SYMPOSIA

S 1

Roles of lipid raft in epithelial Na^+ channel (ENaC) function

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ENaC plays a critical role in homeostasis of fluid content and blood pressure by controlling Na^+ transport in epithelial cells. The amount of ENaC-mediated Na^+ transport is closely correlated to the activity (open probability; Po) of individual ENaC and the number (N) of ENaCs expressed on the apical membrane: these Po and N are regulated through post-translational processing such as proteolysis with GPI-anchored proteases and Nedd4-2-dependent ubiquitination. On the other hand, cholesterol- and sphingolipid-enriched lipid raft is a crucial platform for protein trafficking and signal transduction, and accumulates in the apical membrane more than in the basolateral membrane of epithelial cells. Consequently, lipid raft might be a membrane domain suitable for post-translational processing of ENaC. In this study, we elucidate the role of lipid raft in the apical trafficking of endogenously expressed ENaC and post-translational processing in renal epithelial A6 cells with or without stimulation of aldosterone, and present the importance of lipid raft for ENaC post-translational processing including trafficking. No COI.

S 1

Short Palate Lung and Nasal Epithelial Clone 1 (SPLUNC1) dissociates and internalizes the Epithelial Sodium Channel (ENaC)

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αβγENaC regulates sodium and water absorption across airway epithelia. In cystic fibrosis airways, hyperactive ENaC dehydrates airway surfaces which results in mucus thickening and increased probability of infection. SPLUNC1 is a negative regulator of ENaC but its underlying mechanism of action is unknown. Here, we tested the hypothesis that SPLUNC1 works by internalizing ENaC. Surface biotinylation was performed in HEK293 and Human bronchial epithelial cells (HBECs) to investigate ENaC surface level. Immunoprecipitation, immunostaining and acceptor-photobleaching fluorescent resonance energy transfer (FRET) were performed in HEK293 to investigate ubiquitination, co-localization, and conformational change of ENaC respectively. A Nedd4-2 dominant-negative construct was a gift from Dr. Peter Snyder (UI). SPLUNC1 reduced the %FRET efficiency between βENaC-GFP and γENaC-mCherry from 9.8±1.4 to 5.0±1.1%. SPLUNC1 decreased plasma membrane αENaC by 6.7-fold in HEK293 cells and 2.6-fold in HBECs without affecting the plasma membrane βENaC. When αγENaC was co-expressed, SPLUNC1 did not affect plasma membrane αENaC. SPLUNC1 ubiquitinated αENaC by 4.5 fold, which was abolished when Nedd4-2 ubiquitin ligase function was blocked by αENaC PY-motif truncation or Nedd4-2 dominant negative transfection. Pre-treatment with chloroquine, a lysosome inhibitor, but not MG-115, a proteasome inhibitor, abolished intracellular αENaC degradation without affecting ENaC internalization. Internalized αγENaC by SPLUNC1 co-localized. In conclusion, upon the binding of SPLUNC1 to βENaC, SPLUNC1 allosterically triggers Nedd4-2 mediated αENaC ubiquitination that results in the dissociation of ENaC subunits, internalization and degradation of αγENaC but not βENaC via the lysosomal pathway. No COI.

S 1

CFTR chloride channels as promising therapeutic targets in multiple diseases

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Cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-activated chloride channel expressed in luminal membrane of epithelial cells. Our laboratory has investigated roles of CFTR as drug targets for human diseases and identifies pharmacologically favorable CFTR inhibitors using cross-disciplinary approaches. Using cell-based assays of CFTR function, several novel types of CFTR inhibitors have been identified from collections of both synthetic small molecules and natural compounds including hydrazide-containing compounds, hydrolysable tannins, steviol derivatives, chalcones and xanthones. Furthermore, using the identified compounds to probe CFTR function, roles of CFTR as drug targets for cholera, polycystic kidney disease (PKD) and thalassemia have been validated. Interestingly, we recently demonstrated that existing drugs including diclofenac and pranlukast inhibit CFTR function and may be beneficial in the treatment of secretory diarrheas and PKD, respectively. In addition, our group showed that indirect inhibition of CFTR function by chitosan oligosaccharide, a biomaterial prepared from chitin, reduces intestinal fluid secretion in vivo via AMPK-dependent mechanism. Therefore, CFTR serves as a promising drug target whose functional inhibition, through either direct or indirect mechanism, may be of therapeutic benefit in secretory diarrheas, PKD and/or thalassemia. No COI.

S 1

Ca^{2+} signaling defects underlying exocrine dysfunction

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Radiotherapy for head and neck cancer induces significant acute and long-term by-stander effects on tissues within the treatment area, such as the salivary glands which undergo irreversible loss of fluid secretion. In patients this results in severe dry mouth conditions with debilitating effects. Surprisingly loss of function precedes fibrosis and destruction of glandular tissue, which is a much slower process. The mechanism underlying the loss of salivary gland function is not known. Our earlier studies have identified that the ROS-sensing Ca^{2+} channel, TRPM2, is activated as a consequence of irradiation (IR) and Ca^{2+} entry via this channel contributes to irreversible loss of salivary gland function. $TRPM2^{-/-}$ mice display an initial loss of function that recovers to near normal levels within a month after IR while $TRPM2^{+/+}$ mice show no such recovery. Our data showed that activation of TRPM2 by IR involves increase in PARP activity and treatment of $TRPM2^{+/+}$ mice with PARP inhibitors prior to IR induced significant protection of salivary function. To understand the link between TRPM2 and loss of salivary gland function, we examined Ca^{2+} signaling in glands from IR-mice and in vitro in IR-cells. Our findings show that following IR, store-operated Ca^{2+} entry (SOCE), a critical requirement for fluid secretion, is irreversibly decreased in cells from $TRPM2^{+/+}$ but not TRPM2-/- mice. Importantly, IR leads to compromise of mitochondrial function, caspase activation, and caspase-dependent cleavage of critical proteins involved in SOCE. Together, our studies indicate a novel link between TRPM2 and mitochondria that can account for the loss of salivary fluid secretion. No COI.

S 2

Probing the brain ubiquitome for signs of transport

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Ubiquitylation is a multifaceted post-translational modification that ensures protein levels are subject to tight spatio-temporal control and is a key mechanism of cellular proteostasis. It is a hierarchical process that involves a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin-protein ligase (E3). There are over 600 known E3 ligases that are ultimately responsible for substrate specificity. The topology of the ubiquitin chains also determines outcome. Typically, chains linked via Lys48 on ubiquitin are directed to the proteasome, Lys63 chains modulate protein function while monoubiquitylation regulates endocytosis. Analogous to the regulation of protein phosphorylation by the opposing activities of kinases and phosphatases, ubiquitylation by E3 kinases is also balanced by deubiquitylating enzymes. In the brain, complex ubiquitin–dependent proteostatic networks regulate not only the normal function of the brain by tightly controlling protein levels but also to prevent the accumulation of toxic aggregates in various cellular compartments. The sheer complexity of the ubiquitin pathways has necessitated the development of new proteomic methods to allow an unprecedented view into the ubiquitin-modified proteome (ubiquitome). Mass spectrometry methods using various affinity matrices have started to look at the brain ubiquitome with upwards of 1000 ubiquitylated proteins reported. We have recently developed methods for ubiquitome analysis that use less than 500 ug of tissue. We have been able to detect over 1000 ubiquitylated proteins in embryonic mouse brain, expanding the known brain ubiquitome by ~15%. We have also performed studies in Nedd4$^{-/-}$ mice and have identified ~150 proteins with altered ubiquitylation profiles in the brains of Nedd4$^{-/-}$ embryos compared to wild-type. This presentation will highlight our new data from brain and

S 2

Investigating the role of large neutral amino acids in lymphocyte activation

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Intake of nutrients from the environment is crucial for the activity of most cells. However, some nutrients with hydrophilic trait such as glucose and amino acids cannot pass across the cellular membrane, and transporters are required for the incorporation of these nutrients into cells. LAT1 (L-type amino acid transporter 1) is a transporter which incorporates essential amino acid into cells. LAT1 has been considered to have an important role for cancer growth, but the function of LAT1 in normal tissues remains unsolved due to its extremely low expression level in the normal human body. We characterized LAT1 as a major transporter of amino acid in activated human T cells for immune reactions. Full activation of primary human T cells triggered induction of LAT1 expression. Attenuation of the LAT1 function by a specific inhibitor in human T cells suppressed uptake of essential amino acids and immunological reactions. We also uncovered a previously unknown mechanism by which human T cells put a brake on the cellular metabolism when LAT1 function is disturbed. Our results indicate that LAT1 is employed not only by cancer cells but also by activated T cells, probably because LAT1 has a remarkable ability in the rapid uptake of a large amount of amino acids in metabolically active cells. We also propose the possibility for application of an LAT1 inhibitor as a new drug for therapy of immune diseases. No COI.

S 3

The KLHL proteins, the WNKs, and renal Na^+ transport

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Pseudohypoaldosteronism type II (PHAII) is a hereditary disease characterized by salt-sensitive hypertension, hyperkalemia and thiazide sensitivity. Mutations in with-no-lysine kinase 1 (WNK1) and WNK4 genes were reported to cause PHAII in 2001. Since then, we have identified and characterized novel WNK kinase signaling regulating renal salt handling and vascular tonus. WNK kinases constitute a signaling cascade with oxidative stress-responsive gene 1 (OSR1), Ste20-related proline-alanine-rich kinase (SPAK) and the solute carrier family 12a (SLC12a) transporter, including thiazide-sensitive NaCl cotransporter (NCC). However, although it was clear that the abnormal activation of this signaling cascade is the molecular basis of PHAII, the pathogenic effect of WNK4 mutations in PHAII and the regulatory mechanisms of WNK signaling by various hormonal and dietary factors were poorly understood. Recently, two additional genes, Kelch-like 3 (KLHL3) and Cullin 3 (CUL3), were identified as responsible for PHAII. We and others found that KLHL3 forms an E3-ligase with Cullin3 which ubiquitinates WNK kinases. In PHAII, the disease-causing mutations of WNK4 and KLHL3 affect their interaction, thereby decreasing ubiquitination of WNK4 and leading to the increased levels of WNK4, which strongly activates NCC. We also discuss the involvement of this novel mechanism of WNK regulation by ubiquitination in various pathophysiological situations other than PHAII. No COI.

S 3

IRBIT-mediated synergism in epithelial transport

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Epithelial fluid and HCO_3^- secretion is mediated by basolateral HCO_3^- entry mediated by the $2Na^+$-$1HCO_3^-$ cotransporter NBCe1-B, and luminal HCO_3^- exit mediated by the concerted activities of the Cl^- channel CFTR and the $1Cl^-/2HCO_3^-$ exchanger Slc26a6. Hence, a key step in fluid and HCO_3^- secretion is luminal Cl^- efflux that fuels the Cl^-/HCO_3^- exchange. The two prominent luminal Cl^- channels are CFTR and Ca^{2+}-activated Cl^- channel (CaCC). In secretory epithelia the Ca^{2+} and cAMP signaling synergize to regulate their major function of protein and fluid and electrolyte secretion. In fact, hormonal synergism has been known for more than 80 years, yet we do not know much about the molecular mechanism of synergism. This began to change with the finding of the role of IRBIT (IP3 receptors binding protein released with IP3) in secretory ducts fluid and HCO_3^- secretion. In this presentation, the molecular mechanism of synergism in epithelial transport will be discussed in the context of regulatory processes of Cl^- channel and transporters located in apical membrane of ducts by IRBIT, focused on the IP3 receptor phosphorylation and microdomains. No COI.

