXT neurons identifiably. Taking advantage of this, we examined the difference between the AVP-eGFP neurons and the OXT-mRFP1 neurons in response to glutamate receptors. We focused on the kainate receptors (KARs), and kainic acid (KA)-induced currents between AVP-eGFP neurons and OXT-mRFP1 neurons were compared by using the whole-cell patch-clamp technique. The KA-induced currents in the OXT-mRFP1 neurons were significantly larger than those in the AVP-eGFP neurons. The significant difference in KA-induced currents between the AVP-eGFP neurons and the OXT-mRFP1 neurons was abolished in the presence of the GluK1-containing KARs antagonist UBP302. These findings suggest that there may be a significant difference between the AVP neurons and OXT neurons isolated from the SON in the component of KA-induced currents inhibited by UBP302.

### 3PK-042

#### Hypotonicity-activated Cl<sup>-</sup> currents in the principal cells of isolated rat Kidney cortical collecting Ducts

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The efflux of K<sup>+</sup> and Cl<sup>-</sup> through ion channels plays key roles in the regulatory volume decrease (RVD). We have previously demonstrated that hypotonicity activates the apical BK channel in principal cells of the rat cortical collecting ducts (CCDs). However, the Cl<sup>-</sup> channel involved in the RVD in CCDs has not been identified. In this study, we investigated the hypotonicity-activated Cl<sup>-</sup> currents using whole-cell voltage clamp, and the expression of ClC-3, which is a molecular candidate for volume-regulated Cl<sup>-</sup> channel, using immunofluorescence staining in principal cells of rat CCDs. Even in the control condition with isotonic NMDG-Cl bath solution, Cl<sup>-</sup> channel blockers, NPPB and niflumic acid (NFA) largely inhibited the whole-cell currents. The extracellular hypotonicity increased the amplitude of the whole-cell current, whose reversal potential was close to the Cl<sup>-</sup> equilibrium potential. The increased current was also markedly attenuated by NPPB and NFA. The Replacement of extracellular Cl<sup>-</sup> by I<sup>-</sup> almost abolished the hypotonicity-activated current. Immunofluorescence staining revealed that ClC-3 expression was partly localized in the intercalated cells but not principal cells in rat CCDs. These results suggest that a NPPB and NFA-sensitive hypotonicity-activated Cl<sup>-</sup> channel is expressed in the surface membrane of principal cells of rat CCDs. However, involvement of ClC-3, a possible candidate for the molecular entity responsible for the hypotonicity-activated Cl<sup>-</sup> current, in RVD of the principal cells of CCD could be excluded.

### 3PK-043

#### Subcellular localization of voltage-gated proton channels in primary cultured microglia

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Voltage sensor only protein, VSOP/Hv1, consists of voltage sensor domain lacking pore domain. It functions as voltage-gated proton channels (Sasaki et al, Science, 2006). In phagocyte such as neutrophil and macrophage, VSOP has a crucial role in the production of reactive oxygen species (ROS) by compensating for charge imbalance upon electron extrusion by NADPH oxidase. The previous study suggested that expression of VSOP is specific to microglia in mouse brain. However, subcellular localization of VSOP in microglia and its dynamics have not been well documented. In the present study, the distribution of VSOP was examined by immunocytochemistry in primary cultured microglia. Without any chemical stimulation, numerous small VSOP-positive vesicles were observed in the intracellular compartments of microglia. These signals were not observed in microglia from VSOP knockout mice. When microglia was stimulated by 100 μM ATP, a strong chemoattractant for microglia, VSOP-positive signal was observed in the cell membrane, indicating their recruitment to the cell surface. On the other hand, when phagocytosis of fluorescence labeled zymosan was induced in microglia, the internalized particle was surrounded by VSOP-containing patches. Our present study indicates dynamic mobilization of VSOP-containing vesicles dependent on the physiological state of microglia. The identity of VSOP containing vesicle in microglia is discussed.

### 3PK-044

#### Linoleic acid derivative DCP-LA increases membrane surface localization of $\alpha 7$ ACh receptor in a protein 4.1N-dependent manner

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In the yeast two-hybrid screening, protein 4.1N, a scaffolding protein, was identified as a binding partner of  $\alpha 7$  acetylcholine (ACh) receptor. For rat hippocampal slices, the linoleic acid derivative 8-[2-(2-pentyl-cyclopropylmethyl)-cyclopropyl]-octanoic acid (DCP-LA) increased association of  $\alpha 7$  ACh receptor with 4.1N, and the effect was inhibited by GF109203X, an inhibitor of protein kinases C (PKC), although DCP-LA did not induce PKC phosphorylation of 4.1N. For PC-12 cells, presence of  $\alpha 7$  ACh receptor in the plasma membrane fraction was significantly suppressed by knocking-down 4.1N. DCP-LA increased presence of  $\alpha 7$  ACh receptor in the plasma membrane fraction, and the effect was still inhibited by knocking-down 4.1N. In the monitoring of  $\alpha 7$  ACh receptor mobilizations, DCP-LA enhanced signal intensities for  $\alpha 7$  ACh receptor at the membrane surface in PC-12 cells, which was clearly prevented by knocking-down 4.1N. Taken together, the results of the present study show that 4.1N interacts with  $\alpha 7$  ACh receptor and participates in the receptor tethering to the plasma membrane. The results also indicate that DCP-LA increases membrane surface localization of  $\alpha 7$  ACh receptor in a 4.1N-dependent manner under the control of PKC, but without phosphorylating 4.1N.

### 3PK-045

#### Regulation of neurotransmission through inter-GPCR interplay

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Various guanine nucleotide-binding protein (G protein)-coupled receptors (GPCRs) localized at cytoplasmic membrane of mammalian cells. Recently, there is increasing reports for GPCRs form not only homogeneous but also heterogeneous complex formation which exert signal integration. In previous reports, we show functional interaction between type-1 metabotropic glutamate receptor (mGluR1) and other GPCRs. mGluR1 is a key molecule for inducing synaptic plasticity including long-term depression (LTD) at cerebellar parallel fiber-Purkinje cell synapse, which is crucial for motor learning. We found colocalization and closely association of mGluR1 with metabotropic gamma-aminobutyric acid receptor (GABA<sub>B</sub>R). Furthermore, our electrophysiological analysis showed mGluR1 signaling and mGluR1 dependent LTD is enhanced by activation of GABA<sub>B</sub>R in cultured Purkinje cells. Furthermore, we show signal crosstalk between mGluR1 and adenosine A1 receptor (A1R), each of which plays a different individual role, functionally interact and regulate synaptic plasticity in central neurons. Here we show molecular mechanism and functional interaction of these GPCRs complex in central neurons.

### 3PK-046

#### Kv3.3 channels harboring a missense mutation of spinocerebellar ataxia type 13 alter neuronal excitability and induce apoptotic cell death in cultured cerebellar Purkinje cells of mice

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The cerebellum plays a role in the sensorimotor functions. The output from cerebellar cortex is transmitted by Purkinje cells (PCs), whose impairments cause cerebellar ataxia. Recently, studies have shown that mutations in the voltage-gated potassium channel Kv3.3 are responsible for spinocerebellar ataxia type 13 (SCA13). SCA13 is an autosomal dominant disease and the patients exhibit cerebellar atrophy. In rodent brain, Kv3.3 mRNAs are expressed most strongly in PCs, implying that the mutations severely affect PCs of SCA13 patients. Nevertheless, how the mutant channels alter cell morphology and electrophysiological properties of cerebellar neurons remain unclear.

To investigate these issues, Kv3.3 gene with a missense mutation found in a SCA13 pedigree was expressed in a primary culture of mouse cerebellum. The effects were examined by immunohistochemistry and patch-clamp recording. Whereas PCs expressing GFP or wild-type Kv3.3 showed normal dendritic development at 10 days in vitro (DIV), those expressing mutant Kv3.3 displayed defective dendritic development and apoptotic cell death. Moreover, patch-clamp analysis revealed that PCs expressing mutant Kv3.3 showed decreased outward current, broadened action potentials and altered firing properties at 8-10 DIV. Thus we established a neuronal culture model of SCA13, which would be useful for examining the pathophysiology and drug screening for SCA13.

### 3PK-047

#### Rapid evaluations of optical voltage reporters using field-induced transmembrane potential response

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Externally-applied electric field was used to induce transmembrane potential response in undifferentiated N2a cells with spherical shapes. While angle dependency and magnitude of steady-state responses agreed well with those predicted by Schwan's equation in normal N2a cells, we found significant deviation in cells expressing inwardly rectifying potassium channels. We show that this deviation can be effectively utilized to rapidly evaluate properties of optical voltage reporters without using conventional microelectrode techniques.



### 3PK-048

#### Functional analysis of TRPM7 in odontoblasts

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Transient receptor potential (TRP) ion channels were discovered in *Drosophila* photoreceptors and represent the light-sensitive  $\text{Ca}^{2+}$  influx pathway. In mammals, the TRP family has 29 members. Among TRP channel family, TRPM7 has a unique structure organization that contains a TRP channel domain with 6 transmembrane segments fused to an atypical serine-threonine kinase domain at its C terminus. TRPM7 channel activity was initially identified when the removal of cytoplasmic  $\text{Mg}^{2+}$  with the aid of a metal chelator revealed a steeply outwardly rectifying current. The basal activity of TRPM7 is regulated by millimolar levels of intracellular  $\text{Mg}^{2+}$ , so that TRPM7 is activated by depletion of intracellular  $\text{Mg}^{2+}$ , and is inhibited by high concentrations of  $\text{Mg}^{2+}$ . Although the regulation of TRPM7 channel activity has been extensively studied, physiological function of TRPM7 remains unknown. We found that TRPM7 is highly expressed in odontoblasts in the dental pulp by *in situ* hybridization of mouse embryo. We also confirmed that TRPM7 protein is highly expressed in odontoblasts by immunohistochemistry. To investigate the physical function of TRPM7 in odontoblast, we examined TRPM7 activities in primary odontoblast cells with various external stimulations. These results suggest that TRPM7 plays an important role in odontoblast and may require calcium events in tooth.

### 3PK-049

#### Inhibitory effect of 2-aminoethyl diphenylborinate (2-APB) on a muscarinic receptor-operated cation channel in bovine ciliary myocytes

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In bovine ciliary muscle, stimulation of  $\text{M}_3$ -muscarinic receptors opens two types of non-selective cation channels (NSCCs) with different unitary conductances (35 pS and 100 fS) which serve as major pathways for  $\text{Ca}^{2+}$  entry during sustained contraction. The molecular entities of these NSCCs are still unknown. We studied here the effects of thapsigargin (TG) and caffeine, which promote  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum, on the NSCC currents and intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ). We also examined the effects of 2-aminoethyl diphenylborinate (2-APB), an inhibitor for store-operated  $\text{Ca}^{2+}$  entry, on the NSCC currents.

**Methods** Smooth muscle cells enzymatically isolated from the ciliary body of slaughtered oxen were used.

**Results** In myocytes under whole-cell voltage clamp at -50 mV, in normal saline solution containing 2.4 mM  $\text{Ca}^{2+}$ , superfusion of TG (300 nM) and a following carbachol (CCh) stimulus, caused inward currents, one of which, corresponding to that of 100 fS-NSCC, kept open after CCh-washout. Superfusion of caffeine (5-20 mM) induced opening of 100 fS-NSCC without concomitant activation of 35 pS-NSCC. Pre-incubation of the cells with ryanodine (10  $\mu\text{M}$ ) made the caffeine-induced current irreversible. Both currents of 100 fS-NSCC activated by CCh or caffeine were also blocked at concentrations (>30  $\mu\text{M}$ ) of 2-APB.

**Conclusion** The results of the present experiments suggest the involvement of signals elicited by depletion of intracellular  $\text{Ca}^{2+}$  stores in the activation of 100 fS-NSCC by muscarinic stimulation.

### 3PK-050

#### Classification of long-QT syndrome type-1 mutations by the ability to bind calmodulin

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The cardiac potassium current ( $\text{I}_{\text{Ks}}$ ) contributes to the repolarization of action potentials. The  $\text{I}_{\text{Ks}}$  is an assembly of a pore-forming subunit KCNQ1 and an auxiliary subunit KCNE1 as well as calmodulin (CaM). The CaM confers the  $\text{Ca}^{2+}$  sensitivity on the  $\text{I}_{\text{Ks}}$  and supports the proper folding of KCNQ1. Type-1 long-QT syndrome (LQTS) is caused by loss-of-function mutations in KCNQ1. However, it is still unclear how the LQTS mutations cause reduction in surface expression of the  $\text{I}_{\text{Ks}}$  and how the effect relates to the stability of the  $\text{I}_{\text{Ks}}$  complex. Cytoplasmic C-terminus of KCNQ1 (KCNQ1C) could be expressed in a soluble fraction when CaM was co-expressed. Using this property, the soluble complex of KCNQ1C fused with GFP and CaM were separated by size exclusion chromatography. The complex was eluted at a fraction corresponding to the molecular size estimated as a hetero-octameric complex with a 4:4 subunit stoichiometry. Next, we examined the effect of each of 12 LQTS mutations into the KCNQ1C on the complex formation. A subset of LQTS mutations apparently disrupted or destabilized the complex. These LQTS mutations appear to be located at two discrete regions. We further scanned these regions by introducing Trp to assess the contribution of each residue to stability of the complex. In the first element, the effect of Trp-introduction showed periodic distribution, while, in the second element, all residues were sensitive to the replacement. Specific interaction between KCNQ1 and CaM suggests that the destabilization of the  $\text{I}_{\text{Ks}}$  complex would be in the pathogenesis of LQTS symptoms.

### 3PK-051

#### Properties of GABA<sub>A</sub> receptors in guinea-pig adrenal medullary cells

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GABA is thought to play a paracrine role in adrenal medullary (AM) cells. Thus, the perforated patch clamp technique was used to explore the properties of GABA<sub>A</sub> receptors (GABA<sub>A</sub>Rs) in isolated guinea-pig AM cells. Bath application of GABA produced an inward current at -60 mV in a dose-dependent manner with an  $\text{EC}_{50}$  of 36.4  $\mu\text{M}$ . This dose response curve was shifted in the leftward direction with no change in the maximum by allopregnanolone, a neuroactive steroid which is known to be secreted from adrenal cortical cells. The GABA-induced current was enhanced by various benzodiazepine analogues. Diazepam was three times more potent than zolpidem in enhancing the GABA current, and it was also augmented by L-838,417, which has no action on  $\alpha 1$ -containing GABA<sub>A</sub>Rs. On the other hand, the GABA-induced current was not affected by 100 mM ethanol, which enhances  $\delta$ -containing GABA<sub>A</sub>Rs. The GABA-induced current was suppressed by  $\text{Zn}^{2+}$  in a dose-dependent manner with an  $\text{IC}_{50}$  of 18  $\mu\text{M}$ , whereas it was enhanced by 100  $\mu\text{M}$   $\text{La}^{3+}$ . These pharmacological properties suggest that GABA<sub>A</sub>Rs in guinea-pig AM cells mainly consist of  $\alpha 3$ ,  $\beta$  and  $\gamma 2$  subunits. The paracrine function of GABA in AM cells may be facilitated by allopregnanolone secreting from adrenal cortical cells.

### 3PK-052

#### Upregulation of $\text{Ca}^{2+}$ -sensing receptor in idiopathic pulmonary arterial hypertension

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A rise in  $[\text{Ca}^{2+}]_{\text{cyt}}$  in pulmonary arterial smooth muscle cells (PASMC) is an important stimulus for pulmonary vasoconstriction and vascular remodeling. Increased resting  $[\text{Ca}^{2+}]_{\text{cyt}}$  and enhanced  $\text{Ca}^{2+}$  influx have been implicated in PASMC from idiopathic pulmonary arterial hypertension (IPAH) patients. We examined whether the  $\text{Ca}^{2+}$ -sensing receptor (CaSR) is involved in the enhanced  $\text{Ca}^{2+}$  influx and proliferation in IPAH-PASMC. In normal PASMC superfused with  $\text{Ca}^{2+}$ -free solution, addition of 2.2 mM  $\text{Ca}^{2+}$  to the perfusate had little effect on  $[\text{Ca}^{2+}]_{\text{cyt}}$ . In IPAH-PASMC, however, restoration of extracellular  $\text{Ca}^{2+}$  induced a significant increase in  $[\text{Ca}^{2+}]_{\text{cyt}}$ . The calcimimetic R568 enhanced, whereas the calcilytic NPS2143 attenuated, the response in IPAH-PASMC. Furthermore, the protein expression level of CaSR in IPAH-PASMC was greater than in normal PASMC; knockdown of CaSR in IPAH-PASMC with siRNA attenuated the extracellular  $\text{Ca}^{2+}$ -mediated  $[\text{Ca}^{2+}]_{\text{cyt}}$  increase and inhibited IPAH-PASMC proliferation. In monocrotaline-induced pulmonary hypertensive rats, CaSR expression and function were both enhanced in PASMC, whereas NPS2143 prevented the development of pulmonary hypertension and right ventricular hypertrophy. The extracellular  $\text{Ca}^{2+}$ -induced increase in  $[\text{Ca}^{2+}]_{\text{cyt}}$  due to upregulated CaSR is a novel pathogenic mechanism contributing to the augmented  $\text{Ca}^{2+}$  influx and excessive PASMC proliferation in patients with pulmonary arterial hypertension.

### 3PK-053

#### Capsaicin sensitivities of neonatal rat nodose ganglion neurons under TTX application

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We examined capsaicin sensitivities of vagal primary afferent neurons by using whole cell patch-clamp technique under continuous tetrodotoxin (TTX) application. Nodose ganglion neurons were dissociated from 10- to 11-day-old rats ( $n=16$ ). Extracellular solution contained (in mM): 130 NaCl, 5 KCl, 2  $\text{CaCl}_2$ , 1  $\text{MgCl}_2$ , 10 glucose, and 10 HEPES, at pH 7.4. The intracellular solution contained (in mM): 135 KCl, 5 Mg-ATP, 1  $\text{MgCl}_2$ , 10 EGTA, and 10 HEPES, at pH 7.21. During TTX (1  $\mu\text{M}$ , >5 min) application, firstly the action potential was evoked by two-stepped electrical stimulations (200 and 500 pA, each 5 ms), and then the membrane potential changes in response to an application of capsaicin (1  $\mu\text{M}$ ) were studied. In a total of 11 neurons, 9 neurons (i.e. 82%) changed membrane potential (capsaicin-sensitive), whereas 2 neurons (i.e. 18%) showed no particular changes (capsaicin-insensitive). In addition, we detected from a single capsaicin-sensitive neuron membrane potential changes (current-clamp) and a corresponding inward current (voltage-clamp). In conclusion, a majority of TTX insensitive (i.e. TTX-resistant) vagal primary afferent neurons may contain capsaicin-sensitive transient potential vanilloid receptor subtype-1 (TRPV1) channels. Therefore, TRPV1 channels may have complementary effects on TTX-resistant sodium channels, or vice versa, in these neurons.

### 3PK-054

#### Proton permeation and pH-dependent gating of polytheonamide B

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A peptide from marine sponge *Theonella swinhoei*, polytheonamide B (pTB), shows potent cytotoxic activity. The cytotoxic activity to various types of cells was examined and found that pTB was most effective to eukaryotic cells. We previously examined mechanisms underlying the cytotoxic activities of pTB. The amino acid sequence of pTB is unprecedented, having alternative D- and L-amino acid residues throughout the 48-mer peptide. The alternative chiral sequence suggested the formation of a  $\beta$ -helix similar to gramicidin channels, and planar bilayer experiments were performed. pTB forms monovalent cation-selective channels (the selectivity sequence:  $\text{Cs}^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+ > \text{Li}^+$ ), which is compatible with the inner pore diameter of  $\sim 4$  Å for a  $\beta$ -helical structure. In this study, we examined proton conduction through the polytheonamide B channel. In contrast to the case of other monovalent cations, the gating transition was significantly affected by concentration of the permeating ion for the proton conduction. Our results suggest that protonation of the amino acid residue (s) within the polytheonamide B regulates its gating. Based on our present data, we will discuss the molecular mechanism underlying pH dependent gating of polytheonamide B channel.

### 3PK-055

#### The Effects of $I_{\text{K1}}$ Blockade on the Dynamics of Spiral Wave Reentry in Ventricles of Isolated Rabbit Hearts

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Blockade of  $I_{\text{K1}}$  facilitates termination of ventricular fibrillation/tachycardia (VF/VT), but underlying mechanisms remain unclear. We investigated the effects of partial blockade of  $I_{\text{K1}}$  on the dynamics of spiral wave (SW) reentry, the principal mechanism of VF/VT, in ventricular preparations of isolated rabbit hearts by optical action potential mapping. In some experiments, the membrane potential was recorded from superfused ventricular papillary muscles by using a microelectrode. In papillary muscle,  $\text{Ba}^{2+}$  (10-50  $\mu\text{M}$ ) caused a dose-dependent depolarization of the resting potential. The upstroke velocity of the action potential was unchanged at 10  $\mu\text{M}$   $\text{Ba}^{2+}$  but was significantly reduced at 50  $\mu\text{M}$   $\text{Ba}^{2+}$ . In perfused 2-dimensional ventricles,  $\text{Ba}^{2+}$  (10-50  $\mu\text{M}$ ) caused a dose-dependent prolongation of the action potential duration and a decrease in conduction velocity at 50  $\mu\text{M}$   $\text{Ba}^{2+}$ . SW reentry rotated around a short line of functional block under control conditions, whereas that after 10-50  $\mu\text{M}$   $\text{Ba}^{2+}$  was markedly destabilized: FBL was prolonged and, in some hearts after 50  $\mu\text{M}$ , SW reentry showed a prominent meandering with a cycle of a few rotations, resulting from intermittent conduction block near the rotation center. The space constant, which was estimated from the decay of sub-threshold depolarization, was increased after 10-50  $\mu\text{M}$   $\text{Ba}^{2+}$ . These results suggest that partial blockade of  $I_{\text{K1}}$  enhances electrotonic interactions and destabilizes the rotation center of SW reentry, which could contribute to termination of VF/VT.

### 3PK-056

#### Properties of heat and chemical sensitivity of green anole lizard TRPA1

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Transient receptor potential ankyrin 1 (TRPA1) is a Ca<sup>2+</sup>-permeable nonselective cation channel, and expressed in primary sensory neurons. TRPA1 was reported to be activated by temperature and chemical stimuli, such as allyl isothiocyanate, although mammalian TRPA1's response to cold is still debatable. In the previous study, we found that green anole TRPA1 is activated by heat rather than cold temperature. Our aim is to understand how heat and chemicals activate TRPA1. In this study, we focus on the heat and chemical responses of green anole TRPA1 using a heterologous expression system with HEK293T cells. Green anole TRPA1 was found to be activated by warm temperature and AITC with clear outward rectification in the whole-cell recordings. In addition, single-channel opening was observed by heat and AITC in the inside-out mode. Our results suggest that green anole TRPA1 is directly activated by heat and chemical stimuli.

### 3PK-057

#### Subunit stoichiometry and signal flow analyses in the P2X<sub>2</sub> trimer upon voltage- and [ATP]-dependent activation

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Extracellular ATP gated receptor channel P2X<sub>2</sub> is a homotrimer. We investigated from the view of stoichiometry, how intersubunit ATP binding signal streams down on the trimer to the gate. We introduced mutations at the ATP binding (K308A), pore (T339S) and the linker region between them (D315A) by controlling the number and position of mutations in the trimer by tandem repeat constructs (TTC). (1) TTC with K308A revealed that two intact binding sites are necessary and sufficient for both of the ATP-dependent gating and the voltage-dependent one. (2) D315A showed a biphasic [ATP]-response relationship with two different gating modes. In TTC, the specific phenotype of D315A was observed when more than one site was mutated. (3) T339S makes P2X<sub>2</sub> constitutively active at all membrane potentials. In TTC, with the increase in the number of T339S a gradual shift of conductance-voltage curves to depolarized potentials was observed, suggesting independent contribution of three subunits at the pore level to the voltage and-[ATP]-dependent gating. (4) One D315A (or T339S) mutation was introduced in TTC on the same or neighboring subunit with K308A mutation at which ATP binding is blocked. The effect of D315A mutation was clearly different in these two cases, while that of T339S was not. (5) However, one D315A on the same or neighboring subunit with T339S mutation were similar. Taken together; ATP binding signal at the two binding sites flows down on the same subunit to D315 at linker and then spreads to the three subunits.

## Poster Presentations Cell Physiology, Molecular Physiology (3)

### 3PK-058

#### Expression of N-glycosylated-cell surface antigen-X in tumor-associated endothelial cells

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Accumulating evidence indicates that cancer stem cells (CSCs) of glioblastomas (GBs) cause tumor recurrence based on their resistance to tumor treatment. While searching for a new CSC marker in GBs, we observed that cell surface antigen-X (CSA-X), a stem cell marker, was located in tumor-associated endothelial cells (TECs) expressing endothelial markers such as CD34 or von Willebrand factor in human GB tissues. CSA-X was also expressed in the endothelium of small blood vessels formed in rat brain tumors after the transplantation of rat glioma cells. To assess the utilization of CSA-X monoclonal antibody (mAb) as an anti-angiogenic agent against brain tumors, commercially available-CSA-X mAb were administrated intravenously in glioma model rats. Tumor-associated endothelial vessels in the mAb administrated-group were markedly reduced by the complement system, and severe tumor necrosis was observed. Although CSA-X positive endothelial cells were not present in normal vessels of the brain, CSA-X was expressed in other normal cells, indicating these normal cells may also be targets for CSA-X mAb. We therefore biochemical characterization of CSA-X in TECs, and found that CSA-X in TECs was N-glycosylated. These results suggest that N-Glycosylated-CSA-X may be a good therapeutic target for anti-angiogenic treatment of brain tumors. Therefore, we attempted to purify CSA-X from TECs by gel filtration and lectin affinity column to analyze the molecular structure of N-glycosylated-CSA-X.

### 3PK-059

#### Mechanisms underlying suppressive effects of bromvalerylurea on LPS-activated microglia/macrophages

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Bromvalerylurea (BU) has been used as a hypnotic and sedative agent for a century. However, its effect is weaker than more recently developed agents such as benzodiazepines, and therefore BU is rarely used in clinics. Recently, we found that BU inhibited LPS-induced proinflammatory reactions of microglia in vitro. Furthermore, BU exerted marked therapeutic effects on rat models of Parkinson's disease, traumatic brain injuries and sepsis, in which microglia/macrophages have critical pathogenic functions. Here, we elucidated the mechanism underlying BU protective functions using primary rat microglial cells. Quantitative real-time RT-PCR revealed that BU decreased microglial expression of mRNA for inflammatory mediators such as interleukin (IL)-1 $\beta$ , tumor necrosis factor (TNF)- $\alpha$  and inducible nitric oxide synthase (iNOS), in the presence or absence of LPS. BU inhibited LPS-induced production of nitric oxide (NO) in a concentration-dependent manner. Sodium bromide did not mimic the effects of BU, suggesting that Br<sup>-</sup> is not an essential factor, although Br<sup>-</sup> in the metabolites of BU has been considered responsible for its sedative actions. In addition, BU promoted the expression of neuroprotective factors such as insulin-like growth factor (IGF)-1 and hepatocyte growth factor (HGF) mRNAs by microglia. The effects of BU may be partly similar to those of dexamethasone, a synthetic glucocorticoid. We are investigating whether BU inhibits activation of NF $\kappa$ B-dependent signaling pathways.

### 3PK-060

#### Simple and Rapid Detection of 2-methylthio Modification in tRNA for Diagnostic Application of Type 2 Diabetes

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Post-transcriptional modifications are required for the structural integrity and decoding activity of tRNAs. It is known that defect in some tRNA modifications leads to pathological consequences. Cdk5 regulatory subunit associated protein 1-like 1 (Cdkal1) is associated with type 2 diabetes (T2D). We revealed that Cdkal1 is a tRNA-modifying enzyme that catalyzes 2-methylthio (ms<sup>2</sup>) formation of 2-methylthio-*N*<sup>6</sup>-threonylcarbamoyladenosine (ms<sup>2</sup>t<sup>6</sup>A) at position 37 of cytoplasmic tRNA<sup>Lys</sup>. The pancreatic  $\beta$  cell specific Cdkal1 null mice showed symptoms of T2D including pancreatic islet hypertrophy, reduced insulin secretion, and impaired blood glucose control. Since the ms<sup>2</sup>-modification of ms<sup>2</sup>t<sup>6</sup>A is required for accurate decoding of Lys codon, hypomodified tRNA in the  $\beta$  cell might be associated with development of T2D. Therefore, measuring ms<sup>2</sup>-modification level in cytoplasmic tRNA<sup>Lys</sup> would be a useful biomarker to diagnose T2D. We here report a simple and rapid method for detection and quantification of ms<sup>2</sup>-modification in cytoplasmic and mitochondrial tRNAs by using quantitative reverse transcription PCR. This method was sensitive enough to detect ms<sup>2</sup>-modification in total RNA from human blood specimens. In fact, the decreased ms<sup>2</sup>-modification was observed in cytoplasmic tRNA<sup>Lys</sup> from individuals carrying mutations in cdkal1 gene.

