7

G-Protein-Coupled Receptors

Receptors coupled to heterotrimeric guanine nucleotide-binding proteins, the so-called G-protein-coupled receptors (GPCR), represent the largest set of plasmalemmal receptors ($>$ 800 members in the human genome) . All GPCRs, also called 7-transmembrane and heptahelical receptors, possess a 7-transmembrane (7TM) scaffold that is adapted to different sets of ligands. The 7 membrane-spanning α-helical segments are separated by alternating intra- and extracellular loops.

7.1 Introduction

Ubiquitous G-protein-coupled receptors transmit the majority of signals from various types of messengers, such as hormones, neurotransmitters, and sensors. They can operate as allosteric signaling mediators. Many GPCRs display various levels of G-protein activation.

G-Protein-coupled receptors are actually implicated in diverse physiological processes, such as hormonal signaling, neurotransmission, cognition, chemosensation, photoreception (vision), taste, olfaction (Sect. 6.10), and pain perception, as well as ensure the cardiac and other physiological functions, metabolism, and cell growth, differentiation, and migration, in addition to inflammation and immunity.

7.1.1 Agonists vs. Antagonists

Ligands bind to transmembrane segments and extracellular domains of their cognate receptors. The basal activity of a GPCR can be activated by *agonists* or inhibited by *antagonists*. Agonist binding at the extracellular side triggers a set of conformational changes of the target GPCR at the intracellular side, where a heterotrimeric G protein and other effectors can then tether to initiate the signaling cascade.

A *neutral antagonist* prevents agonist binding, but do not affect GPCR constitutive (agonist-independent) activity; an *inverse agonist* precludes the constitutive activity of its specific receptors.

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Agonist binding does not always cause important conformational changes. Moreover, agonist subtypes can be classified according to their effect on the receptor structure [762]. Some ligands stabilize a given receptor conformation (conformationally selective ligands). Others modify the receptor structure among multiple receptor conformations and shift the dynamical equilibrium.

Human adenosine A_{2A} receptor and β-adrenergic receptors possess binding sites that can bind specifically to agonists rather than antagonists and inverse agonists [763]. Inverse agonists can bind to a given GPCR to prevent the conformational change that primes its activation. An intermediate conformation between the inactive and active states is actually characterized by an occlusion of the G-protein-binding site.

7.1.1.1 Full and Partial Agonists

In addition to neutral antagonists and inverse agonists, full and partial agonists prime various rearrangements of the receptor structure that initiate signaling via G-proteins and/or arrestins (Sect. 7.11). [1] *Full agonists* are capable of maximal receptor stimulation, whereas *partial agonists* are unable to elicit full activity even at high concentration. A given conformation stabilized by a ligand thus determines the efficacy toward a specific pathway.

In summary, a ligand can operate as: (1) a full agonist that is capable of activating the receptor, thereby causing an a priori maximal response; (2) partial agonist that does not activate its cognate receptors thoroughly, hence provoking partial response; (3) inverse agonist that reduces the activity of its receptor by inhibiting their constitutive activity; and (4) antagonist that binds to the receptor, blocks action of agonists, as it prevents access to the receptor by its binding.

7.1.1.2 Dual Agonist and Antagonist Activity

In addition, many GPCRs can stimulate multiple signaling systems, and a given ligand can excite different pathways with different relative efficiency up to opposite activity. For example, agonists of β2-adrenoceptors for the arrestin–MAPK pathway act as antagonists for the Gs–cAMP–PKA axis.

7.1.2 Alternative Splicing of G-Protein-Coupled Receptors

Alternative splicing diversifies the receptorome and augments the number of GPCR types encountered on cell surfaces, hence heterogeneity in GPCR signaling. In humans, airway smooth muscle cells are endowed with multiple types (>350) of G-protein-coupled receptors. More than 190 GPCRs have, on average, 5 different receptor isoforms that can result from various ways of alternative splicing of pre-mRNAs [764] (Vol. 1 – Chap. 5. Protein Synthesis).

1. A huge number ($O[100]$) of GPCR types are coupled to many kinds ($O[10]$) of G proteins and several members ($O[1]$) of the arrestin family.

Table 7.1. Types of subunits of guanine nucleotide-binding (G) proteins and triggered pathways (Source: [765]; ACase: adenylate cyclase; cAMP: cyclic adenosine monophosphate; DAG: diacylglycerol; cGMP: cyclic guanosine monophosphate; GEF: guanine nucleotide-exchange factor; IP_3: inositol (1,4,5)-trisphosphate; PDE: phosphodiesterase; PI3K: phosphatidylinositol 3-kinase; PIP_3: phosphatidylinositol triphosphate; PKC: protein kinase C; PKG: protein kinase G; PLC: phospholipase C; $\ominus\longrightarrow$: inhibition).

Type	Targets and signaling axis
	Gα subunit
$G\alpha_{i1/2/3}$	$\ominus\longrightarrow$ACase
$G\alpha_{i1/2}$	cGMP–PKG–PDE
$G\alpha_{o1/2}$	$\ominus\longrightarrow$ACase
$G\alpha_{t}$	PDE6–cGMP
$G\alpha_{z}$	K^+ channel closure, $\ominus\longrightarrow$exocytosis
$G\alpha_{q}$	PLCβ–IP_3–Ca^{++} PLCβ–DAG–PKC
$G\alpha_{11}$	PLCβ
$G\alpha_{s}$	ACase–cAMP
$G\alpha_{12/13}$	RhoGEF–RhoA
$G\alpha_{14/16}$	PLCβ
	G$\beta\gamma$ dimer
G$\beta\gamma$	ACase–cAMP PLC–IP_3/DAG Rac-cytoskeleton CDC42-cytoskeleton PI3K–PIP_3 GIRK (depolarization) $Ca_V1.2$–Ca^{++} influx

7.1.3 GPCR–G-Protein Coupling

Monomeric GPCR embedded in the lipid bilayer [2] is the minimal, but not necessarily optimal, element required to couple heterotrimeric guanine nucleotide-binding (G) proteins that are composed of a Gα subunit and G$\beta\gamma$ dimer (Vol. 4 – Chap. 8. Guanosine Triphosphatases and Their Regulators; Table 7.1). In humans, at least 18 types of Gα, 5 types of Gβ, and 11 types of Gγ subunits exist.

Binding of GPCR to Gα subunit of G proteins especially targets the receptor-binding region composed of the Ras-like domain and possibly its close sequences on the C-terminal region [766].

2. G$\alpha\beta\gamma$ trimers may interact with: (1) the lipid bilayer via the lipid modifications at the N-terminus of Gα and C-terminus of Gγ as well as (2) the cavity formed in the active GPCR via the C-terminus of Gα [766].

Formation and stability of a GPCR–G-protein complex depend on GPCR agonist efficacy and the presence or absence of cytosolic guanine nucleotides. Conversely, signal deactivation is achieved by Gα-mediated GTP hydrolysis (GTPase activity) that is enhanced by the GTPase-accelerating activity of regulators of G-protein signaling (RGS).

The GPCR receptors and G proteins may exist in a precoupled state, via direct, more or less short-lived GPCR–G-protein interactions or scaffold proteins. Constitutive activity of many GPCRs occurs in the absence of agonists. Whereas neutral antagonists inhibit agonist binding, but do not affect GPCR constitutive (agonist-independent) activity, inverse agonists block the constitutive activity. Inverse agonists can prevent the formation of GPCR–G-protein complex and destabilize preformed complexes.

7.1.3.1 Allosteric Activation

Activation of GPCR happens via molecular switches, such as breaking of a hydrogen bond and electrostatic interactions that stabilize the basal state of the receptor, as well as conformational change of amino acid residues. Some switches are interdependent. Different ligands can activate specific sets of switches, as they disrupt various intramolecular interactions, thereby triggering different receptor conformations and distinct effects on signaling mediators. Receptor conformation destabilization and full activation time scales are O[10 ns] and O[1 ms], respectively.

7.1.3.2 GDP- and GTP-Binding Cycle of G-Proteins

Guanine nucleotide-binding heterotrimers regulate signal transduction via a cycle of GDP- and GTP-bound states. Once the ligand is bound, activated GPCRs serve as guanine nucleotide-exchange factors (GEF) that promote GTP binding to heterotrimeric G proteins, hence activating these G proteins (Table 7.2). Interactions between GPCRs and G proteins represent the signaling interface from which signals are transmitted within the cell.

7.2 GPCR Ligands

G-protein-coupled receptors are activated by multiple agonists (Table 7.3). Responses that result from GPCR activation can integrate several intracellular signaling pathways. Ligands are known for multiple GPCRs [767] (Tables 7.4 to 7.11). G-protein-coupled receptors that do not possess any yet identified endogenous ligand are called orphan GPCRs.

7.3 Adhesion G-Protein-Coupled Receptors

Adhesion G-protein-coupled receptors are plasmalemmal molecules that possess a large extracellular domain coupled to a multitransmembrane region. The G-protein-coupled receptor-proteolytic site (GPS) gives rise to an autocatalytic processing of

Table 7.2. Active, GTP-bound G protein and triggered signal (Source: [765]; AMPAR: AMPA-type glutamate receptor; CFTR: cystic fibrosis transmembrane conductance regulator; CNG: cyclic nucleotide-gated channel; FBP(2)P: fructose (2,6)-bisphosphate 2-phosphatase; PDE: phosphodiesterase; PhK: phosphorylase kinase; PLn: phospholamban; RyR: ryanodine-sensitive Ca^{++} channel; SERCA: sarco(endo)plasmic reticulum calcium ATPase).

Cytoskeleton remodeling
G protein^{GTP}–RhoGEF–Rho
Ion channel modulation
Redox signaling via NOx and ROS
MAPK signaling
PI3K signaling
PLC/PLD signaling
cAMP signaling
G protein^{GTP}–ACase–cAMP–PDE G protein^{GTP}–ACase–cAMP–ABCc4–cAMP efflux
G protein^{GTP}–ACase–cAMP–CNG
G protein^{GTP}–ACase–cAMP–RapGEF3/4–Rap1–PLCϵ
cAMP–PKA axis
Ion fluxes
G protein^{GTP}–ACase–cAMP–PKA–Ca_V1.1/1.2
G protein^{GTP}–ACase–cAMP–PKA–RyR
G protein^{GTP}–ACase–cAMP–PKA–Plb–SERCA
G protein^{GTP}–ACase–cAMP–PKA–CFTR
G protein^{GTP}–ACase–cAMP–PKA–AMPAR
G protein^{GTP}–ACase–cAMP–PKA–PDE
Transcription
G protein^{GTP}–ACase–cAMP–PKA–CREB
Metabolism
G protein^{GTP}–ACase–cAMP–PKA–lipase
G protein^{GTP}–ACase–cAMP–PKA–PhK
G protein^{GTP}–ACase–cAMP–PKA–FBP(2)P

the polypeptide into an extracellular α and a membrane-spanning β chain that associate at the plasma membrane.

7.3.1 EGF-TM7 Class Members

Some adhesion G-protein-coupled receptors contain 3 to 5 consecutive epidermal growth factor (EGF) modules linked via a mucin-like spacer to a 7-span transmembrane class-A G-protein-coupled receptor. Receptors of the EGF-TM7 category are expressed predominantly on immunocytes.

First identified members of the EGF-TM7 class comprise CD97 and EGF-like module-containing, mucin-like, hormone receptor-like proteins EMR1 to EMR4

Table 7.3. Various GPCR ligands activate directly and indirectly cytoplasmic and nuclear signaling mediators using G-protein-dependent and -independent pathways (Source: [768]). Some members of the GPCR superclass (e.g., α- and β-adrenergic, muscarinic cholinergic, angiotensin-2, and endothelin-1 receptors) mediate the effect of various hormones and neurotransmitters (e.g., adrenaline, noradrenaline, acetylcholine, angiotensin-2, and endothelin-1) that control the activity of the cardiovascular and ventilatory systems. The GPCR receptors bind not only to their ligands (also called agonists or stimulators), but also associate with heterotrimeric G proteins to exert signaling and inhibitors serine/threonine GPCR kinases and β-arrestins for signaling termination. Yet, β-arrestins also act as signal transducers and adaptors.