S 3

WNK1-MEDIATED REGULATION OF CFTR

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Human pancreas secretes pancreatic juice which contains as much as 140 mM bicarbonate (HCO_3^-). Recently, we have shown that $[Cl^-]$i-sensitive activation of WNK1-OSR1/SPAK pathway plays a critical role in pancreatic HCO_3^- secretion by increasing the bicarbonate permeability ($PHCO_3/PCl$) of CFTR1). However, how $[Cl^-]$i-sensitive kinases modulate $PHCO_3/PCl$ of CFTR remains elusive. In the present study, we investigated molecular mechanisms that underlie the WNK1-OSR1/SPAK-induced regulation of CFTR anion selectivity. Overexpression and knockdown of each kinase in HEK 293 and epithelial cells revealed that WNK1 is the key molecule that governs overall effect of $[Cl^-]$i-sensitive kinases on the CFTR bicarbonate permeability. Furthermore, experiments with truncated WNK1 indicated that N-terminal parts of WNK1 are required to regulate $PHCO_3/PCl$ of CFTR. Interestingly, WNK1 affects permeability of other anions as well as bicarbonate in patch clamp recordings. Especially, the interval of relative permeabilities (Px/PCl) between each anion was greatly narrowed by WNK1. These findings suggest that WNK1 increases the bicarbonate permeability of CFTR by modulating the polarizability of anion selectivity filter and provide insight into the fundamental question of how ion selectivity of anion channels can be regulated by cytosolic signaling at the molecular level. No COI.

S 4

How are zinc-requiring enzymes activated by zinc transporters in the early secretory pathway?

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Zinc homeostasis in cells is tightly controlled within narrow boundaries through the highly integrated processes of zinc uptake, sequestration and efflux across the cell membrane. Thus, zinc transport proteins are essential to these processes. In general, two SLC transporters, Zn transporter (ZnT) and Zrt, Irt-like protein (ZIP), primarily regulate zinc transport, which enable a variety of zinc-dependent proteins and enzymes to play pivotal roles in numerous and varied biological responses. Of these enzymes, zinc-dependent ectoenzymes receive much attention, because defects of their activities are involved in diseases pathogenesis. Zinc-dependent ectoenzymes are thought to be activated via coordination of zinc during the secretory process, and thus zinc transporters localized to the secretory pathway are indispensable for their activation. However, very little molecular information is available. We have shown that ZnT5-ZnT6 heterodimers and ZnT7 homodimers supply zinc into the early secretory pathway, and activate tissue non-specific alkaline phosphatase (TNAP) in an elaborate two-step mechanism via enzyme protein stabilization followed by enzyme conversion from the apo- to the holo-form. Moreover, we have shown that cooperative functions of ZnT1, ZnT4 and metallothionein (MT) contribute to the activation process of TNAP by regulating cytosolic zinc transfer to ZnT5-ZnT6 and ZnT7 complexes. Here, I will discuss the possibility that cytosolic or luminal zinc chaperone proteins may operate in this enzyme activation process, which will provide insights into the molecular mechanism of the activation of many zinc-requiring enzymes in the cells. No COI.

S 4

Possible role for zinc transporter 3 (ZnT3) in brain developmental disorders

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Zinc deficiency may cause abnormal brain development (Sanstead et al., *J Nutr*, 2000). However, it is unknown whether zinc transporter 3, a protein necessary for the development of synaptic zinc, has a role in brain development. In the present study, we examined whether ZnT3 null mice exhibit abnormalities in the brain development, especially those associated with autism. We examined social behavior, brain size, cytoarchitecture, matrix metalloproteinases (MMP) activities, brain-derived neurotrophic factor (BDNF) expression, and possible relationships among these in Zinc transporter 3 (ZnT3) null mice. At 4-5 weeks of age, compared with wild-type (WT) control mice, male ZnT3 null mice, but not females ones, exhibited autistic behaviors in 3-chamber sociability and social novelty tests as well as marble burying and open field tests. At 5 weeks of age, the size of the frontoparietal cortex and neurite density of ZnT3 null mice, were significantly greater than that of WT mice. Consistent with enhanced neurotrophic stimuli in ZnT3 null mice, the level of BDNF and TrkB in neurons and astrocytes was increased in male ZnT3 null mouse brains. Moreover, activities of matrix MMP 2 and MMP 9 were also increased. Consistent with the role for MMP in BDNF upregulation, megalencephaly, and, autism phenotype, treatment with minocycline, an MMP inhibitor, for 2.5 weeks, significantly attenuated all the above changes seen in ZnT3 null mice. Contrary to our expectation that the ZnT3 null state would reduce free zinc levels, it paradoxically increased free zinc levels in brain cells, which might cause increases in MMP activities and BDNF levels, and induce megalencephaly in male mice. Hence, neuronal zinc dyshomeostasis, rather than synaptic zinc deficiency, during the critical period of brain development, may be an underlying mechanism for the present phenomenon in zinc transporter 3 null mice. No COI.

S 5

Properties and function of the Endolysosomal Two pore channels

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In addition to the ER, acidic organelles such as lysosomes and endosomes store and release Ca^{2+}. Endolysosomal Ca^{2+} homeostasis has several cellular functions; among them are apoptosis, trafficking, energy metabolism and fusion/fission events. The messenger NAADP appears particularly important in mobilizing acidic Ca^{2+} stores and in many cases NAADP-evoked signals are amplified by Ca^{2+} channels on the ER. The molecular basis for triggering of Ca^{2+} release from acidic organelles by NAADP however is unclear. Two endolysosomal channels have been implicated in NAADP-mediated Ca^{2+} release, the two pore channels (TPCs) TPC1 and TPC2. However, properties and function of the TPCs in NAADP-mediated Ca^{2+} release has been controversial and are not fully understood. This presentation will discuss properties of the TPCs channels and their role in the response to NAADP. Both TPC1 and TPC2 are similarly activated by the organellar-enriched lipid $PI(3,5)P_2$. Endolysosomal proteins that interact with $PI(3,5)P_2$ or affect its availability affect channel activity. Both channels are also activated by NAADP. In the case of TPC1 activation by NAADP and $PI(3,5)P_2$ appears to be complementary. Interestingly, although activation of the channels by NAADP does not show any desensitization as was concluded from indirect studies, the concentration dependence for activation of TPC1, but not of TPC2, by NAADP followed a bell-shaped curve that is described well by interaction of NAADP with high affinity activatory site and low affinity inhibitory site. TPC2, but not TPC1, acts as a Mg^{2+} sensor and its activity is modulated by changes in intracellular Mg^{2+} at the physiological range. These findings provide basic information of the properties of TPC1 and TPC2 that should facilitate understanding of their physiological role. No COI.

S 5

Alterations of lysosomal homeostasis in TRPML1 knock-out mice

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In addition to Ca^{2+} stored in the ER, Ca^{2+} storage and release from acidic intracellular organelles affect the overall Ca^{2+} signal and membrane trafficking. TRPML1 is expressed in late endosomes and lysosomes. Mutations in TRPML1 cause mucolipidosis type IV(MLIV) that is characterized by a psychomotor retardation, corneal opacity, retinal degeneration, and achlorhydia. In vitro studies of constitutive membrane trafficking concluded that TRPML1 plays a role in delivery or fusion of late endosomes and lysosomes, resulting in accumulation of material in the lysosomes and induction of autophagy. However, the role of TRPML1 in regulated exocytosis is not known. In the present study, we found enhanced amylase secretion in response to high agonist stimulation, even though there are no apparent effect on saliva fluid secretion. Interestingly, TEM images both of pancreas and parotid gland show enlarged vesicles. Some of the enlarged vesicle shows fusion of the lysosome and vesicles. Moreover, acid phosphatase activity was increased in the whole saliva and pancreatic fluids, and also Trpml1-/- neurons maintain high basal and stimulated exocytosis of the neurotransmitter and neurotoxin glutamate. These features are unique to MLIV and were not observed in another lysosomal storage disease, Niemann-Pick type C1. From these results, we suggest that $Trpml1^{-/-}$ deletion may relate with exocytosis of the secreting vesicles or related with fusion of the vesicles with the lysosome. This would be useful to show new function of $Trpml1^{-/-}$ in secreting cells. No COI.

S 5

Vesicular CLC chloride-proton exchangers: Roles in physiology and disease

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The CLC family of anion transporters comprises both plasma membrane Cl^- channels and vesicular $2Cl^-/H^+$-exchangers that are differentially expressed along the endosomal-lysosomal pathway. Their physiological and medical importance became apparent from human genetic disease and mouse models. ClC-5 is expressed on endosomes, mainly in epithelia. Its mutation in human Dent's disease leads to proteinuria and kidney stones. Our KO mouse model revealed that ClC-5 is important for proximal tubular endocytosis. It disruption leads to hypercalciuria and kidney stones because of defective endocytosis and processing of calciotropic hormones. ClC-7, together with its beta-subunit Ostm1, resides on lysosomes. Disruption of either subunit leads to lysosomal storage, neurodegeneration and osteopetrosis in mice and men. ClC-4 mutations lead to human mental retardation, while disruption of ClC-3 and ClC-6 in mice entail neurodegeneration. The role of these transporters in endosomal/lysosomal function was previously attributed exclusively to impaired vesicular acidification, as these transporters may provide a shunt for the vesicular proton ATPase. While a role of ClC-5 in endosomal acidification has been ascertained, the lysosomal pH of ClC-7 KO mouse is, however, unchanged owing to a parallel cation conductance. We were puzzled by the fact that the vesicular CLCs are $2Cl^-/H^+$-exchangers rather than Cl^- channels. Both are suited, in principle, as shunts for proton pumping. We asked whether chloride/proton exchange is essential for their function and converted ClC-3, ClC-5 and ClC-7 into pure chloride conductances in KI mice. This is possible by single point mutations. Surprisingly, these mice revealed that these mice have almost identical phenotypes as the respective KO mice, suggesting an important role for H^+-exchange dependent vesicular Cl^- accumulation or changes in vesicular voltage. Another ClC-7 mouse model, in which we disrupted its ion transport totally without affecting the expression of the protein, furthermore indicated that the loss of protein-protein interactions explains some aspects of the ClC-7 KO mouse. No COI.

S 6

Vitamin D supplementation and sunlight exposure for the prevention of osteoporosis

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Dermal synthesis of vitamin D after sun exposure is a major source of vitamin D in humans. Recent studies have documented high prevalence of vitamin D deficiency even in tropical countries with abundant sunshine. It is likely that lifestyle and social factors may preclude adequate outdoor sun exposure and bringing about inadequate vitamin D status, especially in urban residents. In addition, air pollution may have a contributory role. Tropospheric ozone is a common urban air pollutant and an efficient absorber of UVB. The phenomenon is likely to be more marked in big cities, and may partially explain the high prevalence of vitamin D deficiency in these areas. Because routine measurement of 25-hydroxyvitamin D [25(OH)D] is not recommended, suggestion on vitamin D intake is provided for achieving desirable 25(OH)D levels (≥ 20 ng/mL in general) in over 97.5% of the population. Available recommendations vary from 200 IU/day to 2,000 IU/day. In the elderly with osteoporosis, higher 25(OH)D levels are warranted to improve muscle function and reduce fracture risk. Adequate vitamin D status in this particular population can achieved by at least 800 IU/day of vitamin D intake. Recommending increased intake of naturally occurring vitamin D-rich food is the ineffective strategy to overcome vitamin D deficiency since there are few food sources that are rich in vitamin D. Frequently, dermal synthesis of vitamin D is unpredictable. Race, calcium intake, renal function, body mass index and polymorphisms in key protein/enzymes involved in the vitamin D metabolism and action can all influence the variability in the increment of 25(OH)D levels after vitamin D supplementation. No COI.