### 3PK-061

#### Involvement of Cytoskeleton Dynamics in the Induction of Hepatocyte Proliferation in Primary Culture

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Adult rat hepatocytes underwent DNA synthesis followed by cell division when they were cultured in a nutrient-rich, low Ca<sup>2+</sup> (< 0.4 mM) medium supplemented with insulin and epidermal growth factor. Addition of colchicine or taxol (10<sup>-6</sup> M each) for 20 hours between 10 and 30 h after plating inhibited DNA synthesis by 80%. Since DNA synthesis began around 30 hours after plating, that culture period corresponds to middle to late G1 period in the cell cycle. Immunofluorescence staining of hepatocytes with antibodies to tubulin revealed that colchicine disassembled microtubules, which were arrayed as thin filaments in non-treated hepatocytes. In contrast, a small number of thick microtubules were observed in taxol-treated hepatocytes. Low concentration of Ca<sup>2+</sup> not only promoted mitoses of hepatocytes, but affected dynamics of microfilaments (MFs). FITC-phalloidin staining showed that elongated MFs (stress fibers) were oriented toward cell periphery in high Ca<sup>2+</sup> medium, but in low Ca<sup>2+</sup> medium, on the other hand, MFs were highly concentrated in cell periphery and were not oriented to any direction. Those results suggest that regulated dynamics of microtubules in middle to late G1 period is necessary for the induction of DNA synthesis and that dynamics of MFs is involved in frequent cell division under low Ca<sup>2+</sup> conditions in primary culture of adult rat hepatocytes.

### 3PK-062

#### Mechanisms of Cell Volume Regulation analysed in a Ventricular Cell Model

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The mechanism for cell volume change consists of many factors: the passive and active transport of ions and substrates through the cell membrane, the water permeability and the diffusion of solute and water in the cytoplasm. In this study, the experimental findings of Sasaki *et al* (1999) were analyzed using the minimum cell model described by Terashima *et al* (2006). Firstly, the water permeability of the membrane was determined by reconstructing cell volume changes in guinea-pig ventricular myocytes induced by various osmotic stimuli. Our model confirmed that the cell volume did not change when the cation channel flux was compensated for by the pipette current through the gramicidin-perforated patch membrane. Surprisingly, the simulation also showed that the cell volume changed depending on direction and amplitude of ionic current when the compensating current was carried by both cation and anion in the ruptured-patch whole cell voltage clamp. This simulation results indicate that the anionic channel flux takes the pivotal role in determining the cell volume change. Finally, the experimental finding of applying a hypotonic solution only to a half of elongated myocyte was well reconstructed. The cell volume increased in the half exposed to the hypotonic test solution, while the other half decreased in the isotonic solution. This result was obtained by assuming a slower water movement than that of the ions in the cytoplasm.

### 3PK-063

#### Suppressive control of N-cadherin mediated cell-cell adhesion by a chondroitin sulfate proteoglycan, NG2

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Cadherins participate in constructing homogenous tissues by mediating cell-cell adhesion between the same types of cells. However, adhesion is dynamic in a living organ, which experiences continuous construction and destruction of adhesion for the maintenance of tissues and organs. In some instances, cadherin adhesion can be modulated by the expression of other species of cadherin molecules. Cadherin adhesion requires calcium ions. Inhibition of cadherins can be mediated by internalization of cadherin proteins, downregulation or mutation of cadherin genes or chelation of calcium ions in vitro. However, apart from dysadherin, a protein that causes downregulation of E-cadherin expression, other molecules that suppress cadherin adhesion have not been identified. Chondroitin sulfate proteoglycan, NG2, is expressed in oligodendrocyte precursor cells, pericytes of blood vessels and macrophage-like cells that are recruited to ischemic brain lesions, although its functions are unclear. We have explored the functions of NG2 with the aim of developing a therapeutic agent for the macrophage-like cells described above for ischemic brain injury, since our previous studies identified these cells to be protective in brain pathology. Here we report the suppressive role of NG2 on N-cadherin adhesion, and discuss the possible biological significance of this NG2 function.

### 3PK-064

#### No allergen-specific IgE production by the 2<sup>nd</sup> s.c. injection of Mite-Dp feces after total IgE production by the 1<sup>st</sup> i.n. injection into mice

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Allergic rhinitis is caused by degranulation after crosslinking of allergen-specific IgE-bound FcεR on effector cells (e.g., mast cells) with the multivalent allergens. However, we know little about early stages of sensitization to allergen. Previously, we reported that IL-4-dependent total IgE induction after an *i.n.* injection of cedar pollen (e.g., cry j) inevitably preceded induction of allergen-specific IgE after the 2<sup>nd</sup> s.c. injection into mice. In the present study, we *i.n.* injected various allergens such as cedar, ragweed, wormwood or hinoki pollen and mite-Dp feces into mice and assessed the amounts of total and allergen-specific IgE in the serum by ELISA. The results showed that time-dependent changes in the amounts of total IgE in the serum after an *i.n.* injection of the allergen were similar one another, whereas no allergen-specific IgE was induced by the 2<sup>nd</sup> s.c. injection of mite-Dp feces. We are under study to clarify the role of total IgE production in the mechanism of allergen-specific IgE production.

### 3PK-065

#### Acidic amino acids located on the N-terminal region of cGMP-dependent protein kinase are responsible for the interaction with TRPC6

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cGMP/cGMP-dependent protein kinase (PKG) signaling pathway plays an important role in vasorelaxation. In this pathway, PKG plays a key role of lowering the intracellular Ca<sup>2+</sup> level and/or Ca<sup>2+</sup> sensitivity of contractile machinery by protein phosphorylation. However, the downstream of PKG is still poorly elucidated. Recently, we reported that a transient receptor potential (TRP) non-voltage-gated cation channel, TRPC6, is a novel PKG substrate and negatively regulated via phosphorylation on its threonine 69 (Takahashi et al. *J Physiol*, 2008). Furthermore, using yeast two-hybrid system to identify responsible binding domains in both TRPC6 and PKG type I proteins, we revealed that two PKG subtypes, both PKG Iα and Iβ, interact with TRPC6 N-terminal domain (the 87th annual meeting of the Physiological Society of Japan, 2010), and that only PKG Iβ showed the cGMP-dependent interaction with TRPC6 N-terminal domain (the 88th annual meeting of the Physiological Society of Japan, 2011). In this study, to determine which amino acids are responsible for the interaction between the PKG Iβ and TRPC6, series of mutations are introduced to the type Iβ PKG N-terminal region, and protein interaction between PKG and TRPC6 was examined by yeast two-hybrid assay. Four mutants (D26K, E27K, E31K and D33K) showed the loss of interaction between type Iβ PKG and TRPC6. These results indicate that acidic amino acids in PKG type Iβ are responsible for interaction with TRPC6 N-terminal region.

### 3PK-066

#### Comparison of the effect of current therapeutic agents for diabetes in Cdkal1-deficient mice

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Genetic variations in the cdk5 regulator associated protein 1-like 1 (cdk1l) gene have been identified as a risk factor for the development of type 2 diabetes (T2D). Pancreatic b-cell-specific cdk1l-deficient (KO) mice show the phenotypes as same as patients with risk variants in cdk1l such as decrease in insulin secretion, no insulin sensitivity or no obesity. Here we examined the effects of current therapeutic agents in KO mice. KO mice were fed with high-fat diet and treated with glibenclamide, exendin-4, liraglutide and sitagliptin everyday for four weeks. Mice were then given glucose-tolerance test and insulin-tolerance test. The expression levels of ER stress markers were examined by quantitative PCR method in the pancreatic islets of mice. The pancreas was stained by H.E. staining and the morphology of the islets was observed. Long-term application of glibenclamide impaired the glucose tolerance and insulin secretion in KO mice. In contrast, exendin-4 and liraglutide, agonists of GLP-1, and sitagliptin, a DPP-4 inhibitor, improved the glucose tolerance and insulin secretion. Moreover, both liraglutide and sitagliptin decreased the expressions of ER stress markers whereas glibenclamide had no effect on the expression. Liraglutide and sitagliptin were most effective on the improvement of diabetes in Cdk1l KO mice, suggesting that the drugs may be the most suitable for the treatment of T2D patients with the risk alleles in cdk1l.

### 3PK-067

#### Involvement of TRPC 6 channel in TGF- $\beta$ 1/SMAD/MAPK/PI3K signaling in human intestinal myofibroblast

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Tumor microenvironment plays a significant role in colitis-associated cancer. Intestinal myofibroblasts reside in the intestinal lamina propria secreting factors known to modulate carcinogenesis; however, their physiological roles and associated signaling pathways remain unknown. Transforming growth factor (TGF)- $\beta$ 1, a major stimulator of colitis-associated cancer, requires TRPC6-mediated  $\text{Ca}^{2+}$  influx for its effect on myofibroblast proliferation and differentiation. TGF- $\beta$ 1 (5ng/ml)-dependent activation of Smad2, p38, and Erk-1/2 in human colonic myofibroblast cell line InMyoFib was examined by Western blot analysis. Specific pharmacologic kinase inhibitors were used to characterize the involvement of MAPK/MEK/Akt-dependent pathways.  $\text{Ca}^{2+}$  influx were examined by  $\text{Ca}^{2+}$  imaging procedure. In InMyoFib, TGF- $\beta$ 1 stimulation induced a rapid and transient activation of Smad2 and Erk, whereas p38 activation was biphasic and sustained. Introduction of TRPC6-specific siRNA and dominant negative TRPC6 DNA significantly suppressed  $\text{Ca}^{2+}$  influx in InMyoFib, but accelerated the activation of Smad2, Erk, p38 signaling by TGF- $\beta$ 1 stimulation. Knockdown of TRPC6 expression also activated the Akt/FOXO1 pathway, which positively regulates TGF- $\beta$ 1 signaling. In addition, calcineurin-NFAT signaling was found necessary and sufficient for these TRPC6 effects.

### 3PK-068

#### Rare sugar D-allose strongly induces thioredoxin interacting protein (TXNIP) expression and inhibits osteoclast differentiation

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Oxidative stress modulates the osteoclast differentiation via redox systems and thioredoxin-1 (Trx) promotes the osteoclast formation by regulating the activity of transcription factors. Receptor activator of nuclear factor kappa-B ligand (RANKL) treatment increased the Trx level during osteoclast differentiation and D-allose dose-dependently inhibited the differentiation in RAW264 cells by up-regulating TXNIP, a negative modulator of Trx in the nucleus. Transcriptional activity of activator protein 1 (AP-1), nuclear factor-kappa B (NF- $\kappa$ B), and nuclear factor of activated T-cells (NFAT), known to be modulated by Trx, was also inhibited by D-allose.

We further examined the effect of D-allose on the ovariectomized mice as an osteoporosis model. Oral administration of D-allose to ovariectomized mice decreased the Trx level and significantly up-regulated TXNIP in the tibial bone marrow cell. Furthermore, D-allose increased the density of total bones in diaphyseal region (control:  $723.4 \pm 46.1$  mg/cm<sup>3</sup>, D-allose:  $769.6 \pm 66.3$  mg/cm<sup>3</sup>;  $p < 0.05$ ) and the quantity of total bones (control:  $1.69 \pm 0.16$  mg/mm, D-allose:  $1.71 \pm 0.10$  mg/mm). Further study would be helpful to understand the effect and mechanism of D-allose on the bone remodeling.

### 3PK-069

#### Increase in cell proliferation by intercellular junctional protein, claudin-2, in lung adenocarcinoma cells

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Claudin-2 is expressed in human lung adenocarcinoma, although it is absent in normal lung tissue. Here we examined the regulatory mechanism of claudin-2 expression and the effect of claudin-2 expression on cell proliferation using human lung adenocarcinoma A549 cells. Claudin-2 protein level temporarily increased at 48 and 72 h after cell inoculation and returned to the basal level at 96 h. In contrast, claudin-1 and E-cadherin levels increased in a time-dependent manner. Claudin-2 expression was decreased by AG1478, an EGFR inhibitor, and U0126, a MEK inhibitor. Furthermore, these inhibitors decreased p-c-Fos and nuclear c-Fos levels. These results indicate claudin-2 expression is up-regulated by a MEK/ERK/c-Fos pathway in lung adenocarcinoma cells. Cell proliferation was decreased by the knockdown of claudin-2 concomitant with a decrease in the percentage of cells in the S phase. The knockdown increased the expression levels of p21<sup>Cip1</sup> and p27<sup>Kip1</sup>, whereas decreased those of cyclin D1 and E1. The nuclear level of ZO-1 associated nucleic acid binding protein (ZONAB) was decreased by claudin-2 knockdown. The knockdown of ZONAB by siRNA decreased cell proliferation concomitant with the increase in p21<sup>Cip1</sup> and p27<sup>Kip1</sup>, and the decrease in cyclin D1 and E1. Taken together, we suggest that abnormal expression of claudin-2 affects the nuclear localization of ZONAB, resulting in the up-regulation of cell proliferation.

### 3PK-070

#### Inhibition of MIC/TRPM7 channel impairs insulin-dependent glucose uptake in adipocytes

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TRPM7 is a non-selective cation channel which permeates  $\text{Mg}^{2+}$  and is involved in regulation of  $[\text{Mg}^{2+}]_i$ . In adipocytes, intracellular  $\text{Mg}^{2+}$  plays a key role in insulin action. We previously demonstrated that white adipocytes isolated from mice functionally expressed magnesium-inhibited cation (MIC) channel whose molecular identity is thought to be TRPM7. In the present study, we first confirmed by patch-clamp recordings that differentiated 3T3L1 adipocytes expressed MIC channel, and then tested the effect of inhibitors of MIC channel on insulin-dependent glucose uptake by using tritium labeled 2-deoxyglucose. Whole-cell patch-clamp recordings revealed that 3T3L1 adipocytes expressed MIC currents that were inhibited by 2-aminoethoxydiphenyl borate (2-APB), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and N-methyl maleimide (NMM). To assess the effect of these inhibitors on insulin-dependent glucose uptake, 3T3L1 adipocytes were pretreated with each inhibitor for 20 min and then stimulated with insulin. Under the control conditions, the rate of glucose uptake was increased 2-fold by insulin application. In the presence of 2-APB or NMM, basal uptake was comparable to control conditions, whereas insulin failed to increase the rate of glucose uptake.  $\text{H}_2\text{O}_2$  itself increased the rate of basal glucose uptake, however, there was no insulin-dependent increase. Thus, it is suggested that MIC/TRPM7 channel activity is required for insulin-dependent glucose uptake in adipocytes, possibly via changes in  $[\text{Mg}^{2+}]_i$ .

### 3PK-071

#### Microelectrode Array Analysis of Ileal Pacemaker Activity in Mice Lacking Interleukin-10

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ICC act as pacemaker cells to generate basic electric rhythms for gut motility. IL-10 deficient mice (IL-10<sup>-/-</sup> mice) suffer from chronic IBD disease. Our aim of this study was to investigate whether basal electric activity including in ICC, occurred any significant changes in IL-10<sup>-/-</sup> mice comparing with wild-type (WT) mice. Ileal musculature containing the myenteric plexus was extracted from both WT and IL-10<sup>-/-</sup> mice. A MEA was employed to measure electric activity of the ileal musculatures. Nifedipine and tetrodotoxin were applied to predominantly measure ICC electric activity in modified Krebs solution. Spontaneous electric activity was synchronized throughout the recording area in both WT mice and IL-10<sup>-/-</sup> mice. The spectral power in the frequency range of 9.4 to 30.0 cpm was estimated in all 64 channels and found no significant difference between these mice. The frequency of oscillation (cpm) estimated from auto-correlation was significantly higher in IL-10<sup>-/-</sup> mice than in WT mice (22.16±4.10 vs 15.72±1.61 cpm, n=21, P<0.001). Also, we performed immunohistochemical analysis for c-Kit on ileal musculature whole mounts from WT and IL-10<sup>-/-</sup> mice, and found normal distribution of ICC (c-Kit) cells in both mice as well. These results suggest that hyper gut activity, in terms of frequency of electric oscillations may reflect inflammation-accelerated ICC pace-making. Further research is necessary to investigate the intrinsic factors, which cause hyper gut activity in IL-10<sup>-/-</sup> mice.

### 3PK-072

#### Physiological Genomics of Amphibian VSP : A Potential Molecular Mechanism of Electrical Block of Fertilization in Vertebrates

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Electrical regulation is involved in the fertilization of many species of vertebrates and invertebrates. In the urodele *H. nebulosus*, polyspermy block is voltage dependent at the level of egg plasma membrane. In contrast, in another urodele, *C. pyrrhogaster*, the block is voltage independent. However, the molecular mechanisms underlying these observations are unknown. VSPs are abundantly expressed in testis and the membrane potential controls their enzymatic activity towards phosphoinositides. VSPs may be capable of translating depolarization of the egg membrane by the fertilizing sperm to establish a fast block to polyspermy. To test this hypothesis, we cloned VSP cDNAs from both salamander and newt testis and studied their molecular properties. The deduced sequence of Hn-VSP (salamander) has 427 amino acids, while that of Cp-VSP (newt) has 511. The sequences show 50% and 60% similarity with Ci-VSP, respectively. Sequence alignment with other known VSPs from *C.intestinalis*, *X.laevis*, *D.rerio*, and *G.galvus* showed that the positively charged arginines are well conserved in the S4 segment. Motion of the voltage sensor and voltage-dependent phosphatase activities are now being examined by electrophysiological and fluorescence imaging techniques using heterologous expression.

### 3PK-073

#### Leptin and cholecystokinin receptor-mediated Ca<sup>2+</sup> transients in murine hypothalamic neurons

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Leptin and cholecystokinin (CCK) are both satiety controllers whereas their receptor localizations and physiological interactions in the hypothalamic satiety centers are not well characterized. In the present study, we analyzed leptin and cholecystokinin-mediated Ca<sup>2+</sup> signaling in the paraventricular nucleus, dorsal-medial hypothalamus, ventral-medial hypothalamus, arcuate nucleus and lateral hypothalamus using *fura-2*-based fluorescent measurements in acutely isolated mouse brain slices. Leptin (100-500 nM) induced Ca<sup>2+</sup> transient most significantly in arcuate nucleus. Parvocellular cells in the paraventricular nucleus, where significant CCK-1 receptors are expressed (Mohammad et al., 2012), also mobilized Ca<sup>2+</sup> following leptin stimulations. However, the leptin response was rather co-localized with CCK-4-induced Ca<sup>+</sup> response and thus presumably coupling to CCK-2 receptors. Real-time PCR assay further demonstrated that largest expression of leptin receptor a (ObRa) in the arcuate nucleus while leptin receptor b (ObRb) was dominant in the paraventricular nucleus. These results reveal that leptin and cholecystokinin receptors may have functioning in identical neurons depending on the hypothalamic nuclei.

## Poster Presentations Sensory Function(3)

### 3PK-074

#### Optogenetic study of whisker-barrel system-cortical electrophysiology and functional MRI

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In one of thy1.2-channelrhodopsin 2 (ChR2)-Venus transgenic rat lines, W-TChR2V4, the ChR2 was expressed in the mechanoreceptive subpopulation of trigeminal ganglion neurons which innervate whisker follicles. It is thus well expected that a whisker-related sensory perception should be induced by the photostimulation of their follicles.

To test this, the barrel cortex responses were examined using electrophysiological recordings and functional magnetic resonance imaging (fMRI). Under anesthesia with urethane, the whiskers were trimmed and connected with optic fibers of which other endings were connected to LEDs. Pulsative irradiation of blue LED light was used as a test and that of red LED light as control.

We found that the blue light irradiation of whisker follicles evoked enhanced unit activities as well as a local field potential in the barrel field of contralateral somatosensory cortex whereas the red light did not. The blue light irradiation also induced a blood oxygenation level-dependent (BOLD) signal response in the barrel field of contralateral somatosensory cortex. It is suggested that the optogenetic whisker stimulation could activate the whisker-barrel cortical pathway of mechanoreceptive signaling. This method would facilitate to study how the spatio-temporal pattern of the whisker mechanoreception would be integrated in the cortex.

All animal procedures were conducted in accordance with the guiding principles of Physiological Society of Japan and NIH.

### 3PK-075

#### Range of motion-dependent mechanical hyperalgesia after lengthening contraction in rats

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Exercise, especially after strenuous lengthening contraction (LC), often elicits delayed onset muscle soreness (DOMS) in a couple of days. Recently, cellular and molecular mechanisms of DOMS are being unveiled while the contractile parameters leading to DOMS have not been clarified yet. The aim of this study was to investigate whether a range of motion (ROM), namely, the length of muscle stretch during LC was a determinant of DOMS. Under inhalation anesthesia with 1.5% isoflurane, ankle extensor muscles of rats were given repetitive LC with a customized device (NDH-1, Bio Research Center) which enables to control contractile parameters of LC variably and precisely. Here the effect of different ROM (30°, 60°, 90°, and 120°) was tested while other parameters were fixed (angular velocity : 200°/sec, repetition : 50 times). Mechanical hyperalgesia (tenderness) was quantified by measuring mechanical withdrawal threshold of the hindpaw before, 3 hours, 1, 2, 3, 4, and 5 days after LC. We found that LC at the ROM of 60°, 90°, and 120° significantly decreased the withdrawal threshold in an ROM-dependent manner while that at 30° did not at all. Stretching of the muscle alone had no effect on the nociceptive threshold at all ROM tested. These results suggest that the length of muscle being stretched during LC is an important factor leading to pain after exercise.

### 3PK-076

#### Modulatory effects of somatosensory inputs on postural sway responses induced by galvanic vestibular stimulation

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Somatosensory and vestibular processes act jointly to control body balance when our postures are disturbed. How these two sensory systems interact and produce appropriate postural responses have not been understood well. We examined the effects of somatosensory inputs on body sway induced by galvanic vestibular stimulation (GVS). Bipolar GVS was applied to mastoid processes while participants were sitting with their eyes closed with or without touching a stable surface lightly with their index fingers. In healthy participants, tilting responses of the body induced by GVS were larger with a finger touch than without a finger touch. To examine the cortical involvement of somatosensory-vestibular interactions, the same experiments were performed in patients with lesions to the right or left posterior insula that constitutes a part of the parietoinsular vestibular cortex. The vestibular stimulation evoked body sway responses from the right-brain-damaged patient, however, no modulatory effect was observed in the finger touch condition. In contrast, the finger touch could evoke tilting responses in the left-brain-damaged patient, though GVS alone did not induce significant responses. These preliminary results were discussed in the light of a hypothesis of a right hemispheric dominance of somatosensory-vestibular interactions in the posterior insula.



### 3PK-077

#### Response pattern of rats amygdaloid neurons to odors

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Amygdaloid area is one of olfactory pathways. However, its detail role in the olfactory pathway system is not well known. In order to investigate the role and character, we searched the odor-responding neurons at the medial amygdale and peripheral portion in rats. We recorded the extracellular responses and checked the odor-responses to several pure substances. Plant origin odors (oaklactone (oak), jasminalactone (jas), trans-2-hexenal (t2h)) and predator origin odors (2,4,5-trimethylthiazoline (TMT), isopentenyl methyl sulfide (IPMS), 2-propylthietane (2PT)) were used. Rats urine and cat odor were also used for odor samples. Thirty-one neurons from twenty male rats responded to at least one odor. Majority of that responded to only one substance. Number of responding neurons to urine, cat, oak and jas were considerably few. In contrast, t2h, TMT, IPMS and 2PT displayed high response ratio. No differences were observed in response ratio between plant odor group and predator odor one. No neurons were found which responded to every predator odors without responses to plant odors. However, 3 neurons responded to more than 2 predator odors without responses to plant odors. This study shows the response pattern of rat amygdaloid neurons to several odors. Generally, the neurons at this area show high selectivity in the odor-response. It remains unclear whether these neurons have central role regarding the integration of the predator odor signals.

### 3PK-078

#### Visual-tactile multisensory integration in primate parietal operculum

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In the well-developed primate brain, the operculum has a unique structural feature that overlays the insula located within the lateral sulcus. Physiological and anatomical experiments in monkeys have shown that the parietal operculum (PO) including the secondary somatosensory area is essentially engaged in the processing of somatosensory information ascending from wide range of bodily surface, whereas the postcentral primary somatosensory area processes rather localized information. Recent human brain imaging studies have revealed that PO receives various neural inputs from different modalities to be involved in multisensory integration and even other cognitive functions such as empathy. To explore PO's multisensory nature at neuronal level, we recorded single-unit activity from PO in two macaque monkeys and examined how they respond to various visual and tactile stimuli. Among 794 isolated neurons, 468 (58.9%) and 207 (26.0%) responded to tactile and visual stimulation, respectively. 63 visual neurons responded to observation of human actions and 40 (63.5%) of them also discharged during the self-initiated movements. 57 neurons responded to visual stimuli presented near the body and half of them were also activated by tactile stimuli. These properties were very similar to those reported earlier in other cortical areas such as ventral premotor and intraparietal areas. These results suggest that the primate PO receives signals from more than one sensory cortices and governs multisensory integration, which may contribute to conducting attentive behaviors to the external world.

### 3PK-079

#### Aging of voltage-gated currents in mouse fungiform taste bud cells

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Taste losses occur as a result from normal aging with apparently intact taste buds. We investigated the action potentials and voltage-gated currents of fungiform taste bud cells in 17-month-old mice in situ, and then immunologically classified these elongated cells into type I, II, and III cells. Here, we show that all of these cell types fire action potentials. Also they generate a variety of voltage-gated currents including tetrodotoxin (TTX)-sensitive and TTX-resistant Na<sup>+</sup> currents, an outwardly rectifying K<sup>+</sup> current, and a hemichannel current. The magnitude of these currents depends on cell types. Although these electrophysiological characteristics are similar to those of 2-month-old mice, quantitative comparisons show that the Na<sup>+</sup> current density of type III cells is significantly smaller in 17-month-old mice, and the hemichannel current density of type I cells is significantly larger in 17-month-old mice. We discuss the effect of these aging on the taste response of old mice. This work was supported in part by JSPS KAKENHI Grant Number 23700469.

### 3PK-080

#### Neuroendocrine response and nocifensive behavior after nociceptive stimulation in TRPV1 and TRPV4 knockout mice

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We investigated neuroendocrine response and nocifensive behavior after formalin test, using transient receptor potential protein vanilloid (TRPV) 1 and TRPV4 knockout mice. We examined Fos like immunoreactivity (Fos-LI) in the paraventricular nucleus (PVN) of the hypothalamus and the dorsal horn of the spinal cord (L4), and also investigated nocifensive behavior during phase I and II after subcutaneous (s.c.) injection of formalin or saline in left hind paw of mice. The number of Fos-LI in the parvocellular division of the PVN in TRPV1 knockout mice was significantly reduced compared with TRPV4 knockout and wild mice after s.c. injection of formalin. In laminae I-II of the dorsal horn in TRPV1 knockout mice the number of Fos-LI was reduced but not significantly compared with TRPV4 knockout and wild mice after s.c. injection of formalin. During phase I, nocifensive behavior was remarkably reduced in TRPV1 and TRPV4 knockout mice, and was significantly reduced in TRPV1 knockout mice but not TRPV4 knockout mice during phase II. These results suggested that both TRPV1- and TRPV4-expressing neurons were activated directly after s.c. injection of formalin and TRPV1-expressing neurons were also activated by inflammation secondary after s.c. injection of formalin.

### 3PK-081

#### Dysfunction of the noradrenergic descending nociception regulatory system in rats with painful diabetic neuropathy

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Hyperalgesia is one of the frequently observed complications in the diabetic patients with neuropathy. Recent clinical studies indicate that serotonin and noradrenaline (NA) reuptake inhibitors such as duloxetine, but not selective serotonin reuptake inhibitors, alleviate pain sensation in patients with painful diabetic neuropathy. This pharmacological specificity suggests that the hyperalgesia in painful diabetic neuropathy might involve dysfunctional noradrenergic system. We analyzed the effect of duloxetine and its dependence on intact noradrenergic systems in streptozotocin (STZ)-induced diabetic model of male Wistar rats. These models showed, in addition to hyperglycemia, mechanical allodynia, thermal hyperalgesia, and surprisingly, a higher concentration of NA in the brainstem and spinal cord. The significant analgesic effect of duloxetine in STZ-treated rats was occluded by prior administration of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4), an agent selectively degenerates noradrenergic axons of locus coeruleus origin. DSP-4 itself significantly lowered nociceptive threshold in non-diabetic animals, but not in STZ-treated rats. Together with the increased NA levels in the STZ-treated rats, these results suggest that aberrant down-regulation of NA release from noradrenergic terminals in the spinal cord would play a role in the persistent hyperalgesia in the painful diabetic neuropathy.

### 3PK-082

#### Voltage sensitive-dye imaging of visual cortical response to suprachoroidal-transretinal electrical stimulation in the retinal degenerated rat

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It was previously shown that a retinal prosthesis-based on suprachoroidal-transretinal stimulation (STS) is feasible for restoring visual function to blind patients with outer retinal degeneration. In the present study, I examined the optimal parameters for STS, such as polarity, and position of a return electrode, in order to evoke visual cortical response efficiently. A single pulse of electrical current was applied to the eyeball of anesthetized retinal dystrophic rats (Royal College of Surgeons rats) via a stimulating electrode attached on the locally fenestrated sclera, and the electrically evoked response of the visual cortical area (VC), stained with voltage-sensitive dye (VSD), was optically recorded. Approximately 15 ms after anodic STS, but not after cathodic STS, a transient and localized excitation in VC was observed, usually followed by wider and longer hyperpolarization. When the stimulating electrode was moved horizontally a distance of 0.8 mm, the center of the response moved  $439 \pm 176.6 \mu\text{m}$  along the anterioposteromedial axis. When an extraocular return electrode was used, the excitatory response apparently decreased. These results provide useful information for the optimal design of electrodes and stimulus parameters.