Biogenic amines	Adrenaline, noradrenaline, dopamine, serotonin, acetylchonine, histamine
Amino acids	Glutamate, γ-aminobutyric acid
Peptides	Angiotensin, bradykinin, thrombin, bombesin, endorphins, follicle-stimulating hormone, leuteinizing hormone, thyroid-stimulating hormone gastrin-releasing peptide, cholecystokinin, neuromedin-B, neurotensin, vasopressin, galanin
Lipids	Lysophosphatidic acid, sphingosine-1-phosphate, prostaglandins, leukotrienes, platelet-activating factor, anandamine
Ions	Calcium
Nucleosides Nucleotides	Adenosine ADP, ATP, UDP, ^{UDP}glucose, UTP
Miscellaneous	Light, odorants, pheronomes

(Vol. 1 – Chap. 7. Plasma Membrane). This class also comprises: (1) brain-specific angiogenesis inhibitor BAI1 to BAI3; (2) cadherin, EGF-like, LAG-like, and seven-pass receptors CELSR1 to CELSR3; (3) latrophilins Lphn1 to Lphn3; and (3) orphan G-protein-coupled receptors GPR56, GPR64, GPR97, GPR110 to GPR116, and GPR123 to GPR128.

Leukocyte-restricted adhesion G-protein-coupled receptors of the EGF-TM7 class include mainly CD97 and EMR1 to EMR4 that undergo alternative splicing. Myeloid-restricted EGF-like module-containing, mucin-like, hormone receptor-like protein EMR2 contains a GPS in the membrane-proximal region. Following the cleavage (Leu517–Ser518), which is independent of the transmembrane domains, the non-covalent association of the resulting extracellular α subunit and transmembrane β subunit can occur [771]. [3]

3. The GPS is necessary, but not sufficient for receptor cleavage, which requires the entire extracellular segment. An alternatively spliced EMR2 isoform with a truncated extracellular segment fails to undergo proteolysis.

Table 7.4. Ligands of G-protein-coupled receptors: (**Part 1**) amino acids, dicarboxylic acids, and biogenic amines (Source: [767]; GABA: γ-aminobutyric acid).

Ligand	Receptor	Gα
	Amino acids	
^{L}Arginine, ^{L}lysine	GPRc6a	aGq/11
Glutamate	$mGlu_{1,5}$	Gq/11
	$mGlu_{2,3,4,6,7,8}$	Gi/o
GABA	$GABA_{B1}$ (binding)	Gi/o
	$GABA_{B2}$ (signaling)	
	Dicarboxylic acids	
α-Ketoglutarate	GPR99	Gq/11
Succinate	GPR91	Gq/11, Gi/o
	Biogenic amines	
Acetylcholine	M_1,M_3,M_5	Gq/11
	M_2, M_4	Gi/o
Adrenaline,	α1a,1b,1d	Gq/11
noradrenaline	α2a,2b,2c	Gi/o
	β1,2,3	Gs
Dopamine	D_1,D_5	Gs
	D_2,D_3,D_4	Gi/o
Histamine	H_1	Gq/11
	H_2	Gs
	H_3,H_4	Gi/o
Melatonin	MT_1,MT_2,MT_3	Gi/o
Serotonin	$5HT_{1A/1B/1D/1E/1F}$	Gi/o
	$5HT_{2A/2B/2C}$	Gq/11
	$5HT_4$,$5HT_6$,$5HT_7$	Gs
	$5HT_{5A/5B}$	Gi/o, Gs
Trace amines	TA_1, TA_2	Gs

The vasculature can experience an organ-specific development. In particular, the specialized vasculature of the central nervous system possesses a strong limitation in molecular permeability through the strongly polarized,[4] tightly sealed endothelial cells, extensive pericyte coverage, and reciprocal interactions with neurons and glial cells. Moreover, it acts as a neural stem cell niche. The blood–brain barrier is able to insulate the central nervous system from substances that can be tolerated by peripheral organs. The pro-angiogenic, endothelial, adhesion G-protein-coupled receptor GPR124[5] is involved in the development of endothelial cells and pericytes of the

4. Molecular carriers are heterogeneously distributed between the luminal and abluminal edges of the plasma membrane.

5. A.k.a. tumor endothelial marker-5 (TEM5). During mouse embryogenesis, GPR124 is expressed in both endothelial cells and pericytes, mainly in the neural tube, and, to a lesser extent, the heart, liver, and kidney, as well as the epithelium of embryonic lung and esophagus

Table 7.5. Ligands of G-protein-coupled receptors: (**Part 2**) ions, nucleotides, and nucleosides (Sources: [767, 769, 770]). Proton-sensing G-protein-coupled receptors sense acidic pH. The GPR4 receptor (or G2 accumulation protein [G2A]) has a low affinity for lysophosphatidylcholine (LPC) and high affinity for sphingosylphosphorylcholine (SPC). On the other hand, GPR132 has a high affinity for LPC and low affinity for SPC. Receptors GPR65 and GPR68 are also called T-cell death-associated gene-8 protein (TDAG8) and ovarian cancer GPCR OGR1 as well as SPC receptor SPC_1, respectively. The GPR4 receptor is involved in angiogenesis primed by SPC in endothelial cells via phosphatidylinositol 3-kinase, protein kinase-B, and vascular endothelial growth factor receptor VEGFR2.

Ligand	Receptor	Gα
	Ions	
Ca^{++}	CaSR	Gq/11, Gi/o
H^{+}	GPR4, GPR65	Gq/11, G12/13
	GPR68, GPR132	Gs
	Nucleotides and nucleosides	
Adenosine	A_1, A_3	Gi/o
	A_{2A} A_{2B}	Gs
ADP	$P2Y_{12}$, $P2Y_{13}$	Gi/o
ADP, ATP	$P2Y_1$	Gq/11
ATP	$P2Y_{11}$	Gq/11, Gs
UDP	$P2Y_6$	Gq/11
^{UDP}glucose	$P2Y_{14}$	Gi/o
UTP, ATP	$P2Y_2$, $P2Y_4$	Gq/11

cerebral vasculature, particularly during CNS-specific angiogenesis that forms the perineural, then periventricular vascular plexi in the forebrain, ventral neural tube, and spinal cord [772, 773]. [6] During adulthood, the brain endothelium remains sensitive to GPR124 receptor.

The recruitment of blood-circulating phagocytes to sites of inflammation and infection begins with activation and interactions of cell adhesion receptors on both leukocytes and endothelial cells. Phagocyte activation is regulated partly by cell-

and mesenchyme. In adult mice, it is exclusively produced in the vasculature with more developed pericyte coating, i.e., in the brain and kidney, pancreas, and corpus luteum [772]. The GPR124 receptor is required for invasion and migration of blood vessels into the neuroepithelium, establishment of the blood–brain barrier, and expansion of the cerebral cortex [773].

6. Deletion of GPR124 causes angiogenesis arrest; overexpression hyperproliferative vascular malformations [772]. The GRP124 receptor regulates CDC42-dependent angiogenic migration and sprouting. Mutations of genes of the Wnt–βCtn and VEGF–Nrp1 pathways, of the helix–loop–helix transcriptional repressors, the inhibitors of DNA binding ID1 to ID3 required to maintain the timing of neuronal differentiation in the embryo and invasiveness of the vasculature [774] (ID1 is an inhibitor of angiogenesis via thrombospondin-1 [775]; ID2 is a target of retinoblastoma protein sufficient and necessary for the production of VEGF [776]), and integrin-α_V and -β_8 also impair angiogenesis in the central nervous system, but without CNS tropism.

Table 7.6. Ligands of G-protein-coupled receptors: (**Part 3**) lipids (Source: [767]; 2AG: 2-arachidonyl glycerol; 5oxoETE: 5-oxo (6,8,11,14)-eicosatetraenoic acid; AEA: ^{N}arachidonoyl ethanolamine (anandamide); G_{Tc}; cone-transducin [G_{T2}]; G_{Tr}: rod-transducin [G_{T1}]; LC-, SCFA: long-, short-chain [<6 carbons] fatty acid; OxER: oxoeicosanoid [OXE] receptor; PAF; platelet-activating factor). Agent 11-cis-retinal covalently binds receptors for light-dependent activation. Agent 5oxoETE is produced by oxidation of 5HETE (HETE: hydroxyeicosatrienoic acid) by 5-hydroxyeicosanoid dehydrogenase (5-lipoxygenase pathway).

Ligand	Receptor	Gα
AEA, 2AG	CB_1, CB_2	Gi/o
11-cis-Retinal	Rhodopsin	G_{Tr}
	Opsins	G_{Tc}
	Melanopsin	Gq/11
	Fatty acids (FA)	
SCFA: C2–C5	GPR41, GPR43	Gi/o, Gq/11
LCFA: C12–C20	GPR40	Gq/11
LCFA: C14–C22	GPR120	Gq/11
5OxoETE	OxER1, GPR170	Gi/o
	Leukotrinenes	
LTB4	BLT	Gi/o
LTC4, LTD4	$CysLT_1$, $CysLT_2$	Gq/11
LXA4	FPRL1 (ALXR)	Gi/o
Lysophosphatidic acid	LPA_1–LPA_3	Gi, Gq/11, G12/13
PAF	PAF	Gq/11
	Prostaglandins	
PGI2	IP	Gs
PGD2	DP	Gs
	$CRTH_2$	Gi
PGF2α	FP	Gq/11
PGE2	EP_1	Gq/11
	EP_2, EP_4	Gs
	EP_3	Gs, Gq/11, Gi
TxA2	TP	Gq/11, G12/13
Sphingosine-1-phosphate	$S1P_1$–$S1P_5$	Gi, Gq/11, G12/13
Sphingosylphosphorylcholine	SPC_1, SPC_2	Gi

surface receptors such as adhesion G-protein-coupled receptors. Epidermal growth factor-like module-containing, mucin-like, hormone receptor-like protein EMR2 is synthesized by neutrophils, monocytes, macrophages, and dendritic cells. It binds to chondroitin sulfate on cells and tissue matrix. It regulates the neutrophil response [777]. It potentiates the effects of numerous pro-inflammatory mediators. Upon neutrophil activation, EMR2 is rapidly translocated to membrane ruffles and the leading edge of the migrating cell and its production can rise. Ligation of EMR2 boosts the activation and recruitment of neutrophils, increases neutrophil adhesion

Table 7.7. Ligands of G-protein-coupled receptors: (**Part 4.1**) peptides and proteins (Source: [767]; CG: chorionic gonadotropin [gonadotrophin] CGRP: calcitonin gene-related peptide; CRF: corticitropin-releasing factor; FSH: follicle-stimulating hormone).

Ligand	Receptor	Gα
Adrenocorticotrophin	MC_2	Gs
Adrenomedullin	AM_1, AM_2	Gs
Amylin	AMY_1–AMY_3	Gs
Angiotensin-2	AT_1 AT_2	Gq/11, G12/13, Gi/o
Apelin	APJ	Gi/o
Bradykinin	B_1, B_2	Gq/11
Calcitonin	CT	Gs, Gq/11
CGRP	$CGRP_1$	Gs, Gq/11
	Chemokines	
CC	CCR1–CCR10	Gi/o
CXC	CXCR1–CXCR6	Gi/o
CX_3C	XCL1, XCL2, CX_3L1	Gi/o
Cholecystokinin (CCK8)	CCK_1, CCK_2	Gq/11, Gs
Complement C3a, C5a	C3a, C5a	Gi/o
CRF, urocortin	CRF_1, CRF_2	Gs
Endothelin-1, -2	ET_A	Gq/11, G12/13, Gs
Endothelin-1, -2, -3	ET_B	Gq/11, G12/13, Gs
FSH	FSH	Gs
Formyl-Met-Leu-Phe	FPR	Gi/o

and migration under both static and flow conditions. Moreover, liganded EMR2 augments superoxide production and proteolytic enzyme liberation, i.e., leukocyte respiratory burst and degranulation [777].

7.3.2 TRPP1 (Polycystin-1)

Polycystin-1 and -2, or transient receptor potential TRPP1 and TRPP2, are encoded by the genes mutated in autosomal dominant polycystic kidney disease. Mutations in the PKD1 (polycystic kidney disease-1, TRPP1, or polycystin-1) and PKD2 (polycystic kidney disease-2, TRPP2, or polycystin-2) genes account for about 85% and 15% of autosomal dominant polycystic kidney disease, respectively. Polycystin-1 (PC1) operates in renal tubule morphogenesis. Proteins TRPP1 and TRPP2 contribute to calcium flux (Sect. 2.3.4.5), regulation of heterotrimeric G proteins, Wnt and STAT signaling, among other functions.