S 6

Periodontal stem cells: molecular aspect and application

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Periodontal ligament (PDL) cells and tissue, located between tooth and alveolar bone, plays roles in tissue connection, distribute and withstand the masticatory force and help maintaining the homeostasis of the periodontal tissue. PDL cells possess the mesenchymal stem cell characteristics. They express several mesenchymal stem cell markers such as CD44, CD73, CD90 and CD105 as well as the embryonic stem cell markers including Rex-1, Oct4 and nanog. Under appropriate condition, PDL cells can differentiate into osteogenic, adipogenic and neurogenic lineages, suggesting the role of PDL cells in periodontal tissue repair and regeneration. Our previous results demonstrated the involvement of Notch signalling pathway in the modulation of differentiation fate of PDL cells in vitro. Moreover, we also found that PDL cells could also be induced to differentiate into insulin secreting cells under the influence of Notch signalling pathway. This ability of PDL cells suggests the high plasticity of PDL cells that might be suitable for stem cell therapy. Beside the role in repair and regeneration, PDL cells could also modulate the immune system through secret interferon gamma and IDO when activating with inflammatory cytokines such as IL-12 or IL-6, indicating the immunosuppressive property of PDL cells. Taken together, PDL cells are one of the potential sources of stem cell for stem cell therapy and tissue engineering. These works were supported by the Research Chair Grant, National Science and Technology Development Agency, Thailand. No COI.

S 6

Advance in pathophysiology of diabetes mellitus-induced osteoporosis

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How diabetes mellitus (DM) affects bone metabolism remains controversial for decades. It was previously believed that type 1 DM led to osteopenia, whereas type 2 DM was associated with increased bone density, suggesting that it might be protective against fragility fracture. However, several recent lines of evidence in both humans and rodents did not support the positive effect of type 2 DM on bone. Both type 1 and 2 DM indeed induce aberrant bone cell function (cellular complication) and abnormal extracellular matrix properties (matrix complication), thereby leading to impaired osteoblast-mediated bone formation, accelerated osteoclast-mediated bone resorption, and poor bone quality. Cellular complications are often caused by insulin resistance and hyperglycemia, while matrix complications, including abnormal structure and alignment of collagen, predominantly result from advanced glycation end products (AGEs). The DM-associated inflammation and pro-inflammatory cytokine release as well as impaired intestinal calcium absorption may also aggravate osteoporosis in diabetic patients, but their underlying mechanisms remain unknown. No COI.

S 7

Myofilament Length Dependent Activation and the Frank-Starling Law of the Heart

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The cellular basis of the Frank-Starling mechanism is sarcomere length (SL) modulation of myofilament Ca2+ sensitivity (LDA). The mechanisms that underlie LDA are unknown, but recent evidence has implicated the giant protein titin as a possible sarcomeric strain sensor. The aim our study was to elucidate the impact of SL on LDA and sarcomere structure in isolated rat myocardium from either wild-type or mutant rats expressing a giant splice isoform of titin. At stretch, wild-type muscles showed reduced increase in passive tension and twitch force, and blunted LDA. Time-resolved small angle x-ray diffraction of intact twitching muscles during diastole revealed at stretch a significant increase in intensity and spacing of myosin M2, troponin T3, and myosin binding protein C C2. These SL dependent changes in sarcomere structure were absent in titin mutant muscles. Cross-bridge radial spacing was significantly reduced upon stretch in wild-type, but not mutant muscles. Equatorial spacings and intensity ratios were similar in both groups of muscles. Electron density reconstruction revealed, only in wild-type, increased mass in both thick and thin filament, and the appearance of an as of yet unidentified moiety spanning the space between the thick and thin filaments at stretch. These results were independently confirmed in skinned myocyte fragments using a fluorescent probe. We conclude that stretch induces structural changes in both thick and thin filaments mediated by titin strain. Moreover, MyoBPC may interact with actin to mediate LDA.

S 7

Disrupt the muscle clock and alter metabolism and fiber type

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Skeletal muscle is a major contributor to whole-body metabolism as it serves as a depot for both glucose and amino acids, and is a highly metabolically active tissue. Within skeletal muscle exists an intrinsic molecular clock mechanism that regulates the timing of physiological processes. A key function of the clock is to regulate the timing of metabolic processes to anticipate time of day changes in environmental conditions. We developed an inducible skeletal muscle-specific Bmal1 knockout mouse model (iMS-Bmal1$^{-/-}$) to test loss of clock function only in skeletal muscle. The results of this study show that skeletal muscle circadian transcriptome was highly enriched for metabolic processes and phase analysis revealed a temporal separation of genes involved in substrate utilization and storage over a 24-h period. Muscle specific loss of Bmal1 resulted in a significant change in many metabolic genes. This was associated with decreased glucose uptake, decreased expression of rate limiting enzymes in glycolysis and subsequent loss of body fat. In addition we also observed a gene signature indicative of a fast to slow fiber-type shift and a more oxidative skeletal muscle in the iMS-Bmal1$^{-/-}$ model. These data provide evidence that the intrinsic molecular clock in skeletal muscle temporally regulates genes involved in the utilization and storage of substrates independent of feeding and cage activity. Disruption of this mechanism caused by phase shifts (that is, jetlag) or night eating may ultimately diminish skeletal muscle's ability to efficiently maintain metabolic homeostasis over a 24-h period. No COI.

S 7

Cardiac contractile activation: sex matters & exercise helps

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Heart failure is the common outcome of many cardiovascular diseases. Despite the many known risk factors of the disease, the underlying mechanisms are incompletely understood and the therapeutic strategies are still on trials and comparisons. A better survival from advanced heart failure in women than in men suggests a sex-specific adaptation of the heart to stress. The rationale of our studies has been based on the well recognizable sex differences in the incidence of cardiovascular diseases which suggest that female sex hormones may exert a protective adaptation but male sex hormones may add a risky effect. We aim to understand the nature, sites, and mechanisms of how sex hormones regulate cardiac muscle physiology especially the cardiac adaptations induced after hormone deprivation or exogenous loading. Our results show that sex matters on cardiac contractile activation through differential responses of both the activity of myofilaments and the handling of intracellular calcium. Significantly, in sex hormone-deprived rat models after gonadectomy regular exercise is able to serve as an alternative to hormone replacement therapy in preventing cardiac contractile dysfunction. No COI.

S 8

Significance of central aortic pressure and arterial stiffness in the assessment of arterial function

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In the circulatory system, large arteries function as conduits for the distribution of blood and cushions to dampen the pulsatile effects of ventricular ejection. Change in dimensions and mechanical properties along the length of the aortic trunk and peripheral branches affect the propagation of the pressure pulse generated in the ascending aorta, such that the pressure pulse increases in amplitude between the central aorta and peripheral sites. Mathematical representation of the transmission characteristics has enabled central aortic pressure to be derived non-invasively from conventional cuff sphygmomanometric measurements and the peripheral pulse. These techniques have shown that central aortic pressure has the potential to discriminate the effects of pharmacological agents that change blood pressure and heart rate on cardiac function. Wave reflection indices determined from the central aortic pressure wave have also been shown to have predictive power for cardiovascular risk. Arterial stiffness is a fundamental parameter that characterizes wave propagation phenomena. Arterial stiffness, as measured non-invasively by aortic pulse wave velocity is a significant predictor of cardiovascular risk and is a major determinant of increased systolic pressure in the elderly. Age-related changes in arterial stiffness occurring predominantly in the central aorta and large arteries have the effect of reducing the arterial elastic non-uniformity between central and peripheral arteries such that the difference between central and peripheral pulse pressure becomes less with age. Non-invasive measurements of central aortic pressure and arterial stiffness have the potential for improved characterization of arterial function and better stratification of cardiovascular risk. No COI.

S 8

Optical sensors for the assessment of vascular biomechanics

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In order to further understand the contribution of venous and arterial effects blood volume to the photoplethysmographic (PPG) signal, recordings were made from twenty healthy volunteer subjects during an exercise in which the right hand was raised and lowered with reference to heart level. Red (R) and infrared (IR) PPG signals were obtained from the right index finger using a custom-made PPG processing system. Laser Doppler flowmetry (LDF) signals were also recorded from an adjacent fingertip. The signals were compared with simultaneous PPG signals obtained from the left index finger. On lowering the hand to 50 cm below heart level, both ac and dc PPG amplitudes from the finger decreased (e.g. 18.70% and 63.15% decrease in infrared dc and ac signals respectively). The decrease in dc amplitude most likely corresponded to increased venous volume, while the decrease in ac PPG amplitude was due to autoregulatory adjustments on the arterial side in response to venous distension. Conversely, ac and dc PPG amplitudes increased on raising the arm above heart level. Morphological changes in the ac PPG signal are thought to be due to vascular resistance changes, predominately venous, as the hand position is changed. No COI.

S 8

A novel method for the assessment of vascular endothelial function

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The gold standard for the non-invasive assessment of endothelial function remains the ultrasonic detection of flow mediated vasodilation (USFMD) but technical difficulties confine this approach to specialist centres, so there is need of a simple and robust method suitable for patient screening. Naka et al. (European Heart J., 2006. 27,302-309.) have shown that the increase in radial artery pulse wave velocity (PWV) following release of an occluding cuff is due to relaxation of vascular smooth muscle and have validated this against USFMD. However, current PWV measurements are technically demanding and prone to movement artefacts. To minimise these problems we have designed a pulse transit-time measurement system using clip-on photoplethysmographic finger probes which detect the pulse arrival time difference (ΔPAT) at the ring finger of each hand. As a preliminary validation we have compared brachial-radial PWV changes during hyperaemic flow with simultaneously acquired ΔPAT values in 48 healthy volunteers and found a significant correlation between the two methods in the maximum hyperaemic response and the time taken to return to baseline. Secondly, we have compared ΔPAT with USFMD in 28 subjects with reasonable agreement in maximum hyperaemic response, although much scatter in the USFMD results. We are currently setting up a more rigorous validation at a centre specialising in USFMD measurements in which subjects will be infused with L-NMMA (a NO synthase blocker) and its inhibitory effect will be measured, assessing if the changes in ΔPAT are endothelially dependent. Ideally measurements of PWV and endothelial function should be non-contact and, to this end preliminary measurements of pulse transit time in the arm using a fast infra-red camera will also be described. No COI.

S 9

Immunity and sympathetic nervous system in hypertension

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The central nervous system determines sympathetic outflow and influenced by various peripheral inputs that are neural, humoral, and immune-inflammatory changes. Recently, we found that inflammatory changes reflected by nuclear factor kappa B in the brain are increased in heart failure associated with sympathoexcitation. We found that toll-like receptor 4 (TLR4) and its adaptor protein, myeloid differentiation primary response protein 88 (MyD88), expression levels are increased in heart failure. Central administration of an AT1R blocker attenuated them. These findings suggest that TLR4/MyD88 pathway as innate immunity is activated in heart failure thereby causing inflammatory responses leading to sympathoexcitation. Activation of TLR4 in the brain in addition to vasculatures has been shown in spontaneously hypertensive rats as well as angiotensin II-induced hypertensive rats from other laboratories. Interestingly, activation of this pathway is involved in cardiac hypertrophy process. Finally, we demonstrated that a decrease in regulatory T cells (CD4+CD8+Foxp3+ cells) proportion is crucial for the development of hypertension and cardiac hypertrophy in genetic hypertensive rats, which is involved in sympathetic neural input to the spleen. Taken together, innate and acquired immunity is closely communicated with the sympathetic nervous system via inflammatory response and play an important role in the pathogenesis of hypertension. No COI.