### 3PK-083

#### The effect of focused and unfocused vision on nystagmus during and after whole-body rotation

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The duration of after-nystagmus, which is caused by whole-body rotation, is shorter in athletes such as skaters and dancers who are required to rotate or spin than non-athletes. In addition, it is reported that rotational training is effective to reduce the duration of after-nystagmus. Meanwhile, to prevent rotational vertigo, skaters and dancers adopt various strategies during rotation, such as gazing at a point and closing their eyes. However, there are few studies that analyzed the effects of how to see the outside world during rotation on after-nystagmus. The aim of our study is to figure out the influence of focused and unfocused vision on the vestibular reflex in non-athlete. We measured rotational nystagmus, which occurs during whole-body rotation and after nystagmus by recording electro oculogram (EOG) signals of subjects sitting on a revolving chair. Subjects were instructed (1) to focus on a 4.1cm diameter ball that was fixed 30cm in front of their eyes (F), or (2) not to focus on any objects on their eyesight (NF), while they are experiencing whole-body rotation which was induced by the revolving chair at a rate of 0.37 rps for 13.5s. EOG signals only the slow component of nystagmus was picked up to obtain the magnitude of vestibulo-ocular reflex in F task and NF task are compared. During the whole-body rotation, the slow component of nystagmus in F task was smaller than that in NF task. In contrast, immediately after the whole body rotation, the slow component of nystagmus was larger than that in NF task.

### 3PK-084

#### Neuronal responses in somatosensory cortex to noxious thermal stimuli in behaving monkey

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To examine functions of the somatosensory cortex in perception of noxious heat stimuli, we trained the monkey to perform the heat stimulus intensity detection task and recorded single neuronal responses in the somatosensory cortex during performance of the task. The monkey performed the task sitting in the primate chair with a thermal probe placed on the whisker pad region. A trial was begun by turning on the light within the button, and the monkey was required to press the button. After keeping button press for 4 s, a heat stimulus (45-47deg C) was applied for 6-10 s (T1 period). Then, temperature of the heat stimulus was slightly increased (0.2-0.8 deg C) (T2 period), and the monkey was required to detect this slight change and to release the button within 3 s to get juice as a reward. As a control trial, the monkey performed similar trials that required it to detect slight changes of brightness of the light. In addition, heat stimulus was applied while the monkey performed light detection to test effects of attention to heat responses. As a result, substantial number of somatosensory cortical neurons with receptive fields that include probe position responded to heat stimuli. Contrary to our expectation, many neurons responded in T1 or T2 period, not in both periods. In addition, responses to heat stimuli in T1 period significantly decreased when heat stimuli were given while monkey was performing light detection. These results suggest that somatosensory cortical neurons play an important role in detection of changes in temperature in noxious heat stimuli.

### 3PK-085

#### The effect of external Na<sup>+</sup> on the electroolfactogram recorded from the goldfish olfactory epithelium submerged in Ringer solution

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Most of reported electroolfactograms (EOG) of freshwater teleosts have been recorded from anesthetized living animals stimulated with odorants in freshwater. While this condition is suitable to reproduce their native habitat, it is difficult to compare such EOG with single-cell recordings in Ringer solution because of their difference in ionic conditions. It has been reported that, in isolated olfactory receptor cells of the frog, a Na<sup>+</sup>-dependent Ca<sup>2+</sup> extrusion contributes the rapid recovery of the odor response and the removal of external Na<sup>+</sup> prolonged the response (Reisert and Matthews, 1998). We previously performed EOG recordings from goldfish olfactory tissue isolated from the body and submerged in Ringer solution. By using this method, in the present study, we tested the effect of external Na<sup>+</sup> on the goldfish EOG. In our recordings, between in the control Ringer solution and in low Na<sup>+</sup> solution, the recovery of the response to IBMX, a bile acid or an amino acid did not show any practical difference. We also tested frog EOG in a similar recording condition to goldfish, and observed that the reduction of the external Na<sup>+</sup> prolonged a cineole response and an IBMX response. These results suggest that the olfactory receptor cells of goldfish may not use the Na<sup>+</sup>-dependent Ca<sup>2+</sup> extrusion in the recovery of the odor response. This might be explained, in part, by a low Na<sup>+</sup> concentration in their habitat and the length of their olfactory cilia and microvilli much shorter than the cilia of frogs. This work was supported in part by KAKENHI (22500351).

### 3PK-086

#### Muscular Nociceptor Heat Sensitivity in Rats After Lengthening Contraction and NGF

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Previous reports showed that mechanical sensitivity of muscle nociceptors increased 2 days after lengthening contraction (LC), when the muscular mechanical hyperalgesia (delayed onset muscle soreness, DOMS) was the strongest, but the heat sensitivity was not different (Taguchi et al., 2005). Injection of nerve growth factor (NGF), which is responsible for DOMS, induced mechanical sensitization of thin-fiber afferents in vitro in 10-20 min (Murase et al., 2010), but it is not known whether NGF sensitizes muscular nociceptors to heat. In this study we examined the heat sensitivities of nociceptors in rats after LC with slowly increasing ramp heat stimulation and after injection of NGF. **Methods** : Single unmyelinated muscle afferents were recorded from muscle-nerve preparations in vitro. Mechanical (0-196 mN in 10 s) and heat (34-50°C in 30s) stimulations were applied on their receptive field. NGF was injected into the muscle and heat stimulations were repeated every 10 minutes for up to 1 hour. **Results** : The mechanical threshold was lower in the LC group (P<0.001), but the heat threshold and response magnitude were similar between groups. After repeated heat stimulations the heat threshold slowly increased with a decreased response magnitude in the control group, the NGF group showed lower threshold (P<0.005) and higher response magnitude over time (P<0.05) when compared with the control group. **Conclusion** : The reason for absence of facilitation of the heat sensitivity after LC despite NGF's capability to facilitate it, might be that NGF produced after LC is not sufficient to sensitize it.

## Poster Presentations

### Behavior Science, Biorhythm(2)

### 3PK-087

#### Removal of photic stimulation impairs estrogen-induced anorexia in rats

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Estrogen reduces food intake specifically during the light phase, which is accompanied with the enhanced neuronal activity of the suprachiasmatic nucleus (SCN). To test the hypothesis that enhanced response to photic stimulation is involved in the mechanism for the estrogen-induced attenuation of food intake and body weight gain, we examined the effects of exposure to a constant dark environment (DD) and estrogen replacement on food intake and body weight change in ovariectomized rats. Female Wistar rats were ovariectomized and implanted with either estradiol (E2) or cholesterol (Veh). Rats were then exposed to DD, and food intake and body weight were measured for 2 weeks. Other rats exposed to the 12 h/12 h-light/dark cycle condition (LD) served as control. Exposure to DD increased food intake during the subjective day, but did not during the subjective night, and consequently increased daily food intake. DD exposure did not alter food intake either during the subjective day or night in the Veh group. DD exposure increased body weight gain in the both E2 and Veh groups compared with LD control. These data indicate that the anorexigenic effect of estrogen is dependent on the light environment. The data also indicate that DD increases body weight gain regardless of E2 replacement.

### 3PK-088

#### Effects of site-specific knockdown of estrogen receptor $\alpha$ or $\beta$ in the medial amygdala on social preference in male mice

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Testosterone (T) is known to play a role in the regulation of male social behavior partly by acting on estrogen receptors (ERs) after being aromatized to estradiol. One of the target brain sites of T is the medial amygdala (MeA), which is involved in processing of social odors. It is still not known about the relative importance of ER $\alpha$  and ER $\beta$ , which are both localized in the MeA, for the regulation of social behavior in male mice. Here, we examined the effects of site-specific knockdown of ER $\alpha$  or ER $\beta$  in the MeA on social investigatory behavior in social preference tests with two different stimuli sets. Adult male ICR/Jcl mice were bilaterally injected with an adeno-associated viral vector that silences ER $\alpha$  (ER $\alpha$ KD) or ER $\beta$  (ER $\beta$ KD), or a control vector into the MeA. They were tested for preference between a receptive female and an ovariectomized (OVX) female (PTFF), and between a receptive female and a gonadally intact male (PTMF). In pre-injection sessions, all mice investigated receptive females more than OVX or male mice. In post-injection sessions, control and ER $\alpha$ KD mice sniffed receptive females longer in both PTFF and PTMF tests. In ER $\beta$ KD mice, sniffing time toward a receptive female was not different from that toward an OVX female in the PTFF test although preference toward receptive females in the PTMF test was not affected. These results suggest that activation of ER $\beta$ , but not ER $\alpha$ , in the MeA may be more crucial for social information processing in male mice. (JSPS DC2 to MN and # 23240057 to SO.)

### 3PK-089

#### Sleep dependent synaptic plasticity in rat barrel cortex

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It is well-known that sleep serves several functions, such as energy saving, detoxification, and brain temperature regulation. Recently, many sleep researchers are interested in the relationship between sleep and synaptic plasticity, and they found that excitatory synaptic transmission is enhanced during wakefulness and decreased during sleep. In this experiment, the rats were divided into two groups: sleep-group and wake-group. The rats in sleep-group were sacrificed at the end of a light period and those in wake-group were sacrificed at the end of a dark period, because sleep and wakefulness are dominant in light and dark periods, respectively. We performed whole-cell patch-clamp recordings from layer II/III pyramidal neurons of barrel cortex in acute slices of the rats to investigate the effect of sleep on excitatory synaptic transmission, especially on synaptic glutamate AMPA receptor composition. Excitatory postsynaptic currents (EPSCs) were evoked by electrical stimulus of layer IV. We found that amplitude of the evoked EPSCs (eEPSCs) was decreased by antagonist of Ca<sup>2+</sup>-permeable AMPA receptor (CP-AMPA) in waking rats. On the other hands, the amplitude of eEPSCs was not changed in sleeping rats. These results suggest that the CP-AMPA receptors are increased during wakefulness decreased during sleep. This study could help to clarify the functions of sleep.

### 3PK-090

#### Demonstration of circadian oscillation in the mouse salivary glands (parotid gland, submandibular gland, and sublingual gland) *in vitro*

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The flow rate and composition of unstimulated saliva show noticeable oscillations that follow the circadian rhythm, suggesting that circadian variations influence the physiological function of salivary glands. In mammals, the central circadian pacemaker is located in the hypothalamic suprachiasmatic nucleus (SCN), but circadian oscillators are also located in the extra-SCN and peripheral tissues. Our aim was to determine whether salivary gland function is regulated by its own circadian oscillations or is driven by the master clock SCN. The oral cavity contains 3 major salivary glands (parotid gland, submandibular gland [SMG], and sublingual gland) and innumerable minor salivary glands. The major salivary glands primarily secrete saliva. We used a PERIOD2::LUCIFERASE fusion protein (PER2::LUC) as a real-time reporter of circadian dynamics and analyzed the bioluminescence of the SMG of mouse *in vitro*. We observed that the SMG showed circadian rhythms *in vitro*, which damped after 2-7 cycles. In addition, we observed the circadian oscillations of the parotid and sublingual glands *in vitro*, which also damped after 2-7 cycles. These results indicate that the circadian rhythms in each of the 3 major salivary glands are damping oscillations and that these glands are modulated via sustaining signals.

### 3PK-091

#### The effect of feeding condition and ambient temperature to sleep-wake pattern

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**Aim** It has been reported that fasting decreases sleep period and its pattern. Although several eating-related peptides in the hypothalamus are thought to be involved in the mechanism, we supposed that fasting-induced hypothermia affects sleep at least in a part. Therefore, we tested the hypothesis that changes in ambient temperature, i.e. a factor affecting body temperature during fasting, modulates sleep. **Methods** Male ICR mice (age, 2-4 m) were individually housed in a plastic cage at the ambient temperature of 27°C with a lighting cycle of 12:12 h (lights-on, 7 am). A radio transmitter device for the measurements of body temperature (T<sub>b</sub>), EEG, EMG, and spontaneous activity was implanted in the abdominal cavity. Mice were placed at 20°C, 27°C or 35°C for 30 h with or without food deprivation. **Results** Fasting at 27°C decreased T<sub>b</sub> by 0.9±0.1°C and 0.7±0.1°C in the light and dark phases, respectively. At 20°C, the reductions were augmented (1.6±0.1°C and 2.1±0.2°C); however, disappeared at 35°C. In mice, fasting itself did not increase total sleep period. Both hot and cold decrease the period; however, fasting had no influence on the period. **Conclusion** Heat and cold modulates sleep and T<sub>b</sub>; however, fasting-induced hypothermia seems not to be the factor affecting sleep.

### 3PK-092

#### The Hippocampus and Brainstem were Activated during REM Sleep in Fear-Conditioned Rats

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Hippocampal theta waves are rhythmic field potentials recorded during particular behavioral activities and REM sleep. The theta wave is known to facilitate induction of synaptic plasticity and memory formation. The theta wave was instantaneously accelerated just before an occurrence of phasic activity in the pontine subcoeruleus region, which is called PGO wave. The mechanism of the memory consolidation during REM sleep is not clear, but it was suggested that the hippocampal theta waves and PGO waves are jointly involved in the memory processes. In this study, we have recorded the theta waves and PGO waves during REM sleep in fear-conditioned rats to clarify the mechanism the memory consolidation during REM sleep. We found that frequency of hippocampal theta waves and PGO wave densities were increased just after the fear conditioning. The results suggest that PGO and theta waves contribute cooperatively to the REM sleep dependent memory consolidation.

### 3PK-093

#### Regulatory mechanism in parental behavior of female mouse by oxytocin receptor

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Studies with mice deficient in oxytocin or oxytocin receptor (Oxtr) genes have suggested that this system has essential functions in the overall regulation of social behaviors. Maternal behavior was an important social behavior of female mice, and Oxtr gene deficient (Oxtr<sup>-/-</sup>) mice showed impaired maternal behavior (Takayanagi et al., 2005). OXTR-Venus knockin mice were generated in order to identify OXTR-expressing neurons. They showed several nuclei with higher expression of OXTR, including the lateral septal nucleus (LS) and medial preoptic area (MPOA), which were thought to be the regions associated with maternal behavior (Yoshida et al., 2009). We observed less increase in the numbers of c-Fos positive cells at the LS than the MPOA of Oxtr<sup>-/-</sup> female mice when exposed to pups compared with Oxtr<sup>+/+</sup> female. We next injected AAV-Oxtr vector to the LS of Oxtr<sup>-/-</sup> female mice and observed rescued maternal behavior after parturition. These data suggested the critical role of OXTR expressed in the LS for maternal behavior. In addition, we developed a new system to facilitate neuron type-specific gene expression. We generated lox71 and loxJTZ17-based AAV vector, in which carried gene was inserted in an opposite direction, and Cre recombinase could activate the gene by one-directional inversion of the inserted gene, flanked by the mutant LoxP elements. Using this system, we could achieve neuron type-specific expression of the fluorescence protein gene.

### 3PK-094

#### Correlation of neuronal activity of the amygdala and blood pressure fluctuation during REM sleep

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The amygdala plays important roles in the regulation of emotion or regulation of physiological responses associated with emotion. Human imaging study has demonstrated that the activity of amygdala increases during REM sleep. During REM sleep, large fluctuations of autonomic signs such as blood pressure, respiration or heart rate occur. Therefore, the amygdala is considered to be involved in fluctuation of autonomic nervous system during REM sleep. However, it remains to be revealed how amygdala is involved in autonomic fluctuation during REM sleep. To address this question, we examined the correlation of amygdala neuronal activity and blood pressure during sleep-wake cycle. Of 45 neurons recorded from the amygdala, 25 neurons (55%) increased their activity during REM sleep. Most of the amygdala neuron exhibited phasic firing during REM sleep. Of 16 neurons examined, 7 neurons showed firing correlated with blood pressure fluctuation during REM sleep. Of them, 4 neurons had positive correlation and 3 neurons had negative correlation with blood pressure fluctuation. Firing changes in 4 neurons (3 positive correlated and 1 negative correlated) preceded blood pressure fluctuations, while those of 2 neurons (1 positive correlated and 1 negative correlated) were delayed to it. These results suggest that some of amygdala neurons drive blood pressure fluctuations during REM sleep.

### 3PK-095

#### Blood pressure fluctuation induced by electrical stimulation of amygdala

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During waking, the amygdala is closely related with regulation of emotion. We have reported that large number of amygdala neurons are highly active during REM sleep and some of them fire in synchronous with blood pressure fluctuation during REM sleep. To locate the area in the amygdala that is closely related with blood pressure fluctuation during REM sleep, effect of electrical stimulation to various parts of the amygdala on blood pressure was examined. In urethane anesthetized male rats, electrical stimulation (50 Hz, 3 sec) was applied to the amygdala and blood pressure was measured telemetrically through the cannula inserted into the descending aorta. Under urethane anesthesia, rats alternatively showed two patterns of EEG; large amplitude slow EEG indicating a state of deep anesthesia (deep sleep), and smaller and faster EEG indicating a state of light anesthesia (light sleep). Basal blood pressure was about 4mmHg lower during light sleep than during deep sleep. The stimulus was more effective during light sleep than during deep sleep. Decrease in blood pressure was induced by the stimulation mainly to the central amygdala and the surrounding basomedial and anterior cortical amygdala. Increase in blood pressure was obtained from the basolateral amygdala and posteromedial cortical amygdala. The results lead to the possibility that the central amygdala plays an important role in the regulation of blood pressure fluctuation not only during waking, but during REM sleep.

### 3PK-096

#### Roles of AVP-producing neurons in the central circadian pacemaker of the suprachiasmatic nucleus

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The suprachiasmatic nucleus (SCN) is the primary circadian pacemaker in mammals and entrains to the environmental light/dark cycle. It is composed of multiple types of neurons, and neuronal network properties are integral to normal function of the SCN. However, mechanisms underlying the SCN neuronal network have remained elusive. As a first step to understand the principle of the SCN network, we generated mice in which *Bmal1*, an essential clock component, is deleted specifically in the neurons producing AVP, one of the primary neuronal types in the SCN (*Avp-Bmal1*<sup>-/-</sup> mice). *Avp-Bmal1*<sup>-/-</sup> mice showed lengthening of circadian period (by approximately 1 hour) and activity period (by approximately 5 hours, splitting-like phenotype) in constant darkness. When exposed to abrupt 8-h advance of light/dark cycle, control mice reentrained progressively to new lighting cycle over approximately 11 days. In contrast, *Avp-Bmal1*<sup>-/-</sup> mice did not show progressive shift of their locomotor activity during the transient cycles and reentrained faster (approximately 7 days) than control mice did. In *Avp-Bmal1*<sup>-/-</sup> mice, expression of *Avp*, Prokineticin 2, and *Rgs 16* was drastically reduced in the dorsomedial region of the SCN, where AVP neurons are located. Thus, circadian oscillators of SCN *Avp* neurons may modulate coupling of clock neurons within the SCN to determine circadian period by regulating transcription of multiple factors important for the function of these neurons in a coordinated manner.

### 3PK-097

#### Oxtr expressed in neurons at the MeA are suspected to control social memory

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Oxytocin/Oxytocin receptor (Oxtr) system in mammalian brain might support social behaviors, such as social memory (1). Oxtr<sup>-/-</sup> mice are ideal sources to elucidate the mechanism of neuronal network controlling social memory, and we tried to locate the causative region in brain, where loss of OXTR in neurons directly impaired social memory. We immunohistochemically stained c-Fos (+) neurons in social behaviors-related nuclei prepared from the tested Oxtr-Venus knockin mice, just after social exposure (2). In the results, we detected elevated numbers of c-Fos (+) neurons and also confirmed the co-localization of both c-Fos and Venus in the medial amygdala (MeA). It showed the importance of expression of OXTR in the MeA for social memory. Next, we carried out region-specific deletion of Oxtr gene in the MeA by injection of AAV-Cre into the brain of Oxtr<sup>fx/fx</sup> mice, and region-specific rescuing experiment by injection of AAV-Oxtr into the same nuclei of Oxtr<sup>-/-</sup> mice. Obtained results strongly suggested that the OXTR expressed in the MeA would be essential for social memory of the mice. To further specify the neurons expressing OXTR and analyze their neuronal circuits in the MeA, controlling social memory, we are now developing new AAV-vector facilitating Cre-dependent expression of WGA, introducing Vgat-Cre mice, and generating Oxtr-Cre mice for anterograde tracing experiment. (1) Takayanagi Y et al., PNAS 16096 (2005) (2) Yoshida M et al., J Neurosci 2259 (2009)

### 3PK-098

#### Pubertal activation of estrogen receptor $\alpha$ in the medial amygdala is essential for the expression of social behavior in adult male mice

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Activation of estrogen receptor  $\alpha$  (ER $\alpha$ ) by estradiol, an aromatized metabolite of testosterone (T) plays a crucial role in the regulation of social behavior in male mice. We previously reported that knockdown of ER $\alpha$  in the medial preoptic area (MPOA) in adult greatly reduced sexual without affecting aggressive behavior, while that in the medial amygdala (MeA) had no effect on either behavior. Recent studies have shown that T stimulation in pubertal period is necessary for full expression of sexual and aggressive behaviors in adulthood. However, it is still not known whether and in which brain region ER $\alpha$  is involved in this developmental effect of T. Thus in this study, we examined the effects of site-specific knockdown of ER $\alpha$  during pre-pubertal period. At the age of 21 days, gonadally intact male mice (ICR/Jcl) were bilaterally injected either with adeno-associated viral vector silencing ER $\alpha$  or a control vector in the MeA or MPOA. All mice were then tested for their sexual and aggressive behaviors starting at 12 weeks old. We found that pre-pubertal knockdown of ER $\alpha$  in the MeA reduced both sexual and aggressive behaviors while that in the MPOA only reduced sexual, but not aggressive behavior. These results suggest that ER $\alpha$  activation in the MeA during pubertal period may be essential for the organizational action of T for the expression of male sexual and aggressive behaviors in adulthood. (Supported by Grant-in-Aid for Scientific Research 23240057 to SO.)

### 3PK-099

#### Early weaning influences vulnerability to binge eating induced by limited access to high palatable food in male rats

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It is known that low levels of maternal care in early life are associated with vulnerability to the later development of stress-related binge eating in rats. However, the effect of maternal care on binge eating induced by limited access to palatable food has not been examined. In the present study, we investigated the effect of early weaning on the anxiety-like behavior and the binge eating and body weight induced by limited access to highly palatable (HP) food in Sprague-Dawley rats. Two groups of rats were prepared. One was weaned from the dam at 16 days of age (early-weaned group; EW) and the other at 30 days (normally weaned group; NW) as a control. At 6 weeks of age, anxiety-like behavior was assessed on the elevated plus maze (EPM). At 6-10 weeks of age, both groups had 2-h access to HP food on Mon, Wed, and Fri. Significant effect of early weaning was not observed in the indexes of the anxiety-like behavior in the EPM. At 9-10 weeks of age, male rats in EW consumed significantly more HP food during 2-h period of availability than male rats in NW and all female rats. The increased consumption of HP food was compensated for the decreased standard diet, and cumulative energy consumption and body weight did not differ between the groups. These results indicate that early weaning could promote vulnerability to binge eating with limited access to HP food in male rats but not to anxiety-like behavior.

### 3PK-100

#### Possible roles of area postrema neurons expressing H-channels in the induction of nausea and vomiting

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The area postrema (AP) is well known to be the chemoreceptor trigger zone for nausea and/or vomiting. AP neurons expressing the hyperpolarization-activated cation channel (H-channel) is the major subclass as has been reported in our previous studies. To clarify the relation between excitation of cells expressing H-channels and induction of nausea, we examined the effects of ZD7288 (an H-channel inhibitor), which was applied 10 minutes before the application of apomorphine HCl, on conditioned taste aversion (CTA) learning to saccharin. In addition effects of the drugs on the neuronal activity were evaluated by the measurement of c-Fos expression in the AP and nucleus tractus solitarius (NTS). Male Wistar rats (250-300 g) were used for the behavioral experiments and same animals were used for the immunohistochemical experiments. In a CTA protocol, we used 0.1% saccharin (conditioned taste stimulus) and subcutaneous injection of apomorphine HCl (a nauseant, unconditioned stimulus). The acquisition of CTA learning was measured by 2-bottles test. Whereas animals in a control group acquired CTA learning to saccharin, rats pretreated with ZD7288 failed to acquire CTA learning. Animals with an injection of ZD7288 showed a significantly small number of c-Fos positive AP neurons as compared with control rats. In the NTS, ZD7288 caused no significant reduction in the number of c-Fos immunoreactive cells. These results suggest that AP neurons expressing H-channels play an important role for drug-induced nausea and/or vomiting.

### 3PK-101

#### Effects of timed physical exercise on phase shifts of human circadian rhythms induced by 8-h advance shift of sleep schedule under bright light conditions

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Previously, we reported that timed physical exercise accelerated phase shift of sleep-wake cycle but not melatonin rhythm, which was induced by 8-h phase advance shift of sleep schedule under dim light condition (<10lx). In the present study, we examined whether the same timed physical exercise accelerated the phase shifts of circadian melatonin rhythms under bright light conditions. Fifteen male subjects spent 14 days in an isolation unit without knowing the time of day. Following 3 days of habitual sleep schedule, sleep phase was advanced by 8-h. The shifted schedule was continued for 4 days and physical exercise was imposed twice a day (exercise group, n=7). During the waking period, bright lights of 5000 lux were given to the subject from the ceiling. Thereafter, they were released into free-running conditions without any time cue under dim light conditions. The circadian melatonin rhythm was measured on the last day of the habitual sleep time, shifted schedule, and free-run under dim light conditions. On the first day of free-run, sleep onset was phase-advanced by 6.3±1.5 h in the exercise group and by 4.4±4.0 h in the control. The phase shift of circadian melatonin rhythm and phase relationship between the sleep-wake cycle and circadian melatonin rhythms will be discussed.

### 3PK-102

#### Persistent Exposure to Low-dose Bisphenol A Increases spontaneous Motor activity in Adult Male rats

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Bisphenol A (BPA) is an environmental endocrine disrupting chemical. Perinatal and postnatal exposure to BPA has been reported to increase locomotor activity in female mice at 30 days after birth and decrease it in male mice at the same age compared with the same-sex controls. In this study, we investigated whether persistent exposure of 8 weeks-old male SD rat to BPA (50 µg/kg/day) causes alterations of spontaneous motor activity. Rats were implanted i.p. with mini-osmotic pumps containing either vehicle (sesame oil) or BPA. Spontaneous motor activity was measured using an animal movement analysis system and monitored over 5 days from day 9 after implantation until day 13. In the whole day (24 h), BPA increased total spontaneous motor activity by 15% at day 9, 18% at day 10, 15% at day 11, 16% at day 12, and 13% at day 13. In the 12-h light phase, BPA increased total spontaneous motor activity by 36% at day 10, 34% at day 11, 48% at day 12, and 29% at day 13. In the 12-h dark phase, BPA increased total spontaneous motor activity by 13% at day 10. Thus, persistent exposure of adult male rats to low-dose of BPA increased spontaneous motor activity during the 12-h light phase.

### 3PK-103

#### Hypothermia is induced by restricted feeding in mice : Effect of time of day

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Restricted feeding (RF) at a fixed time of day is known to induce a circadian rhythm independent of the suprachiasmatic nucleus, the light-entrainable circadian pacemaker, in nocturnal rodents. Recently, hypothermia was reported to associate with RF. To investigate whether hypothermia depends on time of day or on time in the food-no food cycle, C57BL/6J mice were exposed to 3h RF for 10 days starting from Zeitgeber Time (ZT) 2, 8, 14 or 20, where the time of light-on was defined as ZT0 under light-dark cycles of 12h each. A thermometer with data logger was implanted in the abdominal cavity, and the body temperature as well as wheel-running activity was continuously measured. During RF, the running activity was increased in the light phase and decreased in the dark. The body temperature decreased for the first several days to below 30°C. The minimum temperature levels started to increase from around the 5th RF day. The phase of minimum temperature was independent of the meal time in the first half of the RF schedule, except in mice with RF at ZT20, in which the minimum temperature was located at the time immediately before the meal. From these findings, we concluded that in the beginning of RF the time-course of hypothermia was influenced mainly by the LD cycle, whereas in the later stage of RF the time course was mainly determined by the food-no food cycle.

## Poster Presentations Pathophysiology(2)

### 3PK-104

#### The origin and nature of macrophages in experimental glioblastomas of rats

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It has been shown that the dense accumulation of macrophages in gliomas correlates highly with a worse prognosis for patients. We aimed to clarify the nature of macrophages in terms of their origin and roles. C6 glioma cells were transplanted into neonatal rat forebrains. Rats developed visible tumors in the brain within 4 weeks. Rats bearing brain tumors were transcardially perfused with 4% paraformaldehyde in PBS. The fixed brains were thin-sectioned with a cryostat and the sections were subjected to immunofluorescence staining. To investigate the origin of the macrophages, we transplanted bone marrow from transgenic enhanced green fluorescent protein (EGFP) rats to normal rats after irradiation, which were then subjected to C6 cell transplantation 2 months later. Large numbers of Iba1+ macrophages were present in the experimental C6 gliomas, and their density appeared comparable to that of C6 cells. Almost all macrophages expressed EGFP in the bone marrow transplanted rats, indicating that the macrophages present in gliomas were derived from monocytes, and were not brain-resident microglia. Immunohistochemical analysis demonstrated that TREM2+ macrophages accumulated in the peripheral region of the tumor mass. CD68 was expressed by macrophages located around the necrotic area. Macrophages were often densely accumulated close to blood vessels. These results suggest that macrophages may assist the growth, invasion, and vasculogenesis of gliomas.