Protein TRPP1 induces the formation of a complex with tuberin, or tuberous sclerosis complex protein TSC2, and TOR, thereby inhibiting TOR activity, hence unappropriate cell growth and proliferation [778]. The TRPP1–TSC2–TOR complex

Table 7.8. Ligands of G-protein-coupled receptors: (**Part 4.2**) peptides and proteins (Source: [767]; CG: chorionic gonadotropin [gonadotrophin]; GHRH: growth hormone-releasing hormone; GHSR: growth hormone secretagogue receptor; GIP: gastric inhibitory polypeptide; GnRH: gonadotropin-releasing hormone; GRP: gastrin-releasing peptide; LH: luteinizing hormone; LHCGR: luteinizing hormone–choriogonadotropin receptor [a.k.a. lutropin–choriogonadotropin receptor (LCGR) and luteinizing hormone receptor (LHR)]; MCH: melanin-concentrating hormone).

Ligand	Receptor	Gα
Galanin and	GAL_1, GAL_3	Gi/o
galanin-like peptide	GAL_2	Gi/o, Gq/11, G12/13
GIP	GIP	Gs
Gastrin	CCK_2	Gq/11
GRP, bombesin	BB_2	Gq/11
Ghrelin	GHSR	Gq/11
Glucagon	GCGR	Gs
Glucagon-like peptide	GLP_1, GLP_2	Gs
GnRH	GnRH	Gq/11
GHRH	GHRH	Gs
Kisspeptins, metastin	GPR54	Gq/11
LH, CG	LHCGR	Gs, Gi
MCH	MCH_1	Gi/o
	MCH_2	Gq/11
Melanocortins	MC_1, MC_3–MC_5	Gs
Motilin	GPR38	Gq/11

may sense renal insults, possibly by primary cilium-mediated mechanotransduction, and trigger a TOR-initiated repair program.[7]

Proteins TRPP1 and TRPP2 represent an adhesion G-protein-coupled receptor and a mechanosensitive, calcium-permeable, non-selective cation channel of the TRP channel family located at the primary cilium that causes calcium transients at the cilium, respectively. Protein TRPP1 can interact with the C-terminus of TRPP2, thereby regulating the TRPP2 activity.[8]

Protein TRPP1 is a multidomain glycoprotein of 4,303 amino acids. It is made of a large extracellular N-terminal region, a membrane-spanning domain with 11 transmembrane segments, and a cytosolic C-terminal segment (Table 7.12). The last

7. The primary cilium of renal epithelial cells is a non-motile, mechanosensory extensions of the apical (luminal) plasma membrane that bends in response to urine flow and provokes a transient rise in the intracellular calcium concentration. Polycystin-1 links ciliary mechanosensation to changes in gene transcription via flow-regulated proteolytic cleavage of the TRPP1 cytoplasmic tail, its nuclear translocation, and stimulation of STAT6 transcriptional activity [778].

8. The cytoplasmic segment serves in the recruitment to the cell surface and interaction with TRPP2 channel. The TRPP1–TRPP2 complex functions as a cation channel.

Table 7.9. Ligands of G-protein-coupled receptors: (**Part 4.3**) peptides and proteins (Source: [767]; DOR, KOR, MOR: δ-, κ-, μ-opioid receptor; PAR: peptidase-activated receptor; PrRP: prolactin-releasing peptide; PTH: parathyroid hormone; PTHRP: parathyroid hormone-related protein). N-Termini of peptidases released by proteolytic cleavage serves as ligands.

Ligand	Receptor	Gα
Neurokinin-A	NK_2	Gq/11
Neurokinin-B	NK_3	Gq/11
Neuromedin-B, bombesin	BB_1	Gq/11
Neuromedin-U	NMU_1, NMU_2	Gq/11
Neuropeptide-FF, -AF	$NPFF_1$, $NPFF_2$	Gi/o
Neuropeptide-W23, -W30	GRP7, GPR8	Gi/o
Neuropeptide-Y	Y_1, Y_2, Y_4–Y_6	Gi/o
Neurotensin	NTS_1, NTS_2	Gq/11
Opioids	DOR, KOR, MOR, ORL1	Gi/o
Orexin-A/B	OX_1, OX_2	Gs, Gq/11
Oxytocin	OT	Gq/11, Gi/o
PTH, PTHRP	PTH1R	Gs, Gq/11
Prokineticin-1/2	PKR_1, PKR_2	Gq/11
PrRP	PRRP	Gq/11
	Peptidase N-termini	
Thrombin	PAR_1, PAR_3, PAR_4	Gq/11, G12/13, Gi/o
Trypsin	PAR_2	Gq/11

6 transmembrane segments are homologous to TRPP2 and voltage-activated calcium channels.

In renal tubules, bending of the wetted primary cilium by urine flow activates TRPP1 and TRPP2 proteins. It can couple with and activate several heterotrimeric G protein subtypes, such as Gi, Gq, G12/13, and stimulate Jun N-terminal kinase and AP1 transcription factor [779]. Protein TRPP1 regulates the cell cycle, as it upregulates cyclin-dependent kinase inhibitor CKI1a and activates the JaK–STAT pathway [780]. In addition, TRPP1 binds to and stabilizes regulator of G-protein signaling RGS7.

Protein TRPP1, like other adhesion G-protein-coupled receptors, can be cleaved at the *GPCR proteolytic site* (GPS).[9] Protein TRPP1 actually undergoes an autoproteolytic GPS cleavage to form an extracellular N-terminal fragment and a membranous C-terminal fragment that remain non-covalently associated.

Cleavage at GPS is autoproteolytic, but not entirely efficient. Therefore, cleaved and uncleaved adhesion G-protein-coupled receptors coexist. Both full length and cleaved fragment of adhesion G-protein-coupled receptor can be involved in cell signaling.

9. The GPS domain alone is not sufficient to support the cleavage and requires the adjacent receptor for egg jelly domain [781].

Table 7.10. Ligands of G-protein-coupled receptors: (**Part 4.4**) peptides and proteins (Source: [767]; InsL: insulin-like peptide; PACAP: pituitary adenylate cyclase-activating polypeptide; Rln: relaxin; RXFP: relaxin–insulin-like family peptide receptor; TRH: thyrotropin-releasing hormone; TSHR: thyroid-stimulating hormone (thyrotropin) receptor; VIP: vasoactive intestinal polypeptide). Receptors PACAPR1 to PACAPR3 are also named PAC_1 and AdCyAP1R1, $VPAC_1$ and vasoactive intestinal polypeptide receptor VIPR1, and $VPAC_2$ and VIPR2.

Ligand	Receptor	Gα
	Relaxins and insulin-like peptides	
Relaxin	$RXFP_1$	Gs
InsL3	$RXFP_2$	Gs
Relaxin-3	$RXFP_3$	Gi
InsL5	$RXFP_4$	Gi
Secretin	SCTR	Gs
Somatostatin	SST_1–SST_5	Gi/o
Substance-P	NK_1	Gq/11
Thyrotropin	TSHR	Gs, Gq/11, Gi, G12/13
TRH	TRH_1, TRH_2	Gq/11
Urotensin-2	UTS2R	Gq/11
VIP, PACAP	PACAPR1–PACAPR3	Gs
Vasopressin	V_{1A}, V_{1B}	Gq/11
	V_2	Gs

After GPS cleavage, the N-terminal α subunit is anchored to the plasma membrane, as it tethers to transmembrane β subunit or connects to the plasma membrane by itself [782]. In addition, N- and C-termini can be internalized independently. Moreover, α and β subunits of different types of adhesion G-protein-coupled receptors can interact.

However, most of the TRPP1 N-terminal fragment ($TRPP1^{\Delta NT}$) is tethered to the membrane-bound C-terminal fragment ($TRPP1^{\Delta CT}$) in a non-covalent manner (similar to that for long N-terminal class-B GPCR-related 7-transmembrane receptors latrophilin-1 and CD97).

Proteolysis at the GPS that occurs at the endoplasmic reticulum may assist in the transfer to the plasma membrane. However, at least for some adhesion G-protein-coupled receptors such as TRPP1, it is not required for an efficient transport to the plasma membrane [782]. In any case, GPS cleavage is mandatory for a normal receptor functioning. The final, mature, plasmalemmal receptor may be a heterodimer or 2 independent proteins [782]. Membrane-bound N-terminus may either be released in the extracellular medium, or remains connected to the cell surface and bind its cognate ligands and then transmits cues. The C-terminal transmembrane segment subunit may act as a classical GPCR; it can reassociate with the liganded N-terminal subunit [782].

Table 7.11. Ligands of G-protein-coupled receptors: (**Part 5**) sensory receptors (Source: [767]; G_{gust}: gustducin; G_{olf}: olfaction G-protein subunit; G_{Tc} and G_{Tr}: cone- and rod-transducin). Visual signals are sensed by opsins that use a photo-isomerization reaction to translate electromagnetic waves into cellular signals. Opsin signaling is based on the conversion of 11-cis-retinal to all-trans-retinal. Light is an multichromatic electromagnetic wave (visible spectrum wavelength 380–780 nm, i.e. 400–790 THz, with a maximum sensitivity $\sim$ 555 nm). In humans, 4 opsin types exist in addition to rhodopsin. They lodge in different types of cone cells of the retina. They have absorption maxima for yellowish-green, green, and bluish-violet light. Smell sensing is carried out by receptors of the olfactory epithelium that bind odorants (olfactory receptors) and pheromones (vomeronasal receptors). Because of their similar aliases, pheromone receptors V_1R and V_2R should not be mistaken for $V_{1A/1B/2}$ vasopressin receptors.

Ligand	Receptor	Gα
	Light	
Absorption maximum		
$\sim$ 500 nm	Rhodopsin	G_{Tr}
$\sim$ 426 nm	Violet opsin	G_{Tc}
$\sim$ 530 nm	Green opsin	G_{Tc}
$\sim$ 560 nm	Yellow opsin	G_{Tc}
425–480 nm	Melanopsin	Gq/11
	Taste	
Umami	$T_1R1 + T_1R3$	G_{gust}
	mGluR4	Gi/o
Sweet	$T_1R2 + T_1R3$	G_{gust}
Bitter	T_2R ($\sim$25 in humans)	G_{gust}
Odorants ($\sim$ 350 in humans)		G_{olf}
Pheromones	V_1R group (few in human)	
	V_2R group (none in human)	

Table 7.12. Structure of Polycystin-1 (or TRPP1; Source: Wikipedia). Polycystin-1 contains a large extracellular N-terminal domain with a combination of functional motifs, an odd number of transmembrane segments (TM), and an intracellular C-terminal domain. The extracellular segment is composed of the N-terminus (NT), a cysteine-rich segment (CRS), leucine-rich repeats (LRR), wall cell integrity and stress-response component (WSC), polycystic kidney disease repeats (PKD), C-lectin motif (CLec), low-density lipoprotein-A sequence (LDLa), receptor for egg jelly (REJ), and a G-protein-coupled receptor proteolytic site (GPS). The cytoplasmic segment contains a lipooxygenase homolog-2 (LH2), TMs, a coiled-coil domain (CCD), and the C-terminus (CT).

Extracellular part	NT–CRS–LRR–WSC–PKD–CLec–LDLa–PKD–REJ–GPS
Intracellular and cytosolic part	LH2–TM–CCD–CT– –Polycystin-2 (CT–EF hand–TM–NT)

7.4 Proton-Sensing G-Protein-Coupled Receptors

Proton-sensing G-protein-coupled receptors pertain to the subclass A15. They sense acidic pH; they are indeed activated when the extracellular pH falls below 6.8 (acidosis, i.e., increased hydrogen ion concentration). [10]

They include GPR4, GPR65, [11] GPR68, [12] and GPR132. [13] The latter is an immunoregulatory receptor on macrophages for lysophosphatidylcholine, which is a major phospholipid component of oxidized low-density lipoproteins in atherosclerosis. [783]. The GPR132 receptor resides also on lymphocytes [784]. Activation of GPR132 by lysophosphatidylcholine may increase intracellular calcium concentration, cause receptor internalization, and activate extracellular signal-regulated kinases. The GPR65 receptor on the surface of tumor cells facilitates tumor development by sensing the acidic environment [785].

7.5 GPCR Classification

The superclass of G-protein-coupled receptors contains many hundreds of olfactory receptors to detect a wide variety of olfactory (exogenous) ligands and more than 360 endoGPCRs that are targeted by endogenous (non-olfactory) ligands. The GPCR superclass is constituted by several classes.