S 9

Visualizing baroreflex dysfunction by MRI/DTI in animal models

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The baroreflex represents the most fundamental mechanism of cardiovascular regulation, and is responsible for maintaining stable blood pressure and heart rate. Impairment of baroreflex inevitably results in a hypertensive or hypotensive state, and death in extreme case. Based on tractographic evaluations using magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) of the medulla oblongata, our group has successfully visualized baroreflex at work under normal and disease conditions. We found that the connectivity between the nucleus tractus solitarii (NTS) and nucleus ambiguus (NA) is disrupted in an experimental model of neurogenic hypertension, concurrent with impairment of the cardiac vagal baroreflex as detected by radiotelemetry. We further found that the disrupted NTS-NA connectivity is reversible, and is related to oxidative stress induced by augmented levels of NADPH oxidase-generated superoxide in the NTS. In an experimental model of hepatic encephalopathy, we found that the progressive hypotension and loss of baroreflex-mediated sympathetic vasomotor tone indicative of brain death was accompanied by an irreversible loss of the connectivity between NTS and rostral ventrolateral medulla. Intriguingly, the loss of connectivity between NTS and NA that accompanied defunct cardiac vagal baroreflex and bradycardia only occurred before cardiac death. We conclude that tractographic analysis is a new research tool for functional examination of changes associated with dynamic alterations in the connectivity between key neural substrates in the baroreflex circuitry. Our results further suggested that whether the disrupted connectivity is reversible determines whether such changes are pathophysiological or pathological and hence treatable or untreatable. No COI.

S 9

Blood pressure variability in diabetes : from the pig to humans

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Dysfunction of the autonomic nervous system is a common complication in diabetes mellitus. Cardiovascular autonomic neuropathy (CAN) may carry an increased risk of mortality. A model of experimental diabetes could be useful to evaluate. Yucatan miniature pigs were equipped with an arterial catheter for telemetric blood pressure (BP) analysis, and with a venous access. BP and heart rate (HR) oscillated at the respiratory range. Spectral analysis showed this respiratory component was the main determinant of the short-term variability of BP and HR. Atropine increased HR and BP and abolished the respiratory sinus arrhythmia. Propranolol diminished HR and the respiratory peak of HR. Baroreceptor-HR reflex was estimated using injections of phenylephrine and nitroprusside, and by cross-spectral analysis between BP and HR. The data in diabetic pigs demonstrate the dual (vagal and sympathetic) control of HR and the dominant role of respiration in the genesis of oscillations. The spectral and cross-spectral analysis of BP and HR were altered after 3 months of diabetes. The aim of the second study was to estimate the accuracy of a battery of BP and HR variability indexes obtained in different subgroups of diabetic subjects classified according to the conventional laboratory autonomic function tests (Ewing scores). BP was measured continuously at the finger level using a Finapres monitor in the supine position and during standing. The estimates of baroreceptor-HR function provided a powerful tool for assessing CAN at any stage of CAN including the early stage which was not detected by the conventional tests. No COI.

S 10

Physiology of animal toxins : effects on renal ion transport

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Animal toxins compose of enzymes, polypeptides, proteins are chemicals. Injury induced by animal toxins in majority is through hemodynamic changes leading to renal ischemia and partly through direct toxicity. Hemodynamic changes triggered by toxin and vascular inflammatory mediators are integral to injury and do not differ from those observed in sepsis and are characterized by decreased blood pressure, increased renal and systemic vascular resistance with decreased glomerular filtration rate and renal blood flow. By isolated renal perfusion which reflect direct toxin effects on vascular ion channels renal vascular resistances is either increased or decreased. Fractional excretion of Na is increased. The role of vascular ion channels including DEG/ASIC/ENac, Ca and K channels are considered. Renal tubular ion channels and transporters targeted by animal toxins are reviewed, focusing on Na and K transport. In most cases due to blockage of single channel clinical manifestation related to renal ion channel block may not be apparent. The effects are of only physiologic interest. At the clinical level, scorpion and bee envenoming with blockage of multiple channels or transporters are good models. No COI.

S 10

On the role and mechanism of action by which Russell's viper venom induces acute renal failure

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Russell's viper (Daboia russellii siamensis) envenoming is well known as a cause of an acute kidney injury. The pathophysiological mechanism for the acute effect of Russell's viper venom(RVV) on renal function has not been fully elucidated. The studies in vivo in experimental animals for the mechanisms of venom action on renal functions have been clarified in relation to changes in either extrarenal factors or/and intrarenal factors. Experimental animals injected with crude RVV and a major class of venom fractions especially phospholipase A2 ($PLA_{2)}$ and metalloprotease (MP) show changes in the cardiovascular system and renal hemodynamics. The initial drops in blood pressure and bradycardia are consistent with a tendency to increased total peripheral resistance and renal vascular resistance and restoration of blood pressure following these transient decreass are compatible with vasoconstrictor hormones stimulation as a compensatory mechanism. An increase in renal vascular resistance and decreases in renal blood flow and glomerular filtration rate are still apparent after envenomation. An in vivo study is complicated by the presence of proinflammatory cytokines and vasoactive mediators from the host during envenomation. *In vitro* studies can be evaluated the direct effect of venom action on changes in renal physiology without the influence of extra renal factors. A number of studies for the direct action of RVV have been performed e.g. in the isolated perfused kidney, changes in the characteristic polarization of the renal tubular cell membrane, changes in mitochondria activity and changes in Na,K-ATPase activity of the renal tissues after envenomation. Accumulating evidence demonstrates that a major class of enzymatic activities of venom fractions for PLA_2 and MP perform main functions as a cause of an acute kidney injury, but their precise roles and mechanisms of action on renal hemodynamic and renal tissues have to further elucidate. The true effects of, PLA_2 and MP should be evaluated by isolated renal perfusion study which will allow insight into how RVV are involved in the pathophysiology of acute renal failure. The recent studies in isolated perfused kidney will be described on how crude venom and its fractions act directly on the kidney function without extra-renal factors. The possible ion channel inhibitors/blockers are used to block some of the renal effects caused by the RVV venom and its fractions in isolated perfused kidney. No COI.

S 10

Microvascular changes associated to LPS and possible treatments

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Among various indications of intensive care unit hospitalization, sepsis is highlighted by its high incidence, morbidity, mortality, and cost to healthcare system. In sepsis syndrome, inflammatory response is associated with a state of oxidative stress that results in impairment of microcirculatory function. Several factors are related to this impairment, such as systemic hypotension, vasoconstriction, stiffness of red blood cells, increased leukocyte-endothelium interactions (adhesion and aggregation), and platelet/fibrin clot formation (leading to microthrombosis). Ischemia, impaired tissue perfusion, organ dysfunction, and death may occur depending on the severity and duration of microcirculatory dysfunction.Accordingly, drugs that assist in the reversal of microcirculatory changes could be decisive in sepsis treatment. Due to limitations involving the study of these drugs in human subjects, the development and evaluation of new treatments requires the use of reliable experimental models. Although no model is capable of reproducing in its entirety the complexity of sepsis in humans, LPS-induced endotoxemia is a well-established experimental model that reproduces many of the clinical features of sepsis syndrome and allows homogeneous and reproducible *in vivo* studies of the microvascular function. In fact, using this model, we have already tested several drugs with microvascular action, such as inotropics, sedatives, and anticoagulants, achieving promising results.Thus, the aim of my lecture is to present the microvascular changes associated with the LPS-induced endotoxemia model and our experience with possible treatments. No COI.

S 11

Flow-mediated the activation of integrin α5 requires its translocation to membrane lipid rafts in vascular endothelial cells

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Focal distribution of atherosclerotic lesions reflects a significant role of local hemodynamic forces in atherogenesis. Sensing mechanical forces, membrane lipid rafts are crucial for maintaining endothelial function. To investigate whether lipid rafts mediate the effects of different shear stress on endothelial cells (ECs), we compared translocation of proteins in lipid rafts under laminar (LSS) and oscillatory shear stress (OSS), and further investigated the contribution of these proteins in the development of atherosclerosis. After isolation of lipid rafts with sucrose density gradient centrifugation, quantitative proteomics and bioinformatics analysis revealed that more than 100 proteins redistributed in lipid rafts under different flows, among which integrin α5 was significantly elevated in the lipid rafts of ECs exposed to atheroprone OSS (4+/-0.5 dyne/cm2 for 2h) than atheroprotective LSS (12+/-0.5 dyne/cm2 for 2h). Moreover, western blot showed that OSS increased integrin α5 activity. Both knockdown caveolin-1 and disruption of cytoskeleton prevented disturbed flow-induced integrin α5 translocation, suggesting that integrin α5 translocated depending on caveolin-1 and cytoskeleton. Further, OSS activated integrin α5 could induce NLRP3 inflammasome in ECs, which, in turn, caused endothelial activation. In vivo, integrin α5 activation, ICAM-1 and VCAM-1 expression were observed in the atheroprone aortic areas and in partially ligated carotid arteries of $LDLR^{-/-}$ mice. As well, interference by shRNA adenovirus of integrin α5 greatly retarded the EC activation at OSS zones of partially $LDLR^{-/-}$ mice. In conclusion, proteins translocation in lipid rafts causally contributes to distinct features of ECs under different flows. Atheroprone flow induces integrin α5 activation through lipid rafts anchoring and activates NLRP3 inflammasome in ECs, which revealed a novel mechanism for endothelial activation. No COI.

S 11

Mechanical regulation of histone deacetylases and microRNAs in vascular endothelial pathophysiology in response to disturbed flow

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In atherosclerotic lesions, synthetic smooth muscle cells (sSMCs) induce aberrant microRNA (miR) profiles in endothelial cells (ECs) under flow stagnation. Increase in shear stress induces favorable miR modulation to mitigate sSMC-induced inflammation. We addressed the role of miRs in sSMC-induced EC inflammation and its inhibition by shear stress. Coculturing ECs with sSMCs under static condition causes transient increases of 4 anti-inflammatory miRs (146a/708/451/98) in ECs. Shear stress (12 dynes/cm(2)) to cocultured ECs for 24 hours augments these 4 miR expressions. In vivo, these 4 miRs are highly expressed in neointimal ECs in injured arteries under physiological levels of flow, but not expressed under flow stagnation. Silencing either Nrf-2 or miR-146a led to increased neointima formation of injured rat carotid artery under physiological levels of flow. Overexpressing miR-146a inhibits neointima formation of rat or mouse carotid artery induced by injury or flow cessation. In addition, application of oscillatory shear stress (OSS, 0.5 ± 4 dynes/cm(2)) to cultured ECs sustainably up-regulated class I and II HDACs class I and II histone deacetylases (HDAC-1/2/3 and HDAC-5/7, respectively) and their nuclear accumulation, whereas pulsatile shear stress (PSS, 12 ± 4 dynes/cm(2)) induced phosphorylation-dependent nuclear export of class II HDACs. Intraperitoneal administration of the class I-specific HDAC inhibitor valproic acid into bromodeoxyuridine (BrdU)-infused rats inhibited the increased EC uptake of BrdU at poststenotic sites. Our findings demonstrate the important roles of different groups of HDACs and miRs in regulating the oxidative, inflammatory, and proliferative responses of ECs to different patterns of flow. No COI.

S 11

Shear Regulation of MicroRNA Transportation and Targeting in Vascular Homeostasis

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The local flow patterns acting on the arterial wall play a crucial role in determining whether the endothelium is protected from or predisposed to atherosclerosis. Recent research demonstrated that microRNAs (miRs) originated from vascular endothelial cells (ECs) mediate extracellular communication via transportation to the underlying smooth muscle cells (SMCs). In this study, miR profiling was performed both in ECs in response to oscillatory shear (OS, the main feature of atheroprone flow) vs. pulsatile shear (PS, the main features of atheroprotective flow) and in the perfusates. The expressions of endothelial-enriched miR-126 inside the cells were quite stable between OS and PS; however, the levels of miR-126 in the perfusates differ. Coculture of SMCs with ECs or treatment of SMCs with conditioned media from static ECs (EC-CM) increased SMC miR-126 level and SMC turnover; these effects were abolished by inhibition of endo-miR-126 and by the application of PS to ECs. RNA sequencing assays in sheared ECs indicated that the expressions of Soluble NSF Attachment Protein Receptor (SNARE)-associated proteins were differentially regulated by PS vs. OS. SMC miR-126 did not increase when cocultured with ECs subjected to inhibition of exocytosis/SNAREs. Endo-miR-126 represses its target mRNAs in the cocultured SMCs, indicating the signaling and functional roles of the transmitted endo-miRs. Systemic depletion of miR-126 in mice or pharmaceutical inhibition of endocytosis in the arterial smooth muscle inhibited neointimal lesion formation of carotid arteries induced by interference of blood flow. Our study suggests that atheroprotective and atheroprone flows modulate distinct miR transportation and targeting to result in beneficial or detrimental outcomes for the vasculature. No COI.