### 3PK-105

#### ROS – sensitive TRPM 2 channels accelerate the growth of tumor

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Malignant tumor is known to have various characteristics including unlimited proliferation, metastasis and angiogenesis. Inflammatory microenvironment of solid tumor has newly been found to affect these tumor behaviors and contribute to its progression. Although many observations indicate that tumor-associated macrophages (TAMs) are key links between inflammation and cancer, the mechanism of how inflammation enforces protumoral activity of TAM is not fully understood. Here we show that the growth of tumor is promoted by the TRPM2 that is sensitive to reactive oxygen species, major proinflammatory mediators, and is responsible for macrophage functions such as cytokine and chemokine production. TRPM2 is functionally expressed in TAM and controls angiogenic cytokines production. Immunohistochemical examination of the tumor cryosection shows structural alterations of the intratumoral microvessels in *Trpm2* knock-out (KO) mice bearing sc-implanted B16F10 cells; total vessel density is increased but average cross-sectional area of each vessel is reduced. In addition, the microvessels in the tumor of *Trpm2* KO mice have lower pericyte coverage, which is the characteristics of immature and unstable vessel. Consistent with these observations, *Trpm2* KO mice display reduced tumor growth and prolonged survival after tumor inoculation. Thus, it can be concluded that TRPM2 is responsible for vessel formation in tumor by modulating the functions of TAM.

### 3PK-106

#### Bromvalerylurea, an outdated hypnotic/sedative, exerts curative effects on rats with 6-OHDA-induced Parkinsonism

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Damaged neurons express damage-associated molecular patterns (DAMPs) such as heat shock proteins and HMGB-1, which can activate microglia to induce proinflammatory reactions that further aggravate neuronal damage. Therefore, such vicious cycles should be prevented either by inhibiting neuronal damage or microglial activation. We previously attempted to suppress the activation of microglia to ameliorate neuronal damage. Recently bromvalerylurea (BU), an outdated hypnotic/sedative, was found to suppress nitric oxide (NO) release by lipopolysaccharide (LPS)-activated microglial cells in a concentration-dependent manner. Inducible nitric oxide synthase (iNOS) expression by LPS-activated microglial cells was suppressed at mRNA and protein levels as revealed by real-time RT-PCR and immunoblotting. A rat model of Parkinson's disease (PD) model was induced by administering 6-OHDA into the right striatum to cause a substantial loss of dopaminergic neurons in the substantia nigra pars compacta. BU dissolved in drinking water was administered to the PD model rats at a dose of 50 mg/kg body weight/day. BU administration prevented dopaminergic neuron loss and microglial activation. Furthermore, BU ameliorated motor function of the rats as revealed by Rota-rod test. Thus, BU may be a promising agent for the treatment of PD by suppressing microglial activation.



### 3PK-107

#### The alteration of glutamate clearance in astrocytes derived from MeCP2-null mouse

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Rett syndrome (RTT) is a neurodevelopmental disorder that is the leading cause of mental retardation in females. The classical RTT cases are caused by mutations in the methyl-CpG-binding protein 2 (MeCP2) gene. We cultured astroglial cells from the MeCP2-deficient RTT model neonatal mouse brains and examined astroglial gene expression and the capacity of glutamate (Glu) clearance in comparison with those from wild type mouse brains. When high extracellular Glu was added to the astrocyte cultures and incubated, a time-dependent decrease of extracellular Glu concentration occurred due to Glu clearance by astrocytes. Although the shapes of the profiles of Glu concentration versus time for each strain of astrocytes were grossly similar, Glu concentration in the medium of MeCP2-null astrocytes were lower than those of control astrocytes at 12 and 18 h. In addition, MeCP2 deficiency impaired downregulation of excitatory amino acid transporter 1 and 2 (EAAT1/2) transcripts, but not induction of glutamine synthetase (GS) transcripts, upon high Glu exposure. In contrast, GS protein was significantly higher in MeCP2-null astrocytes than in control astrocytes. These findings suggest that MeCP2 affects astroglial genes expression in cultured astrocytes, and that abnormal Glu clearance in MeCP2-deficient astrocytes may influence the onset and progression of RTT.

### 3PK-108

#### A possible anti-metastasis therapy based on analyses of pre-metastatic microenvironmental reconstruction in target lymph nodes upon squamous cell carcinoma metastasis

Shiota, Kohei; Guilian, Alejandro Mayorca; Yano, Hajime;

Sugimoto, Kana; Takahashi, Hisaaki; Tanaka, Junya (Molecular and Cellular Physiology, Ehime Univ. Ehime, Japan)

Metastasis is a critical threat to the life of cancer patients. Multicellular organisms, including humans, maintain their tissue organization through many mechanisms to prevent the destruction of tissues and organs and the progression of cancer. Therefore, achieving metastasis is not easy, even for cancer cells. Here we found pre-metastatic tissue reconstruction in tumor-draining lymph nodes, induced by tumor cells in a squamous cell carcinoma (SCC)-xenograft metastasis model, with vasculogenesis and mimicry of inflammation at primary tumor sites. Vasculogenesis was induced by injection of conditioned medium from SCC, but inflammation was not. These results strongly suggested that soluble factors played roles in vasculogenesis. Lysyl oxidase-like factor 2 (LOXL2), known to play a role in tissue remodeling, was identified by gene expression analyses as a possible factor responsible for the tissue reconstruction. Furthermore, we found over-expression of Sodium Ion/Proton Exchanger-1 (NHE1), which we had previously identified as a factor responsible for cellular invasion in a highly metastatic SCC, SASL1m, which was used in the xenograft model above. We would like to discuss the possibility of developing combinatorial "anti-metastatic therapy" with anti-invasion and anti-tissue reconstruction factors.

### 3PK-109

#### Analysis of the mode of action of 5-(N-ethyl-N-isopropyl)-amiloride (EIPA) on the inhibition of glioma cell invasion

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Sugimoto, Kana; Takahashi, Hisaaki; Tanaka, Junya (Molecular and Cellular Physiology, Ehime Univ. Ehime, Japan)

One of the critical threats of gliomas is their insidious invasion into the brain parenchyma, by which therapeutic outcome is greatly reduced. NHE1 regulates intracellular pH, functions as an anchor point for the actin-cytoskeleton on the plasma membrane, and participates in the maintenance of cellular motility and polarity. Previously, we found aberrant over-expression of NHE1 in gliomas, correlated with high invasive activity. Moreover, an NHE1 inhibitor, EIPA, suppresses glioma invasion in vitro and in vivo, implying that NHE1-inhibition is a novel "anti-invasion therapy" for gliomas. In order to establish this "anti-invasion therapy" for gliomas, we attempted to elucidate the mode of action of EIPA to obtain basic knowledge about the mechanisms of inhibition of glioma cell invasion. Morphological analysis of EIPA-treated glioma cells revealed prominently hampered reorganization of the actin-cytoskeleton. We investigated several factors in these cells and observed a decrease in Rac1 activity and unaltered Arf6 activity. Focusing on molecular events, we observed a decrease in the molecular weight of NHE1 from approximately 100kDa to 74kDa. We would like to discuss how these changes can contribute to the alteration of pH regulation and Rac1 activity.

### 3PK-110

#### Na<sup>+</sup>/H<sup>+</sup> Exchanger 1 (NHE1) as a molecular target to suppress glioblastoma invasion

Yaguchi, Haruna; Shimoda, Takefumi; Shiota, Kohei;

Sugimoto, Kana; Takahashi, Hisaaki; Yano, Hajime; Tanaka, Junya (Molecular and Cellular Physiology, Ehime Univ. Ehime, Japan)

Glioblastoma is characterized by insidious invasion of cerebral parenchyma. We found that human and rat glioblastoma cells expressed NHE1 at higher levels than rat astrocytes. We investigated the effect of NHE1 knockdown on glioblastoma cells. NHE1 knockdown suppressed glioblastoma cell invasive activity by over 90% as assessed by matrigel invasion assay. Furthermore, the invasion activity was inhibited by 5-(N-ethyl-N-isopropyl)-amiloride (EIPA), which disturbs intracellular pH control by NHE1. The invasive activities of various glioblastoma cells were evaluated using the matrigel assay using serum as a chemoattractant. Serum strongly attracted most cells, but it suppressed the migration of other cells. EIPA attenuated all types of invasive migration of glioblastoma cells, suggesting that EIPA may be utilized as an anti-invasion agent for treatment of glioblastomas. Rat glioblastoma cells (C6) were transplanted into the brains of nude mice to investigate the in vivo effects of EIPA as an anti-invasion agent for glioblastoma. Since C6 glioblastoma cells insidiously and widely invade the brain parenchyma, this in vivo glioblastoma model is considered valid for the study of glioblastoma cell invasion. Using this model, EIPA was found to suppress the insidious invasion of C6 glioblastoma cells by 75% in vivo. We are currently determining the maximum suppressive effect of EIPA and the most effective duration of administration. In conclusion, EIPA may be a potential anti-invasive agent for the treatment of glioblastomas.

### 3PK-111

#### Inhibition of VEGFR signaling suppresses $\beta$ -cell injury and diabetes development in SDT rats

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Objective Spontaneously Diabetic Torii (SDT) rat, a model of non-obese diabetes, exhibits vascular abnormalities within islets at pre-diabetic stage (8 weeks old), followed by  $\beta$ -cell loss. We investigated the relationship between  $\beta$ -cells and vasculature within islets and its contribution to pathogenesis of diabetes in SDT rats. Methods SDT rats (5 weeks), which do not show any morphological change in islets, were injected with Habu snake venom (HSV), streptozotocin, or a VEGF receptor (VEGFR) inhibitor Sunitinib. Results HSV injection induced hemorrhage in islets in SDT rats, whereas islets in control SD rats showed less bleeding. Co-injection of soluble VEGFR apparently ameliorated HSV-induced hemorrhage in SDT rat islets. Although saline-treated SDT rats at 13-weeks-old showed hemorrhage and inflammation in islets,  $\beta$ -cell depletion induced by Streptozotocin resulted in neither hemorrhage nor inflammation in islets. VEGF secretion from islets was increased in SDT rats compared with in SD rats. Five weeks Sunitinib treatment inhibited hemorrhage in islets,  $\beta$ -cell loss, and diabetes development in SDT rats. Conclusion Enhanced VEGF signaling in islets contributes to islet vascular failure and consequential  $\beta$ -cell dysfunction in SDT rats

### 3PK-112

#### Effects of TGF $\beta$ 1 on microglia in the penumbra of ischemic rat brain

Sugimoto, Kana; Nishioka, Ryutarō; Mise, Ayano; Ohhara, Yudai; Shiota, Kohei; Takahashi, Hisaaki; Yano, Hajime; Tanaka, Junya (Molecular and Cellular Physiology, Grad Sch of Med, Ehime Univ, Japan)

Resident activated microglia were present in the penumbra of the ischemic brains of rats that were subjected to transient right middle cerebral artery occlusion. Some of these microglia were found to express NG2 chondroitin sulfate proteoglycan (NG2). Compared to resting microglia in the contralateral hemisphere, NG2<sup>+</sup> microglia in the penumbra were characterized by short thick processes and enlarged somata. Furthermore, they expressed a phagocyte marker, CD68, and a triggering receptor expressed on myeloid cells 2 (TREM-2), which may be responsible for the recognition of apoptotic neurons. These proteins are not expressed by resting microglia. Observation with confocal laser scan microscopy showed that NG2<sup>+</sup> microglial cells were located in the close vicinity of degenerating neurons. NeuN<sup>+</sup> material was found in the somata of some NG2<sup>+</sup>/CD68<sup>+</sup>/TREM2<sup>+</sup> microglia, suggesting that these microglia are engaged in the elimination of degenerating neurons. TGF $\beta$ 1, TGF $\beta$ 1R1, and TGF $\beta$ 1R2 mRNA expression increased in the ischemic brains, as revealed by quantitative real-time RT-PCR. TGF $\beta$ 1 increased the expression of NG2 protein and TREM-2 mRNA by primary cultured microglia. These effects were abolished when the cells were incubated with a TGF $\beta$ 1 inhibitor. In conclusion, TGF $\beta$ 1 stimulated microglial cells in the penumbra to express NG2, leading to elimination of degenerating neurons in the penumbra of the ischemic brain.

### 3PK-113

#### Treadmill exercise as rehabilitation for stroke model ; its effects on brain edema and glial reactions

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Although rehabilitation may be the most effective therapy for stroke, mechanisms underlying the curative effects are not fully elucidated. In this study, we addressed the short-term effects of treadmill exercise on ischemic brain edema and glial cell reactions. Wistar rats were subjected to transient (90 min) right middle cerebral artery occlusion that produced large stroke lesion. The area of the lesion was measured with magnetic resonance imaging on the next day or 1 day-post reperfusion (1 dpr), and only rats with substantially large ischemic lesion were grouped into exercise and non-exercise ones. Treadmill was horizontally set and its speed was at 4 m/sec for 1 and 2 dpr and 6 m/sec for 3 and 4 dpr. The rats ran only for 10 min/day. On the 5 dpr, rats were transcardially perfused with paraformaldehyde and fixed brains were dissected out. Cryosections were made and subjected to immunohistochemical staining with antibodies to GFAP and nestin. The nestin<sup>+</sup> and GFAP<sup>+</sup> areas in the penumbra in the cerebral cortex were measured. To evaluate the brain edema induced by the ischemic events, total area of right and left hemisphere were independently measured. Consequently, the brief and light exercise caused amelioration of brain edema and concomitant increase of expression of GFAP and nestin. The present study suggests that such brief and light exercise exerts ameliorating effects in the sub-acute phase of stroke. The ameliorating effects may possibly be through the enhanced gliosis.

### 3PK-114

#### The involvement of ryanodine receptors on ischemic preconditioning

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Ischemic preconditioning (IPC) is induced by a variety of insult to the brain such as nonfatal ischemia and provides strong neuroprotective effect. Although its mechanisms are still obscure, Ca<sup>2+</sup> is regarded as one of key mediators of IPC. The purpose of the present study is to investigate the involvement of ryanodine receptor (RyR) as Ca<sup>2+</sup> release channel in IPC. IPC was induced by 2-min occlusion of bilateral common carotid artery of the gerbils and neuroprotective effect of IPC against fatal ischemia (5-min occlusion) was evaluated by survival CA1 cell count in the hippocampus. An administration of dantrolene (25 mg/kg, ip; RyR antagonist) just before the induction of IPC was significantly suppressed the protective effect of IPC, while caffeine (100 mg/kg, ip; RyR agonist) potentiated it. In vitro experiment was also performed by using rat hippocampal cell culture. IPC was induced by 10-min oxygen-glucose deprivation (OGD) in the culture cells and its protective effect was suppressed by RyR antagonist as well as in vivo experiment. Furthermore, the changes in three RyR isoforms of culture cells after IPC were examined by real-time PCR and both RyR type 1 and 2 significantly decreased 24hr after 10-min OGD. These results indicate that RyR contributes to both induction and retention of ischemic tolerance.

### 3PK-115

#### Response of glial cells in and around ischemic lesion of rats that were subjected to transient middle cerebral artery occlusion : the involvement of IL-18

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Response of glial cells in and around ischemic brain lesions were investigated in this study, using a rat stroke model, in which middle cerebral artery was transiently (90 min) occluded. Blood-borne macrophages massively accumulated in the core of ischemic brain lesions and their number reached the maximum around 7 days-post reperfusion (7 dpr). The macrophages have been shown engaged in scavenging cell and tissue debris and also to express neuroprotective factors such as IGF-1 and HGF. Interleukin-18 (IL-18)-mRNA was found highly expressed in the ischemic core around at 7 dpr as revealed by real-time RT-PCR, and immunohistochemical investigation demonstrated that macrophages in the core expressed IL-18. IL-18 produced in the core may presumably diffuse into the penumbra region, affecting glial cells there. To examine the effects of IL-18 on glial cells, mixed glial cultures started from the neonatal rat forebrains were incubated with recombinant rat IL-18. IL-18 markedly enhanced expression of interferon  $\alpha$  and  $\beta$  by glial cells, suggesting that the recombinant IL-18 normally worked. Furthermore, IL-18 elevated the expression of mRNAs encoding HGF, nestin and NG2 in a dose-dependent manner. These results suggest that IL-18 may regulate the response of glial cells in the penumbra. Since mixed glial cell culture contained astrocytes, microglial cells and NG2 glial cells, a study is currently conducting to determine which glial cells respond to IL-18.

### 3PK-116

#### Increases in Breath Carbon Oxide during Night Sleep are associated with Deterioration of Mental Health Conditions

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Numerous kinds of measures to assess mental conditions have been proposed. Most of them are questionnaires or chemical analysis of blood, urine or saliva. In the present study, we examined whether breath carbon monoxide can be a biomarker to evaluate mental stress and or psychological health status. Seventy five adults volunteered to this study. Current smokers and drinkers were excluded. Mental stress, depression and neurosis levels were evaluated by the General Health Questionnaires (GHQ), Self-Depression Scale (SDS) and Cornell Medical Index (CMI), respectively. Before sleep and after rising, end-tidal breath was collected into bags. Breath CO was analyzed by gas chromatography with a semiconductor sensor. Differential changes in breath CO during the night sleep (dCO) were calculated. There was a significantly positive correlation between GHQ and CMI scores. The dCO was significantly correlated to the scores of CMI and GHQ, but not to SDS score. In addition, dCO had significant correlations with % body fat, item scores of snoring, daytime fatigue and napping. Both GHQ and CMI scores were significantly correlated to fatigue score after rising and during daytime. These results suggested that dCO could be an available biomarker to assess mental health conditions, although differential diagnosis of the obstructive sleep apnea is needed.

### 3PK-117

#### Alterations of hydrogen exhalation and carbon monoxide production in monocrotaline-induced pulmonary hypertension rats

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Hydrogen molecules ( $H_2$ ) are produced by colonic fermentation. There is a growing body of evidences that  $H_2$  weakly but selectively scavenges hydroxyl radicals. Carbon monoxide (CO) plays key roles as a gaseous mediator in cardiovascular, nervous and inflammatory systems. This study explored their interactive relationship and distribution characteristics in monocrotaline (MCT)-induced pulmonary hypertension model. Male specific-pathogen free SD rats aged with 6 weeks were randomly divided into two groups with or without injections of 60 mg/kg MCT. After 3 weeks, arterial blood and exhaled air were collected under anesthesia and artificial ventilation. Thereafter, colon, liver, lung and heart were immediately excised and washed in saline solution. Each organ was separately incubated in a sealed aluminum bag with a constant volume of purified air. Concentrations of  $H_2$  and CO were analyzed by gas chromatography. There was not significant difference between  $H_2$  releases from the colon in both groups. In MCT rats, breath CO and hepatic CO releases were significantly increased, whereas hepatic  $H_2$  release was not changed. Interestingly, breath  $H_2$  indicated a significantly inverse correlation to the ratio of breath  $H_2$ /colonic  $H_2$ , suggesting that CO production in the liver was associated with the reduction of breath  $H_2$  originating from colonic fermentation.

### 3PK-118

#### Expression of sodium ion/proton exchanger 1 (NHE1) in macrophage-like cells recruited into the ischemic brain lesion

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Sodium ion/proton exchanger 1 (NHE1) exports proton from cytoplasm to extracellular space by that contribute to regulate intracellular pH to be alkaline, as well as anchoring actin cytoskeleton onto the plasma membrane, and hence participates in the maintenance of cellular morphology, motility, and polarity. In rat brain, NHE1 distributes to vascular endothelial cells and presynaptic membrane of neurons. Upregulation of NHE1 in penumbra region of ischemic brain lesion, possibly on endothelial cells, is also reported. We have elucidated that NHE1 play a role in the invasions of gliomas, a kind of brain tumor. Here we examined the NHE1 expressions in cells recruited to ischemic brain lesion, and found the expression in macrophage-like cells recruited to ischemic core region. We also observed the NHE1 expression in primary cultured microglia, and also the high level of expression in core of the ischemic brain lesion compared with penumbra and non-ischemic hemisphere. Roles of the microglia and macrophage-like cells in brain diseases are still controversial. For example in brain tumor, the cells possibly participate, at least partly, in progression of the tumor, while are brain protective in ischemic brain injury. We would like to discuss about the role of NHE1 expressions in microglia as well as macrophage-like cells.

### 3PK-119

#### Role of alpha<sub>2</sub>-antiplasmin on repair responses after brain damage

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Brain damage caused by ischemic stroke triggers repair processes including microglial accumulation and astrocyte activation. In addition, the accumulated microglia form a granulation layer at the damage surroundings, which is invaded by new vessels. Previously we reported that mice with gene deficient of plasminogen showed less clearance of damage tissue. Plasminogen is an inactive precursor of plasmin which is an extracellular protease involved in tissue remodeling. Based on these findings, we studied the role of alpha<sub>2</sub>-antiplasmin ( $\alpha_2$ -AP), a physiological inhibitor of plasmin, on these responses after ischemic stroke. Ischemic brain damage was induced in mice with gene deficient of  $\alpha_2$ -AP ( $\alpha_2$ -AP<sup>-/-</sup>) and their control wild type mice ( $\alpha_2$ -AP<sup>+/+</sup>) by a photochemical reaction. In this model, both size and location of induced damage are highly reproducible among individual mice. Then we evaluated the damage size, microglial accumulation and vessel invasion in the granulation layer. The induction of glial-fibril acidic protein (GFAP), which induction is associated with astrocyte activation, was also evaluated. On day 4, damage size was comparative between  $\alpha_2$ -AP<sup>+/+</sup> mice and  $\alpha_2$ -AP<sup>-/-</sup> mice. However, the induction of GFAP in ipsilateral hemisphere was remarkable higher in  $\alpha_2$ -AP<sup>-/-</sup> mice than in  $\alpha_2$ -AP<sup>+/+</sup> mice. Furthermore, the number of invaded vessels in the granulation tissue was higher in  $\alpha_2$ -AP<sup>-/-</sup> mice than  $\alpha_2$ -AP<sup>+/+</sup> mice, although the microglia accumulation was comparative between these mice. These findings indicate that  $\alpha_2$ -AP is involved in the brain repair processes after ischemic stroke.

### 3PK-120

#### Blood flow response to carbon dioxide in human internal and external carotid arteries during hyperthermic condition

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The purpose of this study was to assess carbon dioxide (CO<sub>2</sub>) reactivity in the internal carotid (ICA) and external carotid (ECA) arteries during normothermic and hyperthermic conditions. Five young healthy subjects aged 23±3 yrs dressed tube-lined suit and rested in a supine position. Skin and esophageal temperatures were controlled by changing water temperature perfusing the suit. Both ICA and ECA blood flow were measured by ultrasonography (Vivid-e; GE healthcare, Tokyo, Japan) before and during whole body heating. CO<sub>2</sub> reactivity in both arteries was identified as the % increase in ICA and ECA blood flow per mmHg change in partial pressure of end-tidal CO<sub>2</sub> during standardised hypo/hyperventilatory challenges. Esophageal temperature was increased from 37.0±0.3 to 38.5±0.3°C during whole body heating. The heat stress decreased ICA blood flow and increased ECA blood flow by -29±6 and +109±38%, respectively (P<0.005). CO<sub>2</sub> reactivity in the ICA tended to decrease from 3.9±1.3 to 2.8±0.7%/mmHg. In contrast, CO<sub>2</sub> reactivity in the ECA increased from 0.5±0.6 to 1.5±1.3%/mmHg, but not significantly (P=0.268). Blood flow and CO<sub>2</sub> reactivity in both the ICA and ECA were affected differently by the hemodynamic challenges imposed by heat stress, the functional significance of which warrants further investigation.

### 3PK-121

#### Glutamate in orexin neurons is important for stress-induced thermogenesis

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A subset of orexin neurons expresses vesicular glutamate transporter-2 (vGLUT2), a marker of glutaminergic neurotransmission. We hypothesized that glutamate co-localized in the orexin neurons is important for stress-induced thermogenesis because prior treatment with glutamate receptor antagonists but not with orexin receptor antagonists markedly inhibited thermogenesis induced by successive injection of a pyrogen, PGE<sub>2</sub>. To test our hypothesis, we made a mouse model (ORX-Cre; vGLUT2<sup>fl/fl</sup>) in which vGLUT2 is deficient only in orexin neurons using Cre/loxP system. As compared to the control (vGLUT2<sup>fl/fl</sup>) mice, phenotypes of the ORX-Cre; vGLUT2<sup>fl/fl</sup> mice were summarized as follows. 1) Stress-induced thermogenesis was blunted. 2) Stress-induced tachycardia was also attenuated, as was the case in orexin knockout mice. 3) Locomotor activity was not difference between the genotypes during both nighttime and daytime. From these results, we conclude that putative neurotransmitter glutamate in orexin neurons plays an important role in stress-induced autonomic changes but has only a minor role, if any, in sleep/wake cycles. Stress-induced tachycardia is caused by both of glutamate and orexin in orexin neurons.

## Poster Presentations Environmental Physiology(2)

### 3PK-122

#### Comparison between palm and forearm skin blood flow responses to arterial and venous occlusions

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The blood flow responses in palm and forearm skin were compared by measuring the blood flow velocity in the thenar area by laser speckle flowgraphy and in the forearm skin by both laser speckle flowgraphy and laser Doppler flowgraphy before during and after 5-min arterial (50 mmHg above systolic blood pressure) and 10-s venous (30 mmHg) occlusions in 11 healthy young males. Heart rate and blood pressure were recorded continuously. Arterial occlusion decreased the skin blood flow observed by laser speckle flowgraphy significantly more in the palm ( $-57 \pm 3\%$ , mean  $\pm$  SE) than in the forearm ( $-37 \pm 2\%$ ). The increase in skin blood flow immediately after arterial occlusion (flow-mediated dilatation) was significantly less in the palm ( $+94 \pm 13\%$ ) than in the forearm ( $+128 \pm 8\%$ ). Venous occlusion significantly decreased the two blood flows by similar amounts ( $-18 \pm 4\%$  in the palm,  $-16 \pm 3\%$  in the forearm). There were no significant differences in data obtained by laser Doppler flowgraphy and speckle flowgraphy. No significant change occurred in heart rate or blood pressure. These results suggest that the skin vasculature dilates less in palm skin than in forearm skin during flow-mediated dilatation, and contracts more in palm skin than in forearm skin during arterial occlusion. Supported by KAKENHI 24650352.

### 3PK-123

#### Effect of static exercise on the blood flow response in the posterior cerebral artery to visual stimulation

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We have reported that the intensity of dynamic exercise has no effect on neurovascular coupling (NVC) as assessed by the increase in blood flow in the posterior cerebral artery (PCA) to visual stimulation. The effect of static exercise on NVC was investigated by measuring the blood flow velocity in the PCA (PCAv) by transcranial Doppler ultrasound flowmetry during rest and 2-min isometric handgrip exercise at 30% maximal voluntary contraction in 17 healthy males. NVC was estimated as the relative change in PCAv from the mean value during 20 s of eye closing to the peak response to 40 s of visual stimulation involving looking at a reversed checkerboard. The conductance index (CI) of the PCA was calculated by dividing PCAv by the mean arterial pressure. PCAv was significantly higher than the resting baseline during static exercise, and significantly higher during visual stimulation than the eye-closed baseline during either rest or exercise, indicating that the PCAv response to visual stimulation was not significantly affected by static exercise. Exercise significantly increased the pressor response to visual stimulation [ $3.7 \pm 1.1$  mmHg (mean  $\pm$  SE) at rest,  $9.8 \pm 1.2$  mmHg during exercise] but significantly inhibited the CI response ( $8.4 \pm 1.6\%$  at rest,  $1.9 \pm 1.3\%$  during exercise). These results may indicate that static exercise does not affect the magnitude of NVC to visual stimulation but does affect the relative contributions of the pressor response and vasodilatation to NVC. Supported by Kozuki Foundation.

### 3PK-124

#### The role of primary somatosensory cortex in chronic pain

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There is increasing evidence that plastic changes of neuronal circuits are important for chronic pain. We previously reported that the neuronal activity, spine turnover and motility were increased in somatosensory cortex (S1) under chronic pain conditions. However, it remains to be clarified whether the S1 really contributes to the chronic pain behavior. In the present study, therefore, we studied the activity of neuronal and astrocytic cells in S1 using in vivo 2-photon Ca<sup>2+</sup> imaging under chronic pain conditions which was made by partial ligation of the right sciatic nerve (PSL) of mouse. As previously reported, neuronal activity of contralateral S1 was increased by PSL. Interestingly, astrocyte activity was increased in not only contralateral but also ipsilateral S1. In ipsilateral S1, the activity of inhibitory neuron also increased. These ipsilateral activity was inhibited by TTX injection into contralateral S1, indicating involvement of callosal input. Surprisingly, low concentrations of gabazine applied to ipsilateral S1 increased the spine motility and turnover in excitatory neurons S1 and reduced the mechanical withdrawal threshold of left hindpaw. The results suggest that concomitant increase of neuronal and astrocytic activities may produce reorganization of neuronal circuit of S1, thereby causing chronic pain.