On the basis of their sequence and structural similarity, ligand interaction, and phylogeny, GPCRs have been originally grouped into A to F or 1 to 5 classes. [14] Phylogenetic analysis of the human repertoire yields the alternative GRAFS classification with 5 families: glutamate (G), rhodopsin (R), adhesion (A), Frizzled–Taste-2 (F), and secretin (S) [788].

The number of elements in each class varies according to literature data, and especially publication date, as new proteins are discovered (Table 7.13).

The class of rhodopsin GPCRs is the largest set with about 672 members in the human genome ($\sim$388 olfactory receptors). Its members are often encoded by single exons or genes with a small number of introns. Isomerization of 11-cis-retinal

10. Acidosis is usually detected in blood (acidemia; arterial pH $<$7.35).

11. A.k.a. T-cell death-associated gene-8 (TDAG8) and psychosine receptor. It is overexpressed in various tumor cell types.

12. A.k.a. ovarian cancer G-protein coupled receptor-1 (OGR1).

13. A.k.a. cell cycle phase G2 accumulation protein (G2A).

14. The families comprise Rhodopsin (class A with more than 270 members), Secretin (at least 15 receptors) and Adhesion (33 members; class B), metabotropic glutamate (class C; 15 receptors), fungal mating pheromone receptors (class D), cAMP receptors (class E), and Frizzled–Smoothened–Taste-2 (class F; 24 members) classes [786, 787]. The very large Rhodopsin class A is subdivided into 19 groups (A1–A19). Class-B GPCRs include both Adhesion and Secretin families. Class-B GPCRs are also called class-2 GPCRs. Taste-2 receptors sense bitter substances. Another classification scheme of GPCRs is based on class 1 Rhodopsin-like, class 2 Secretin-like and Adhesion, class 3 Glutamate receptor-like, class 4 Frizzled–Taste-2, and class 5 Miscellaneous (Others, including unclassified GPCRs).

Table 7.13. Estimated GPCR number per class in humans (Source: [789]). Rhodopsin consists of the protein moiety opsin that is reversibly, covalently bound to its cofactor retinal. The latter is produced in the retina from vitamin-A. Photo-isomerization of retinal is the primary vision event that creates photosensory signals via retinal interactions with other proteins.

Class (Group)	Number
Glutamate	22
Rhodopsin-α	101
Rhodopsin-β	43
Rhodopsin-γ	64
Rhodopsin-δ	63
Adhesion	33
Frizzled	11
Taste-2	25
Secretin	15
Pheromone V_1R	3
Olfactory	388
Others	23

Table 7.14. Visible light spectrum.

Color	Wavelength (nm)
Violet	380–450
Blue	450–475
Cyan	476–495
Green	495–570
Yellow	570–590
Orange	590–620
Red	620–750

into all-trans-retinal by light induces a conformational change in opsin that activates the associated G protein and triggers a signaling cascade. In humans, several opsin types exist in addition to rhodopsin. They reside in different types of cone cells of the retina. They have absorption maxima for yellowish-green ($\sim$ 560 nm), green ($\sim$ 530 nm), and bluish-violet ($\sim$ 426 nm) light (Table 7.14).

The class of adhesion GPCRs that comprises 33 members is the second largest GPCR set in humans. Mammalian adhesion GPCRs are encoded by large, complex genomic structures with several introns that give rise to alternatively spliced variants.

The class of secretin GPCRs members have a peptide hormone-binding domain. They likely originates from ancestors to the class of adhesion GPCRs.

Class-1 GPCRs, the largest and most diverse class of GPCRs, encompass more than 400 sensory receptor types involved with the detection of taste, odor, or light, as well as more than 250 non-sensory receptor types for transmitters or chemical messengers, in addition to orphan receptors.

Class-2 GPCRs comprise 20 receptor types that are activated by peptides, in addition to orphan receptors. Their ligand peptides include transmitters, adrenomedullin, calcitonin gene-related peptide, vasoactive intestinal peptide, and urocortins.

Class-3 GPCRs include 11 metabotropic receptors that are activated by glutamate and γ-aminobutyric acid as well as orphan receptors. These receptors localize presynaptically to perivascular nerves, but are generally not expressed in endothelia and smooth muscles.

Class-4 GPCRs incorporate receptors of the Frizzled and Smoothened families that are implicated in vascular remodeling.

Many GPCR classes can be decomposed into GPCR subclasses and families (Tables 7.15 and 7.16). Each GPCR category contains a variable number of receptors.

7.6 Structure and Function

Synthesis of GPCR and their activity are tightly controlled. Availability of GPCRs relies not only on its production and exocytosis, but also on desensitization via endocytosis and resensitization by endosome recycling. Responsiveness of GPCRs depends on associated regulators, such as G proteins, kinases (feedback loops mediated by PKA and PKC) and phosphatases, as well as membrane partners.

Ligand binding on G-protein-coupled receptors can be voltage sensitive, as GPCRs can serve as sensors for both transmembrane potential and external compounds [791]. Voltage-sensitive G-protein-coupled receptors are triggered by the voltage across the plasmalemma.[15] Gating currents act on structural GPCR components.

7.6.1 GPCR Structure

G-protein-coupled receptors share a common structure of 7 membrane-spanning helices (transmembrane domains TM1–TM7) connected by 3 loops on each side of the membrane (intra- [IL1–IL3] and extracellular [EL1–EL3] loops, with an extracellular N- and intracellular C-terminus. Extra- and intracellular loops serve as binding sites for agonists (activators) and G proteins, respectively.

The structure, conformation, and specificity of the G-protein binding site can depends on the ligand type. The ends of the third intracellular loop are involved in G-protein activation and selectivity of GPCR–G-protein interactions, as well as interactions with G-protein-coupled receptor kinases, arrestins, and other signaling molecules.

Ligand binding causes a conformational change in the GPCR cytoplasmic domain. Class-A GPCR activation leads to an outward displacement of transmembrane helix 6 that opens for G-protein binding [792]. The activation rate of α2a-adrenergic

15. The resting potential difference across the 3-nm-thick plasmalemma is equal to about 70 mV. It can affect the conformation of membrane proteins.

Table 7.15. Classes of GPCRs. (**Part 1**) class A (GRH: gonadotropin-releasing hormone; TRH: thyrotropin-releasing hormone; Source: [790]). Platelet-activating factor (PAF) receptor initiates cell response to its phospholipid ligand that has potent platelet aggregating and inflammatory effects and elicits smooth muscle contraction.

Sub-class	Ligand family
Amine	Cathecolamines, dopamine, histamine, acetylcholine (muscarinic), serotonin
Cannabinoid	2-Arachidonoyl glycerol
Peptide	Adrenomedullin, angiogenin-like, angiotensin, bombesin, C5a-anaphylatoxin, galanin-like, interleukin-8, chemokine, bradykinin, cholecystokinin, endothelin, melanocortin, melanin-concentrating hormone, neuromedin-U-like, neuropeptide-Y, neurotensin, orexin and neuropeptides-FF, prokineticin, proteinase-activated-like, somatostatin, tachykinin, vasopressin-like, urotensin-2
GRH	
Hormone	Follicle-stimulating hormone, lutropin-choriogonadotropic hormone, thyrotropin, gonadotropin-1 and -2
Leukotriene-B4	
Lysosphingolipid	Lysophosphatidic acid, sphingosine 1-phosphate
Melatonin	
Nucleoside Nucleotides	Adenosine Purines (ADP, ATP), pyrimidines (UDP, UTP)
(Rhod)opsin	
Olfactory	
PAF	
Prostanoid	Prostaglandin, prostacyclin, thromboxane
TRH	

receptor and parathyroid hormone receptor are 40 ms and 1 s, respectively. The kinetics of the GPCR–G-protein complex formation depends on the cell concentration of G proteins.

Table 7.16. Classes of GPCRs (**Part 2**; Source: [790]).

Class	Types
B	Brain-specific angiogenesis inhibitor
	Calcitonin
	Corticotropin-releasing factor
	Diuretic hormone
	Gastric inhibitory peptide
	Growth hormone-releasing hormone
	Glucagon
	Latrophilin
	Methuselah-like proteins
	Parathyroid hormone
	Secretin
	Vasoactive intestinal polypeptide
	Very large G-protein-coupled receptor
C	Bride of sevenless proteins
	Calcium-sensing-like
	$GABA_B$
	Metabotropic glutamate
	Orphan GPCR5
	Orphan GPCR6
	Putative pheromone receptors
	Taste receptors
D	Fungal pheromone
E	cAMP receptors
F	Frizzled
	Smoothened

7.6.2 GPCR Signaling

G-protein-coupled receptors interact with guanine nucleotide–binding (G) proteins (Vol. 4 – Chap. 8. Guanosine Triphosphatases and Their Regulators) to transduce signals transmitted by messengers in the cardiovascular and ventilatory apparatus among others physiological systems. [16] Ligand-activated receptors catalyze the GDP–GTP exchange at a coupled G protein, and thereby promote the activity of effectors (second messenger–producing enzymes and ion channels). G-protein-coupled receptors thus act as guanine nucleotide-exchange factors (GEF) for G protein subunits. Release of GDP is the rate-limiting step in G-protein activation. After receptor activation, G protein binds to the receptor, the subsequent conformational change decreasing the affinity of the G protein for guanosine diphosphate.

16. On the cardiomyocyte sarcolemma, angiotensin-2, endothelin-1, noradrenaline, and prostaglandin-F2α activate receptors coupled to a Gq subunit.

Most receptors are able to activate more than one type of G protein. They thus trigger several signal transduction cascades. However, some receptors interact only with G-protein isoforms of the same class.

Many GPCRs have a complex signaling behavior. In cardiomyocytes, β2-adrenoceptors couple to both stimulatory ($G\alpha_s$) and inhibitory ($G\alpha_i$) subunits that target adenylate cyclase as well as, upon phosphorylation by a G-protein-coupled receptor kinase, arrestins to signal via MAPK pathways (Vol. 4 – Chap. 5. Mitogen-Activated Protein Kinase Modules) in a G-protein-independent manner. Desensitization of GPCRs involves multiple processes, such as receptor phosphorylation and arrestin-mediated endocytosis followed by receptor recycling or lysosomal degradation. Furthermore, GPCR oligomerization and localization to specific membrane compartments influence GPCR activity.

G-protein-coupled receptors signal not only via heterotrimeric G proteins, but also various small monomeric GTPases to regulate the activity of effectors (Tables 7.17 to 7.19). Subunit Gα is composed of a GTPase and a helical domain [792]. The GTPase domain hydrolyzes GTP and provides the binding site for Gβγ dimer, GPCR, and effectors. It contains 3 flexible loops. The helical domain comprises 7 α-helices. Post-translational modifications of Gα regulate membrane location and between-protein interactions. All Gα, except $G\alpha_t$, undergo palmitoylation at their N-termini. Certain $G\alpha_i$ are also myristoylated at their N-termini. Binding of GTP on a Gα subunit induces a structural rearrangement of the GPCR–G-protein complex that destabilizes this complex to interact with effector; some complexes dissociate, but not all.

Subunits Gβ and Gγ form a functional unit. Several Gβγ dimers can interact with the same Gα isoform. Subunit Gβγ binds GPCRs and helps stabilize the GPCR–Gα interface. Subunit Gβ has a 7-bladed β-propeller structure with an α-helical N-terminus that forms a coiled-coil with N-terminus of Gγ [792]. The C-terminus of Gγ binds to blades 5 and 6. All Gγ undergo post-translational isoprenylation of their C-terminus with either a farnesyl (Gγ1, Gγ8, and Gγ11) or geranylgeranyl moiety.

Subunits Gα and Gγ interact sequentially with activated GPCR to dock G protein onto the receptor. Activated Gα and Gβγ proteins positively or negatively regulate various effectors such as phospholipase-A, -C, and -D,[17] adenylate and guanylate cyclases, phosphoinositide 3-kinases, phosphodiesterases, protein kinase-C, and ion channels (Table 7.20, Fig. 7.1).[18]

17. Phospholipases PLD1 and PLD2 regulate cytoskeletal organization and endo- and exocytosis. G-protein-coupled receptor signaling directs stimulation of PLD1 by PKC.

18. Transiently activated Gs-coupled β-adrenergic receptors cause a calcium influx through Ca_V1 channels that are phosphorylated by cAMP-dependent protein kinase-A [793]. (Mediator cAMP is produced in response to Gs activation.) On the other hand, sustained activation of β-adrenergic receptors causes their phosphorylation by GPCR kinases for recruitment of β-arrestin-1 and endocytosis. Additional activation of Gi by β2-adrenergic receptors explains the difference in signaling by β1- (the predominant subtype in cardiomyocytes) and β2-adrenergic receptors. Subunit Gi slows down the heart rate and atrioventricular conductance. Isoform Gi2, the major Gi subunit in cardiomyocytes, represses the activity of Ca_V1 channels.