S 12

Recent Advances in Viral Hepatitis B

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Hepatitis B virus (HBV) infection is a major public health problem, with more than 350 million carriers estimated worldwide. HBV, a member of the family Hepadnaviridae, is a relaxed circular double-stranded DNA virus that shows remarkable genetic variability. Chronic HBV infection is associated with a diverse clinical spectrum of liver disease ranging from asymptomatic carriers, chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Current data have suggested that host and viral factors may have important influences on the clinical outcome and treatment response. Universal vaccination against HBV leads to a marked decrease in the incidence of HCC. In addition, reduction of HBV-associated complications can be achieved by antiviral therapy, which includes nucleoside/nucleotide analogues (NA) and pegylated interferon-alpha (PEG-IFN). However, current therapies have some limitations and HBV cure is rarely achieved. NA therapy is generally well tolerated but prolonged or indefinite duration of treatment is needed. In contrast, PEG-IFN therapy is accompanied by a higher rate of sustained response but its use is compromised by frequent side-effects. Recent efforts have been focused on developing strategies to eliminate the virus and improving the cure rate for infected individuals.

S 13

Epigenetic mechanisms of visceral hypersensitivity in irritable bowel syndrome

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Irritable bowel syndrome (IBS), charactered by recurrent abdominal pain with altered bowel movement in the absence of an overt pathology, is one of the most common functional gastrointestinal disorders. The pathophysiology of visceral pain in IBS remains unknown. Hydrogen sulfide (H2S), a third gaseous modulator/mediator, has become recognized as an important endogenous molecular. H2S is synthesized from L-cysteine primarily via cystathionine-β-synthetase (CBS) and cystathionine-γ-lyase, and has been reported to be involved in nociceptive signaling and inflammation. However, the molecular and epigenetic mechanisms of CBS-H2S signaling in visceral nociceptive processing are not fully understood. Here we showed that neonatal colonic inflammation produced a significant upregulation of CBS expression in dorsal root ganglia (DRG). Intraperitoneal administration of CBS antagonist aminooxyacetic acid (AOAA) attenuated visceral hypersensitivity. In addition, application of AOAA reversed the hyperexcitability and decreased total sodium currents of DRG neurons innervating the colon, and reduced upregulation of voltage-gated sodium channel $Na_V1.7$ and $Na_V1.8$ in DRGs rats with visceral hypersensitivity. Methylation specific PCR and bisulfate sequence analysis demonstrated that promoter region of cbs gene was less methylated in DRG samples from rats with visceral hypersensitivity than that from controls. The expression of thymine DNA glycosylase was significantly upregulated in DRGs from IBS-like rats while the expression of DNA methyltransferases was not greatly altered in DRGs from IBS-like rats. Our results suggest that epigenetic regulation of CBS gene may contribute to the visceral hypersensitivity, thus identifying a potential therapeutic target for the treatment of chronic visceral pain in patients with IBS. No COI.

S 13

Pancreatic dopamine-evoked somatostatin release and blood glucose increase was cAMP-dependent and dopamine receptor 2 mediated

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Pancreas can produce dopamine which has been measured in the pancreatic juice. Dopamine receptor 2 (D2R) is able to regulate the level of blood glucose. But the mechanism is unclear. We have reported that D2Rs were constant expressed in the somatostatin secreting δ cells, but not in the insulin secreting β cells in rat and human islets. We hypothesized that dopamine may regulate insulin secretion through regulating somatostatin release from δ cells by binding with D2Rs. Immunofluorescence, HPLC/MS, radioimmunoassay, ELISA, pancreatic tissue incubation, and islets isolation etc. were employed. The results indicated that dopamine was abundantly existed in the pancreatic tissue, D2Rs and somatostatin receptor 2 (SSTR2) were respectively distributed in the δ and β cells in rat and human islets. In in-vivo study, activating D2Rs increased blood glucose, decreased insulin in serum and pancreatic tissue, and increased pancreatic somatostatin. In the isolated pancreatic tissue and islets, activating D2Rs inhibited insulin secretion, promoted somatostatin secretion and elevated intracellular cAMP level. The D2 agonist-induced inhibition of insulin secretion was blocked by SSTR2 antagonist. This study demonstrates that pancreatic dopamine is able to elicit a somatostatin-dependent inhibition of insulin secretion through activating D2Rs and intracellular cAMP pathway. Key Words: D2 receptors, somatostatin, insulin secretion No COI.

S 13

The inhibitory effect of oxytocin on mast cell degranulation mediated by Ca^{2+}-NOS-NO pathway

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To investigate the effects of OT on visceral hypersensitivity/pain and mast cells degranulation and the underlying mechanisms. The expression of oxytocin receptor (OTR) on mast cells was assessed with immunofluorescence assays and Western blot. The colon perfusion of TNBS was to used to induce visceral hypersensitivity. The extent of visceral hypersensitivity was quantified by abdominal withdrawal reflex (AWR) scores of colorectal distension. Tissue sections were stained by toluidine blue for mast cell counting. The levels of histamine were measured by a commercial ELISA kit. The electrical properties of mast cells were tested by whole-cell patch clamp recording. The intracellular Ca^{2+} was determined by fluorescent microscopy. Nitric oxide (NO) was detected with the fluorescent nitric oxide probe DAF-FM diacetate. OTR was expressed in colonic mast cells in human and rat, as well as in human and mouse mast cell lines, HMC-1 and P815. OT decreased TNBS-induced visceral hypersensitivity, colonic mast cell degranulation and histamine release in rats and attenuated the C48/80-evoked histamine release and inward currents in HMC-1 and P815 cells. Pretreatment of OTR antagonist atosiban significantly reversed the inhibitory effect of OT on TNBS-induced visceral hypersensitivity in rats. Pretreatment of atosiban or L-NMMA significantly attenuated the inhibitory effect of OT on C48/80-evoked histamine release and inward currents. OT produced a concentration-dependent increase in intracellular Ca^{2+} in HMC-1 and P815 cells. OT increased the production of NO in HMC-1 cells. In conclusion, OT might exert the antinociceptive effect on colonic hypersensitivity through inhibition of mast cell degranulation via Ca^{2+}-NOS-NO pathway. No COI.

S 14

Essential role of nuclear receptors in metabolic and fluid regulation

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Nuclear receptors are ligand-activated transcriptional regulators of many physiological and pathophysiological processes. A large body of evidence demonstrates that nuclear receptors control a large variety of metabolic processes including lipid metabolism, adipogenesis, drug disposition, bile acid homeostasis, insulin sensitivity, blood pressure regulation as well as inflammation, fibrosis, cell differentiation, and tumor formation. Among dozens of nuclear receptors, peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs) and farnesoid X receptors (FXRs) have been identified and attracted enormous attention due to the key role these receptors play in metabolic regulation. Dysfunction of these nuclear receptors and genetic variants may contribute to the pathogenesis and progression of the metabolic syndrome, including insulin resistance, glucose intolerance or type 2 diabetes, obesity, dyslipidemia, hypertension, and albuminuria. This places PPARs, LXRs and FXRs into the frontline for novel therapeutic approaches for a broad range of metabolic disorders and diseases. For example, PPAR s (agonists including antidiabetic thiazolidinedione (TZDs) and PPAR clinically proved to be effective for improving insulin resistance and hyperlipidemia, respectively. In addition, it has been recently shown that some of these nuclear receptors are also essential in renal physiology and play important roles in the maintenance of fluid homeostasis. We will discuss the physiological and pathophysiological roles of PPARs, LXRs and FXRs with particular emphasis on the therapeutic potential of their ligands in the metabolic syndrome and disorders associated with water metabolism. No COI.

S 14

PPARγ Integrates energy metabolism, fluid metabolism, and cardiovascular function

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Peroxisome proliferator-activated receptor- (PPAR) is a nuclear hormone receptor and PPARγ is a ligand-activated transcription factor promoting adipogenesis and energy storage, representing a novel target for antidiabetic therapy due to its role in sensitizing the action of insulin. Accumulating evidence shows that PPARγ is profoundly involved in water and sodium metabolism in the kidney. Activation of PPARγ in the kidney promotes Na+ reabsorption and fluid retention by upregulating expression of aquaporin 2 (AQP2) and epithelial sodium channels, two primary targets for vasopressin (AVP) regulation of collecting duct water permeability and sodium reabsorption. Systemic knockout of PPARγ in mice causes polyuria and urine concentrating defect with blunted response to AVP but normal AVP levels. Interestingly, protein expression of AQP2 and phosphorylated-AQP2 in the collecting ducts is unchanged in these mice, indicating a unique pathway independent of AVP by which PPARγ regulates water and sodium transport in the kidney. In addition, emerging evidence suggests that PPARγ in the vascular endothelial cells control adipose-specific capillary permeability. Paradoxically, activation of PPARγ lowers blood pressure likely via suppression of synpathetic activity. Taken together, PPARγ appears to play an important role in integrating energy metabolism, fluid metabolism, and cardiovascular function. No COI.

S 14

miRNAs in Nfat5 signaling, osmoregulation and urine concentration

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Nuclear factor of activated T cell-5 (also called tonicity response element binding protein or osmotic responsive element binding protein, Nfat5/TonEBP/OREBP) is a transcriptional factor playing important roles in renal osmoregulation as well as inflammatory responses. We have explored epigenetic mechanisms especially miRNAs in Nfat5 signaling, osmoregulation and urine concentration. Following a high NaCl hypertonicity (550 mOsmol/kg H_2O) exposure for 2 h in mIMCD3 cells, 21 miRNAs were significantly up-regulated and 12 miRNAs were down-regulated by at least 2 folds, which included the significantly downregulated miR-200b-3p and miR-717 and significantly up-regulated miR-466(a/e)-3p and their close relatives. Transfection studies indicated miR-200b-3p and miR-717 were capable of targeting *Nfat5* post-transcriptionally, thereby contributing significantly to hypertonicity-induced induction of *Nfat5*. Although in response to hypertonicity exposure miR-466(a/e)-3p were upregulated in vitro in cultured mIMCD3 cells, these miRNAs were found to be significantly down-regulated when mIMCD3 cells were cultured in the presence of arginine vasopressin or in vivo in renal tissues of water deprived mice. *Sfmbt2*-hosted miR-466(a/e)-3p were also found to be capable of silencing Nfat5 post-transcriptionally. In vivo, transgenic overexpression of miR-466a-3p was found to be associated with significant down-regulation of renal Nfat5 and other osmoregulation-related genes. On the other hand, sustained transgenic overexpression of miR-466a-3p was shown to cause significantly altered renal morphology, the development of polyuria and polydipsia, and disturbed ion homeostasis in mice. In conclusions, miRNAs such as miR-200b-3p, miR-717 and miR-466(a/e)-3p are important epigenetic regulators of Nfat5 signaling, osmoregulation and urine concentration. No COI.