### 3PK-125

#### Thermal sensation during hyperthermia is modulated by postural change in men

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Increase in skin and esophageal temperature ( $T_{sk}$  and  $T_{es}$ ) induces autonomic and behavioral thermoregulation with the sensation of both temperatures. We assessed whether the sensation of both temperatures was modulated by postural change from supine (SUP) to sitting (SIT) during hyperthermia (HT) which attenuates autonomic thermoregulation. Methods: Fifteen healthy young men underwent measurements of noticeable increase/decrease ( $\pm 0.1^\circ\text{C}/\text{sec}$ ) of  $T_{sk}$  (warm/cold threshold) at forearm and chest by using a thermode (6.25 cm<sup>2</sup>), and of whole body thermal sensation (visual analogue scale) in SUP and SIT during normothermia (NT,  $T_{es}$ ;  $36.6 \pm 0.2^\circ\text{C}$ ) and HT ( $T_{es}$ ;  $37.3 \pm 0.1^\circ\text{C}$ , lower legs immersion in  $42^\circ\text{C}$  of water).  $T_{es}$ ,  $T_{sk}$ , blood pressure, cutaneous vascular conductance (CVC) and sweat rate (SR) at forearm and chest were measured continuously. Results: Pulse pressure in SIT was lower than SUP during both NT and HT ( $P < 0.05$ ). Cold threshold at both sites were lower during HT than NT in SIT ( $P < 0.05$ ) but not in SUP, with interactive effects of temperature (NT vs. HT)  $\times$  posture (SUP vs. SIT) (chest,  $P = 0.08$ ; forearm,  $P < 0.05$ ) in two-way ANOVA, indicating that attenuation of cold threshold with HT was greater in SIT than SUP. Whole body thermal sensation was higher in SIT than SUP during both NT and HT. Conclusions: Thermal sensation during hyperthermia is modulated by postural change.

### 3PK-126

#### Facial circulatory responses evoked by singing

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The facial skin color characteristically responds to various emotions, suggesting underlying changes in the facial blood flow. We observed responses in facial circulation to emotions evoked by singing. Fourteen healthy subjects (12 males and 2 females) sang a children's song for 90 seconds in front of an experimenter. The facial blood flow (BF) and vascular conductance index (CI) were measured using laser speckle flowgraphy before, during, and after singing. The heart rate (HR) and mean arterial pressure (MAP) were recorded continuously. The CI in the face was calculated as the ratio of BF to MAP. Subjective emotion scores were measured immediately after singing. During singing, the HR and MAP, the BF's in the eyelid, cheek, and upper lip, and the CIs in the eyelid and upper lip were significantly higher than the corresponding baseline values. The singing task evoked significant subjective emotions of embarrassment, amusement, strain, and arousal, but these emotion scores were not significantly correlated with the BF and CI responses. These results suggest that singing characteristically elicits regional changes in facial circulation that are not directly related to subjective emotion scores. Supported by KAKENHI 24650352.

### 3PK-127

#### Ocular blood flow responses to an acute decrease in blood pressure in resting humans

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Autoregulation to acute fluctuations in blood pressure in ocular vessels is unclear. The present study aimed to characterize dynamic autoregulation in the ocular circulation during an acute decrease in blood pressure. Blood flows in the superior nasal and inferior temporal retinal arterioles (SNRA and ITRA) and in the retinal and choroidal vessels (RCV) were measured by laser speckle flowmetry before and immediately after an acute decrease in blood pressure in 20 healthy subjects. The blood pressure was measured continuously. Acute hypotension was induced by rapidly releasing bilateral thigh occlusion cuffs that had been inflated to 220 mmHg for 2 min. The dynamic autoregulation in the ocular vessels was estimated by calculating the ratio of the relative change in ocular blood flow to the relative change in mean arterial pressure (MAP). Immediately after releasing the thigh cuff, MAP and the blood flows in the RCV, SNRA, and ITRA significantly decreased from the baseline values obtained before releasing the cuff (by  $-13 \pm 1\%$ ,  $-20 \pm 2\%$ ,  $-19 \pm 2\%$ , and  $-18 \pm 2\%$ , respectively; mean  $\pm$  SE). The ratios of the relative changes in ocular blood flows to that in MAP all exceeded 1% / % mmHg ( $1.4 \pm 0.1$  -  $1.6 \pm 0.1$  / % mmHg). Thigh cuff deflation decreased ocular blood flows more than the MAP, which implies that ocular blood vessels do not exhibit dynamic autoregulation to short-time decreases in MAP in resting humans. This work was supported by grant-in-aid for JSPS Research Fellow 247022 (to T. Ike-mura).

### 3PK-128

#### Hibernation-specific high performance of hamster brown fat under extreme hypothermia

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Brown adipose tissue (BAT) of hibernator animals is indispensable thermogenic organ particularly when they arouse from severe hypothermic state of hibernation. BAT temperature increases initially among other body organs when animal starts arousal from hibernation. Treatment of hibernating hamsters with  $\beta_3$ -adrenergic receptor antagonist inhibits or postpones arousal from hibernation while  $\beta_3$ -agonist promotes the arousal. Thus, predominant signal pathway controlling BAT thermogenesis is considered to be  $\beta_3$ -adrenergic in hibernating hamster also. These observations imply that  $\beta_3$ -receptor of BAT functions under severe hypothermia in hibernator. In a series of studies, we investigated  $\beta_3$ -receptor-mediated BAT thermogenesis under extreme hypothermia conditions in hibernator. In vitro measurements of oxygen consumption using fragments of dissected BAT from warm-adapted, cold-adapted, or hibernating animals indicated that BAT of hibernating animals has more thermogenic potency than that of warm-adapted group when measured at 12°C, though measurement done under normothermia (36°C) indicated no difference. Direct stimulation to adenylyl cyclase with forskolin at 12°C evoked equivalent BAT responses in these two groups. Expression of  $\beta_3$ -receptor mRNA in hibernating BAT was slightly smaller than that in warm-adapted animals. This hibernation-specific  $\beta_3$ -mediated high performance BAT thermogenesis under extreme hypothermia condition may be due to an improved efficiency of signal transduction between receptor and adenylyl cyclase activation.

### 3PK-129

#### Microglial IL-1 $\beta$ -induced serotonin transporter (5-HTT) expression in astrocytes in immunologically induced fatigue rats

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Immunologically induced fatigue was introduced in rats by intraperitoneal injection of synthetic double-stranded RNA, polyriboinosinic : polyribocytidylic acid (poly I : C). An injection of poly I : C decreased the daily amounts of spontaneous running wheel activity to  $\sim 60\%$  of the preinjection levels until day 7. Both microglia and astrocytes were activated in the prefrontal cortex (PFC) until 72 hrs after the injection of poly I : C. Pretreatment with minocycline (MC), an inhibitor of microglial activation, blocked the poly I : C-induced decrease in the running wheel activity as well as glial activation. Following poly I : C injection, the immunofluorescence for IL-1 $\beta$  was markedly increased in microglia, but not in astrocytes, in the PFC, which was also blocked by pretreatment with MC. The poly I : C-induced fatigue was blocked by intracerebroventricular injection of IL-1 $\beta$  neutralizing antibody for IL-1 $\beta$ . In primary glial cell culture studies, application of IL-1 $\beta$  induced an expression of 5-HTT in astrocytes but not microglia. Finally, direct application of poly I : C to cultured microglia enhanced IL-1 $\beta$  expression and systemic poly I : C injection induced expression of mRNAs for TLR3 and interferon regulatory factor 3 (IRF3) and IRF7, downstream transcription factors of TLR3. These findings, taken together, suggest that systemic injection of poly I : C induces IL-1 $\beta$  in microglia through TLR3, which in turn enhances expression of 5-HTT in astrocytes, resulting in the development of the immunologically induced fatigue.

### 3PK-130

#### Association of diet-induced thermogenesis with mastication in humans

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The present study examined the effects of eating speed and amount of mastication on diet-induced thermogenesis (DIT). The subjects masticated 100-kcal of solid food for as long as and many times as they could before swallowing in a slow eating trial, while they swallowed the fractured food with 100 mL of pure water in a rapid eating trial. Eating time, the amount of mastication (quantified as the number of chewing movements), and gas exchange variables were measured in six males and three females before and after eating. The amount of mastication increased with the eating time [slow eating vs rapid eating : eating time, 147±22 s vs 41±4 s (mean±SE), amount of mastication, 175±21 times vs 40±4 times,  $P<0.05$ ]. ANOVA revealed an interactive effect of time and trial on gas exchange variables ; postprandial oxygen uptake ( $V_{O_2}$ ) and minute ventilation ( $V_E$ ) were significantly greater in the slow eating trial than in the rapid eating trial 0-15 min after the end of swallowing (e.g. slow eating vs rapid eating :  $V_{O_2}$ , 253±14 mL/min vs 238±14 mL/min,  $V_E$ , 9.1±0.6 L/min vs 8.3±0.4 L/min at 10-15 min,  $P<0.05$ ). These results suggest that the DIT is associated with the characteristics of mastication.

### 3PK-131

#### Effects of lowered skin temperature with whole body surface cooling prior to exercise on lactate threshold in cool environment

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Lowered skin temperature (T<sub>sk</sub>) with whole body surface cooling before exercise enhances aerobic performance in the heat, while the effects of this procedure on aerobic performance in a cool condition remain unknown. The procedure increases sympathetic nerves activity and glycolysis, therefore we hypothesized that the procedure increases plasma lactate concentration ([Lac]p) and reduces lactate threshold (LT) during exercise in a cool environment. Methods : Six healthy adults performed a graded maximal cycling exercise after pre-cooling (60 min) in 3 conditions. The temperatures of room air (T<sub>a</sub>) and water perfusion suit (T<sub>w</sub>) were controlled at 10°C and 10°C in Cool-Cool (CC), 25°C and 10°C in Mild-Cool (MC), 25°C and 34°C in Mild-Neutral (MN) conditions, respectively. [Lac]p, heart rate, blood pressure, expired gas, esophageal (T<sub>es</sub>) and T<sub>sk</sub> were measured and LT was determined. Results : After pre-cooling, T<sub>es</sub> was not different among conditions while T<sub>sk</sub> for CC and MC was lower than MN. [Lac]p after pre-cooling and during each workload were higher and LT was lower in the order of CC, MC, and MN. Conclusions : LT is decreased by lowered skin temperature with whole body surface cooling prior to exercise in a cool environment.

### 3PK-132

#### The effect of hypergravity exposure for two weeks on propofol anesthesia in rats

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General anesthesia in space flight is a big challenge for now. To achieve this, understanding the influence of gravitational change on central nervous system (CNS) modulation is required, however it is still unclear. The vestibular system is known to be a highly plastic organ. Previous studies reported that the hypergravity exposure induced plastic alteration of the glutamergic and GABAergic system via the vestibular system. We hypothesized that hypergravity exposure-induced CNS modulation might change the effectiveness of anesthetic agent. To clarify this hypothesis, we examined the effect of the intravenous propofol anesthesia in rats reared in 1 G and 3 G environments for 14 days. We measured electroencephalogram (EEG), electrical stimulation-induced pressor response, propofol metabolism, and GABAA  $\beta 2/3$  subunits in the brain. EEG data showed that the hypnotic effect of propofol (20mg/kg) in 3 G rats was prolonged (43.7±2.9 min) than that in 1 G (26.0±1.0 min) rats. This effect was completely abolished by vestibular lesion (29.7±1.6 min) in 3 G rats. Since there was no difference in propofol metabolism among groups, hypergravity exposure might alter the sensitivity of the propofol via vestibular system.

### 3PK-133

#### Estrogen suppresses psychological stress-induced pressor response by inhibiting the activation of the renin-angiotensin system in ovariectomized rats

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We examined whether chronic estrogen replacement in ovariectomized rats has the beneficial effect of attenuating psychological stress-induced pressor response by suppressing the activation of the renin-angiotensin system (RAS). Female Wistar rats aged 9 wk were ovariectomized and implanted with radiotelemetry devices for blood pressure (BP) measurements. After 4 wk, the rats were assigned either to a placebo-treated (Pla ; n=13) group or a group treated with 17 $\beta$ -estradiol (E2 ; n=13) subcutaneously implanted with either placebo or 17 $\beta$ -estradiol (2.5 mg/90-day release) pellets. These rats underwent cage-switch stress after 4 wk of estrogen or placebo treatment. The stress rapidly and continuously elevated the BP and heart rate both in the Pla and E2 groups. However, these responses to the stress were attenuated significantly in the E2 group compared with the Pla group. Similarly, the stress induced elevations of plasma renin activity and angiotensin II concentration in Pla group, but not in E2 group. In addition, an angiotensin II type 1 receptor inhibitor losartan, given in drinking water reduced the difference in the stress-induced diastolic BP response between the two groups. However, intravenous angiotensin II infusion increased BP similarly in both groups. These results suggest that estrogen replacement may attenuate psychological stress-induced pressor response by suppressing RAS in the ovariectomized rat.

### 3PK-134

#### Comparison of monoaminergic neurotransmitters in the hypothalamic area under several environmental conditions

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Brain monoaminergic neurotransmitters such as serotonin (5-HT), dopamine (DA) and noradrenaline (NA) has been involved in the regulation of several physiological functions and behaviours. In addition, it has been reported that these neurotransmitters change by an environmental condition such as ambient temperature, light, breeding and exercise. Although there are many researches that observed each neurotransmitter change to an environmental condition, the research that compared three neurotransmitters (5-HT, DA, NA) in the viewpoint of balance is not found. The present study was designed to observe the change or balance of 5-HT, DA and NA in the hypothalamic area under several environmental conditions by using homogenate technique. Male wistar rats were bred under each environment (isolation, exercise, low or high ambient temperature) with food and water ad libitum. After 1 month, rats' brain was quickly taken out and each hypothalamic area (preoptic area : PO, paraventricular nucleus : PVN, ventromedial hypothalamus : VMH, dorsomedial hypothalamus : DMH and posterior hypothalamus : PH) was homogenized. Monoamines in the each hypothalamic area were analyzed by HPLC. We found the various patterns of neurotransmitters according to each environment. For example, the amount of 5-HT and DA but not NA in the PO of the isolated rats increased as compared with a control group.

### 3PK-135

#### Strain - dependent mice social behavior in partial gravities using parabolic flight paradigm

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Social behaviors play important roles in group adaptation to novel environments and to establish sustainable habitat. Making habitat in Moon and Mars is one of important goals of space technology, therefore adaptive social functions in low gravity conditions need to be elucidated. In this study we evaluated social behaviors of paired mice in partial gravity conditions such as Moon (0.15G) and Mars (0.3G) using parabolic flight paradigms. We used three strains, C57BL, ICR and obese db/db mice. In 0.3 G and 0.15G, all mice showed startle stretch response and distance between paired mice did not differ. Tail flip response of these strains did not differ in 0.3G but decreased in C57BL in 0.15 G. In 0.01 G, mice floated with vigorous limb and tail movements when a floor is smooth but they were rather stable if a floor is cover by carpet. Obese mice contacted each other for longer duration with a partner. ICR showed the least social contact. When they returned to the home cage after the flights, obese mice started to eat sooner without restless behavior, while C57BL and ICR mice showed restless behavior without eating initially. Obese mice returned to resting condition faster than the control. The present study proposes useful experimental protocols to evaluate social functions in partial gravity conditions such as Mars and Moon. Obese animal may have higher adaptability to low-gravity conditions.

## Poster Presentations

### Autonomic Nervous System(2)

### 3PK-136

#### The role of glutamate as a co-neurotransmitter from orexin neurons in methamphetamine-induced physiological response

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Methamphetamine has a central nervous system stimulant action and causes hyperactivity and activation of the sympathetic nervous system. In our previous study, in orexin-deficient mice, an administration of methamphetamine caused less hyperactivity comparing to control wild type mice and biphasic response in body temperature. In orexin neuron, several neurotransmitters co-exist with orexin. Among them the importance of glutamate in autonomic physiology responses is suggested. The aim of this study is to explore a role of glutamate as co-transmitter from orexin neurons in methamphetamine-induced physiological responses. We used genetically modified mice, in which vesicular glutamate transporter 2 is deficient only in orexin neurons and thus glutamate cannot be released from the neurons (vGLUT2-KO, n=5). We measured body temperature, locomotor activity and ECG with a pre-implanted telemetry probe under freely moving condition. In vGLUT-KO mice, methamphetamine (2mg/kg, i.p.) increased locomotor activity by  $1324 \pm 112$  (arbitrary unit), which was significantly larger than the corresponding value in wild-type mice ( $P < 0.05$ ). Body temperature was increased by  $1.1 \pm 0.2^\circ\text{C}$  in vGLUT-KO, but not in wild type. These results suggest that glutamate as a co-transmitter in orexin neurons has suppressive action to an increase in locomotor activity and body temperature in methamphetamine-induced response, while orexin has promotive actions.



### 3PK-137

#### Hepatic sympathetic nerve is not involved in rat anaphylactic portal hypertension

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Hepatic venoconstriction occurs in rat anaphylactic hypotension, where the sympathetic nervous system is activated. However, hepatic sympathetic nerve activity and its role in anaphylactic hepatic venoconstriction remain unknown, and therefore were determined. Anesthetized ovalbumin-sensitized male Sprague-Dawley rats were randomly allocated to the following pretreatment groups (n=6/group): (1) anaphylaxis control (non-pretreatment), (2)  $\alpha_1$ -adrenoceptor antagonist prazosin, (3) non-selective  $\alpha$ -adrenoceptor antagonist phentolamine, and (4) hepatic sympathectomy. The systemic arterial pressure (SAP), central venous pressure (CVP), portal venous pressure (PVP) and portal venous blood flow (PBF) were measured, and splanchnic (Rspl : (SAP-PVP)/PBF) and portal venous (Rpv : (PVP-CVP)/PBF) resistances were determined. Separately, we measured efferent hepatic nerve activity. In the anaphylaxis control group, PVP increased 3.2-fold and PBF decreased by 70% at 25 min after an intravenous injection of antigen, with resultant 23.3-fold increases in Rpv, along with systemic hypotension. Immediately after antigen, Rspl decreased only transiently, and increased 1.5-fold later than 10 min. Pretreatment with phentolamine or prazosin, or sympathectomy did not affect the antigen-induced increases in PVP and Rpv, whereas both  $\alpha$ -adrenoceptor antagonists inhibited the increase in Rspl. Hepatic sympathetic nerve activity did not increase but tended to decrease after antigen. Thus, hepatic sympathetic nerve is not involved in rat anaphylactic hepatic venoconstriction.

### 3PK-138

#### Changes in renal sympathetic nerve activity during development of renovascular-hypertensive rats

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The aim of the present study was to explore a potential role of renal sympathetic nerve activity (RSNA) in initial development of renovascular hypertensive rats (2-kidney, one-clip model) by measuring time course of changes in RSNA and arterial pressure. Wistar rats were chronically instrumented with a bipolar electrode for the measurement of RSNA and with a telemetry transmitter for mean arterial pressure (MAP) and heart rate (HR). At least 7 days after the implantation of the electrode and telemetry, RSNA, MAP and HR were measured simultaneously and continuously over three weeks. After 4 days control period, two-kidney and one clip hypertension was produced by constricting the right renal artery with silver clip under isoflurane gas anesthesia and then measurements were carried on again. We observed two step increases in MAP. At the first step, MAP increased sharply with in 24 hours while RSNA decreased and also HR decreased. At the second step (6 days after clipping), MAP increased gradually while RSNA and HR tended to recover to the control level and then RSNA tend to increase compared with the control level. Plasma norepinephrine concentration was decrease immediately after the clip and then it increased. These data suggest that baroreflex mediate the sharp reductions in RSNA and HR during the first few days after the clipping and the subsequent increases in RSNA may be involved in the increase in MAP observed at the second step.

### 3PK-139

#### Enhancement of colorectal motility by dopamine through an activation of lumbo-sacral defecation center in rats

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It has been demonstrated that dopaminergic neurons in the enteric nervous system play a role in regulating gastrointestinal motility. However, it remains unclear whether dopamine (DA) acting in the central nervous system is involved in the regulation of intestinal motility. The aim of this study was to examine effects of DA injected into the defecation center at the lumbo-sacral cord (L6-S1) on colorectal motility in rats. Rats were anesthetized and cannulated to the colorectum, and colorectal intraluminal pressure and propelled liquid volume were recorded in vivo to evaluate colorectal motility. Intrathecal application of DA to L6-S1 region in the spinal cord elicited an increase in intraluminal pressure and increased fluid output through the anal cannula. This effect was inhibited by pretreatment with a D2-like receptor antagonist. Then, action of dopamine on parasympathetic preganglionic nuclei neurons in the lumbo-sacral cord was examined using whole-cell patch-clamp technique. Under voltage-clamp conditions, application of DA elicited either an inward or outward current. In the presence of tetrodotoxin, the dopamine-induced currents were still evoked. These results suggest that DA in the lumbo-sacral defecation center enhances propulsive motility of the colorectum by acting on the spinal parasympathetic preganglionic nuclei neurons through D2-like receptors.

### 3PK-140

#### An analysis of sympathetic nerve activity on the mesenteric nerve in rats

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We have been trying to develop a new experimental model for simultaneous measurements of mesenteric sympathetic nerve activity (SNA), arteriolar diameter, and blood flow in small intestinal arterioles of Sprague-Dawley rats, to elucidate a quantitative relationship between SNA and arteriolar vasomotion for blood pressure control. Using intraperitoneal urethane (1.2 g/kg), the small intestine was exteriorized through a midline abdominal incision and placed on a dish. The mesenteric nerve was exposed between the mesenteric artery and vein under a dissecting microscope. Bipolar silver electrodes were put under the nerve to record. Mesenteric SNA was recorded with a power lab system. Arteriolar blood flow was recorded using a noncontact laser-Doppler flowmeter. Mesenteric SNA and arteriolar blood flow at the small intestine was recorded before and after cutting (or using Lidocaine) the proximal side of the mesenteric nerve. Mesenteric SNA decreased after cutting (or using Lidocaine) the proximal side of the mesenteric nerve, and blood flow at the small intestine significantly increased immediately after cutting the nerve. However, the quantitative relationship between mesenteric SNA and arteriolar vasomotion cannot be analyzed in various ways. These results suggest that the mesenteric nerve includes both efferent and afferent fibers. We must try to sort efferent and afferent signals from mesenteric SNA.

### 3PK-141

#### Effects of self-talking on the darts performance in the young beginners

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We observed effects of each of several kinds of self-talks (ST's) on the performance of the darts playing in male student beginners. Subjects were informed of the task load for being a subject beforehand, but no experimental aim at all. In the first experiment, each of ten subjects carried out two experimental runs such that first a positive ST and then negative one, interposed between both by one or two days. Either experiment started with 15 min rest with sitting on a chair and then repeated 8 rounds, each of which was composed of 1 min ST and 3 darts-throwing with standing. We used a normal darts board. But we, as our own rule, gave ten as a score when the arrow stuck the small central circular area colored red and green, or all black-colored areas. We measured ECG during resting and experimental periods, and stored into a computer through a 1kHz ADC. Scores (Mean±SD) of positive ST run and negative one were 18.1±2.80 and 12.1±2.53, respectively, showing the statistically high score in the positive ST ( $t(9)=6.35$ ,  $p=0.0004$ ). In this experiment, HF powers evaluated by consecutive DFT analyses of RRI also showed significant augmentation in the positive ST runs. In the second experiment, then we examined by using 15 male student beginners the hypothesis that the positive ST could affect on darts performance mainly through the parasympathetic nerve system, by comparing with autogenic training ST. Our results partially supported the above hypothesis.

### 3PK-142

#### Pancreatic polypeptide and peptide YY<sub>3-36</sub> directly activate vagal afferent neurons

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Pancreatic polypeptide (PP) and peptide YY<sub>3-36</sub> (PYY) are released upon meal intake and participate in inducing satiety. Peripheral injections of PP and PYY decrease food intake, in which the rank order of the potency is PP>PYY. These anorexigenic effects of PP and PYY are abolished in the vagotomized rodents, indicative of the involvement of the vagus nerve. In this study, we examine whether PP and PYY directly act on the vagal afferents, by measuring cytosolic Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>i</sub>) in the single nodose ganglion neurons (NGNs) isolated from the mice. PP and PYY increased [Ca<sup>2+</sup>]<sub>i</sub> in NGN in a dose-dependent manner. At 10<sup>-11</sup> M, PP but not PYY induced [Ca<sup>2+</sup>]<sub>i</sub> increases in a significant population of NGNs. PP at 10<sup>-10</sup> and 10<sup>-8</sup> M, the submaximal to maximal concentrations, increased [Ca<sup>2+</sup>]<sub>i</sub> in approximately twice greater population of NGNs than PYY. Additionally, PP-responsive NGNs responded to cholecystokinin, the anorectic peptide known to inhibit feeding via directly activating the vagal afferents. In conclusion, PP more potently than PYY activates vagal afferent NGNs including cholecystokinin-responsive neurons. This action of PP could be linked to the regulation of feeding.

### 3PK-143

#### Acute administration of 17-beta estradiol relieves the responses of arterial pressure to electrical muscle stimulation in ovariectomized rats

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We have shown that the responses of arterial pressure to electrical stimulation of the muscle are enhanced in ovariectomized (OVX) rats, an animal model of postmenopause, than in the sham operated rats. The present study aimed to elucidate whether acute administration of 17-beta estradiol relieves the responses. Experiments were performed in urethane anesthetized, artificially ventilated rats. Arterial pressure was recorded from the common carotid artery. Electrical muscle stimulation was delivered to the posterior tibial muscle for 30 s at a frequency of 80 Hz with an intensity of 1.5 mA. 17-beta estradiol was administered intravenously and the effect was examined for 135 min after the onset of the administration. 17-beta estradiol significantly reduced the responses of arterial pressure between 45-135 min after the onset of administration. On the other hand, the administration of 17-beta estradiol showed no effects on the basal (prestimulus) values of the arterial pressure. Control saline administration had no significant influence on both the responses and the basal values of the arterial pressure. The present results suggest that augmentation of the responses of arterial pressure to electrical muscle stimulation in the OVX rats is mainly caused by the lack of estrogen.

### 3PK-144

#### The renal afferent nerve activity induced by local infusion of hyperosmotic saline

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Kidney, the organ which has a significant role in the long term control of arterial pressure, includes autonomic and sensory innervation as many other internal organs. The property and the function of renal sensory nerve are still unclear compared with the renal sympathetic nerve. To examine this, we measured the renal afferent nerve activity (RANA) in response to local infusion of hyperosmotic saline in rats. The catheter for infusion was implanted into the renal artery via the adrenal artery. The central side of renal nerve was ligated after the electrode placement. The RANA was increased in a dose-dependent manner, and the response was significantly suppressed by local administration of gadolinium chloride. These results suggest that kidney watch plasma electrolytes or osmolality, and transmit the information to central nervous system by afferent nerve to utilize for elaborate control of systemic body fluid components.

### 3PK-145

#### Pathophysiology of hemilateral hyperhidrosis

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Localized hyperhidrosis is sometimes compensatory for the anhidrotic parts, but little is known on the pathophysiological mechanism. We analyzed it by defining the sweating distribution using starch iodine methods, skin temperature using infrared thermography, skin blood flow using laser Doppler flowmetry, and magnetic resonance imaging. Results : We can classified it as follows : 1) segmental hemilateral anhidrosis from face to the cervical innervation area, accompanied by the contralateral face flushing (harlequin sign) when heated. The lesion of three male infants (mean age 3) would be in cervical sympathetic trunk on the anhidrotic side. Since cervical traction side for cephalic presentation at delivery corresponded with disorder side, the child-birth delivery might be involved in the etiology : 2) systemic unilateral hypohidrosis. The cases (mean age 49) with this symptom had cervical spinal disc herniation, protruding to the median site of the spinal cord, without abnormal intramedullary signal by MRI. The causes might be minor circulatory disturbance of the central artery of spinal cord on compressed side : 3) segmental well demarcated anhidrosis along the cervical innervated areas. The cases with this symptom, the mean age 57, had cervical disc herniation with the most protruding approximately 3mm from the midline, with unknown pathogenesises : 4) other symptomatic segmental anhidrosis. In conclusion, defining the sweat distribution and skin temperature are the prerequisite for investigating the pathophysiological mechanisms of hemifacial hyperhidrosis.

### 3PK-146

#### Transient bradycardia induced by restraint stress is differently controlled by autonomic nervous system between newborn mouse and rat

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Sudden infant death syndrome (SIDS) is unknown-cause, sleep-related death. SIDS mostly occurs within 2-4 months of age, the period during autonomic shifts occur (autonomic instability). A number of rat/mouse studies have been conducted to investigate the cause of SIDS. However, there has been a lack of fundamental knowledge of autonomic instability, which is prerequisite to analyze various responses to autonomic challenges during the early developmental stages in mice and rats. In the present study, we found that mice and rats show similar heart rate (HR) responses of transient bradycardia (TB) to restraint stress during the second postnatal week. However, it was also found that the mechanism how the autonomic nervous system (ANS) controls the similar HR responses may be different between them. In mice, both atropine and metoprolol (2mg/kg; parasympathetic and sympathetic receptor blocker, respectively) attenuated the magnitude of TB while dual blockade completely removed it, indicating a synergistic control by both sides of ANS. In contrast, in rats, both atropine and metoprolol removed the TB; tachycardia (507±14 b/m) under parasympathetic blockade and bradycardia (261±45 b/m) under sympathetic blockade. Interestingly, dual blockade unexpectedly elevated the HR to 339±9 b/m, which can be best explained by "accentuated antagonism".

### 3PK-147

#### The effect of local infusion of hyperosmotic saline on the expression of arginine vasopressin in the paraventricular nucleus

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It is well known that the kidney includes autonomic and sensory innervation as many other internal organs. The property and the function of renal sensory nerve are still unclear compared with the renal sympathetic nerve. Previous study from our laboratory demonstrated that the renal afferent nerve activity was increased to local infusion of hyperosmotic saline in a dose-dependent manner. However, the effect of local infusion of hyperosmotic saline on the central nervous system is still unknown. In the present study, we examined the expression of Fos and arginine vasopressin (AVP) in the paraventricular nucleus in response to chronic local infusion of hyperosmotic saline. The expression of AVP in the contralateral side was significantly increases compared with ipsilateral side. Since the hyperosmotic saline was diluted by the water infusion in the central vein, the systemic osmolality was not increased. Accordingly, this data indicated that the renal afferent nerve might participate in the fluid volume control via the AVP.