Table 7.17. Subunit Gα of trimeric guanine nucleotide-binding proteins and their distribution and effectors (**Part 1**; Source: [767]; ACase: adenylate cyclase; AC*i*: adenylate cyclase isoform *i*; Ca_V: voltage-gated Ca^{++} channel; GIRK: Gβγ-regulated inwardly rectifying K^+ channel [$K_{IR}3$]; G_{Tc}, G_{Tr}: cone and rod transducin; PDE: phosphodiesterase). Gα subunits define the basic properties of a heterotrimeric G protein. They can be classified into 4 families, $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$, and $G\alpha_{12/13}$. In addition to Gs, 2 transcripts that encode $XL_{\alpha s}$ and neuroendocrine secretory protein NESP55 are generated by promoters upstream of the GNAS (Gs-encoding) promoter of the GNAS complex locus. Extra-large protein Gs_{XL} that has a limited expression pattern (adrenal gland, heart, pancreas, brain, and pituitary pars intermedia) is able to bind to Gβγ dimer and provoke cAMP synthesis. The GNASXL gene also produce ALEX that inhibits Gs_{XL}. In addition, exon 3 of the GNAS gene is alternatively spliced to give rise to long (Gs_L) and short (Gs_S) Gs isoforms.

Type	Distribution	Effectors
	$G\alpha_s$ class	
Gs	Ubiquitous	ACases (+: all types)
Gs_{XL}	Neuroendocrine cells	ACases (+)
$G\alpha_{olf}$	Olfactory epithelium, brain	ACases (+)
	$G\alpha_{i/o}$ class	
Gi1	Wide	AC1/3/5/6/8/9 (−)
Gi2	Ubiquitous	AC1/3/5/6/8/9 (−)
Gi3	Wide	AC1/3/5/6/8/9 (−)
Go	Neurons, neuroendocrine cells	Ca_V (−), GIRK (+) (via Gβγ)
Gz	Neurons, platelets	AC5/6 (−), Rap1GAP
$G\alpha_{gust}$	Taste and brush cells	PDE (+)
G_{Tr}	Retinal and taste cells	PDE6 (+)
G_{Tc}	Retina	PDE6 (+)

Subsequently, these effectors activate or inhibit the production of second messengers (cAMP, cGMP, diacylglycerol, inositol trisphosphate, phosphatidylinositol

Go-Coupled muscarinic channels M_2 also inhibit Ca_V1 channels. In enteroendocrine cells, bitter taste receptors (T_2R) and $G\alpha_{gust}$ of the Gi/o group increase Ca^{++} influx via activated Ca_V1 channels [794]. In the hippocampus, activated Gi/o-coupled adenosine receptor A_1 suppresses serotonin release, as it inhibits $Ca_V2.2$ and $Ca_V2.1$ channels (more precisely $Ca_V2.2$–PKC–syntaxin and $Ca_V2.1$–PKA–synaptobrevin axes) [795]. On the other hand, activated Gs-coupled A_2 stimulates serotonin release, as it stimulates $Ca_V2.1$ channels ($Ca_V2.1$–PKA–synaptobrevin axis). The 3 Gi subtypes (Gi1–Gi3) activate K^+ channels $K_{IR}3$ (K_{ACh} current) of atriomyocytes (Gβγ dimer is inactive) [796]. Gi/o-Coupled glutamate receptor mGluR7 is able to inhibit $Ca_V2.1$ channels via Gβγ dimer. Upon Ca^{++} influx, Ca^{++}–calmodulin binds to macrophage myristoylated alanine-rich C-kinase substrate (macMARCKS) that is tethered to mGluR7 [797]. It then displaces macMARCKS and Gβγ from mGluR7 receptor. Gβγ Dimer can then inhibit $Ca_V2.1$ channel. In sympathetic neurons, Gβγ inhibits $Ca_V2.2$ channels upon stimulation by noradrenaline and somatostatin [798].

Table 7.18. Subunit Gα of trimeric guanine nucleotide-binding proteins and their distribution and effectors (**Part 2**; Source: [767]; BTK: Bruton Tyr kinase; HAX1: hematopoietic cell-specific Lyn substrate [HCLS1]-associated protein-X1 [anti-apoptotic]; HSP: heat shock protein [chaperone]; JNK: Jun N-terminal kinase; PP: protein phosphatase; RasA2: Ras P21 protein activator-2 [RasGAP2]). Subunits G15 and G16 are the murine and human ortholog, respectively. Enzymes PLCβ1, -3, and -4 are the major effectors of Gq/11 and PLCβ2 is a minor effector. Subunits G12 and G13 are often activated by Gq/G11-coupled receptors (crosstalk). Cadherins (a portmanteau word for calcium-dependent adhesion) are type-1 transmembrane proteins and constituents of adherens junction. They are connected to nearby actin filaments via α-actinin and cortical actin via catenin and vinculin. Radixin is a cytoskeletal linker of actin to the plasma membrane. Members of the $G\alpha_i$, $G\alpha_q$, and $G\alpha_{12/13}$ families cooperate to regulate Ca^{++} signal and nitric oxide (NO). Subunit $G\alpha_q$ targets phospholipase-C and causes Ca^{++} influx without the assistance of NO. Subunit $G\alpha_{13}$ supports Ca^{++} influx by a mechanism dependent on NO, guanylate cyclase, and cGMP (a process blocked by activated adenylate cyclase, the cAMP–PKA axis preventing Ca^{++} influx and NO production). NO may relieve inhibition of adenylate cyclase caused by Gi subunit via adpribosylation of Gi subunit.

Type	Distribution	Effectors
		$G\alpha_{q/11}$ class
Gq	Ubiquitous	PLCβ1–PLCβ4 (+)
G11	Very wide	PLCβ1–PLCβ4 (+)
G14	Lung, kidney, spleen	PLCβ1–PLCβ4 (+)
G15/16	Hematopoietic cells	PLCβ1–PLCβ4 (+)
		$G\alpha_{12/13}$ class
G12	Ubiquitous	RhoGEF1, RhoGEF11, RhoGEF12, RasA2, BTK, cadherin
G13	Ubiquitous	RhoGEF1, RhoGEF11, RhoGEF12, radixin
G12/13		PLA2, Na^+–H^+ exchanger, JNK, PP5, HAX1, HSP90, AKAP3

(3,4,5)-trisphosphate, and arachidonic and phosphatidic acids), and promote calcium influx, and opening or closure of various ion channels.

A relatively small number of G proteins transduce signals from a large number of GPCRs. Each member of the G-protein family is thus able to interact with many different GPCRs. In addition, many GPCRs can activate multiple G proteins. Suitable signal transduction relies on specific interactions. Each contact site of Gα subunits, as well as other molecular regions, contribute to coupling specificity [792]. In addition, specific isoforms of Gβ interact preferentially with specific GPCRs. [19] The

19. Subunits Gβ4 and Gβ5 have the strongest and the poorest coupling ability with β1-adrenergic and A_{2A} adenosine receptors. Subunits Gβ1 and Gβ4 have a similar coupling capacity with β1-adrenergic receptor, but Gβ1 has 20-fold lower coupling capacity than Gβ4 for adenosine receptor.

Table 7.19. Subunits Gβ and Gγ of trimeric guanine nucleotide-binding proteins and their effectors (Source: [767]). In the absence of signaling, Gα subunit is tethered to its associated Gβγ dimer. This dimer is assembled from a repertoire of 5 Gβ (Gβ1–Gβ5) and 12 Gγ (Gγ1–Gγ5, Gγ7–Gγ8, and Gγ10–Gγ14) subunits. Subunit Gβ5 only binds weakly to Gγ and links to regulators of G-protein signaling. It resides mainly in the brain. Subunits Gβ1 to Gβ4 as well as Gγ2, Gγ4 to Gγ6, and Gγ10 to Gγ12 are widely distributed. Subunits Gγ1, Gγ3, and Gγ13 lodge in the central nervous system. Subunit Gγ8 resides in the olfactory epithelium and Gγ3 also in blood cells. The Gα and Gβγ subunits stimulate various effectors, such as adenylate and guanylate cyclases, phosphodiesterases, phospholipase-A2 and -C (PLC), phosphoinositide 3-kinases (PI3K), besides guanine nucleotide-exchange factor (GEF), thereby activating or inhibiting the production of multiple second messengers such as cAMP, cGMP, diacylglycerol (DAG), inositol (1,4,5)-trisphosphate (IP_3), Ca^{++} ions, phosphatidylinositol (3,4,5)-trisphosphate (PIP_3), arachidonic and phosphatidic acids, and opening or closing of numerous ion channels. Mediators PLC and PI3K via different pathways activate the MAPK module.

Target type	Components
Adenylate cyclases	AC1 (−), AC2/4/7 (+)
Ion channels	$Ca_V2.1$–$Ca_V2.3$, $Ca_V3.2$ (−) $K_{IR}3.1$–$K_{IR}3.4$ (GIRK) (+)
Kinases	G-protein-coupled receptor kinases GRK2/3 (+) Phosphatidylinositol 3-kinase-β/γ(+) (Src–SHC–GRB2–SOS–Ras–cRaf–MAPK cascade)
Phospholipases	PLCβ1–PLCβ3 (+) (in increasing order of potency) (DAG–PKC–cRaf–MAPK, IP_3–Ca^{++}–RasGRF–Ras–cRaf–MAPK, cAMP–PKA–RapGEF3/4–Rap–bRaf–MAPK axes)

subtype of Gγ influences the linkage of the Gβγ dimer. [20] Furthermore, homologous Gα binding to GPCRs depends on the GPCR ligand. [21]

Proteic constituents of ubiquitous primary cilia are required for the localization of G-protein-coupled receptors, at least on central neurons [799]. Certain GPCRs such as somatostatin receptor SstR3 and serotonin receptor-6 are specifically located in neuronal cilia. Ciliary localization of somatostatin receptor SstR3 (neurotransmission) and melanin-concentrating hormone receptor MchR1 (feeding regulation) in neurons needs ciliary disorder Bardet-Biedl syndrome BBS2 and BBS4 proteins.

G-protein-coupled receptors also act as scaffolds for the formation and location of signaling complexes in the cell. The response properties are determined by the relationship among the ligand, receptor, G protein, and associated proteins. Recep-

20. Dimers Gβ1γ5 and Gβ1γ7 can mediate binding to M_2 muscarinic receptor, but not Gβ1γ2.

21. Activated peptidase-activated receptor-1 functions according to ligand type. For example, thrombin favors $G\alpha_q$ rather than $G\alpha_{12/13}$.

Table 7.20. Gα effectors (Source: [768]). Trimeric G proteins are made of Gα and Gβγ subunits (IP_3: inositol (1,4,5)-trisphosphate; RhoGEF: Rho-guanine nucleotide exchange factor). Gα Subunits are grouped into 4 main families: Gs, Gi, Gq, and G12. Activated Gα proteins positively or negatively regulate various effectors, such as phospholipases PLA2, PLCβ, PLD, adenylate (ACase) and guanylate (GCase) cyclases, phosphoinositide 3-kinases (PI3K), protein kinase-C (PKC), phosphodiesterases (PDE), Rho GTPases, and ion channels, such as G-protein-regulated inward rectifier potassium channels (GIRK). Both Gα subunit and Gβγ dimer activate or inhibit their effectors. Subunit Gs stimulates adenylate cyclase that primes phosphorylation by protein kinase-A of cAMP response element-binding protein and corresponding transcription. On the other hand, Gi inhibits adenylate cyclase, whereas its associated Gβγ dimer activates mitogen-activated protein kinases via small GTPase Ras. Gi-coupled receptors stimulates phospholipases-C via Gβγ. Family Gq members stimulate phospholipase-C that elevates intracellular calcium concentration and activates PKC to stimulate nuclear factor of activated T cell and MAPK. Subunits G12 and G13 target serum response element-dependent transcription mainly via small GTPase RhoA. Yet, GPCR can signal not only via heterotrimeric G proteins, but also Src kinases, according to ligand level.