S 14

High salt and chronic renal diseases

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High salt is a primary cause of hypertension. Immune cells especially T cells and monocytes/ macrophages have recently been identified to be an important sensor of high salt and may act via production of inflammatory and vasoactive mediators to disrupt the functional and structural homeostasis of kidney and vasculature, subsequencially leading to hypertension and the progression of chronic kidney diseases (CKD). Glomerular diseases, characterized clinically by proteinuria and hypertension are major cause of CKD. Both hypertension and proteinuria are key players in CKD progression. Our current findings indicate that high salt may not only contribute to hypertension but also increases proteinuria. High salt loading rapidly elevated proteinuria in CKD humans and animals but not in normal controls. Inflammatory mediators including IL-13 were induced by high salt in T cells and glomerular cells and may play a role in proteinuria. Suppression of inflammation by calcineurin inhibitor largely prevented high salt induced proteinuria, whereas suppression of renin-angiotensin system had minimal effect. NFAT5/TonEBP, a transcription factor that senses high osmolarity was involved in high salt induced inflammatory mediators production and proteinuria. Moreover, high salt caused a stress response in cells especially the glomerular podocytes that may alter organization of cytoskeletons and microtubules in cells, resulting in inflammation and proteinuria. Further studies are needed to explore the underlying cellular and molecular mechanism(s) for high salt mediated proteinuria. Nevertheless, since salt intake is a modifiable factor for blood pressure and proteinuria control, clincial practice may proceed from now. No COI.

S 15

Development and regulation of gonadotropin-releasing hormone (GnRH) release during the prepubertal period

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Pulsatile GnRH release is the ultimate signal for central neuronal control of pituitary gonadotropin release and thereby fertility. Based on gonadotropin release, GnRH release before puberty is thought to be low, but actual measurements of GnRH release have not been made during the neonatal and early prepubertal period. We used the electrochemical method fast-scan cyclic voltammetry (FSCV) to monitor GnRH release in the median eminence (ME) in brain slices from gonad-intact male mice. In adults, the frequency of GnRH release in this preparation is similar to the luteinizing hormone (LH) pulse pattern in vivo. High-frequency GnRH release in ME occurred as early as embryonic day 18, peaked on the day of birth and remained elevated through 7-9d postnatal. By 2wks of age, release was minimal. We studied the regulation of GnRH release frequency at 1wk of age. GnRH release persisted in kisspeptin knockout mice, thus release at this age is kisspeptin independent. Testosterone given in vivo 4hr before slice preparation reduced spontaneous GnRH release frequency at 1wk, as did GnIH applied via the bath during recording. Blocking the GnIH receptor (GPR147) reversed the inhibitory effect of testosterone. Blocking GPR147 also increased GnRH release at 2wk of age. Exogenous GnRH failed to increase serum LH in 1wk old mice, when endogenous GnRH release was high frequency, but did in mice aged ≥2wk, after frequency was reduced. These data indicate high frequency GnRH release in early prepubertal male mice may result from lack of endogenous inhibition of the GnRH network. We postulate high-frequency GnRH release is needed for development of appropriate synaptic inputs, and that pituitary non-responsiveness at 1wk of age protects the reproductive system from premature activation. No COI.

S 15

Functional and Molecular Evolution of Kisspeptin Systems in Social Behaviours

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An evolutionarily conserved hypothalamic neuropeptide, kisspeptin (encoded by Kiss1 gene) and its receptor (Kiss-R=GPR54) signal a key role in vertebrate reproduction and puberty. In non-mammalian vertebrates, Kiss1 and its isoform Kiss2, and two Kiss-R types have been identified. Kiss2 gene appears to have been lost in the mammalian linage. Recent studies in mammalian and non-mammalian vertebrates have implicated potential additional roles for kisspeptin based on its expression in brain regions such as the medial amygdala, hippocampus and the habenula. Recently, we have shown that kisspeptin modulates the release of 5-HT to inhibit alarm substance-evoked fear response in the zebrafish. These results suggest that kisspeptin may subserve an additional role in social behaviours to maintain emotional aspects of reproductive capability such as sexual motivation and arousal. No COI.

S 15

Regulatory mechanisms of seasonal reproduction in vertebrates

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Animals living in temperate zones use changes in day length to adapt to seasonal changes in environment, but mechanisms underlying seasonal (photoperiodic) time measurement are not fully understood. Japanese quail is an excellent model for the study of these mechanisms because of its rapid and dramatic response to changes in photoperiod. We have demonstrated that local thyroid hormone catabolism within the mediobasal hypothalamus (MBH) by thyroid hormone-activating enzyme (type 2 deiodinase: DIO2) regulates photoperiodism. Functional genomics analysis in quail demonstrated that long day stimulus induces thyrotropin (thyroid stimulating hormone: TSH) production in the pars tuberalis (PT) of the pituitary gland, which triggers DIO2 expression in the ependymal cells of the MBH. In mammals, nocturnal melatonin secretion provides an endocrine signal of the photoperiod to the PT that contains melatonin receptors in high density. We have also demonstrated the involvement of TSH signaling pathway in mammals by using the TSH receptor null mice. Well known function of TSH derived from pars distalis (PD) of the pituitary gland is stimulation of thyroid gland. However, the mechanisms by which PT- and PD-TSH exert distinct functions within the body remained mystery. We found TSHs from two anatomical sources undergo different glycosylation and this tissue-specific glycosylation imparts different functions on a single hormone. No COI.

S 16

Tonic inhibition of locus coeruleus neurons by GABAB receptors

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The locus coeruleus (LC) nucleus contains noradrenergic (NAergic) neurons that provide the major norepinephrine (NE) supply to the forebrain. It has been shown that the release rate of NE in forebrain directly correlate to the discharging rate of NAergic LC neurons; accordingly, exploring how LC neuron firing rate is regulated is essential to understand the operation of brain functions associated with LC-NE system, such as the regulation of sleep-wakefulness cycle. Here we focus on the role of GABAB receptor (GABABR), a G-protein-couple receptor. Our recent electron microscopy observations show that in LC most of the postsynaptic GABABRs locate at extrasynaptic sites. These extrasynaptic GABABRs are continuously activated by ambient GABA, resulting a tonic current of ~ 10 pA, which accounts for about 13% of total GABABR- mediated whole-cell current in LC NAergic neurons. These results suggest that there is still plenty of room for GABABR to regulate the excitability of LC NAergic neurons through with manipulating ambient GABA. In support of this speculation, inhibiting GABA reuptake can effectively tuning the firing rate of LC neurons. The GABABR- mediated tonic inhibition is not unique to LC NAergic neurons, it also occurs in NAergic A7 neurons. Most importantly, it is not only exists in brain slice preparation, but also in urethane anaesthetized condition. Since ambient GABA is reported to be higher in sleep than in waking condition using microdialysis method, our results suggest that GABABR-mediated tonic inhibition of LC neurons might be a molecular mechanism underlying higher LC neuronal activity in waking than in sleep condition (Supported by research grants from MOST, Taiwan).

S 16

Orexinergic mechanisms in drug addiction and pain modulation

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Orexin neuropeptides have a remarkable role in drug addiction and pain modulation. The locus coeruleus (LC) receives dense orexinergic fibers and express mainly the orexin receptors type-1 (OXR1) and is involved in opioid tolerance and dependence as well as pain modulation. However the role of orexinergic transmission at the LC nucleus in these situations is unclear. Central administration of OXR1 antagonist (SB-334867) inhibits the development of morphine analgesic tolerance. In other words, OXR1 blockade by SB-334867 prevents the development of morphine analgesic tolerance. Moreover, the results indicate that intra LC microinjection of SB-334867 prior to each morphine injection or prior to naloxone administration reduce the severity of naloxone-induced morphine withdrawal symptoms. The whole-cell patch clamp recording results showed that in vitro application of orexin-A increases LC spontaneous firing rate and paired-pulse ratio (PPR). It also decreases spontaneous excitatory postsynaptic currents (sEPSCs) frequency of LC neurons, but did not change the sEPSCs amplitude. Our electrophysiological data indicate that orexin-A application decrease evoked excitatory postsynaptic currents (eEPSCs) and evoke inhibitory postsynaptic currents (eIPSCs) in LC neurons synapses. It is concluded that orexinergic transmission in the locus coeruleus appears to be involved in the drug addiction and pain modulation. Also, the in vitro results provide supporting evidences for a critical role of orexin signaling in LC neurons. It can be deduced that these changes in excitatory synaptic transmission may be elicited by presynaptic rather than presynaptic mechanisms. No COI.

S 16

Excitation of LC neurons by anesthetics

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The locus coeruleus (LC) regulates the activity of widely distributed cerebral networks through release of noradrenaline and other catecholamines in a one-to-many manner. Such "global" regulation is of particular importance in many physiological situations in which coordinated control of various functions implemented in many distributed networks is essential. Arousal and nociception are among such functions. This feature of the LC is also important in understanding mechanism of drugs. For example, dexmedetomidine, an alpha2-adrenoceptor agonist, exerts sedative effects by reducing conscious level and elevating nociception thresholds through suppressing LC neuron activities. General anesthetics are the most frequently and widely used central acting drugs that exert robust inhibitory effects in human and animals. However, sevoflurane, one of the most frequently used volatile anesthetics, has been shown to cause aberrant agitation in human patients, especially in children and at the emergence. As this "emergence agitation" is prevented by dexmedetomidine, we hypothesized that LC plays a key role in this sevoflurane-induced aberrant excitation. In brain slices from young rats, 5% sevoflurane induced an early-phase inward current in most of LC neurons even in the absence of synaptic inputs, in a manner inhibited by a gap junction inhibitor. Such excitatory current was not observed with nonvolatile general anesthetics and in non-LC neurons examined. It is thus expected that a potent drug effect on arousal and nociception would involve its direct effects on LC neuron excitability. In collaboration with Yutaka Yasui and Eiji Masaki. No COI.

Symposium 17 **Deactivation of excitatory neurons in the contralateral prelimbic cortex via Cdk5 promotes pain sensation and anxiety**

S 17

Empathy for pain in rodents

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Empathy, a basic prosocial behavior, is referred to as an ability to understand and share other's emotional state. Empathy is also a basis of altruism. In contrast, anti-empathy may be associated with autism, narcissism, alexithymia, personalality disorders, schizophrenia and depression. Thus study of the brain mechanisms of empathy has great importance to both scientific and clinical advances. However, less is known about the molecular and cellular mechanisms of empathy due to lack of laboratory animal models in that only human and non-human primates have been considered to have such unique emotional sharing. Recently, a group from McGill university and we independently found that both mice and rats have empathy for pain. We also mapped out that the medial prefrontal cortex including the anterior cingulate cortex, prelimbic cortex and infralimbic cortex is involved in pain empathy in rats, suggesting existence of a neural network associated with development of pain empathy in the CNS. In the present lecture, I would like to give a brief outline of the advances in study of empathy for pain in rodents, try to provide with a bio-psychosocial-behavioral model for studying pain and its emotional comorbidity with anxiety and depression. No COI.

S 17

Involvement of the anterior cingulate cortex in pain-related negative emotion

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The anterior cingulate cortex (ACC) is thought to be key neural substrates underlying emotional responses. Using pain-related conditioned place avoidance models (CPA), we observed that bilateral excitotoxic lesions of the ACC suppressed the formalin-induced CPA (F-CPA). Intra-ACC NMDA receptors antagonist, AP5, but not AMPA/KA receptors antagonist DNQX, F-CPA was effectively eliminated. NMDA receptor activation induced pPKA, pERK and pCREB in rACC slices. Blockade of PKA and ERK activation in the rACC prevented the induction of F-CPA. Thus, NMDA-PKA-ERK-CREB pathway activation in the rACC is required for the induction and expression of pain-related negative affect. Estrogen has been reported to enhance NMDA-mediated synaptic activity in the hippocampus and cortex. We demonstrated that estrogen acutely enhanced the EPSCs in rACC slices by increasing the ratio of NMDA–EPSCs to AMPA–EPSCs and presynaptic glutamate release. Intra-rACC injection of estrogen receptor (ER) inhibitor ICI 182, 780 or inhibitor of aromatase androstatrienedione completely blocked F-CPA, suggesting that estrogen in the rACC facilitated NMDA receptor-mediated synaptic transmission to drive pain-related negative emotion. Using a chronic constriction injury (CCI) model of neuropathic pain, we profiled gene expression in the rat brain and identified sip30, which was upregulated in the rACC after CCI. Knockdown of SIP30 by intra-rACC injection of shRNA targeting the rat sip30 gene suppressed the frequency of mEPSCs in rACC slices. Inhibition of CCI-mediated induction of SIP30 reduced neuropathic pain-evoked place escape/avoidance paradigm (PEAP), suggesting that SIP30 in the rACC mediates neuropathic pain-evoked negative emotion via modulation of glutamate release and excitatory synaptic transmission. No COI.