### 3PK-148

#### Evidence of centrally-induced cholinergic vasodilatation in skeletal muscle during voluntary one-legged cycling

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We recently reported that central command contributes to increased blood flow in non-contracting vastus lateralis muscle (VL) at the onset of voluntary one-legged cycling (Ishii et al. J Appl Physiol, 2012). The centrally-induced vasodilatation may be mediated by sympathetic cholinergic mechanism as previously reported in conscious cats (Komine et al. Am J Physiol Regul Integr Comp Physiol, 2008). To test whether this hypothesis is also true in humans, seven subjects were asked to perform voluntary one-legged cycling before and after intravenous injection of atropine sulfate. The relative concentrations of oxygenated- and deoxygenated-hemoglobins (Oxy- and Deoxy-Hb) in bilateral VLs were measured as an index of muscle blood flow with near-infrared spectroscopy. Oxy-Hb in bilateral VLs increased at the onset of voluntary one-legged cycling. Atropine not only blunted the initial increase in Oxy-Hb of contracting VL but also decreased Oxy-Hb of non-contracting VL during voluntary one-legged cycling. Regardless of injection of atropine, the Deoxy-Hb in bilateral VLs did not change at the onset of voluntary one-legged cycling. These results suggest that centrally-induced cholinergic vasodilatation contributes to the initial increases in blood flow of not only non-contracting but also contracting muscle during voluntary one-legged cycling.

### 3PK-149

#### Possible role of Afferent Vagal Nerve in Effects of glutamate on autonomic nerve activity

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In the present study, the effects of intragastric injection of glutamate on the efferent sympathetic nerve outflow to the kidney and the white adipose tissue in rats or mice were analyzed. Although the sympathetic nerve activities of the renal and white adipose tissues were not affected after intragastric glutamate injection in rats or mice that were fasted for 3h, these activities were significantly accelerated after intragastric glutamate injection that were fasted for 48h. Moreover, to test the role of the afferent neural pathway on sympathoexcitation caused by glutamate injection, the effects of vagotomy, or sympathetic denervation, were also examined. In vagotomized rats, the sympathetic nerve activities of the renal and white adipose tissues were eliminated after glutamate injection, but these activities remained unaffected in rats that underwent sympathetic denervation. In addition, intra-hypothalamic pre-treatment with SB334867, orexin receptor (OX1R) antagonist, blocked renal sympathetic response to glutamate injection in rats. Thus, the stimulating effects of glutamate administration on the sympathetic nerve activities of the renal and white adipose tissues could depend on the length of the fast and the vagal afferent pathway and central orexinergic system.

### 3PK-150

#### Epithelium of Seminal Vesicle Confers Stretch Sensitivity and Modulates Noradrenaline-Induced Contraction

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The entity of ejaculation is peristaltic contraction of seminal vesicle (SV), which is triggered by sympathetic nerve activity. In addition to this regulation, we previously reported that SV possessed intrinsic response to mechanical stretch. To explore its mechanism, we developed a reversed ring preparation (RRP; ~4 mm long) dissected from SV of guinea pig, and measured its isometric tension. Like intact SV preparation, spontaneous contraction was induced by stretching RRP. This was not blocked by 1  $\mu$ M tetrodotoxin. Removal of epithelium from RRP completely abolished stretch-induced contraction. Application of noradrenaline (NA 0.01-90  $\mu$ M) to epithelium-free RRP induced tonic contraction in dose dependent manner. NA also induced spontaneous oscillatory contraction, but its magnitude was less dependent on NA dose. NA-induced tonic contraction was also observed in the control RRP (with the epithelium), although NA sensitivity was significantly lower: The half-maximal concentrations were 4  $\mu$ M (epithelium-free) and 12  $\mu$ M (control). The maximal response induced by 90  $\mu$ M NA was also lower in control RRP. NOS inhibitor L-NAME (200  $\mu$ M), and NO scavenger, C-PTIO (300  $\mu$ M) were without any effects on NA-induced contraction of control RRP, suggesting that NO-cGMP system was not involved in this regulation. We concluded that the epithelium of SV possessed unexpected regulatory function on the contraction, although its mechanism remains unsolved.

### 3PK-151

#### The characterization of a cytoskeleton-related protein identified as the novel downstream target of Fyn in SPC/Fyn/ROK pathway mediating abnormal vascular smooth muscle contraction

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Rho-kinase (ROK)-mediated  $Ca^{2+}$ -sensitization of vascular smooth muscle (VSM) plays a critical role for abnormal VSM contractions such as vasospasm. Previously we identified sphingosylphosphorylcholine (SPC)/Fyn/ROK pathway as a novel signaling pathway for abnormal VSM contraction. Furthermore, focused proteomics in human VSM identified 6 cytoskeletal proteins as possible downstream targets of Fyn. To narrow down the candidate proteins for real mediators downstream of Fyn in abnormal VSM contraction, we developed an original unbiased and high-throughput screening system, consisting of siRNA-mediated knockdown of the candidate protein and an automated cell imaging analysis by original algorithm and ArrayScanV. This system enabled us to identify two cytoskeleton-related proteins as novel downstream targets of Fyn. In this study, we focused on one of the two targets, V1, and analyzed its interaction with Fyn. HaloTag pull-down assay revealed that V1 bound to Fyn in human VSM, which was enhanced by SPC stimulation. Interestingly, SPC induced upward shift of the V1-interacted Fyn protein band in SDS-PAGE. Furthermore, such shifted bands did not react with the primary antibodies against phosphorylated Fyn at known phosphorylation sites (Y214 or Y420). These findings suggest that Fyn interacts with newly-discovered V1 to undergo novel post-translational modification upon abnormal VSM contraction.

## Poster Presentations Muscle Physiology(2)

### 3PK-152

#### Mechanisms of Blebbistatin Induced Smooth Muscle Relaxation

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Blebbistatin, a potent myosin II inhibitor, suppressed force development of skinned taenia cecum strips from guinea pig at any given  $Ca^{2+}$  concentration but had little effects on the  $Ca^{2+}$ -induced phosphorylation of myosin regulatory light chain (MLC20). Also blebbistatin inhibited high  $Mg^{2+}$ -induced, "MLC-phosphorylation independent" tension development (Watanabe et al. *Am J Physiol Cell Physiol* 298 : C1118-26, 2010). Also we found that blebbistatin at 10  $\mu$ M and higher accelerated relaxation time courses by removing  $Ca^{2+}$  from contracting muscle preparations (Watanabe, *J Physiol Sci* 62 : S160, 2012). These results suggest that blebbistatin suppressed skinned smooth muscle contraction through disturbing function and/or conformation of myosin heavy chain by the agent. To explore the mechanisms of blebbistatin induced smooth muscle relaxation, we analyzed kinetics of the relaxation time courses of the skinned taenia cecum and carotid artery in detail. The relaxation time courses were well fitted in a double exponential manner, and the double exponential decay of the force could be explained as a portion of fast detaching cross-bridges to transfer to latch-bridges dissociating very slowly. Our present results suggested that, 1) blebbistatin suppressed transferring from fast detaching-cross bridges to slow detaching (latch)-bridges, and also 2) blebbistatin accelerated dissociation of the latch-bridges.

### 3PK-153

#### The role of JAK2/STAT3 signaling pathway on muscle regenerative process

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**PURPOSE:** We have previously shown that JAK2/STAT3 signaling pathway-induced downstream genes such as Cyclin D1, SOCS3 protein is an important regulator for satellite cell proliferation. Thus, JAK2/STAT3 signaling pathway may be a key factor for muscle regeneration. The purpose of this study was to determine the role of JAK2/STAT3 signaling pathway on muscle regenerative process. **METHODS:** 10-week-old male C57BL/6J mice ( $n=23$ ) were used in this study. All mice were injured by injection of cardiotoxin (CTX) or equal volume of PBS (CON :  $n=4$ ) was injected into tibialis anterior (TA) muscles to induce muscle damage. All mice were anesthetized 2 (D2 :  $n=5$ ), 5 (D5 :  $n=4$ ), 7 (D7,  $n=5$ ), 12 (D12 :  $n=5$ ) days after muscle damage, and the TA muscles were sampled to analyze satellite cell marker Pax7, total-STAT3 (t-STAT3), phosphorylation of STAT3 (p-STAT3), Cyclin D1, p27 and SOCS3 protein level. **Results:** Pax7 was significantly higher in D5 than CON ( $p<0.05$ ), suggesting that satellite cell proliferation was caused until D5. Cyclin D1 is necessary for proliferation of myogenic cells. CyclinD1 was significantly higher in D2 than CON ( $p<0.05$ ). Whereas, cell cycle inhibitor p27 and SCOS3 (which provides a negative feedback loop controlling the activation of STAT3) level were significantly higher in D5, D7 than CON ( $p<0.05$ ). **Conclusions:** These results suggest that JAK2/STAT3 signaling pathway-induced downstream gene may play critical roles in skeletal muscle regeneration.

### 3PK-154

#### Essential roles of paxillin and its binding to the active Fyn in actin stress fiber formation induced by sphingosylphosphorylcholine

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Actin stress fibers (SF) play important roles in many cellular functions, including cell adhesion and motility. We previously found the involvement of Fyn tyrosine kinase in Rho-kinase (ROK)-mediated SF formation in fibroblasts induced by sphingosylphosphorylcholine (SPC). However, the molecular mechanisms between Fyn and ROK have not been clarified yet. To search for the downstream molecule of Fyn, we performed pulldown assay with halotag constitutively active Fyn (CA-Fyn) and dominant negative Fyn (DN-Fyn) in human vascular smooth muscle cells (VSMCs). Subsequently, MALDI-TOF MS enabled us to identify paxillin as a novel downstream molecule of Fyn, which bound to CA-Fyn, but not DN-Fyn. Immunoprecipitation assay showed that paxillin directly bound to endogenous Fyn activated by SPC in VSMCs. Immunocytochemical study revealed that both the overexpressed CA-Fyn, but not DN-Fyn, and endogenous Fyn activated by SPC co-localized with paxillin at the ends of actin SF. In addition, the siRNA-mediated knockdown of paxillin prevented SPC from inducing SF formation and cell migration, which was rescued by full-length paxillin, but neither N-terminus nor C-terminus ones. These results demonstrate that paxillin, as the binding partner of active Fyn, is a novel signal mediator of the SPC-induced SF formation and cell migration in human VSMCs.

### 3PK-155

#### Involvement of TRPM7/MIC channels in $Mg^{2+}$ influx in rat ventricular myocytes

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We measured free  $Mg^{2+}$  concentration ( $[Mg^{2+}]_i$ ) in rat ventricular myocytes using a fluorescent indicator fura2/AM (fura-2). Upon  $Mg^{2+}$  depletion (in a  $Mg^{2+}$ -free  $Ca^{2+}$ -free high- $K^+$  solution)  $[Mg^{2+}]_i$  decreased from  $\sim 0.9$  mM to 0.2-0.5 mM, and subsequent recovery of  $[Mg^{2+}]_i$  was caused by  $Mg^{2+}$  influx in  $Ca^{2+}$ -free Tyrode's solution that contained 1 mM  $Mg^{2+}$  [*J Physiol Sci* 62 : S138, 2012]. To study  $Mg^{2+}$  influx pathways in the present study, we used  $Ni^{2+}$  that is known to permeate TRPM7/MIC channels with higher permeability than  $Mg^{2+}$ . When extracellular  $Mg^{2+}$  of the  $Ca^{2+}$ -free Tyrode's solution was substituted with  $Ni^{2+}$ , fura2/AM fluorescence intensity at 350 nm excitation (an isosbestic wavelength for  $Mg^{2+}$ ) measured from  $Mg^{2+}$ -depleted cells decreased rapidly, which could be attributed to quenching of fura2/AM fluorescence by  $Ni^{2+}$  that entered the cells. Coexistence of 1 mM extracellular  $Mg^{2+}$  and  $Ni^{2+}$  reduced the initial rate of the decrease in fluorescence intensity by 62%, suggesting permeation of  $Mg^{2+}$  and  $Ni^{2+}$  through the same channels. 2-Aminoethoxydiphenyl borate (2-APB), a known inhibitor of TRPM7/MIC channels, inhibited the initial rate by 85% at 100  $\mu$ M, and by 49% at 20  $\mu$ M. This concentration dependence is consistent with  $IC_{50}$  of 17  $\mu$ M previously estimated for 2-APB inhibition of the  $Mg^{2+}$  influx rate. We also measured MIC currents using whole cell patch-clamp, and confirmed that 100  $\mu$ M 2-APB inhibited the inward current by 52% at -120 mV. These results support our previous conclusion that TRPM7/MIC channels serve as a major physiological pathway of  $Mg^{2+}$  influx in cardiac myocytes.

### 3PK-156

#### Roles of Epac in muscle hypertrophy and fiber-type transition induced by $\beta_2$ -adrenoceptor agonist in masseter muscle

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Chronic stimulation of  $\beta_2$ -adrenoceptor ( $\beta_2$ -AR) with clenbuterol (CB), a  $\beta_2$ -AR agonist, induces skeletal muscle hypertrophy and slow-to-fast fiber-type transition in mammalian species, but their molecular mechanisms have not been well understood. Recently, Epac (exchange protein directly activated by cAMP) has been reported to induce cardiac hypertrophy in vitro. However, its roles in the pathogenesis of masseter muscle hypertrophy and fiber-type transition have not been well explored. Here, we examined the effects of chronic CB treatment (i.p., 2mg/kg/day for 3 weeks) on muscle mass, fiber diameter and myosin heavy chain (MyHC) composition in masseter muscle (the principal jaw closer in rodents) of wild-type controls (WT) and Epac1 knockout mice (KO). Masseter mass and muscle fiber diameter were significantly increased by CB treatment in WT while not in KO. On the other hand, CB treatment significantly increased the proportion of MyHC IIb at the expense of that of MyHC IIc/x in both WT and KO, indicating that disruption of Epac1 did not affect the MyHC transition towards faster isoforms. Putting together, Epac plays an important role for the development of muscle hypertrophy but not for the MyHC transition induced by  $\beta_2$ -AR stimulation in masseter muscle.

### 3PK-157

#### Spin-spin relaxation of $^1\text{H}$ NMR signals from myofibril suspension of rabbit skeletal muscle with or without ATP

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We observed spin-spin relaxation process of  $^1\text{H}$ -NMR signals from suspension of myofibrils prepared from rabbit psoas muscle. As was the case in tissue skeletal muscle, decomposition analysis of the relaxation process could be well represented by the summation of several exponentials indicating that water molecules in the suspension could be conveniently grouped into several components based on the relaxation time constant (T<sub>2</sub>). The slowest two components dominated over faster relaxation components at the myofibril concentration ranges studied. With increase in the concentration of myofibrils, water component that relaxed with T<sub>2</sub> around 0.15 s progressively replaced the slowest component of T<sub>2</sub>>0.4 s. An equivolumic point for these two components was found at 12 mg/ml and 20mg/ml myofibril concentration at 20°C in the absence and presence of MgATP respectively. Water components that relaxed more rapidly existed at small fractions. Since the average separation between the myofibrils is estimated to be 1.72  $\mu\text{m}$  at the myofibril concentration of 10 mg/ml, myofibril affects water molecules within a significant distance from its surface differently from water molecules in the bulk solution.

### 3PK-158

#### Novel fish-derived peptides which induce endothelium-dependent vasorelaxation

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Endothelium-dependent vasorelaxation (EDR) not only regulates physiological vascular tone but also counteracts abnormal vascular contractions leading to vasospasm and hypertension. Therefore, deterioration of these EDR functions of vascular endothelial cells, which is caused by various pathological states such as aging, oxidative stress and lipid disorders, increases risk of vascular diseases, including heart attack and stroke. For the reliable prevention of such acute and lethal vascular diseases caused by endothelial dysfunctions, we attempted to discover foods or food components which help vascular endothelial cells induce EDR. After extensive screening, we finally found fish-derived peptide fragments as the candidates. The peptide fragments were obtained by eatable enzyme digestion of a fish protein and showed strong activities of both endothelium-dependent and -independent relaxations of the porcine coronary arteries. Pharmacological studies with an inhibitor of nitric oxide synthase suggested the involvement of nitric oxide in EDR. Peptide sequencing of the fish-derived peptide fragments by tandem mass spectrometry (MS/MS) identified six peptides with previously unreported sequences responsible for EDR. The synthesized peptides with identified amino-acid sequences actually induced EDR with a potency similar to the original peptide fragments. These results suggest that identified peptides are main and novel components in fish-derived peptide fragments which induce EDR.

### 3PK-159

#### Effect of intravenous anesthetic propofol on $\text{Ca}^{2+}$ -activated force in skinned porcine left ventricular muscle

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**BACKGROUND :** Propofol (2,6-Diisopropylphenol) is an intravenous anesthetic agent widely used for induction and maintenance of general anesthesia. The drug, however, occasionally exhibits a cardiotoxic effect in clinical settings. In the present study, we investigated whether and how propofol exerts its cardiodepressant effect at the sarcomere level by using skinned myocardial preparations.

**METHODS :** Porcine left ventricular preparations were skinned in relaxing solution containing 1% (w/v) Triton X-100 (as in Terui et al., 2008, 2010). We tested the effects of propofol on force-pCa (= -log [Ca<sup>2+</sup>]) curves as well as on maximal Ca<sup>2+</sup>-activated force at short (1.9  $\mu\text{m}$ ) and long (2.3  $\mu\text{m}$ ) sarcomere lengths (SLs).

**RESULTS :** Propofol at a clinically high concentration of 100  $\mu\text{M}$  shifted the mid-point of the force-pCa curve rightward by  $\sim 0.05$  pCa units at both SLs. Likewise, it lowered maximal Ca<sup>2+</sup>-activated force by  $\sim 10\%$  at both SLs.

**CONCLUSION :** Propofol may exert its acute cardiodepressant effect by a relatively small magnitude (cf. Mio et al., 2002, 2004 for bupivacaine) via decrease in myofibrillar Ca<sup>2+</sup> sensitivity when used at high doses.

### 3PK-160

#### Endothelin-1 Induces Sustained Constriction of the Rat Renal Afferent Arteriole Via Diphosphorylation of the Myosin Regulatory Light Chains

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Endothelin-1 (ET-1) elicits long-lasting constriction of rat afferent arterioles, in contrast to transient constriction induced by angiotensin II. In general, vasoconstriction is evoked by myosin regulatory light chain (LC<sub>20</sub>) phosphorylation at Ser19 by myosin light chain kinase (MLCK), which can be enhanced by Rho-kinase (ROCK)-mediated inhibition of myosin light chain phosphatase. LC<sub>20</sub> can also be phosphorylated at Ser19 and Thr18 by integrin-linked kinase and zipper-interacting protein kinase, resulting in reduced rates of dephosphorylation and relaxation. In this study, we tested the hypothesis that the sustained vasoconstriction induced by ET-1 is due to LC<sub>20</sub> diphosphorylation.

Methods: Isolated rat afferent arterioles were stimulated with angiotensin II (ANG) or ET-1, and LC<sub>20</sub> phosphorylation was quantified by Phos-tag SDS-PAGE and highly sensitive western blotting.

Results: ANG increased monophosphorylation of LC<sub>20</sub> in a concentration dependent manner. This increase was attenuated by a Rho-kinase inhibitor, H1152. In contrast, ET-1 increased diphosphorylation of LC<sub>20</sub> as well as monophosphorylation. H1152 attenuated the diphosphorylation, but not the monophosphorylation. Pre-treatment with the ET<sub>B</sub> receptor antagonist BQ788 abolished LC<sub>20</sub> diphosphorylation

Conclusion: ET-1-induced sustained constriction of rat afferent arteriole is likely due to LC<sub>20</sub> diphosphorylation via ET<sub>B</sub> receptor stimulation.

### 3PK-161

#### Bovine ciliary muscle shows no force-inhibiting effect of okadaic acid

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Purpose: Ciliary muscle is a smooth muscle with parasympathetic innervations and characterized by a rapid response to muscarinic receptor stimulation and sustained contraction. We recently reported that in bovine ciliary muscle, myosin phosphorylation does not change irrespective of the state of contraction or relaxation. In order to address the regulatory mechanisms of ciliary muscle contraction, we examined the effect of okadaic acid, a phosphatase inhibitor, on ciliary muscle contraction and myosin phosphorylation. Okadaic acid has been reported to cause contraction or relaxation in a concentration-dependent manner in other smooth muscles.

Methods: Smooth muscle strips were excised from bovine ciliary body and guinea pig taenia cecum. Muscle strips were contracted with carbachol and isometric tension was recorded. Various concentrations of okadaic acid were administered to contracted or relaxed muscle strips. Myosin phosphorylation was measured by phos-tag electrophoresis and western blotting.

Results: Low concentration of okadaic acid (5  $\mu$ M) impaired carbachol-induced contraction in taenia cecum, but not in ciliary muscle. Furthermore, the myosin phosphorylation in ciliary muscle was not altered by okadaic acid.

Conclusion: These results raise the possibility that ciliary muscle has a unique regulatory mechanism of contraction which may not involve myosin phosphorylation.

### 3PK-162

#### Effects of polyethylene glycol on contraction in skinned skeletal muscle

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It has been considered that solution of macromolecules such as Dextran T-500 (Mw. 500,000) osmotically dehydrate skinned skeletal muscle because they are sterically excluded from sarcomeres. However, it was recently found that small organic molecules such as di-ethylene-glycol (di-EG; Mw. 106) also dehydrate skinned skeletal muscle partially penetrating into the sarcomere space. Their dehydration was not due to excluded volume effect but might be related to hydrophobic effects exerted by their carbohydrate scaffolds. They affected x-ray diffraction patterns of skinned muscle differently from macromolecules. To further characterize the dehydrating effects of small organic molecules, we studied contractile properties of dehydrated skinned muscle prepared from sartorius muscle of *Rana Catesbeiana*. Dehydrated with Dextran T-500, Ca<sup>2+</sup>-sensitivity for force development slightly increased with an increase in maximally Ca<sup>2+</sup>-activated force until hydration state of intact sarcomere was restored. On the other hand, PEG 3350 (Mw. 3,350) decreased Ca<sup>2+</sup>-sensitivity with a significant decrease in maximally activated force. Since Chinn et al. (2000) reported that PEG potentiated the binding of myosin to actin with a smaller effect on dissociation of the former cross-bridges, the present results suggest that PEG suppressed cross-bridge former to reduce the maximally activated forms. Additionally, different effect on Ca<sup>2+</sup> sensitivity suggest that PEG removes different water groups in the sarcomere from those removed by Dextran.

### 3PK-163

#### Characterising Ca<sup>2+</sup> mobilisation mechanisms during the generation of spontaneous activity in the guinea pig prostate

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Benign prostatic hyperplasia (BPH) is commonly associated with irritative lower urinary tract symptoms (LUTS) partially due to an enhanced prostatic smooth muscle tone. The present study examines the contributions of Ca<sup>2+</sup> mobilisation mechanisms in generating spontaneous contractions of the guinea-pig prostate that may give rise to the enhanced smooth muscle tone. Similar to action potentials recorded in other hollow smooth muscle organs, action potentials of the prostate are nifedipine-sensitive but not the underlying slow wave component. High concentrations of Ni<sup>2+</sup> (100 $\mu$ M) significantly reduced slow wave frequency. Spontaneous contractions recorded simultaneous to electrical activity reveal their modulation is heavily dependent on Ca<sup>2+</sup> entry via L- and T- type Ca<sup>2+</sup> channels with the contractile amplitude reduced to 15% ( $P < 0.05$ ,  $n = 3$ ). Additionally, intracellular Ca<sup>2+</sup> release forms an essential component in modulating slow waves as CPA (10  $\mu$ M) in the presence of nifedipine (1 $\mu$ M) abolishes both spontaneous slow wave and contractile activity ( $n = 5$ ). Disrupting mitochondrial function with 2-deoxy-D-glucose (10mM) and oligomycin (5 $\mu$ M) also abolished spontaneous activity (both  $n = 4$ ). It is clear that external Ca<sup>2+</sup> entry plays a significant role in the modulation of spontaneous activity in the guinea-pig prostate; however, it is intracellular Ca<sup>2+</sup> release from the internal stores that is essential in generating spontaneous contractions.

## Poster Presentations

### Neurochemistry(2)

#### 3PK-164

##### Generation of ramified microglia-like cells from leptomeninges in vitro

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Although del Rio Hortega originally reported that leptomeningeal cells are the direct source of ramified microglia in the developing brain, recent views seem not to pay much attention to this notion. In this study, in vitro experiments were conducted to determine whether leptomeninges generate ramified microglia. The leptomeninges of neonatal rats containing Iba1+macrophages were peeled off the brain surface. Leptomeningeal macrophages expressed CD68 and CD163 strongly, but not microglia in the brain parenchyma. Leptomeningeal macrophages expressed epidermal growth factor receptor (EGFR) as revealed by RT-PCR and immunohistochemical staining. Cells obtained from the peeled-off leptomeninges were cultured in a serum-free medium containing EGF for 4 or 6 d resulting in the formation of large cell aggregates, in which many proliferating macrophages were present. In contrast to EGF, macrophage-colony stimulating factor (M-CSF) did not enhance the generation of Iba1+cells from the leptomeningeal culture. The cell aggregates generated ramified Iba1+ cells in the presence of fetal calf serum, which express CD68 and CD163 at much lower levels than primary microglia isolated from a mixed glial culture. Therefore, the leptomeninge-derived cells resembled parenchymal microglia better than the primary microglia. This study suggests that microglial progenitors expressing EGFR reside in the leptomeninges and that there is a population of microglia that grow independently of M-CSF.

#### 3PK-165

##### The sucrose preference regulating mechanism of body weight in mice placed under chronic food restriction

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Our previous work has shown that sucrose (Suc) preference was formed when mice, whose body weight (BW) was reduced to 80% of original BW by chronic food restriction, were alternately exposed to 0.15 M Suc and 5 mM sodium saccharin (Sac). Suc consumption abruptly dropped to Sac levels (Saltatory suppression of Suc preference, SSSP) as soon as the mice regained their original BW by sufficient food supply or by loading an extra-weight, and the lesion of the somatosensory cortex of the limbs (S1FL/S1HL) suppressed the SSSP. In the present experiment, we examined whether the abrupt BW loss by removal of the extra-weight which was previously embedded increases Suc preference after the SSSP induced by the sufficient food supply or not. The result showed that the abrupt BW loss also induced a sudden increase of Suc preference, suggesting the possibility that S1FL/S1HL contributes to the control of the Suc preference by the BW loss as well as the BW gain. Based on an assumption that the BW influences on the reward value induced by the caloric supply through Suc consumption via S1FL/S1HL and reward system including the hypothalamus and nucleus accumbens because of unchanged Sac consumption, we might be able to report how c-fos expression changes with alteration of the Suc preference and the BW in these brain regions or not.

#### 3PK-166

##### Immunohistochemical study on reactions of microglial cells, astrocytes and NG2 glial cells in the brains of rats with 6-OHDA-induced Parkinsonism

Aono, Hitomi; Higaki, Hiromi; Sugimoto, Kana; Takahashi, Hisaaki; Yano, Hajime; Tanaka, Junya (Graduate School of Medicine, Ehime University, Ehime, Japan)

Microglia are activated in response to even minor pathologic changes in the brain. Astrocytes and NG2 glial cells, two other major types of glial cell, also change phenotype during brain pathology. In this study, we investigated morphological changes of these three types of glial cells in the brains from 6-OHDA-induced Parkinsonism model rats. 6-OHDA was administered into the right striatum to induce dopaminergic neuron loss in the substantia nigra pars compacta (SNpc). Neuronal loss was prevented by administration of a cytokine mixture containing GM-CSF and IL-3 or with bromvalerylurea. An immunohistochemical study was conducted to identify morphological changes in glial cells from the brains of these different treatment groups. Microglial activation, characterized by enlarged somata and thickened processes, was observed in the SNpc 2 days post-lesioning (dpl) with 6-OHDA (2 dpl) treatment and it was increased at 7 dpl indicating dopaminergic neuron loss. Activated microglia are densely populated in the SN pars reticulata. Activation of astrocytes was not marked at 2 dpl, but increased GFAP-staining was observed in the SNpc at 7 dpl. NG2 cell activation (or proliferation) was not observed in the degenerated SN, but was observed in the SNpc when the cytokine mixture was administered at 7 dpl. These results suggest the dynamic involvement of glial cells in the pathogenesis of Parkinson's disease.



### 3PK-167

#### Noradrenaline and adrenergic agonists protect neuronal cells by suppressing microglial proinflammatory reactions

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The locus coeruleus contains many noradrenergic (NAergic) neurons, which are the targets of degeneration in neurodegenerative disorders such as Parkinson's disease (PD). Decreased NA levels induce proinflammatory activation of microglia (MG), leading to aggravation of neuronal degeneration. We examined the neuroprotective effects of NA by suppressing microglial proinflammatory functions. As revealed by quantitative real-time RT-PCR, rat primary MG expressed mRNAs encoding alpha1, alpha2 and beta2 adrenergic receptors. We incubated lipopolysaccharide (LPS)-stimulated MG with NA, alpha1, alpha2, beta1 and beta2 adrenergic agonists (phenylephrine : Phe, clonidine : Clo, dobutamine : Dob, terbutaline : Ter, respectively). As a result, NA, Phe, Dob, Ter suppressed nitric oxide (NO) production from MG. When MG were cultured with cortical primary neurons in the presence of LPS, neurons underwent degeneration, which could be prevented by treatment with adrenergic agonists. The neuroprotective action of Dob was due to signaling through the beta2 receptor. These results indicate that NA protects neurons from the toxic actions of NO released by activated MG through binding to alpha1 and beta2 receptors. In addition, Ter, a beta2 agonist, could suppress MG functions at the lowest concentration used. Because Ter cannot pass through the blood-brain barrier (BBB), we are investigating the effects of another beta2 agonist, clenbuterol with an ability to cross the BBB in a rat 6-hydroxydopamine-induced PD model.