G subunit	Effectors
$G\alpha_s$	ACase (+), PKA Na^+ and Cl^- channels (+) $Ca_V2.1$ (+)
$G\alpha_i$	AC1/3/5/6/8/9 (−), phospholipases (+), PDEs (+), K^+ and Cl^- channels (+) Ca_V1 (−), Ca_V2 (−), Ca_V3 (−)
$G\alpha_q$	PLCβ(+), diacylglycerol, PKC, IP_3, Ca^{++}
$G\alpha_{12}$	Rho, RhoGEFs
Gβγ	PLCβ1–PLCβ3 (+) ACase: AC2, AC4, AC7 (+), AC1 (−) GIRK (+), GRK (+), PI3K (+), NRTK (+) Ca_V (−)

tor signaling is regulated by both endogenous and exogenous actions. Activation of G-protein-coupled receptors can be sensitive to the plasmalemma lipid composition. Local cholesterol and sphingolipid concentrations affect the ligand binding and receptor transport.

Although ubiquitous GPCRs classically signal via heterotrimeric G proteins, they can also signal via β-arrestins, even without the participation of G proteins. β-Arrestin adaptors can control receptor signaling, desensitization, and trafficking. Most GPCRs use both β-arrestins and G proteins. However, some GPCR receptors that fail to activate G protein can act as *coreceptors* or *decoy receptors* that eliminate ligands. Agonist binding can prime β-arrestin association with decoy receptor CXCR7, hence β-arrestin-dependent signaling via activation of mitogen-activated protein kinase in the absence of G-protein. Therefore, GPCRs can signal exclu-

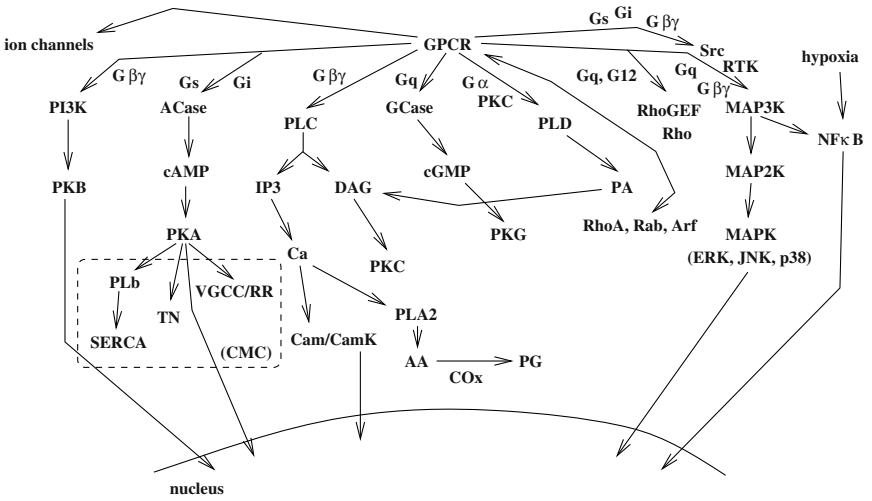

Figure 7.1. Effectors of GPCRs with their associated molecular triggers: Gα (Gs, Gi, Gq, and G12), Gβγ, PKC, Src, and RTK.

sively via β-arrestins (*non-canonical pathway*). Ligand CXCR7 stromal-derived factor SDF1α initiates β-arrestin-2-mediated endocytosis, whereas another ligand, interferon-inducible T-cell α chemoattractant fails to activate Gi and Gq, but promotes vascular smooth muscle cell migration [800].

7.6.3 GPCR Basal Activity

Many G-protein-coupled receptors exhibit a *basal activity* in the absence of their agonists with a variable magnitude according to GPCR type. G proteins can interact with GPCRs before ligand binding (GPCR–G-protein precoupling). The ligand-independent constitutive activity allows the conformation change to an active state upon stimulation, owing to interactions with the plasmalemmal lipids. The plasmalemma tension can then modulate the GPCR activity. The GPCR stimulation then catalyzes G-protein activation.

7.6.4 GPCR Oligomerization

The ligand–receptor complex is supposed to constitute the functional unit of GPCR signaling. However, G-protein-coupled receptors can form homo- or heterodimers. Moreover, some GPCRs form higher-order oligomers upon homo- or heterodimerization during their exocytosis, activation, inactivation, and/or endocytosis. Transient or sustained di- and oligomerization may explain the negative or positive receptor cooperativity. Therefore, G-protein-coupled receptors operate as monomers as well as unstable dimers or oligomers.

Agonist of a GPCR then must choose one protomer of the GPCR complex, its binding influencing the activity of associated protomers, among which at least one

functional unit activates signaling effectors. The history of GPCR protomer association and dissociation from synthesis to degradation on cell signaling may play a role.

Heterodimerization can lead to signaling complexes that have a single ligand binding. However, only one GPCR of the dimer can be active in signaling. Two subunits of a receptor dimer coupled to G protein differ in their conformation [801]. A receptor dimer with a single ligand-bound subunit can trigger G-protein activation. The interaction of G protein with the receptor dimer prevents a symmetrical functioning of the dimer and explains the negative cooperativity in ligand binding on GPCR dimers.

Both class-A and class-C GPCRs (Sect. 7.5) can act as oligomers [802]. Metabotropic glutamate receptors assemble into strict dimers. γ-Aminobutyric acid receptor-B spontaneously forms dimers of heterodimers. Homo- and heterodimerization of GPCRs exist for class-C receptors, not only metabotropic γ-aminobutyric acid ($GABA_B$) and glutamate (mGluR) receptors [803], [22] but also taste heterodimeric-(T_1R1–T_1R3) and calcium-sensing receptors, for which only dimers are involved in signal transduction. Whereas, at least, some class-A GPCRs may function as monomers, other class-A GPCRs for glycoproteic hormones, such as luteinizing, follicle-stimulating, and thyroid-stimulating hormone, may transduce their signal in vitro as di- and/or oligomers by transactivation. In vivo, intermolecular cooperation can rescue GPCR defective in binding as well as signaling capacity [804]. Intermolecular cooperation and di- or oligomerization between mutant binding- and signaling-deficient luteinizing hormone receptors, indeed, re-establish luteinizing hormone effect in the absence of functional wild-type receptors.

7.6.5 GPCR Function in the Vasculature

In the cardiovascular system, G-protein-coupled receptors regulate numerous functions, such as heart frequency and contractility as well as vascular tone. More than 100 different GPCRs are involved in the cardiovascular system, such as (muscarinic) acetylcholine, adenosine, adrenergic, angiotensin-2, bradykinin, endothelin, lysophosphatidic acid, serotonin, sphingosine 1-phosphate, and vasopressin receptors.

7.6.5.1 Blood Vessels and Vasomotor Tone

G-protein-coupled receptors abound in the vasculature, particularly those of class-1 (Vol. 5 – Chap. 7. Vessel Wall). In blood vessels, class-1 GPCRs, once they

22. Metabotropic glutamate receptors are constitutive dimers that do not form larger oligomers. Like receptor Tyr kinases and receptor guanylate cyclases, agonist stimulation of mGluR dimer with a protomer that has its extracellular agonist-binding domain uncoupled from its G-protein-activating transmembrane region causes a symmetric activation of twin transmembrane domains with the same efficiency [803]. Agonist binding to one Venus flytrap (VFT) extracellular domain not only cis-activates the transmembrane domain of the same subunit, but also trans-activates that of the other protomer owing to an intersubunit rearrangement.

Table 7.21. Mediators of vasodilation and vasoconstriction (MLCK: myosin light-chain kinase; MLCP: myosin light-chain phosphatase; PKA: cAMP-dependent protein kinase-A; PLC: phospholipase-C; RoCK: Rho kinase). Subunits of the Gq/11 family prime a fast, transient response, whereas those of the G12/13 family causes a slower, but sustained, tonic contraction.

Vasodilation	Vasoconstriction
MLC	MLC^P
Ca^{++} outflux	Ca^{++} influx
MLCP	MLCK
PKA (+; Gs)	PKA (−; Gi)
	RoCK (G12/13–RhoA)
	PLCβ (Gq/11)

are activated by chemical messengers that comprise peptides act directly as vasoconstrictors or vasodilators or indirectly as vasodilators.[23]

Smooth muscle tone is controlled by the phosphorylation state of the regulatory myosin-2 light chain (MLC). Phosphorylation of MLC, hence the vasomotor tone, depends on intracellular calcium concentration. Vasodilation results from: (1) low intracellular calcium concentration by Ca^{++} export (egress from cytosol by reuptake into the sarcoplasmic reticulum and expulsion across the plasma membrane) through Ca^{++} carriers and (2) MLC dephosphorylation by myosin light chain phosphatase (MLCP) under the control of RoCK kinase (Table 7.21).

Conversely, vasoconstriction results from increased Ca^{++} concentration that, in cooperation with calmodulin, activates Ca^{++}–calmodulin-dependent myosin light chain kinase (MLCK). The latter stimulates cross-bridge cycling of actin–myosin stress fibers.

Vasodilation can be primed by: (1) trans-plasmalemmal hyperpolarization (e.g., on elevated activity of ATP-sensitive K^+ channel) that precludes the activity of voltage-gated calcium channels (mechanism used by adenosine) and (2) cAMP and cGMP signaling that stimulates protein kinase-A and -G, respectively, that both phosphorylate MLCK (procedure utilized by prostaglandins [PGD2, PGE2, and PGI2] and nitric oxide, respectively). PKA Kinase also hampers the activity of RhoA GTPase.

Vasoconstriction can be intiated by: (1) trans-plasmalemmal depolarization that promotes the activity of voltage-gated calcium channels (this process can be further enhanced by voltage-gated Na^+ channels and is typically triggered by ATP and tension); (2) decline in PKA activity by Gi subunit; and (3) elevated PLC activity by Gq subunit that causes, via IP_3, Ca^{++} influx, hence MLCK activation, as well as RhoA stimulation (thus RoCK excitation that hinders MLCP action).

23. Blood pressure-lowering drugs target a limited number of class-1 GPCRs. Such drugs include angiotensin-2 receptor and adrenoceptor antagonists.

Vasoconstrictors include adrenaline, angiotensin-2, apelin, adenosine triphosphate, endothelin-1, motilin, neuromedin-U, neuropeptide-Y, sphingosine 1-phosphate, thrombin, thromboxane-A2, urotensin-2, and vasopressin [805].

Vasodilators encompass extracellular adenosine, extracellular ADP and ATP, bradykinin, ghrelin, histamine, natriuretic peptides, nitric oxide, noradrenaline (via β2-adrenergic receptors), platelet-activating factor, some prostaglandins, as well as substance-P ghrelin. Ghrelin targets growth hormone secretagog receptor and nociceptin.

On vascular smooth muscle cells, GPCRs are predominantly coupled to Gs. They thus activate adenylate cyclase and cause direct vasodilation. Among class-2 GPCRs that are activated by peptides, all those with vasoactivity directly operate as vasodilators. They are targeted by adrenomedullin and urocortins. [24]

On vascular endothelial cells, GPCRs can generate and release: (1) nitric oxide; (2) arachidonic metabolites such as prostacyclin; or (3) endothelium-derived hyperpolarizing factor that target adjoining smooth muscle cells, where they excite soluble guanylate cyclase, Gs-coupled prostanoid IP_1 receptors, and induce hyperpolarization, respectively. All these processes create vasodilation.

7.6.5.2 Heart and Its Nervous Command

Cardiac regulation by the sympathetic nervous system is carried out by β-adrenergic receptors that are coupled primarily to Gs. Produced cAMP targets hyperpolarization-activated, cyclic nucleotide-gated channels and activates protein kinase-A. The latter phosphorylates Ca_V channels, phospholamban, and troponin-I [767] (Vol. 5 – Chap. 5. Cardiomyocytes). These events explain positive chronotropic (C+; increased heart rate), dromotropic (D+; elevated conduction velocity through the nodal tissue), lusitropic (L+, enhanced cardiomyocyte relaxation), and inotropic (I+; improved contractility) effects of the sympathetic command.

On the other hand, the parasympathetic nervous system has negative chronotropic (C−) and dromotropic (D−) effects. It operates via muscarinic acetylcholine M_2 Gi/o-coupled receptor. In addition to the inhibition of cAMP production, this receptor activates atrial G-protein-regulated inward rectifier K^+ channels (GIRK; $K_{IR}3.1$ and $K_{IR}3.4$) via released Gβγ dimer [767]. Muscarinic receptors also inhibit voltage-gated Ca_V channels.

7.6.6 Airway Smooth Muscle Tone

Like in vascular smooth muscle cells, the tone of airway smooth muscle cells is mainly regulated by members of the Gq/11 and Gs families of G-protein subunits that cause bronchoconstriction and bronchodilation, respectively.