S 18

Subcellular Purkinje neuron translatome at rest and during plasticity

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Two major networks underlay brain complexity: neuronal connections and molecular signaling pathways. Until recently, the diversity and complexity of the spatially intermingled neurons posed a serious challenge to the exhaustive identification and quantification of components, in a neuron-type specific manner. This is an obstacle especially for our understanding of the protein-synthesis dependent late phase of synaptic plasticity. To address this problem, we present a novel approach to identify ribosome-associated translating mRNAs, from selected sub-neuronal domains of Purkinje cells (PC) in the rat cerebellum. We combined microdissection, Translating Ribosome Affinity Purification (TRAP) and quantitative nanoCAGE RNA deep-sequencing, we obtained snapshots of RNAs bound to cytoplasmic or rough endoplasmic reticulum (rER)-associated ribosomes, in the PC and its dendrites, at different time points following plasticity induction. We show that the approach yield selective enrichment in PC markers, identifies transcripts not previously known to be enriched in PCs and some hitherto uncharacterized mRNAs. We introduced an improved detection of translating mRNAs for membrane receptors and ion channels, allowing estimate of relative synthesis rate. The late phase of plasticity was specifically correlated with altered translation of an mRNA subset, associated with markers of synaptic plasticity and cell signaling. No COI.

S 18

Developmental plasticity in spatial coding impacts on navigational behaviour

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We asked if synaptic plasticity of excitatory and inhibitory transmission in the developing circuitry of the vestibular nucleus (VN) impacts on behavioural outcome. We demonstrated that LTP of glutamatergic synapses at interneurons in the VN enabled postnatal emergence of graviceptive behaviour in rats. Furthermore, GABAergic transmission in the VN was excitatory in the first postnatal week but switched to inhibitory in the second postnatal week. During this period, LTD of GABA transmission in the VN could be modulated by neuromodulators such as endocannabinoid or BDNF. With neonatal administration of GABA-A receptor agonist to the VN, we found that the proportion of LTD-expression neurons was reduced and the emergence of graviceptive behaviour was advanced. Perturbation of synaptic plasticity in the postnatal VN impacted on spatial map formation in the adult brain with effects on spatial navigation. Taken together, tuning the vestibular circuitry for spatial coding during a postnatal period of plasticity is critical to the recruitment of effectors for orienting behaviours in the adult animal. (HKRGC-GRF 761711, 761812, 762313, N_HKU735/14). No COI.

S 19

Multiple actions of endocrine disrupting chemicals on thyroid hormone-mediated brain development

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Thyroid hormone (TH) plays critical role on brain development. Deficiency of TH during perinatal period causes abnormal brain development known as cretinism in humans. On the other hand, there is a potential risk of exposure of toxic substances through food during perinatal period. In fact, several chemicals disrupt TH-action in the developing brain. Such chemicals are known as endocrine disrupting chemicals (EDCs). They affect TH systems through different mechanisms. For example, although hydroxylated polychlorinated biphenyl (PCB), polybrominated diphenyl ether (PBDE) and perfluoro-octanesulfonate (PFOS) suppress TH-mediated dendrite arborization of cerebellar Purkinje cell in culture, PCB and PBDE suppress TH-mediated transcription by dissociating TR from TH response element, whereas PFOS suppresses deiodination of thyroxine (T4) by inhibiting type 2 iodothyronine deiodinase activity. On the other hand, several isoflavones such as genistein and daizein activate TH-mediated dendrite arborization by augmenting TH-mediated transcription through recruiting coactivators. It should be noted that the effects may be induced at low dose exposure. Perinatal administration of PCB or PFOS during pregnancy or lactating period induces hyperactivity in the open field, motor coordination defect on the rotarod in their offsprings. These results indicate that various EDCs affect brain development at least in part through modulating thyroid hormone action. Thus, care must be taken not to intake synthetic chemicals particularly during pregnancy and lactating period. No COI.

S 20

Leptin's role in cardiorenal function

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Adipocytes secrete a number of bioactive molecules that maintain homeostasis via the activation of key cellular signalling pathways. The adipokine leptin signals via the leptin receptor and megalin in the hypothalamus, adipose tissue, liver, kidney, heart and placenta via a tissue-specific and receptor-specific manner. Most of the current knowledge concerning the role of leptin in cardiorenal function is focused on studies where plasma leptin concentrations are elevated to mimic the obese state. Chronic leptin exposure in animals via intravenous infusion results in increased arterial pressure and heart rate, while hyperleptinemia in humans has been shown to be associated with hypertension via the modulation of both systolic and diastolic blood pressure. Recent research has also demonstrated that leptin controls neuronal circuits specifically in the dorsomedial hypothalamus to modulate blood pressure. Further, the link between leptin and blood pressure may in part be controlled by the kidney. Acute exposure to leptin increases sodium excretion and urinary output in animal models. In addition, in isolated glomerular mesangial cells, leptin induces hypertrophy via the activation of phosphoinositide 3-kinase and mitogen-activated protein kinases. This in turn increases the amount of filtered protein and albumin reaching the tubules, which activates profibrotic and inflammatory pathways. Recently, we have established that exposure to elevated leptin in vitro increases the expression of a number of downstream signalling targets which modulates metabolic activity and increases fibrotic mediators which leads to a reduction in albumin handling by the proximal tubules. Thus leptin appears to play multiple roles in the modulation of cardiorenal function. No COI.

S 20

Adverse effects of leptin on sperm parameters

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Leptin, a 167 amino-acid product of the ob gene, was first identified in 1991 after a 40-year search that began following the emergence of a mutant strain of mice with hyperphagia, early on-set obesity, and delayed sexual maturation. Since then, leptin has been shown to be involved in the regulation of appetite and body weight, immune and reproductive functions, CNS development during the neonatal period, bone growth and development. Although it was its deficiency that first led to its discovery, it now appears that its clinical significance also lies when it is in excess. Emerging evidence from animal studies is implicating leptin in infertility and in a numerous obesity-related diseases. Chronic leptin administration to non-obese rats decreases sperm count, increases the fraction of sperm with abnormal morphology, increases sperm DNA fragmentation and alters its histone: protamine ratio. These changes are accompanied by the up-regulation of respiratory chain enzyme genes and down-regulation of the anti-oxidant enzyme genes in the testes of leptin treated rats. Expressions of TNF-α, p53, p21 and AIF are also up-regulated while the expression of Bcl2-like-1 is down-regulated following leptin treatment. Most of these changes are however reversible upon cessation of leptin treatment and recovery is near complete in 7-8 weeks. These changes are also prevented by concurrent administration of melatonin. It appears that the adverse effects of leptin involves increases in free radical activity that then induce DNA fragmentation and a necrotic-like cell death of sperm and seminiferous tubular cells through activation of TNF and JNK pathway. No COI.

Symposium 21 **Advances in reproductive physiology for wild life conservation**

S 21

Biodiversity and Reproductive Endocrinology in Mammals

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Pregnancy with placentation and lactation are the peculiarity of mammals. A remarkably growth of fetus occurs in uterus during the late stages of pregnancy in any mammals. The most important factor is rapid elongation and cylindrical changes of uterus to maintain fetal survival in the stage of rapid fetal growth during the late stages of pregnancy. Progesterone, estrogen, prolactin and relaxin play key roles in the implantation of embryos, maintenance of pregnancy and the induction of parturition. Although the synergistic action of progesterone and estrogen is generally essential for most mammals in maintaining a successful pregnancy, estrogen plays a dominant role in the maintenance of uterine elongation when the fetus grow rapidly in the late pregnancy. However, the origin and mechanism responsible for secretion of a large amount of estrogen in the late pregnancy are different among species. There are two types of groups. The first group is the ovary and placenta unit type which can be seen in rat, mouse, golden hamster, rabbit, dog, goat, cow and elephant. For the first group, the mother's ovary is an essential endocrine organ throughout pregnancy. The second group is the fetus and placenta unit type which can be seen in guinea pig, cat, sheep, horse, monkey and human. In this latter type, mother's ovary is not essential in the late stages of pregnancy. The currently known aspects of the unique biodiversity of endocrine mechanism in pregnancy and the comparison between those two groups of animals will be reviewed in this paper. No COI.

S 21

Shp2 is essential for normal embryo implantation in mice

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Ovarian steroid hormones and their nuclear receptors PR and ER coupled with locally produced signaling molecules have been well demonstrated essential for embryo implantation, however, the hierarchical landscape of the molecular pathways that govern this process remains largely unexplored. The crosstalk and interaction between the RTK and steroid hormone receptor has been well studied in the caner related research, but whether this happens in the physiological implantation process remains unclear. Shp2 is a positive signal transducer of RTK signaling and its systemic knockout would cause the embryo lethal. We show herein that uterine conditional deletion of Shp2 by utilizing PR-Cre mouse model derails normal uterine receptivity leading to a complete implantation failure. Unexpectedly, this compromised uterine receptivity exhibiting progesterone resistance upon Shp2 depletion is seeded by limited ERα activation and thus reduced PR expression in uterine stroma independent of ERK pathway activation. Further analysis reveals that nuclear Shp2, rather than cytosolic Shp2 physically interacts with ERα, facilitating ERα binding to DNA sequence of targeting PR promoter and subsequent cofactor recruitment for ERα transcriptional activation in peri-implantation uteri. Besides uncovering a novel regulatory mechanism, this study could be clinically relevant to dysfunctional ERα-caused endometrial disorders in women. No COI.

S 21

Using science to understand factors related to zoo elephant reproduction and welfare

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Questions have been raised about whether environmental and social needs are being met for elephants in zoos. Lack of reproductive function has long been considered an indicator of reduced welfare and poor sustainability of zoo elephants. A large, epidemiological study was conducted in the U.S. that involved 250 elephants at 64 zoological institutions to identify how social, facility, management, keeper factors affect ovarian function in elephants. Rates of normal cycling, non-cycling and irregular cycling were 73.2, 22.5 and 4.2% for Asian, and 48.4, 37.9 and 13.7% for African elephants, which differed between species. Several management factors were related to ovarian cycle status. Enrichment diversity was found to increase the chance that a female African elephant will cycle normally, possibly due to elephants making a positive appraisal of the quality of the zoo environment. Social experience was also associated with ovarian cycling for female African elephants. Females that are socially separated, even if they have access to one or more other elephants through a barrier, have a decreased chance of cycling. Although a larger percentage of female Asian elephants had normal ovarian cycles, univariate analyses indicated that more time spent in spaces with free choice of being indoors or outdoors was associated with an increased likelihood of normal cycles. Thus, there are many factors in the captive environment that can be managed to improve reproduction and improve welfare of elephants under human care. No COI.