### 3PK-168

#### Neuroglobin as a regeneration associated molecules in zebrafish retina after optic nerve injury

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Neuroglobin (Ngb) is known as a new member of vertebrate heme protein which is originally discovered in the central nervous system. Mammalian Ngb is involved in neuroprotection under oxidative stress conditions, such as ischemia and reperfusion insults. After optic nerve injury, fish retinal ganglion cells can survive and regenerate their axons unlike mammals. In this study using zebrafish, we found out that Ngb was increased in damaged retina after optic nerve injury. Therefore, we investigated the functional role of Ngb as one of the regeneration associated molecules. In normal adult zebrafish retina, Ngb mRNA and protein were detected in the innermost layer of the inner nuclear layer. To identify which type of cells express the Ngb in retina, we performed double staining of Ngb and several cell marker proteins. Among of these markers, Ngb expressing cells were merged with NADPH diaphorase (NADPHd) positive amacrine cells and they were also merged with neural nitric oxide synthase (nNOS) positive cells. Ngb mRNA was significantly increased in the amacrine cells 3 days after optic nerve injury. To elucidate a role of Ngb during fish optic nerve regeneration, we are now in progress to study the effect of recombinant Ngb protein on neurite outgrowth in retinal explant culture system.

### 3PK-169

#### Low doses of dexamethasone suppress dopaminergic neuron loss by modulating microglial functions in a rat model of Parkinson's disease

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Activated microglia are one potential cause of neurodegeneration in Parkinson's disease (PD). They release factors that are detrimental to neurons, such as reactive oxygen/nitrogen species and proinflammatory cytokines. In this study, the suppressive effects of ten agents on lipopolysaccharide (LPS)-activated rat primary microglial cells were investigated. Dexamethasone (Dex), aldosterone, adrenergic beta-agonists, terbutaline and isoproterenol significantly suppressed LPS-induced nitric oxide synthesis and expression of the pro-inflammatory cytokines. Only Dex enhanced mRNA expression of the neuroprotective factors hepatocyte-growth factor and insulin-like growth factor 1. Co-culture with LPS-activated microglial cells caused degeneration of rat cerebrocortical neurons. Dex was administered subcutaneously to rats with 6-OHDA-induced Parkinsonism and was highly effective in preventing neuronal degeneration and suppressed nitric oxide release. Dex treatment also inhibited dopaminergic neuron loss and suppressed microglial activation in the substantia nigra of rats treated with 6-OHDA. Dex at a dose of 0.003 mg/kg/day prevented dopamine content decrease in the striatum and dopaminergic neuron loss at 50 days post-6-OHDA treatment. The present findings indicate that glucocorticoid treatment may be useful in the suppression of progressive dopaminergic neuron loss in PD. Our results also show the critical involvement of activated microglial cells in a rat PD model.

### 3PK-170

#### Analyses of structure-activity correlations of bromvalerylurea that exerts anti-inflammatory effects on LPS-activated microglial cells

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Microglia elicit neurotoxicity by producing and releasing inflammatory mediators such as interleukin (IL)-1beta, tumor necrosis factor (TNF)-alpha, and nitric oxide (NO) in various brain diseases. Microglial cells express inducible nitric oxide synthase (iNOS) causing neuronal cell death in neuron-microglia cocultures in response to a TLR4-ligand, lipopolysaccharide (LPS). In the present study, we show that bromvalerylurea (BU; a classical hypnotic and sedative drug) suppressed LPS-induced iNOS expression by microglial cells and prevented neuronal cell death. BU is a low molecular weight compound with a simple structure, possessing a Br atom, which may cause acute/chronic poisoning. Therefore, we investigated the structure-activity correlation of BU using LPS-treated primary rat microglial cells, by comparing it with other low molecular compounds which can cross the blood brain barrier. We identified candidate compounds that suppress iNOS expression in LPS-treated microglial cells. It is thought that Br-ion release from metabolized BU is critical for its sedative actions by replacing Cl-to suppress neuronal depolarization. However, sodium bromide, which releases Br-in culture medium, did not display any effects on microglial cells. These results suggest that BU and related compounds suppress NO release in LPS-treated microglia at a transcriptional level.

### 3PK-171

#### Sensory neurons in dorsal root ganglion can survive after spinal cord injury in adult zebrafish

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Unlike mammals, zebrafish spinal cord can regenerate and recover its motor function after spinal cord injury (SCI). We have previously reported that upper motor neurons in brainstem, which regulate motor function, can survive via activation of IGF-1/PI3K/Akt survival pathway within 7 days post-injury (dpi) and regrow their descending axons across the injury site within 30-60 dpi. Next we focused on the survival and regenerative capacity of sensory neurons in dorsal root ganglion (DRG), because spinal cord fibers are composed of both descending and ascending axons, and they are completely cut by SCI. The spinal cord of adult zebrafish was severed at the position 2 mm caudal to the brainstem-spinal cord junction, and a retrograde tracer was applied to the injury site to label sensory neurons in DRG. At 2 dpi, about 20 neurons of DRG within 2 and 6 mm caudal to the injury site were successfully labeled. At 7 dpi, when injured mammalian central neurons normally die, we could detect labeled neurons in DRG, and the number of labeled DRG neurons was comparable to that at 2 dpi. Thus the injured DRG neurons could survive after SCI in adult zebrafish. We are now investigating the survival and regenerative properties of sensory neurons in adult zebrafish DRG following SCI.

### 3PK-172

#### Invasion of monocytes/macrophages into ischemic lesion of rat brain : involvement of chemokines and their receptors

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A number of macrophages accumulate in the ischemic core of a rat brain subjected to transient middle cerebral artery occlusion (MCAO) for 90 min. These cells may play a neuroprotective role and modulate pathophysiologic processes of stroke lesion. The macrophages are derived from circulating monocytes but not resident microglia. It is still to be elucidated how monocytes invade into the lesion. In this study, chemokine-based mechanisms underlying the invasion of monocytes were investigated. We identified high expression of mRNA encoding CCR2 and CX3CR1 by isolated brain macrophages and that of mRNA encoding their ligands, MCP-1 and fractalkine by astrocytes. Recombinant MCP-1 and/or fractalkine induced the migration of brain macrophages *in vitro*. mRNA for MCP-1, fractalkine, CCR2 and CX3CR1 was expressed in the ischemic core during the acute phase of the ischemic event. Immunohistochemical studies revealed that MCP-1 localized in vascular endothelial cells in the ischemic core during the acute phase. Astrocytic endfeet surrounding blood vessels in the ischemic lesion expressed fractalkine. These results suggest that CCR2<sup>+</sup> monocytes first attached to activated MCP-1<sup>+</sup> endothelial cells and migrate toward fractalkine<sup>+</sup> astrocytic endfeet through the disrupted blood-brain barrier. Thus, chemokines may play a critical role in the accumulation of neuroprotective BINCS.

### 3PK-173

#### Plasmalogens drive postnatal neurogenesis

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Plasmalogens (PLs) are unique glycerophospholipids that contain vinyl ether bond at the sn-1 position of the glycerol moiety, not only constituting cellular membranes, but also playing significant roles in membrane fluidity and cellular processes like vesicular fusion and signal transduction. In the present study, we investigated the possible role of PLs in the promotion of neurogenesis. We compared the total number of double cortin (DCX)-positive cells in the dentate gyrus (DG), as marker for young immature neurons, in mice given PLs containing food. Quantitative analysis revealed a significantly increase in the number of DCX-positive cells measured 4 weeks after PLs-diet significantly increased compared with those given control diet. We further examined effects of PLs on the neurogenesis using a cultured neuronal cell line. Retinoic acid (RA) is known to induce neurogenesis, characterized by enhanced axonal outgrowth on day 1 and 2, in mouse neuronal cell line (Neuro2A) incubated with the reduced fetal bovine serum (FBS)-containing DMEM medium. When Neuro2A cells are pretreated with PLs, axonal outgrowth was accelerated compared with control group. In addition, PLs enhanced the formation of filopodia-like protrusions of the dendrites, which must grow dendritic spines, and branching of dendrites on day 2 after RA treatments. Our present results strongly suggest that PLs have neuroprotective as well as neurogenesis-promoting effects on neuronal cells.

### 3PK-174

#### Hippocalcin protects hippocampal neurons against MLK3-mediated excitotoxin damage

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Hippocalcin is a member of the neuronal calcium sensors (NCS) family predominantly expressed in the hippocampal pyramidal cells. We have found that hippocalcin binds to C-terminal region of Mixed Lineage Kinase (MLK) 3 and inhibits its kinase activity in calcium-dependent manner. Here we analyzed MLK3 activities and kainic acid-induced neuronal degeneration in hippocalcin deficient mice. Kinase activity of immunoprecipitated MLK3 was examined using bacterially expressed MKK4, one of the substrate of MLK3. The resting MLK3 activity in the hippocampus of the hippocalcin deficient mice was higher than that in wild type. MLK3 activating stimulation was subjected to hippocalcin deficient mice by intraperitoneal injection of kainic acid (30 mg/kg), which is known to cause neuronal damage via glutamate receptor GluR6 subunit. We first evaluated the time-course of changes in phospho-MLK3 and -MKK4 after kainic acid injection using substrate specific antibodies. Significant increases in the amounts of phospho-MLK3 and -MKK4 were observed in the hippocampus of the hippocalcin deficient mice within 1 hour after injection. Histological studies using cresyl violet and TUNEL staining revealed that apoptotic neuronal degeneration in hippocampal CA1 region was highly increased in the hippocalcin deficient mice. These results indicate that hippocalcin protects hippocampal neurons against glutamate toxicity by inhibiting MLK3 activation.

### 3PK-175

#### Optogenetic induction of bursting activity of hilar mossy cells

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In the dentate gyrus (DG) of hippocampus, a hilar mossy cell (HMC) receives excitatory inputs convergently from a group of granule cells (GCs) and sends excitatory outputs divergently to another group of GCs. Based on this anatomical feature GCs may have positive feedback association with other GCs through HMCs ("GC association" hypothesis). The activity of hippocampal neurons generally follows theta rhythm of 4-10 Hz. Here we tested whether the magnitude of GC association is dependent on the rhythmic activity using optogenetics.

A slice of hippocampus was made from a transgenic rat which expresses channelrhodopsin-2 (ChR2) under regulation of thy1.2 promoter (W-TChR2V4, 4-5 weeks old). One of HMCs was identified under microscopy with its localization and morphology and served to the conventional whole-cell patch clamp. The dentate gyrus was optogenetically stimulated with a spatio-temporal pattern of irradiation generated by a projector-managing optical system (MiLSS).

Under current clamp, spontaneous action potentials were observed at low frequency. However, bursts of high-frequency action potentials were often generated in synchronous with rhythmical irradiation of DG at 10 Hz. The bursting activity continued for 10-60 s after termination of irradiation. These bursting activities were almost negligible in the presence of D-AP5 (25  $\mu$ M). It is suggested that the GC association of DG neurons were enhanced by the theta-rhythmic activity through a mechanism dependent on NMDA receptors.

### 3PK-176

#### The developmental changes in prefrontal dopamine and serotonin release with neonatal habenula lesion in rats

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The habenula is a relay between limbic system and midbrain monoamines such as dopamine (DA) and serotonin (5HT). Neonatal habenula lesion (NHL) given at postnatal days (PD) 6-8 increases locomotor activity in an open field chamber at PD28-35 but not at PD56 or older in rats (Lee and Goto, 2011). This is similar to a core feature of attention deficit/hyperactivity disorder (ADHD). In this study, we compared DA/5HT release in the medial prefrontal cortex (mPFC) in juvenile (PD26-38) and adult (PD56 or older) rats with NHL. We measured DA/5HT release during locomotor activity/rearing for 2 days (d1 or d2) using microdialysis and HPLC assay. In juvenile rats, as we have previously reported, DA and 5HT release increased upon placement in the chamber on both days, with significant attenuation on d2 compared to d1 in both juvenile NHL and sham rats; however, extracellular basal DA/5HT release were higher in the NHL compared to sham controls. These results suggest that a certain ceiling-like control mechanism associated with NHL may underlie DA/5HT release in juvenile rats. We will report details of analyses including behavioral data for adult NHL rats at the meeting to further elucidate a possible relationship between early habenula disruption and ADHD pathogenesis.

### 3PK-177

#### Evaluation of non-alcohol beer intake by elevated plus-maze

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Using a elevated plus-maze, effects of alcohol-intake was evaluated. Male mice (C57BL6N, 7 week-old) were subjected to drink beer. We found that they drank beer ad libitum more than 10 gm per day after overnight water deprivation. Thereafter, they have given beer every other day for two weeks. As a control, another group of mice were given the tap water for the same period. The behavior of mice on the elevated plus-maze was recorded by a video camera for 10 min. Using recorded images, the number of entrance and time spent in each arm of the maze were measured. We found in control group that the mice with beer drinking following the pretreatment with tap water exhibited longer staying in the open arm of the maze when compared with those treated with non-alcohol beer. The number of entering times into the open arm is significantly larger in the former than latter (1.41 times larger,  $P < 0.05$ ). The results indicate that the beer intake reduced the fear of elevation in maze. Intriguingly, in the group of mice that had been pretreated with beer for two weeks before the maze task, the difference between alcohol and non-alcohol beer drinking was abolished. These results suggest following two possibilities that are not mutually exclusive: i) alcohol intake for two weeks exhibits prolonged suppressive effects of fear, or ii) non-alcohol beer following beer intake for two weeks evoked the emotional memory of pre-treatment of beer, relieving them from emotional fear on the elevated plus-maze.

## Poster Presentations

### CNS Function(2)

### 3PK-178

#### Single unit activity in the monkey orbitofrontal cortex related to reward value processing during the decision-making schedule task

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We often choose one of alternatives by considering their values and efforts to obtain them. To understand the neuronal mechanism of such decision-making process, we developed a decision-making schedule task and recorded single unit activity from monkey orbitofrontal cortex (OFC) which has been reported to be one of the important brain area for reward-guided behaviors. The monkey was initially trained to perform a reward schedule task which consists of 1, 2 or 4 trials of visual discriminations to earn 1, 2 or 4 drops of liquid reward. After the monkey learned this task, we introduced the decision-making schedule task in which two kinds of choice target (CT) whose brightness and length indicated reward amount and required number of the trial, respectively, were sequentially presented, then, these two CTs were simultaneously reappeared side by side (firstly presented CT was on the left, the second one was on the right). If the monkey chose one of them successfully, the chosen reward schedule started. Over 60% of the recorded neurons increased their firing in the right target presented period. Some neuronal activity was modulated by the difference of values between the 2 CTs. This result suggests that OFC neurons play an important role in the decision-making by reward value information processing.

### 3PK-179

#### A gaze control model based on saliency of the face while viewing video stories

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When typical adults watch TV programs, they show surprisingly similar gaze behaviors (Nakano, et al., 2010). To elucidate the rules that control stereotypical gaze behaviors, we examined temporo-spatial gaze patterns in 27 typical adults, while they viewed 12 short video clips, each of which featured one to several main characters. We found that participants spent 75% of time viewing one of the faces in each scene. We estimated the time required for face selection by comparing gaze patterns during replays in the normal and reversed directions. The estimated time for face selection was ~200 ms on average and increased with the number of faces from 150 ms (n=1) to 350 ms (n=4). We then compared three models of gaze control, based on 1) the physical saliency, 2) random face selection, and 3) the "saliency" of the face that was defined according to the size and location of the face. We used multidimensional scaling to summarize similarities of the gaze patterns, and found that the one yielded by the salient face selection model fell in the cluster of the actual gaze patterns, but those yielded by the other two models fell apart from the cluster. The results suggest that we do not count much on the physical saliency of the scene in controlling our gazes, but choose one of the most salient faces in the scene in about 150 to 350 ms, depending on the number of faces in the scene.

### 3PK-180

#### Dissociable Anterograde Amnesic Effects of Retrosplenial Cortex and Hippocampal Lesions on Spontaneous Object Recognition Memory in Rats

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The amnesic effects of excitotoxic lesions of the rat retrosplenial cortex (RS) and hippocampus (HPC) in the spontaneous object recognition (SOR) performance were investigated. The SOR test consisted of the sample-exposure session (s) and a test session. First, to test retrograde amnesia, rats received four sample-exposure sessions within a day at 4 weeks and 1 day before the surgery, respectively. In the test sessions conducted 1 week after the surgery, both lesion groups showed a temporally ungraded retrograde amnesia. Second, to test anterograde amnesia, 1- and 4-week retention intervals were inserted between the four sample-exposure sessions and the test session. The RS-lesioned rats showed a retention interval-dependent impairment in the test sessions, while the HPC-lesioned rats showed an impairment regardless of the retention interval. Finally, to test short-term recognition memory, 5- or 30-min delay was interposed between the single sample-exposure session and the test session. Both lesion groups performed normally irrespective of the delay length. These results suggest that both the RS and HPC are important for long-term object recognition memory, but these areas have different roles in it.

### 3PK-181

#### Effect of calligraphy on change in cerebral blood volume

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Calligraphy (shodou, the way of writing) is the art of writing beautifully. Most children learn calligraphy in elementary school. It is a popular hobby among adults, too. On the other hand, Chinese researchers reported that calligraphy enhanced cognitive function in older people with mild cognitive impairment. The purpose of this study was to evaluate the usefulness of calligraphy as a tool of rehabilitation. Calligraphy is composed of some processes such as rubbing stick of sumi, perception of characters, writing by brush, and so on. In this study, we investigated the effect of rubbing stick of sumi on the cerebral blood volume in ten healthy young volunteers. They rubbed stick of sumi with (R+S) or without (R) smelling its flavor and smelt simply without rubbing stick (S) for one minute. Near-infrared spectroscopy (NIRS) was used to monitor concentration changes of oxyhemoglobin (delta [O 2Hb]) and deoxyhemoglobin (delta [HHb]) in the frontal cerebral cortex. Change in total Hb (THb) was calculated and used as an index of change in regional blood volume. The values of THb were increased immediately after starting R trial and S trial. On the other hand, THb's value in R+S trial also increased immediately and the magnitude of increment was more than two times compared with either of R, or S trial. The result indicated that rubbing and smelling had the synergistic effects on the augmentation of the cerebral blood volume. Present finding suggests that normal processes of calligraphy could activate cognitive brain functions.

### 3PK-182

#### Relationship between food intake and brain plasticity

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During food intake (FI) aFGF and leptin are released from the ependymal cells in the third cerebral ventricle and adipose tissue respectively and enter into the hypothalamus and hippocampus. Both of them suppress FI and facilitate the hippocampal plasticity. Now we demonstrate the glucose (G) increased in the brain during FI also facilitates this. During FI 3 mM G in CSF becomes twice. When 6 mM G injected into the rat hippocampal CA1 region behavioral learning and memory are facilitated. In CA1 slice with 3 mM G in Ringer solution EPSPs amplitudes generated by the Schaffer collateral/commissural stimulation markedly increased in 6 mM G. The paired-pulse facilitation experiments indicated augmentation of transmitter release by 6 mM G. The postsynaptic EPSPs amplitudes were significantly increased in 6 mM G associated with the augmentations of the phosphorylations of CAMKII and PKC. The increased EPSPs amplitudes were also due to the increase of the presynaptic synapsin phosphorylation. Transmitter evoked postsynaptic currents were measured in CA1 neurons by electrophoretic applications of NMDA and AMPA to the apical dendrites of the pyramidal neurons. NMDA and AMPA evoked currents were significantly augmented by elevation to 6 mM G. Notably high frequency stimulation of the Schaffer pathway failed to induce LTP in the CA1 region in 3 mM G but facilitated LTP in 6 mM G. The LTP induction in the 6 mM G was associated with further increase in CAMKII and PKC autophosphorylations. Taken together FI is valuable for the brain plasticity.

### 3PK-183

#### Occlusion of vision during tactile temporal order judgment with arms crossed

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When people cross their arms over the body midline, subjective rank ordering of successive unseen tactile stimuli delivered to both arms is confused (often being reversed) (Yamamoto & Kitazawa, 2001). In a former study we found that the confusion due to arm crossing is more obvious in the eyes closing condition than in the eyes opening condition (Wada & Kansaku, 2012). This study investigated the effect of occlusion of vision on the confusion during tactile temporal order judgment with arms crossed. In a psychophysical experiment, participants were required to judge temporal order of two tactile stimuli that were delivered to their both ring fingers in the range of  $\pm 1500$ ms stimulus onset asynchronies ( $n=12$ ). We prepared a liquid crystal (LC) shutter to occlude vision. The participant's eyes were either closed or opened, and each participant experienced two arm positions: arms crossed and arms uncrossed. To evaluate judgment probabilities of the participants, degree of reversals of their judgment was calculated as the sum of differences between correct response rates of the arms crossed condition and those of the arms uncrossed condition. The occlusion of vision during tactile TOJ showed arm crossing effect ( $P < 0.05$ , Wilcoxon signed rank test), and the effect was not significantly different with the effect of closing eyes ( $P > 0.05$ ). Both closing eyes and occlusion of vision showed significant effect on enhancing the confusion during tactile TOJ with arms crossed. The lack of visual information of the participant's own body/hands may have an important role in the arm crossing effect.

### 3PK-184

#### Properties of the Hippocampal sharp wave ripple events during delayed reinforcement task

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Sharp-wave ripples are component of the local field potential in the hippocampus that characterized short oscillatory activity at high frequency (about 100-250Hz) during slow wave sleep. At the neuronal level, reactivation of awake experience in the hippocampus during sharp-wave ripple events has been proposed as a neural mechanism for memory consolidation. In recent study, the sharp-wave ripple events are also observed during the awake quiescent state just before or after the learning task. However, the mechanism of the ripple activities during learning stage has not been established. In this study, we recorded the hippocampal local field potential during the delayed reinforcement task as a kind of memory retention test and analyzed the sharp-wave ripple events. After the recording, the ripple events were detected from local field potential and occurrence of the ripple was calculated in delay period of the task. In the correct trials, the occurrence of the ripple just before reward cue during delay period was increased. The peaks of the occurrence were correlated with performance of the task significantly. In contrast, the occurrence of ripples during incorrect trials showed no significant change. In addition, the frequency properties of the ripples that correlated with performance were analyzed. The ripples before reward cue may reflect the neuronal activities for prediction of a reward involving memory recall.

### 3PK-185

#### Cooperative task facilitates social and sexual behaviors in the rhesus monkey

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Although higher rank primates have been shown to perform cooperative behaviors, it is not known whether macaque monkeys have ability to perform cooperative behavior. Since social contact affects emotional and physiological functions, cooperative behavior may play some roles in physiological and emotional processes. In the present study, we trained female and male rhesus monkeys (*Macaca mulata*) to perform a simple cooperative button-push or bar-press task to get food reward and examined the effects of the cooperative task on sexual and social behaviors among them. In the cooperative task, two monkeys needed to push a button or press a bar at the same time to get reward, while in the solo task, they independently perform a task to get their own reward. One pair of monkeys performed the solo task for five successive days at 1st, 3rd and 5th week and did the cooperative task at 2nd and 4th week. Sexual behaviors (male's touching, mounting and thrusting and female's presenting) and social behaviors such as allogrooming and aggressive behavior were evaluated before and after task performance for 30 min each. The performance of cooperative task selectively facilitated sexual behavior between sexually active male and receptive female and allogrooming between sexually inactive male and female. The solo task did not affect either social behavior or sexual behavior. The results suggest that the macaque monkey has ability to perform cooperative task which facilitate sexual and social functions.

## Poster Presentations Nutrition, Metabolism, Thermoregulation(2)

### 3PK-186

#### High fat diet feeding duration and time dependent impairment of energy homeostasis and peripheral clock phase

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It is known that the overeating at dinner and/or at night snack time, and breakfast skipping, all things lead to obesity in humans. Thus, meal timing and meal frequency may affect body weight and body fat gain, and be associated with obesity. In this experiment, we gave high fat diet (HFD) or normal diet (ND) to mice at active period or at inactive period in 2 meals per day schedule, and amount of food was regulated by feeding time (2-12hrs per half day). The body weight and body fat were higher in 8 and 12hrs groups than 4hrs group. Although 2hrs group kept small food intake than 4hrs group, the body weight and body fat were higher in 2hrs group than 4hrs group. It is suggested that speed-eating is bad feeding habit. We also measured peripheral clock phase in liver using In Vivo Imaging System (IVIS). In the groups of HFD feeding at inactive period, their liver peripheral clock phases were advanced for about 5 hours as compared with other groups such as mice fed HFD at active period. Feeding large volume of HFD at inactive period induced the phase advance of peripheral clock. In the next, we gave HFD at inactive period for 2 hours, and then ND at active period for several hours (6 or 12hrs). The body weight and body fat were compatible to free feeding of ND, suggesting that short period of feeding time (2hrs) for night snack does not affect body weight, body fat and peripheral clock phase.

### 3PK-187

#### Combination food of carbohydrate and protein entrains mouse peripheral clocks through insulin secretion

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It is well known that insulin secretion may be important step for the entrainment of peripheral clock by restricted feeding (RF) (Hirao et al., 2009; Thara et al., 2011), because fast digestible carbohydrate possesses the stronger effect than slow digestible it. In addition, we found fish oil containing rich DHA/EPA could facilitate the RF-induced entrainment by the activation of insulin secretion through GPR120 located on the lower ileum and upper large intestine. As the role of carbohydrate and oil components of food became clear, however we have not yet found the role of protein on RF-induced entrainment. We prepared 6% casein (6C) and 20% casein (20C) groups, and 24-hr fasting mice were fed with 6C or 20C. After 1 hr, we sacrificed them and measured insulin secretion. Although total amount of carbohydrate is 20C<6C but, 20C group showed higher concentration of insulin than 6C group. Two day intake of 20C food at day time caused a phase-advance of liver and kidney clock compared with 6C food group using in vivo imaging system (IVIS). Thus, we focused on difference of composition of basic amino acid in protein components of food. We will compare among proteins such as casein, gluten, or soy protein evaluated by insulin secretion and IVIS. Finally, we will find the best combination of nutrients for entrainer.

### 3PK-188

#### Effects of ambient temperature on behavioral thermoregulation assessed by a simple experimental apparatus allowing alternating floor temperature changes

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We tested the behavioral thermoregulation of mice under different ambient temperatures (Ta). The apparatus was composed of two hollow stainless-steel plates (plates A and B, 20×5×1 cm), which had an inlet and an outlet connected to temperature-controlled water baths. Automatically controlled pumps to alternate the water supply were inserted between the plates and bath. The whole system was installed in a human neonate incubator. The measurements of thermoregulatory behaviors started after the 2-week recovery of six ovariectomized female mice with a temperature telemetry device implanted in the abdominal cavity. We placed a mouse on plates A and B at 25 and 30°C, respectively. The temperature of the incubation chamber, that is, Ta, was set at 25°C. After 1-hour habituation, we alternated the water supply between plates A and B every 7.5 min for one hour. On the second and third days, mice were starved with free access to water, and similar experiments were performed. The rate of staying on plates of 30°C significantly increased 75 to 81% with a body temperature decreasing from 37.7 to 36.2°C after starvation. However, when the Ta were 15 and 38°C, the mice remained on one of the plates with rolling and stretching their bodies, respectively. Starvation was ineffective in these cases. These results demonstrate that behavioral thermoregulation changes depending on Ta.

### 3PK-189

#### Seasonal differences in plasma adipocytokines and hormones concentration in obese subjects

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Obesity has been increasing in the world during the past several decades. In Japan, 30.4% of men and 21.1% of women are obese, being defined by a BMI of 25 kg/m<sup>2</sup> or over. Obese people have an increased incidence for developing cardiovascular, renal, and hormonal diseases and sleep disorders. Adipose tissue has not only the physiological function for the conserving energy as triglyceride but also the function of secretion for adipocytokines such as leptin, TNF- $\alpha$ , free fat acid (FFA) and adiponectin. Especially, adiponectin have an important role for anti-developing effects in arteriosclerosis and insulin resistance. There are some reports that the metabolic syndrome develops in winter, inducing higher incidence of cardiovascular events. We investigated the seasonal variations of adipocytokines and hormones concentration in obese subjects in Japan. Five obese (BMI, 32.0 $\pm$ 4.9 kg/m<sup>2</sup>) and 5 non-obese (BMI, 23.2 $\pm$ 2.9 kg/m<sup>2</sup>) men participated in this study in the summer and winter in a climatic chamber at 26°C with a relative humidity of 50%. The average ambient temperature during the experimental period was 29.1 $\pm$ 0.3°C in the summer and 3.3 $\pm$ 0.4°C in the winter. Blood samples were analyzed for adiponectin, TNF- $\alpha$ , free fat acid (FFA), leptin, ghrelin, insulin and glucose concentrations in obese and non-obese in each season. The seasonal differences of adipocytokines and hormones in obese and non-obese will be discussed.