24. G-protein-coupled receptors on vascular smooth muscle cells that provoke vasoconstriction can be linked to more than one signaling cascade via different families of G-protein subunits (Gi/o, Gq/11, and/or G12/13). Subunits of the Gi/o, Gq/11, and/or G12/13 families activate primarily, but not exclusively, phospholipase-C and small monomeric GTPase Rho and inhibit adenylate cyclase, respectively [805].

Acetylcholine released from postganglionic parasympathetic nerves intervenes mainly via Gq/11-coupled M_3 receptors. Gq/11-coupled receptors in airway smooth muscles also comprise H_1 histamine, B_2 bradykinin, ET_B endothelin, and $CysLT_1$ leukotriene receptor [767]. Subunit Gi impedes the relaxation of airway smooth muscle cells.

Gs-coupled receptors provoke relaxation of contracted airway smooth muscle cells, such as β2-adrenoceptor, EP_2 prostaglandin, and IP prostacyclin receptor [767].

7.6.7 Platelet Activation

Platelet adhesion and activation is initiated by interaction with components of the subendothelial matrix, such as collagen and von Willebrand factor (Vol. 5 – Chap. 9. Endothelium). Collagen is able to cause a firm adhesion of thrombocytes to the subendothelial layer. Additional platelets are recruited by diffusible mediators, such as ADP, ATP, and thromboxane-A2, that are secreted by activated thrombocytes.

G-Protein-coupled receptors operate in platelet aggregation. Twelve most abundant platelet GPCRs include: (1) thrombin receptor, i.e., peptidase-activated receptors PAR_1 and PAR_4;[25] (2) adenosine receptors A_1, A_{2A}, and A_{2B}; (3) nucleotide receptors $P2Y_1$, $P2Y_{12}$, as well as $P2Y_{10}$; (4) GPR183[26] (5) succinate receptor-1 (SucnR1 or GPR91); (6) lysophosphatidic acid receptors LPA_1, LPA_3, LPA_4 (GPR23), and LPA_5 (GPR92); (7) α2a-adrenergic receptor; (8) glutamate $mGlu_3$ and $mGlu_4$; and (9) serotonin receptors $5HT_{1F}$ and $5HT_4$ [836].

Agent ADP activates the G-protein subunits Gq and Gi via $P2Y_1$ and $P2Y_{12}$ receptors, respectively. Thromboxane-A2 targets its cognate Gq-coupled receptor TP. Thrombin binds to peptidase-activated receptors that are coupled to Gq, G12/G13, and in some cases to Gi [767]. Adrenaline connects to Gz-coupled α2-adrenergic receptors to potentiate the effect of other platelet activators.

7.6.8 Leukocyte Migration

Leukocytes move according to a concentration gradient of chemoattractants. They can then enter into lymphoid organs to mature and, then, exit for antigen surveillance. They can also reach sites of infection.

Lymphocyte chemoattractants include chemokines, sphingosine 1-phosphate, and lysophosphatidic acid. Chemokine receptors act mainly via Gi subunits. Lysophospholipid receptors activate Gi, G12/G13, and Gq/G11 subunits. Hematopoietic cells are able to synthesize Gq, G11, and G16 subunits. Members of the G12/13 family target RhoA GTPase.

Neutrophils respond to diverse chemoattractants, such as N-formyl Met-Leu-Phe (fMLP), C5a, platelet-activating factor, or interleukin-8. Complement C5a element

25. Activated receptor PAR_1 is more potent than PAR_4.

26. A.k.a. Epstein-Barr virus-induced gene product-2 EBI_2. Epstein-Barr virus-induced gene product-1 (EBI_1) is the (CCL19 and CCL21) chemokine receptor CCR7. Up- and down-regulation of GPCRs and their ligands result from viral infections.

operates via Gi2, Gi3, and Gβ2 subunits to stimulate PI3Kγ as well as PLCβ2 and PLCβ3. In addition, PIP_3-dependent Rac exchanger-1 (PREx1) that acts as a guanine nucleotide-exchange factor for small GTPases Rac, is boosted by Gβγ dimer.

7.6.9 Mastocyte Activity

Mastocytes also express various GPCRs that intervene during allergy and anaphylaxis (with vasodilation, bronchoconstriction, etc.). Ligands, such as anaphylatoxins C3a and C5a, leukotrienes, sphingosine 1-phosphate, and platelet-activating factor mediate anaphylaxis, acting as transmitters within mastocytes and extracellular medium, including circulating biofluids. Pulmonary mastocytes express a huge number of GPCRs, such as [837]: (1) complement component receptors C3aR1 and C5aR1; (2) cysteinyl-leukotriene receptor $CysLT_1$; (3) sphingosine 1-phosphate receptor $S1P_1$; (4) corticotropin-releasing hormone (CRH) receptor CRF_1; (5) platelet-activating factor receptor (PAFR); (6) cadherin, EGF LAG seven-pass receptor CELSR1;[27] (7) Zn^{++}-activated GPR39; (8) progestin and adiponectin, C1q, and collagen domain (adipoQ)-containing receptor family member PAQR5; (9) lysophosphatidic acid receptor LPA_5; (10) succinate receptor-1 (SucnR1); and (11) GPR12.

Acupuncture is an old medical technique in traditional chinese medicine. In acupuncture, the internal organs are assumed to be interconnected by meridians, i.e., pathways in which the vital energy (Qi) flows throughout the body. Thin needles are manipulated (using lifting, thrusting, and rotation) to stimulate specific points (acupoints) of the body to restore the balance between Yin and Yang energy by removing blocks in the flow of Qi [806].[28] The effects of acupuncture can be explained by interactions between the nervous, circulatory, endocrine, and immune systems. The subcutaneous connective tissue is composed of cells embedded in the extracellular matrix mainly constituted by collagen and elastic fibers in a gel of glycoproteins and proteoglycans. Mastocytes can be presumed to play a major role in acupuncture. They are scattered, but localize predominantly near blood and lymphatic vessels and nerves. Their density is higher near acupoints [809].

A mechanical stress field results from local deformations of the connective tissue imposed by the needle motions. This stress field is sensed by the local population of mastocytes that react, in particular, by degranulation. Released molecules include calcitonin gene-related peptide (CGRP), heparin, histamine, leukotrienes (LTb4, LTc4, LTd4, and LTe4), platelet-activating factor, prostaglandin-E2, serotonin, substance-P, and thromboxane-A2 (Table 7.22). Mastocytes also secrete pep-

27. A.k.a. Flamingo homolog-2.

28. The acupuncture needle is inserted in the subcutaneous connective tissue and manipulated until a special sensation is obtained, which is supposed to prove that the needle has been placed in the proper location. The same acupoints can also be stimulated by other methods, such as moxibuxion, acupressure, and electroacupuncture. Moxibustion relies on heating acupoints by burning a moxa stick made of Artemisia vulgaris (common mugwort) to stimulate the blood circulation and produce a smoother flow of Qi [807]. Acupressure can be referred as needleless acupuncture by applying finger pressure. Electroacupuncture relies on the electrical stimulation of acupoints [808].

Table 7.22. Released molecules by the mastocyte and their effects. Acupuncture can be modeled by an immediate and a late response. Nerves and mastocytes exchange chemical messengers such as substance-P. The latter stimulates histamine and nitric oxide (NO) release. Calcitonin gene-related peptide (CGRP) causes a vasodilation; nitric oxide cooperates with CGRP to increase its positive inotropic effect that raises the local blood flow in dilated vessels. Histamine is quickly catabolized, thereby acting near the site of release. Resulting vasodilation and increased vessel wall permeability support the transfer of chemical mediators into the blood circulation. The NO concentration rises and enhances the vasodilation. Serotonin has a biphasic effect, as it triggers a vasoconstriction and promotes NO release, hence a subsequent vasodilation. Nerve growth factor (NGF), tumor-necrosis factor (TNF) and interleukins (IL) are potent mastocyte chemoattractants. Mastocyte chemotaxis is supported by matrix degradation by secreted peptidases.

Agent	Effects
CGRP	Vasodilation, positive chronotropy, inotropy, and lusitropy, mastocyte degranulation
Heparin	Blood clot prevention
Histamine	Vasodilation (directly and via NO), nerve stimulation
Leukotrienes	Vasodilation, vascular permeability elevation
IL, NGF, TNF	Chemotaxis
Prostaglandin-D2	Nerve stimulation
Prostaglandin-E2	Vasodilation, inhibition of mediator release
Serotonin	Vasoconstriction followed by NO-mediated vasodilation
Thromboxane-A2	Vasoconstriction, platelet aggregation
Tryptase, chymase	Matrix degradation for enhanced cell migration

tidases (e.g., tryptase), growth factors (e.g., FGF, gmCSF, and NGF), and cytokines (e.g., interleukins and tumor-necrosis factor). Nerve endings are stimulated and release substance-P that further activates mastocytes and triggers the production of nitric oxide.

Meridian can be assumed to be a neurovascular signaling tract. A compartmental model can then be designed. Compartment 1 is the acupoint region with its 3 components: (1) mastocytes, (2) blood and lymph vessels, and (3) nerves. Chemoattractants augment the mastocyte population (auto-amplification); autocrine signals bestow a self-sustained response. Nerves and mastocytes exchange cues. Compartment 2 is related to signal transmission to the central nervous system, either very rapidly via nervous impulses, or delayed via messenger convection through the blood circulation. A feedforward loop associated with elevated cardiac function enables an increased blood flow for a relevant material transport. Compartment 3 deals with signal processing with a quick and late responses corresponding to fast and delayed inputs. Compartment 4 represents outputs sent from the central nervous system and the body's response.

7.7 Crosstalk and Transactivations

G-protein-coupled receptors not only act via heterotrimeric G proteins, but can also function in a G-protein-independent manner. β2-Adrenergic receptors signal via either Gα or via tyrosine kinase Src according to the ligand concentration, low or high, respectively [810].

Activated Gi-coupled receptors often contribute to inositol phosphate signal triggered by Gq-coupled receptors (synergistic receptor crosstalk). For example, Gi-coupled adenosine A_1 and α2c-adrenergic receptors collaborate with Gq-coupled bradykinin B_2 and P2Y receptors. Gβ1γ2 Dimer as well as combinations of Gβ (Gβ1–Gβ3) with Gγ (Gγ2–Gγ7) increases potency of bradykinin or UTP messenger without interfering with $G\alpha_q$ signaling [811]. This GPCR crosstalk results from Gβγ exchange between Gi- and Gq-coupled receptors.

Certain GPCR effectors do not depend on G proteins. Crosstalk between GPCRs and small GTPases exists.[29] GPCR signaling through G proteins can activate Ras and Rho GTPases. Activated Ras triggers the mitogen-activated protein kinase cascade. RhoA, Rab, Arf, and ArfGEF can directly associate with GPCRs. GPCRs can also function as guanine nucleotide-exchange factors for small GTPases.

The interactions between GPCRs and small GTPases is required for cellular transport and cell migration. GPCRs are synthesized and modified in the endoplasmic reticulum, then transported to the Golgi body for additional changes, lastly to the plasmalemma. Rab GTPases are implicated in exocytosis. Rab GTPases also regulate GPCR endocytosis into early endosomes, GPCR targeting to lysosomes for degradation, or GPCR recycling from early endosomes. Arf1 and Arf6, with Arf GEFs (adpribosylation factor nucleotide-binding site opener [ARNO]) and β-arrestin, also control GPCR endocytosis [812].

Activated Gi-coupled chemoattractant receptor, the N-formylmethionyl-leucyl-phenylalanine receptor (fMLPR), leads to both Ras-dependent and -independent activation of Ral GTPase. Ral GEF RalGDS interacts with Ras, which activates Ral, hence, actin cytoskeleton reorganization (Ras-dependent mechanism). Plasmalemmal translocation of complexes made by β-arrestin and RalGDS allows binding to fMLPR and uncoupling from G protein, thus activates membrane-bound Ral (Ras-independent mechanism) [812].