S 22

Role of Thai Traditional Medicine in the Health Care System of Thailand

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Humanity has long been faced with a variety of illnesses, and are determined to find a way to relieve suffering caused by them. Knowledge has continuously evolved and has been passed down from generation to generation. Medical knowledge from the western world has been accepted for its advanced methods and technology and has become mainstream medicine in most parts of the world. But the advancement of western medicine has also created problems, such as the burden of the increasing cost of the health care system, and many patients still suffer from diseases for which western medicine has yet to develop successful treatments. Society is now beginning to realize that the advances of western medicine may not be the answer for all problems, and wants to learn more about whether traditional medicine can be integrated with modern medicine to help solve or at least reduce these problems. In Thailand, attempts have been made to conserve and make use of Thai traditional medicine. Work related to Thai traditional medicine, such as education, health services and herbal medicine production, has been improved. At present, some university hospitals and most hospitals under the Ministry of Public Health provide services in Thai traditional medicine; these services cover diagnosis and treatment with herbal medicine, traditional maneuvers including massage, hot herbal compresses, herbal steam baths and postpartum treatment. Some development of the works from the Faculty of Medicine Siriraj Hospital, Mahidol University, the oldest hospital and medical school in Thailand, established 127 years ago will be presented. No COI.

S 22

Thai Traditional Medicine: Moving Towards Precision Medicine

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In order to accelerate the integration of Thai traditional medicine into the health care system, Thai government has established the national essential herbal drug list comprising traditional drugs and herbal items for many health conditions since 1999. Although the prescribed items can be reimbursed from the government under the universal health coverage scheme, only a small fraction of modern physicians agreed to prescribed some products on the list, but most of the physicians asked for scientific evidences on safety and efficacy. Therefore, the recent trend in researches on Thai traditional medicine has shifted to clinical trials assessing the efficacy and safety of herbal medicines rather than in vitro screening of interested pharmacological properties which was performed extensively in the past. Thai traditional practitioners have been asked to perform documentation on the actual uses of Thai traditional herbal remedies, so called Actual Use Research with the hope that the information will be evidence to support the usage and ensure the safety of the remedies. It can also be used as basic information to investigate pharmacological mechanisms and help in the explanation of the effects of such remedies to human body. A new approach of Multi-omics analysis (genomics, proteomics, metabolomics, phenomics and etc.) are used to create databases with the ultimate aim to generate massive data network of Thai traditional medicine in patients and reaching out towards disease mechanisms, precision diagnosis and treatment for individual patient, calling precision medicine. This approach will help not only creating evidence-based Thai traditional medical practices, with rational use of traditional drugs as personalised medicine, but also help to conserve Thai traditional medical knowledge, one of the wisdoms of mankind. No COI.

S 22

Thai Traditional Massage: Explaining the Art of Healing with Modern Scientific Knowledge

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Court-type Thai traditional massage (CTTM), an art of healing, is a popular alternative treatment for musculoskeletal disorders nowadays. Researches have been done in this field to reveal the effects of CTTM in terms of evidence-based medicine. The main characteristic of CTTM is the use of thumbs, fingers or palm pressing with gentle force on points along basic massage lines in the initial phase and later on pressing some specific body points, called major pressure point or major signal point (MaSP). There are 50 MaSPs all over the body. Thai traditional practitioners believe that by pressing these MaSPs, the blood and heat would be regulated to specific body parts to cure the symptoms or ailments. Anatomical positions of MaSPs have been studied by various groups, the result of 15 MaSPs on neck and upper extremities will be presented. Surface landmarks can be used to distinguish location of each point in cadaver as well as in practice. Muscles were the main anatomical structure underneath MaSPs together with their nerves and arterial supplies. Physiological effects of the massage on these 15 MaSPs were studied in healthy volunteers to determine blood flow and thermal skin using Duplex ultrasound and thermographic camera, respectively. After pressing each MaSP for 30 seconds, blood flow increased immediately and lasted for 60 seconds. Thermal skin was also conformed to blood flow, skin temperature increased immediately but lasted longer for 3 minutes. In clinical practice, practitioners who perform CTTM usually massage along the basic massage lines and on specific MaSPs related to the symptom for a period of 45 minutes. This study demonstrated that massage of the MaSPs may increase local blood circulation and improve muscle relaxation via the increase of blood flow and thermal skin. No COI.

S 23

Systems Biology of Vasopressin Signaling in Kidney

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Vasopressin regulates water excretion by controlling the water channel aquaporin-2 (AQP2) in two ways: 1) regulation of aquaporin-2 (AQP2) trafficking to and from the apical plasma membrane; and 2) regulation of transcription of the Aqp2 gene. We are using large-scale proteomics (LC-MS/MS) and deep sequencing of DNA to identify the signaling mechanisms involved. Phosphoproteomics studies in collecting duct cells identified several hundred phosphorylation sites that increase with vasopressin (including Ser-256, 264 and 269 of AQP2) and a similar number of sites that decrease with vasopressin (including Ser-261 of AQP2). The former have sequences predicted to be targets of basophilic kinases (AGC and CAMK families), while the latter are predicted to be phosphorylated by proline-directed kinases (CMGC family). Of the 521 protein kinases present in the rat genome, we have identified 217 that are expressed in rat collecting duct (RNA-seq analysis). These protein kinases were ranked in terms of likelihood of phosphorylating each the four phosphorylation sites in AQP2 by employing Bayes' rule to integrate data from multiple sources (transcriptomics data, proteomics data, data from reductionist studies of vasopressin signaling, kinase inhibitor data and kinase target specificities). We are systematically deleting these kinases using CRISPR-Cas9 followed by phosphoproteomics to identify which of these candidate kinases play critical roles in vasopressin signaling. To identify upstream kinases in the relevant signaling cascades, we have determined the effects of 7 broad spectrum protein kinase inhibitors on the phosphoproteome of mpkCCD cells. The result is a vasopressin-activated signaling network responsible for regulation of AQP2 phosphorylation. No COI.

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Systems Biology of Renal Epithelium

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The success of the "human genome project" has marked the beginning of 21st century inter-disciplinary field of study so-called "Systems Biology" that focuses on complex interactions within biological systems, using a more holistic perspective approach to biological and biomedical research. Currently, all of the elite research institutes around the globe have set their missions toward this new approach in science. It is now clear that systems biology has brought us a magnitude of information that never before attained in the history of biological science. Systems biology studies require an integration of basic and clinical science standpoints, large-scale "omics" technologies, and high-level computational methods for data analysis and interpretation. Many laboratories have successfully applied the state-of-the-art methods in systems biology viz. protein mass spectrometry, microarrays, next-generation sequencing, and bioinformatics to investigate the fundamental mechanisms involved in the regulation of water and solute transport by the kidney. In this symposium, we will discuss the development of systems biology approaches in the investigation of physiology and pathophysiology of renal epithelium. Numerous new findings from these studies that help us understand more about the underlying molecular mechanisms of vasopressin-related disorders such as the syndrome of inappropriate secretion of antidiuretic hormone (SIADH), diabetes insipidus, and polycystic kidney disease will be highlighted. Finally, we will explore approaches to investigate the heterogeneity of phenotypic responses down to the single-cell resolution. No COI.

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Investigation of heterogeneity in cellular phenotypic responses at the single-cell resolution

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How cells convert the extracellular cues to intracellular signals and commit on a specific phenotypic outcome has still been an active area of research. Using time-lapse microscopy and the construction of live reporter proteins, we can now begin to investigate how cells respond heterogeneously at the single-cell level. Using live reporters of ERK activity and the translocation reporter of FoxO3a protein, we investigated how the activity of these two proteins temporally vary upon stimulation with six different growth factors at varying doses. In addition to confirming the pulsing characteristics of ERK activity, we demonstrated that FoxO3a translocations are also truly dynamic, involving both early synchronous phase and the late asynchronous pulsatile response. Co-expression of ERK and FoxO3a reporters showed for the first time how ERK activation contributes towards the pulsing of FoxO3a translocation. We then explored the AKT/ERK/FoxO3a connectivity in different breast epithelial and breast cancer cell lines and showed that FoxO3a can be distinctly regulated by AKT and ERK pathways. Our discovery of FoxO3a pulsatile translocation is consistent with the already reported pulsing dynamics of other transcription factors including p53, NFκB and NFAT4. Such temporally dynamic cellular responses cannot be identified using conventional bulk assays, emphasizing the necessity to reinvestigate other common physiological processes using these novel technologies. No COI.

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Thermoregulation in the tropics

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It has been reported recently that humans have developed such a remarkable system for endurance exercise in the heat, that we could outrun almost all other mammals, including horses. The key for maintaining exercise in the heat may reside in controlling both core temperature and systemic blood pressure simultaneously. Thus, thermoregulatory researches, specially, during exercise in the heat, are critical to understanding human adaptation to the tropic conditions. During exercise, heat loss (via sweating and skin blood flow) is regulated by two main factors; thermal (core and skin temperature) and work factors (non-thermal; central command, muscle metab-mechanoreceptors etc). Although it is well known that increases in thermal factors have a positive effect on sweating and cutaneous blood flow response, the effects of non-thermal factors on the heat loss responses are not well understood. Previous research indicates that afferent signals from working muscles (muscle metaboreflex) are one of the non-thermal factors, which increases sympathetic nerve activity and are important in controlling cardiovascular responses during exercise. Therefore, it has been suggested that this muscle metaboreflex also modulates heat loss responses during exercise. Thus, we will discuss an integrative control of heat loss responses during exercise based on muscle metaboreflex. No COI.

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Exertional heat stroke in the tropics

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With the influence of global warming and increasing surface temperature about 1.8- 4oC by 2100 (IPCC 2007), heat related illness (HRI) in the tropical countries is likely to be more prevalent. Exertional heat stroke (EHS) is a key concern for athletes, military recruits, workers under outdoor and indoor without air conditioning even following heat acclimatization. The risk of HRI is dependent on work intensity and heat exposure time. The incidence of EHS in each tropical country may be under-diagnosed as symptoms are non-specific. The incidence of EHS can be minimized with education to increase awareness. Furthermore, the strategies for heat mitigation at work/drill settings including exercise and work/rest guidelines, identifying individuals with high heat strain, fluid and electrolyte replacement and effective cooling should be made known to the supervisors. If EHS is detected, rapid ice immersion with immediate hospital evacuation is the most effective way to reduce poor prognosis. In the Royal Thai Army, heat related injuries in new recruits during military training are still a health problem. The prevention program provided includes health education, display color flags in accordance with environmental temperature and humidity, water replacement with exercise/rest guidelines, measurement of tympanic temperature and urine color daily. Research is being undertaken to develop more effective preventive method such as real time monitoring. As the trend of EHS is increasing for high risk populations in the tropics, in addition to awareness through education, heat preventive policies and appropriate effective preventive methods need to be implemented in work places. No COI.

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Endurance performance in the tropics

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Excessive heat stress compromises endurance performance and increases the risk of heat stroke. The rapid rise of body core temperature (Tc) when exercising in the heat often results in an impairment of exercise capacity and performance. As such, coaches and employers apply various heat mitigation strategies to counteract the debilitating effects of heat strain in their athletes and workers, respectively. These strategies include behavioural alteration, aerobic fitness, heat acclimation/acclimatisation, pre-exercise cooling and fluid ingestion. An ideal heat mitigation strategy would: (*i*) lower Tc before exercise; (*ii*) attenuate the rise of Tc during exercise; and (*iii*) extend Tc at the end of exercise within safe limits. Through these alterations of Tc, an athlete would increase his capacity for heat storage during exercise, and therefore optimise performance in the heat. Current understanding in this field of research is largely based on data collected from non-heat acclimatised cohorts and therefore its validity remains unanswered for tropical natives where the exposure to heat is higher. For example, a conventional heat acclimatisation programme that is usually effective when employed on Caucasians did not induce any physiological adaptation in tropical natives. This lecture will have practical implications for individuals seeking to optimise performance and health in the heat by making informed decisions when choosing the appropriate heat mitigation to employ. No COI.