### 3PK-190

#### Effect of feeding rhythm on hypothalamic regulation of energy metabolism

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Feeding rhythm influences whole body insulin sensitivity, however, it is not elucidated the effect of the rhythm on hypothalamic regulation of energy metabolism. In this study, C57BL/6J mice were given lab chows freely during dark phase (ZT12-24, Control group), first 4-hour in dark phase (ZT12-16; Morning group), or last 4-hour in dark phase (ZT20-24, Evening group) for 8 weeks. Mice in Evening group showed impaired whole body insulin sensitivity despite mice in the group ingested the smaller food intake than that of Control group, while mice in Morning group showed normal insulin sensitivity. We observed higher triglyceride (TG) content, increased gene expression of fatty acid synthase (FAS) and reduced insulin sensitivity in skeletal muscle in Evening group compared to other group. On the other hand, mRNA expression of agouti-related protein (AgRP), an endogenous antagonist for melanocortin receptor, was increased in hypothalamus in Evening group while mRNA expression of POMC, a precursor of endogenous agonist for melanocortin receptor, was decreased. Acute and/or chronic ICV-injection of AgRP increased FAS expression and TG content in skeletal muscle. These results indicate that feeding rhythm like as ingestion only in the evening impairs insulin sensitivity because of TG accumulation in skeletal muscle. This phenomenon may be partly mediated by hypothalamic melanocortin system.

### 3PK-191

#### The effect of head cooling on body core temperature and thermoregulation in humans

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**Aim** A mechanism that selectively cools the brain during hyperthermia exists in some animals, while it remains controversial if humans have such mechanism. Tympanic temperature have often been used to estimate brain temperature, however it is reported to be affected by facial skin temperature. In the present study, we investigated the effect of head cooling on tympanic and esophageal temperature in normothermic condition. **Methods** Healthy male subjects sat on a chair, and the skin temperature of the periphery and torso were maintained with a water-perfusion suit at 32°C. After 10-min baseline period, facial fanning was conducted for 15 min. Esophageal and tympanic temperatures were monitored with thermocouples. Thermal sensation was also assessed. **Results** During facial fanning, while facial skin temperature was significantly decreased, there was no significant difference between changes in esophageal and tympanic temperature. As for thermal sensation, facial fanning produced slight change. **Discussion** These results suggest that tympanic temperature was not affected by facial skin temperature. These methods should make it possible to estimate selective brain cooling in humans.

### 3PK-192

#### Effects of intake of $\alpha$ -glucosylhesperidin on the edema of the lower leg in human

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4'- $\alpha$ -glucopyranosylhesperidin (Hsp-G) was synthesized from hesperidin by transglucosylation using cyclodextrin glucanotransferase. It has been reported that Hsp-G had the ability to attenuate capillary permeability. The main aim of our study was to test the effects of intake of Hsp-G on the edema of the lower leg in human. Nine female volunteers, age 43.4 $\pm$ 2.6 yrs, completed in our study. After ingestion of either a Hsp-G (1000mg) or placebo (Pla) drinking water of 100 mL with pure water of 100 mL, all subjects kept the sitting position for six hours to cause edema of the lower leg. Of the nine subjects, we analyzed the six subjects observed reduction of edema by walking at a rest during experiment. Impedance of lower leg was decreased by keeping the sitting position for six hours at the both conditions, but it tended to be suppressed by a Hsp-G drinking (P=0.053). Increase of perimeter of ankle (Hsp-G : 101.8 $\pm$ 1.5% vs Pla : 103.3 $\pm$ 0.8%, P=0.004) and perimeter of calf (Hsp-G 101.4 $\pm$ 0.7% vs Pla : 102.9 $\pm$ 1.3%, P=0.043) was significantly suppressed by the ingestion of drinking Hsp-G. We assumed that the Hsp-G strengthened blood vessel walls and prevented capillary permeability, therefore edema of lower leg induced by the sitting position for six hours was suppressed.

### 3PK-193

#### Estrogenic regulation of T-cell protein tyrosine phosphatase (TCPTP) in the hypothalamus controlling feeding behavior

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Recently, particular attention is devoted to understanding how the protein tyrosine phosphatases are involved in cellular functions. Among them, T-cell protein tyrosine phosphatase (TCPTP) (encoded by *Ptpn2*) is a ubiquitous tyrosine-specific phosphatase and dephosphorylates JAK1/3, STAT3 but not JAK2. Using cell-based approaches, we revealed that *Ptpn2* +/- mice exhibited lower fasted blood glucose and decreased hepatic glucose output in high fat-induced diabetic mouse by increasing the phosphorylation of STAT3. The same is true in the brain specific *Ptpn2* +/- mice. Thus, these results led us to speculate TCPTP dephosphorylates STAT3 in proopiomelanocortin (POMC) neurons in the brain. Female rats were ovariectomized and used for two weeks later. We firstly confirmed by western blotting that the expression of TCPTP was increased by a high-fat diet for 4 weeks. Surprisingly, we found that 2 weeks estrogen treatment significantly increased the expression of TCPTP in the hypothalamus, when food intakes and body weights were decreased. We then examined the distribution of TCPTP-immunoreactive cells in the hypothalamus. We revealed that the expression was found to be medial part of the arcuate nucleus where NPY neurons are located. Some NPY neurons in the arcuate nucleus also expressed estrogen receptor  $\alpha$ . We therefore hypothesized that NPY neurons were target for anorectic effects of estrogen in addition to POMC neurons.

### 3PK-194

#### Rare sugar D-psicose prevents diabetes development and excess fat accumulation in type 2 diabetes OLETF rats

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Prevalence and consequences of obesity have been increasing alarmingly, mostly due to energy imbalance; excess calorie intake and insufficient physical activities. The circumstance demands health screening and age-adjusted balanced food intake on obese-tendency subjects from early life. D-psicose, a rare sweet sugar having zero calorie, has been developed and produced in Kagawa University Rare Sugar Research Center and has been proven to control blood glucose levels and excess fat accumulation in growing T2DM OLETF rats. Long-term feeding of 5% D-psicose in drinking water controlled food intake, reduced body weight gain, and prevented excess fat accumulation. D-psicose improved insulin resistance through constant maintenance of blood sugar and serum lipid levels and thus prevented diabetes development. These data demonstrate that D-psicose might be a promising non-toxic food-additive to control obesity or prevent diabetes in the obese-tendency individuals.

## Poster Presentations Motor Function(2)

### 3PK-195

#### Diabetic polyneuropathy targets gamma motoneurons of the gastrocnemius muscle in STZ rats

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Alterations in the number and size of motoneurons were studied in the medial gastrocnemius (MG) motor nucleus of type I diabetic rats and controls. MG motoneurons were retrogradely labeled by dextran-fluorescein and the number and size of cell bodies were examined. Additionally, morphological alterations of muscle fibers of MG muscles were examined. Significantly fewer labeled MG motoneurons were found in the diabetic rats as compared with control animals. The mean soma diameter of MG motoneurons was significantly smaller in the diabetic animals. The distribution of soma diameters of control animals was bimodal; larger cells were presumed to be alpha-motoneurons and those with smaller cells were presumed to be gamma. Compared to control animals, the number of smaller cells was reduced in 12-week diabetic animals. By 22 weeks, virtually no small cells were found in diabetic animals and the size distribution was unimodal. Additionally, morphological changes of intrafusal muscle fibers were observed in MG muscle of diabetic animals, although structures of extrafusal muscle fibers were preserved. We conclude that there is a significant decrease in the number and size of MG motoneurons in diabetic rats with the possibility that the decrease occurred predominantly among the gamma-motoneurons.



### 3PK-196

#### NR2B antagonist ameliorates L-DOPA induced dyskinesia via suppressing hyperactivity of subthalamic neurons in hemi-parkinsonian rat

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The antiparkinsonian actions of NR2B selective NMDA receptor antagonist has been studied. However, the effect of NR2B antagonist in the model of Parkinson's disease (PD) or patients with PD is still controversial. Because of the functional difference between glutamatergic receptor and dopaminergic receptor, we thought that the effect of drug is restricted to a part of the antiparkinsonian effects of L-DOPA. To examine the possibility, we first compared the effects of ifenprodil, NR2B antagonist, on motor dysfunctions with those of L-DOPA. Next, we evaluated whether co-application of ifenprodil and L-DOPA would improve the effects of L-DOPA. For the present study, hemi-parkinsonian rats were used. We performed cylinder test to investigate forelimb-use asymmetry (FUA) as a parameter of motor coordination and total forelimb-use (TFU) as motor activity. The administration of ifenprodil showed no significant improvement on either FUA or reduced TFU at all doses. The antiparkinsonian effect of L-DOPA was significant on TFU, but not on FUA. When administrated with a combination of ifenprodil and high dose of L-DOPA, ifenprodil markedly ameliorated L-DOPA-induced dyskinesia represented by different FUA. In the further investigation by using c-Fos immunohistochemistry, the ifenprodil significantly suppressed the L-DOPA-induced c-Fos expression in neurons only in the subthalamic nucleus (STN).

### 3PK-197

#### Arm posture modulates the excitability of cervical spinal cord in sedated monkeys

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We can reach to the same target from different initial postures of upper limb. Central nervous system (CNS) need to know the initial state of limb for generating proper motor command, but neural mechanism for compiling this information is not known. To address this question, we stimulated the spinal cord of the primates at different upper limb postures, and examined if there are systematic change of evoked muscle response depending on the postures. For the stimulation, floating microelectrode arrays (12 or 32 ch) were chronically implanted at the C6 level of macaca mulatta (n=3). Evoked EMGs were recorded with paired EMG wires implanted in the left shoulder, elbow and hand muscles (n=11 or 12). Posture of upper limb was changed systematically by fixing the wrist position at 7 points in the coronal plane (8×8 cm grid). Stimuli through 71.4% of electrode induced the evoked response at least one muscle. We found that 79.2% and 75.0% of evoked responses changed their amplitude and onset latency, respectively, depending on the arm posture (p<0.01, ANOVA). This posture dependency was more frequently observed in the wrist and elbow than shoulder muscles (p<0.05,  $\chi^2$  test). These results suggest that initial arm posture modulates excitability of the CNS and it could be used as a calibration signal for generation of proper motor command.

### 3PK-198

#### Accuracy in isometric ballistic force generation of elbow flexion

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Ballistic contraction is regarded as being controlled with feed-forward mechanism which does not utilize sensory signals from the periphery. This means that the command output from the cortical motor center is directly reflected in performances. We investigated how accurately the motor command from the brain was tuned by analyzing the accuracy in the ballistic exertion of elbow flexion force at 30, 50, 70% of maximum voluntary contraction (MVC). We also analyzed the training effects on the accuracy. Six participants completed sessions which were held in five consecutive days. Isometric contractions of elbow flexors were performed at three targeted intensities as quickly as possible 30 times in each condition. For the evaluation of participants' performance in accuracy of force production, the mean of the difference between exerted force and the target was utilized ERROR. For the analysis of training effects, SD of difference between the exerted force and the target in the first session and fifth session were compared. ERRORS at target intensities of 30, 50 and 70% were 2.0±0.4%, 0.2±0.6%, and -2.0±0.9%, respectively. Standard deviation of errors in three conditions on the first and the fifth days were 6.4±2.5% vs. 5.1±1.3% at 30%, 6.5±2.6% vs. 5.7±2.0% at 50%, and 5.3±1.0% vs. 4.7±1.0% at 70%. It seems likely that the accuracy in ballistic force generation tended to be improved with 5-day training.

### 3PK-199

#### Effects of movement variability of dominant and non-dominant arm on adaptation to visual disturbances

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We can flexibly adapt to changes in an environment and our bodies to produce accurate movements. Many experimental studies have suggested that adaptation to a novel environment occurs acquiring an adequate internal model driven by errors. There are two sources of errors, external and internal ones. The former is caused by changes in the environment and the body. The latter is caused by so-called movement variability. Movement variability is induced by uncertainty of internal model and noises in a perception and execution system. Thus, when we adapt to the novel environment, the movement variability may affect the adaptation through the internal error. Also, we can empirically imagine that amount of the internal errors maybe different between dominant and nondominant arms. In this study, we investigated effects of the internal errors, or the movement variability, on adaptation to visual disturbances in goal directed arm movements. We measured trial by trial adaptation to random visual disturbances with each arm and found that the movement variability and the size of adaptation of the nondominant arm tended to be larger than those of the dominant arm. In addition, the size of adaptation is correlated with the movement variability of the dominant arm but not with that of the nondominant arm. These findings suggest that adaptation in the dominant arm occurs depending on uncertainty in movements and that in the nondominant arm does not.

### 3PK-200

#### Relationship between saccades and via-points in free drawing of a circle like closed curve in the monkey

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It is important to detect via-points to draw a complex trajectory. Drawing a shape composed of lines such as a triangle can be segmented into reaching movements at each vertex. On the other hand, drawing a closed curve like a circle cannot be segmented because it does not have precise vertices. To estimate via-points during free drawing of a circle like closed curve, we investigated interactions between gaze points and a hand trajectory during the drawing in two Japanese monkeys. They performed the drawing with two different rotation directions and 8 different starting points, which were placed every 45° on a circle with a 100 mm-radius. We obtained the following results regardless of the rotation direction, starting point of the drawing movement and individuals. The saccade endpoints were distributed widely in a first half of drawing trajectory and were concentrated at around the goal point. On the other hand, curvature of the hand trajectory was not constant but changed in the time domain, indicating that the trajectory was not a circle. The saccade tended to occur around local minimum points of curvature of the drawing trajectory. Finally, the saccade slightly preceded the hand movement. The findings suggest that a local minimum point of curvature of the drawing trajectory may be regarded as a via point of the hand drawing movement.

### 3PK-201

#### The simultaneous movements of neck and both elbows affect the tonic vibration reflex in the arms

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Tonic vibration reflex (TVR) was elicited by vibration to the muscle of the triceps brachii. This study examined whether rotation of the neck affected misperception of movement of the elbow in TVR. Fifteen healthy subjects actively flexed their elbows from 0° to 90° for 3 s with their eyes closed. During the time that the elbow was flexed, vibratory stimulation (100 Hz) was applied to the tendon of the right triceps brachii. In the first experiment, only the right elbow was flexed (one-arm experiment), whereas in the second experiment both elbows were flexed simultaneously (two-arm experiment). In the two-arm experiment with vibratory stimulation, the mean ( $\pm$ SD) angle of the elbow was  $63.2 \pm 11.2^\circ$  with neck rotation at 0°, which decreased significantly to  $53.0 \pm 15.5^\circ$  ( $p < 0.05$ ) when the neck was rotated back to 0° from a position of maximal right rotation. This findings suggested that the effect of TVR was enhanced by neck rotation and the simultaneous movement of both elbows.

### 3PK-202

#### Motor primitive for walking in human lumbar spinal cord

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Coordinated movement pattern of legs was produced by locomotor network in lumbar spinal cord during walking. However, it is unclear how the network formulates alternative and coordinated movement patterns between right and left legs in human. We have reported that voluntary controlled non-invasive magnetic stimulus over the lumbar spinal cord induced the walking behavior. The behavior induced by this stimulus may be partly or entirely produced by a neural module controlling a muscle group in walking. To tackle this issue, we investigate how the behavioral pattern depends on the activation site by stimulating several spinal levels. The intervertebral segment at lumbar cord was stimulated by voluntary controlled magnetic stimulation. Stimulus site was also positioned on right and left with respect to midline at each intervertebral level. The right-left alternating walking like movement was induced when the stimulus probe positioned at L1-L2, L2-L3 and L3-L4 intervertebral segments. The forward step induced by controlled burst stimulation was always ipsilateral to the stimulation site, whereas the backward movement was induced in contralateral side. This mirror-like behavioral pattern induced by right and left sided stimulus indicates that two distinct neural modules interact and formulate alternative movement pattern between right and left leg. These neural modules may be a part of motor primitive for walking.

## Poster Presentations Others(3)

### 3PK-203

#### Effect of human concentration on background conversation by Fm $\theta$ of EEG

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We examined the effect of human concentration on silence and hearing background conversation by measuring EEG. Degree of concentration of attention was evaluated by Fm $\theta$  (4-7Hz) which is appeared in center of forehead (frontal-midline) during concentration of consciousness. In order to concentrate attention calculation test was performed for 3 min by single digit multiply two digits. During calculation test we measured EEG without and with background conversation in familiar language in Japanese or not in English. For this experiment we made own 12ch EEG apparatus with band-pass and hum filter. All data were taken by data logger and transferred to computer. Then EEG data during calculation test were analyzed by fast Fourier transform and compared a power spectrum of Fm $\theta$  without and with Japanese or English conversation. Especially we focused differences in the degree of concentration hearing Japanese and English. We also examined the number of the answers to calculation test by silence and hearing conversation. As the result, both the number of the answers of calculation test and power output of Fm $\theta$  increased more during silence than hearing conversation. Power output of Fm $\theta$  increased more in the case of hearing English than that of Japanese. These results show that in quiet circumstance concentration increase, and in familiar conversation concentration decrease unconsciously tended to hear.

### 3PK-204

#### Intestinal myofibroblast TRPC4, C6 channels confer fibrogenic potential for human stenosis

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The myofibroblasts represents an activated mesenchymal cell and plays a key role in extracellular matrix synthesis and inflammatory/fibrogenic cytokine secretion and wound contraction. Available evidence suggests that part of myofibroblastic function may reflect altered Ca<sup>2+</sup> homeostasis. We thus explored a potential role of canonical transient receptor potential (TRPC) channels expressed therein by using human colonic myofibroblast cell line InMyoFib. Expression of TRPC channels, collagen I $\alpha$ 1 and the myofibroblast transdifferentiation marker alpha smooth muscle actin (alpha-SMA) was quantitatively analyzed by real-time RT-PCR, Western blot analysis and immunofluorescence cytochemistry. TGF-beta1 (5ng/ml) induced differentiation of InMyoFib, and this was paralleled by increase in TRPC4 and TRPC6 expression. siRNA knockdown of TRPC6 alone markedly reduced TGF-beta1 associated increases in alpha-SMA expression. Similarly, enhanced collagen I $\alpha$ 1 synthesis, interleukins secretion, and myofibroblast migration, which promote fibrogenic changes, were all significantly accelerated by TRPC4- or TRPC6-siRNA treatment. In addition, an important cell adhesion protein cadherin interacted with TRPC6 strongly is augmented by TGF-beta1 stimulation. From these results, we suggest that myofibroblast TRPC channels including TRPC4, C6 could act as important mediators for fibrogenesis in the colon.

### 3PK-205

#### Changes in Na<sup>+</sup> metabolism and its effect on nutrients absorption in claudin 15 knockout mice

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It is shown that the claudin family of tight junction proteins is critical in determining the paracellular ionic permeability and selectivity. We have shown that loss of claudin 15 resulted in decreased of luminal Na<sup>+</sup> concentration and glucose malabsorption in the small intestine. In order to get more insight into the relationship between Na<sup>+</sup> metabolism and nutrition absorption induced in the loss of claudin 15, we have investigated the site of absorption of electrolytes and nutrient in the claudin 15 knockout (cldn15KO) mice and compared with wild-type under in vivo condition. Mice were fed a powdered diet supplemented with <sup>14</sup>C-polyethylene glycol (PEG) as nonabsorbable marker. After 3 h, small intestines were isolated and divided into six segments, then luminal contents were collected for analysis of Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup> concentration and <sup>14</sup>C-PEG. The gastric emptying time by measuring <sup>14</sup>C-PEG was decreased in cldn15KO compared to wild type. Total amount of luminal contents were increased in cldn15KO and the retention time of digesta was ~3 times increased in upper jejunum. Robust Na<sup>+</sup> secretion and absorption rate was observed in upper jejunum in wild type mice and this was attenuated in cldn15KO. K<sup>+</sup> absorption rate was increased in cldn15KO lower ileum. Luminal glucose concentration was increased in cldn15KO ileum. These results suggested that the sites of absorption and secretion of electrolytes and glucose were changed to the adaptation of loss of claudin 15.

### 3PK-206

#### Role of PTEN in the development of inner ear

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PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a tumor suppressor gene that regulates various cell processes including proliferation, growth, synaptogenesis and the dynamics of the actin-myosin cytoskeleton. We studied the expression pattern of PTEN in the mouse inner ear during development and explored its function by analysis of PTEN heterozygous null mice. Immunolabeling revealed that PTEN is expressed primarily in differentiating sensory neurons and hair cells, coinciding with the temporal and spatial gradients of hair cell differentiation. In heterozygous null mice, the sensory epithelial progenitors withdraw later from the cell cycle than wild type and this is associated with an increase in hair cell number. Disorganization and loss of hair bundles is uniquely associated with the formation of ectopic hair bundles on the inner pillar cells. These results show that PTEN plays an important role in regulating the proliferation, differentiation and innervation of mammalian cochlear hair cells. PTEN signaling pathways provide potential therapeutic targets for regeneration of mammalian inner ear sensory epithelia.

### 3PK-207

#### Physical and psychological effects of hot spring bathing in elderly people

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The spa treatment is very popular therapy for promoting health and relieving symptoms of certain diseases, and is often used for elderly people as part of the social welfare services. On the other hand, it is reported that elderly people often have accidents while taking a bath. Therefore, the present study aimed to examine the efficacy and safety of spa treatment in the elderly people. Eleven elderly male volunteers (mean age : 77.2 years) served for this study. The body temperature, heart rate, heart rate variability, blood pressure, cardio ankle vascular index (CAVI), and salivary chromogranin A (CgA : as an indicator of the psychological stress) were measured from the subjects before and after taking bath as they like for 10 minutes. As a control, the same subjects were also measured in the corresponding time on different days without taking a bath. The results show that the heart rate and body temperature slightly increased after bath compared to the control (without bathing) ( $p < 0.05$ ). Other parameters showed no significant changes with bathing. From these results, short-lasting hot spring bathing was both physically and psychologically less stressful, and was relatively safety in elderly people.

### 3PK-208

#### Central command selectively inhibits the cardiac component of aortic baroreflex during spontaneous motor activity

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We have reported using electrical stimulation of the baroafferent nerves that central command provides selective inhibition of the cardiac limb of aortic baroreflex with preserving carotid sinus baroreflex during exercise (Matsukawa et al. Am J Physiol, 2012). To confirm differential effects of central command on the arterial baroreflexes, we surgically separated the baroreflex systems and identified the relationship between the changes in mean arterial pressure and heart rate in response to brief occlusion of the abdominal aorta given before and at the onset of spontaneously-occurring motor activity in paralyzed, decerebrate cats. Baroreflex bradycardia elicited by aortic occlusion was compared among the three conditions of aortic baroreflex (AOR) alone, carotid sinus baroreflex (CS) alone, and intact baroreflexes (INT). When the aortic occlusion was given at the onset of spontaneous motor activity, the baroreflex bradycardia was markedly reduced and the baroreflex sensitivity was blunted in INT and AOR conditions, as compared to the control before exercise. In contrast, the reduction in the baroreflex bradycardia and sensitivity were much less in the CS. Taken together, it is confirmed using mechanical stimulation of baroreceptors that central command provides selective inhibition for the cardiomotor component of aortic baroreflex at the onset of exercise, which in turn contributes to instantaneous increase in HR.

### 3PK-209

#### Binding of thrombin-activated platelets to a rigid fibrin network is essential for phosphatidylserine exposure on their cell surface

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Exposure of phosphatidylserine (PS) on the platelet outer membrane leaflet promotes thrombin formation. Recently, by employing intravital confocal microscopy, we demonstrated that platelets expose PS only when they exist in the center of the thrombus but not when they are in its periphery. To address the question how exposure of platelet anionic phospholipids is regulated within the thrombus, an *in-vitro* experiment was employed, in which the fibrin network was formed using platelet rich plasma. PS exposure on the platelet surface was then analyzed using Confocal Laser Scanning Microscopy. Almost all platelets exposed PS after treatment with tissue factor, thrombin or ionomycin. An attenuation of either fibrin mesh formation or platelets' binding to fibrin scaffold by several pharmacological methods including FK 633, an  $\alpha$ Ib $\beta$ 3 antagonist, and cytochalasin B suppressed surface exposure of PS in platelets evoked by thrombin. Gly-Pro-Arg-Pro amide abrogated fibrin network formation and reduced PS exposure on platelets without suppressing platelet binding to fibrin/fibrinogen. These results suggest that outside-in signals in platelets generated by their binding to the rigid fibrin network are essential for PS exposure after thrombin treatment.

### 3PK-210

#### A role of Ca-sensing receptor (CaSR) expressed in type B intercalated cells along the mouse kidney collecting duct

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The renal collecting duct (CD) serves the fine-tuning of water, electrolytes, and acid-base homeostasis by stimulation of Ca-sensing receptor (CaSR). Therefore, we investigated the renal segmental distribution and cellular localization of CaSR mRNA and protein to characterize its function in the kidney. Methods : Localization of CaSR mRNA and protein in mouse (C57B/6J, male, 10 weeks) kidney nephron was assessed by a double-staining technique (IHC) with the anti-AQP2, AE1, AE4 (markers of principal cell (PC), IC-type A (IC-A), IC-type B (IC-B) in sequence) and CaSR antibodies. By using a quantitative *in situ* hybridization and IHC, we evaluated changes in the CaSR expression along the CD after (1) CaCl<sub>2</sub>-loading, (2) acidosis (Ac), and (3) alkalosis (Al) induced, respectively, by NH<sub>4</sub>Cl- and NaHCO<sub>3</sub>-intake (6-d). Results : CaSR mRNA and protein were expressed in TAL, DCT, and IC-B through CD, but not in either PC or IC-A. CaSR protein was localized in the basolateral membrane of IC-B. Although its level of staining was unchanged during Al, it was significantly decreased under the conditions of Ac and CaCl<sub>2</sub>-loading. Plasma [Ca<sup>2+</sup>] was significantly increased by CaCl<sub>2</sub>-loading (6-d), but was within normal ranges during Ac and Al. Plasma pH was decreased significantly under the conditions of Ac and CaCl<sub>2</sub>-loading, but was unchanged during Al. Conclusions : CaSR in IC-B may inhibit urinary excretion of HCO<sub>3</sub><sup>-</sup> by decreasing the level of the expression during metabolic acidosis.

### 3PK-211

#### OXTR and AVPR1A polymorphisms modulation of prefrontal activations of mothers and fathers in response to their own infant's smiling

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Changes in hormone levels during pregnancy to parenthood seem to make both women's and men's brain maternal and paternal, respectively. We examined whether the prefrontal cortex (PFC) activations in response to mothers'and fathers'own infants'smiling are candidate to reflect their maternal and paternal love. Furthermore, we examined whether OXTR and AVPR1A polymorphisms influence on such PFC activations. Forty three mothers and 41 fathers were participated, and their infant smile was videotaped. Then, they underwent near-infrared spectroscopy (NIRS) while observing own-infant smile compared with unfamiliar-infant smile. We found that the neural activations of the right ventromedial PFC (vmPFC) of mothers. However, these activations were not observed in A-carrier mothers of the rs2254298 of the OXTR, but the opposite hemisphere was activated. Although the PFC activations were not observed in all participant fathers, fathers who have not the 334 allele of the RS3 of the AVPR1A showed the left vmPFC activations. These results suggest that mothers have certain abilities that the left vmPFC compensates for the right and fathers are divided into two types, the one being highly devoted fathers and the other less so.

### 3PK-212

#### Miniaturized wireless calcium recording system for freely moving mice

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Methods for monitoring neural activity from freely moving animals are vital for understanding a neural basis of animal behavior. Here we describe a wireless calcium recording system for freely moving mice. The system consists of two main devices : 1) a fluorescence detector with an LED, single photo diode, excitation and emission filter, dichroic mirror and a gradient index (GRIN) lens (with dimensions 10 x 10 x 7.75 mm and 2.0 gram). 2) A circuit board that was divided into two parts : one with batteries, an amplifier, power supply circuitry and a light control IC with dimensions 20 x 22 mm. The other consists of a transmitter and a microcomputer with dimensions 20 x 20 mm. The total weight of the circuit board is 7.7 g. For calcium recordings, the calcium-sensitive dye (Oregon green 488 BAPTA-1 AM) was pressure-injected into layer 2/3 of hindlimb area of the somatosensory cortex. The fluorescence detector was positioned on surface of the injected site and attached to skull with dental cement. The system could monitor population calcium activity from freely moving mice, and transmit data to a PC that also monitors mouse behavior with web cameras. Using this system, we recorded cortical calcium activity and mouse behavior during a tactile preference test.

### 3PK-213 (SPK-1)

#### BDNF secretion regulated by secretory vesicle-associated protein CAPS2

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Calcium-dependent protein for secretion 2 (CAPS2) is known to be associated with the secretion of dense-core vesicles (DCVs) that contain peptides, hormones and proteins. Brain derived neurotrophic factor (BDNF) is one of the most critical protein which is involved in the neural generation, proliferation, differentiation, network construction and plasticity, which is thought to be contained in DCVs. Through the use of CAPS2 knockout (KO) mice, the present study analyzed the role of CAPS2 in BDNF secretion. CAPS2 KO mice had reduced hippocampal BDNF levels, and overexpression of exogenous CAPS2 significantly increased frequency, amplitude, and kinetics of depolarization-induced BDNF vesicle exocytosis in CAPS2 KO hippocampal neurons. The CAPS2 KO hippocampus displayed impaired GABAergic interneuron systems, including decreased GABAergic neuronal numbers in the juvenile stage, decreased number of synaptic vesicles in inhibitory synapses, and reduced frequency and amplitude of miniature inhibitory postsynaptic currents. Moreover, the CAPS2 KO mice exhibited reduced late-phase long-term potentiation (L-LTP) in CA3-CA1 synapses, decreased hippocampal theta oscillation frequencies, and increased anxiety-like behavior. These results suggest that CAPS2 promotes activity-dependent BDNF secretion, which is critical for the formation of a hippocampal GABAergic interneuronal network and their related behavior.