Ligand binding to GPCRs activates small GTPases either directly or indirectly.[30] Rho-dependent responses can be activated by Gq and G12 (Fig. 7.2). Furthermore, G12 can bind and activate Rho-specific guanine nucleotide-exchange factors [813]. Mutant Gs-induced cAMP can activate small GTPases Rap1 and B-Raf that, in

29. The small GTPase superfamily includes at least five families: Ras GTPase subfamily (Ras, Rap, and Ral) of regulators of cell signaling, Rho GTPase subfamily (Rho, Rac, and Cdc42) of regulators of actin cytoskeleton, Rab GTPase subfamily of regulators of vesicular transport, Arf GTPase subfamily (Arf, Arl, and Sar) of regulators of vesicular transport, and Ran GTPase subfamily of regulators of microtubule organization and nucleocytoplasmic transport.

30. GPCRs for lysophosphatidic acid and thrombin induce stress fibers, focal adhesions, and cell rounding through Rho-dependent pathways [813].

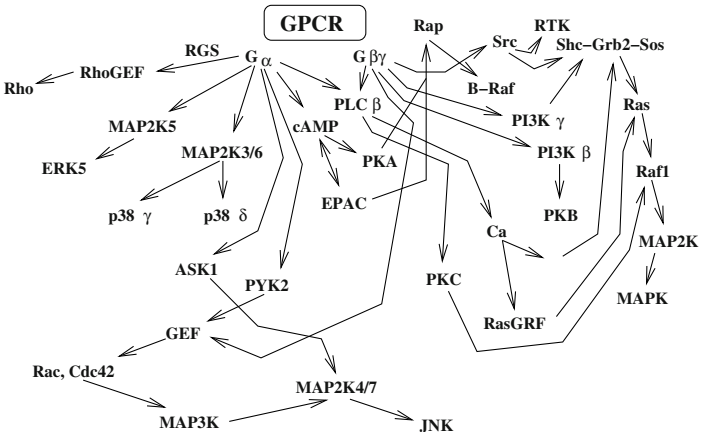

Figure 7.2. Interactions between GPCRs, small GTPAses and MAPKs. G-protein-coupled receptors (GPCR) activate mitogen-activated protein kinase (MAPK) using many pathways. G$\beta\gamma$ can stimulate Ras by the activation of receptor and non-receptor tyrosine kinases, thereby recruiting Sos to the plasmalemma. Activated Gα_q can stimulate: (1) Raf1 via phospholipase-Cβ (PLCβ) and protein kinase-C (PKC) and (2) Ras via PLCβ and activation of (2a) Ca^{++}-dependent Ras guanine nucleotide-releasing factor (RasGRF) and (2b) Ca^{++}- and PKC-regulated proline-rich tyrosine kinase-2 (PYK2) of the focal adhesion kinase family. GPCR stimulation also leads to phosphorylation of Src homology and collagen (SHC), which subsequently forms complexes with growth factor receptor-bound-2 (GRB2), and activation of tyrosine kinases acting on Son of sevenless (Sos). PI3Kγ can then act on the SHC–GRB2–Sos–Ras pathway. PI3Kβ stimulated by GPCRs drives activation of Rac and p21-activated kinase to enhance Raf activity. Gi and Gs, via the binding of cAMP to exchange protein activated by cAMP (EPAC) Rap1GEF EPAC and phosphorylation of Rap1 by PKA, can use the Rap1 pathway, which stimulates B-Raf. Gq, via PYK2, with adaptor Crk and paxillin, can activate GEFs for Rac and Cdc42, leading to the activation of MAP2K4/7 and JNK. G12 can stimulate MAP2K4/7 via apoptosis signal-regulating kinase-1 (ASK1). (8) G12/13, with regulators of G-protein signaling (RGS) also targets RhoGEFs for Rho activation. G12/13 and Gq can stimulate ERK5 (or big mitogen-activated kinase BMK1) via MAP2K5. G12, Gq, and G$\beta\gamma$ can activate P38MAPKα in cooperation with Btk and Src kinases. Subunits G12/13 and Gq can also activate P38MAPKγ and P38MAPKδ via MAP2K3/6 (Sources: [768, 812]).

turn, stimulate MAP2K and MAPK. G$\beta\gamma$ activates the MAPK pathway using a Ras-dependent process [814]. Gq-coupled receptors use both PKC-dependent and -independent pathways to stimulate the MAPK pathway.

The transactivation of receptor tyrosine kinases by GPCRs encompasses: (1) the activation of receptor Tyr kinases via non-receptor Tyr kinases; (2) formation of complexes between GPCRs and RTKs; and (3) release of RTK ligands.

Stimulation of GPCRs can stimulate receptor Tyr kinases, such as epidermal growth factor receptors, via: (1) proteolytic cleavage by ADAM metallopeptidase and release of EGF-like ligands, such as heparin-binding EGF-like growth factor, and (2) NRTK activation that can phosphorylate EGFR tyrosine residues.

Activation by GPCRs of the Ras–MAPK pathway uses alternate RTK-dependent and -independent pathways according to cell types. Angiotensin-2 in thoracic aortic smooth muscle cells requires RTK activity to signal to ERK, but not in smooth muscle cells of the renal microvasculature [815].

β2-Adrenergic receptor mediates extracellular signal-regulated kinase activation via assembly of a multireceptor complex with the epidermal growth factor receptor following co-internalization of both receptors into clathrin-coated vesicles [816]. Several NRTKs (CSK, Lyn, BTK, proline-rich tyrosine kinase-2, and focal adhesion kinase; Vol. 4 – Chap. 3. Cytosolic Protein Tyr Kinases) could mediate MAPK activation by Gi and Gq [768]. Src or Src-like kinases can phosphorylate SHC on stimulation by Gβγ and α-adrenoceptors, after recruitment of β-arrestin and GRK2 kinase. Kinase Src can be activated also by interaction with Gi and Gs or β3-adrenoceptors.

Subunit Gq can stimulate components of the MAPK module via: (1) a PKC-dependent, Ras-independent mechanism; (2) PKC- and Ras-dependent process; or (3) PKC-independent, Ras-dependent procedure, depending on the cell type and receptor expression level.

Isoforms of PI3K kinase can be required for GPCR-induced MAPK activation (Fig. 7.2). Subtype PI3Kγ is activated by direct interaction with Gβγ subunit. It can then act on the SHC–GRB2–SOS–Ras pathway. The Gβγ dimer activates PI3Kβ, which then stimulates PKB [817].

Arrestins bind many phosphorylated GPCRs for endocytosis. β2-Adrenoceptors can associate with the Na^+–H^+ exchanger regulatory factor (NHERF); angiotensin receptors AT_{1A} with Janus kinase JaK2; metabotropic glutamate receptors with Homers, which can complex with IP_3 receptors; muscarinic acetylcholine M_3 receptors and AT_{1A} with GTPases Rho and ARF [768].

GPCR ligands, such as lysophosphatidic acid, endothelin-1, platelet-activating factor, and thrombin can induce a rapid transient phosphorylation of epidermal growth factor receptors in fibroblasts and in vascular smooth muscle cells. Catecholamines, angiotensin-2, and endothelin-1 act in cardiomyocytes where they can activate heparin-binding epidermal growth factor with members of a disintegrin and metallopeptidase (ADAM) family and cause GPCR-induced cardiac hypertrophy (Fig. 7.3). Different types of G proteins (Gi, Gq, and G12), different metallopeptidases of the ADAM family (ADAM10, ADAM12, and ADAM17), and different EGF-like ligands (heparin-binding epidermal growth factor, transforming growth factor-α, and amphiregulin) are involved in inter-receptor crosstalk (or transactivation) [818].

7.8 Regulators of G-Protein Signaling

Regulators of G-protein signaling are GTPase-accelerating proteins that activate the GTPase activity of α subunits of heterotrimeric G proteins, thereby inactivating G protein and rapidly switching off the GPCR signaling pathway.

All the RGS proteins contain an RGS domain. Small RGS proteins, such as RGS1 and RGS4, are slightly greater than the RGS domain. Other RGSs contain additional

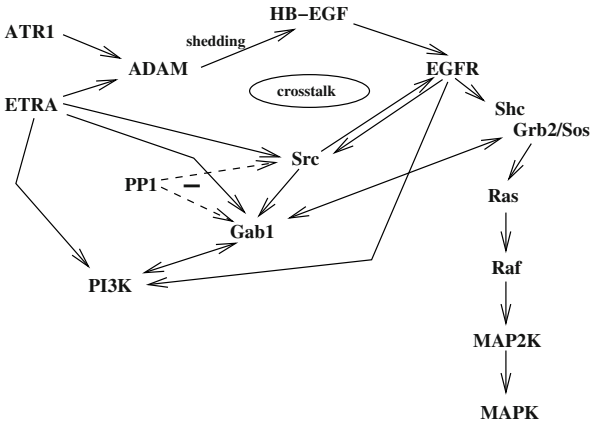

Figure 7.3. Crosstalk between receptors of angiotensin-2 (AT_1) and endothelin-1 (ET_A, or ETRA) and epidermal growth factor receptor (EGFR) in the cardiomyocyte. Transactivation of EGFR requires metallopeptidases of the ADAM family, particularly ADAM12, heparin-binding EGF-like growth factor, docking molecule GRB2-binding protein GAB1, Src Tyr kinase, and phosphoinositide 3-kinase. Transactivation of EGFR leads to MAPK activation.

domains that confer further functionality, such as DEP for membrane targeting, GGL (G-protein-γ subunit-like) for binding Gβ subunits, GoLoco for guanine nucleotide inhibitory activity, PH for guanine nucleotide-exchange stimulating effect, and PDZ for GPCR, PTB for phosphotyrosine, PX for phosphatidylinositol, and RBD for Ras binding.

Regulators of G-protein signaling can also speed up the onset of signaling, in addition to accelerating deactivation without changing amplitude or sensitivity of the signal. This paradoxical activity has been attributed to enzymatic (GAP) or scaffolding (non-GAP) functions. Yet, fast GTPase activity of RGSs may suffice to explain the activation kinetics and agonist sensitivity, i.e., increased onset, deactivation rates, and blunting of sensitivity [819].

7.9 G-Protein-Coupled Receptor Kinases

Agonist–GPCR binding strongly enhances interactions between GPCRs and G proteins, G-protein-coupled receptor kinases (GRK), and β-arrestins. Agonists of GPCRs can simultaneously activate G-protein- and β-arrestin-dependent pathways. Agonist-activated GPCRs are also phosphorylated by G-protein-coupled receptor kinases. Phosphorylation of GPCRs by GRKs decreases GPCR interactions with G proteins and increases GPCR binding of β-arrestins (Sect. 7.11).

β-Arrestins can then further desensitize GPCR-mediated signaling by several mechanisms: (1) receptor hindrance due to β-arrestin attachment; (2) recruitment of second messenger-degrading enzymes; and (3) scaffold proteins that facilitate receptor endocytosis. However, β-arrestin binding can initiate additional signaling

programs via recruitment of signaling mediators such as mitogen-activated protein kinases to activated receptors.

Therefore, interaction of GPCRs with β-arrestins prevents GPCR signaling via G proteins, but can simultaneously prime other signaling pathways as well as associate active receptors to clathrin-coated pits to facilitate receptor endocytosis that controls GPCR desensitization and resensitization, in addition to their endocytosis-mediated activity.

Ubiquitous G-protein-coupled receptor kinases constitute a 7-member family (GRK1–GRK7). These kinases that have different distribution patterns among the body's tissues as well as distinct binding preferences for some receptors. They can yield functional specialization. Isoforms of GPCR kinases determine β-arrestin function, as they can lead to functionally distinct pools of β-arrestin, possibly by phosphorylating distinct receptor sites. Enzymes GRK5 and GRK6 are required for β-arrestin-mediated MAPK signaling.

G-protein-coupled receptor kinases, particularly GRK2 isoform, not only target G-protein-coupled receptors, but also numerous other substrates, such as receptor Tyr kinases (PDGFR and EGFR), as well as members of the GIT family of GTPase-activating proteins of adpribosylation factor (ArfGAP), DM2 ubiquitin ligase, and catalytic subunit P110 of PI3K, PKB, MAP2K, and P38MAPK kinases. They also have many interacting proteins. The GRK2 subtype can attenuate PDGF-dependent proliferation of smooth muscle cells, but favors mitogenic signaling induced by EGF in osteoblasts and the Hedgehog receptor Smoothened in fibroblasts [820]. In addition, increased GRK2 concentration potentiates migration of epithelial cells directed by fibronectin and sphingosine 1-phosphate.

G-protein-coupled receptor kinases possess their own regulators. Calcium-binding recoverin is a neuronal calcium sensor that localizes to the retina and serves as a Ca^{++}-dependent regulator of GRK1 kinase. At high Ca^{++} concentration, recoverin sequesters GRK1, whereas at low Ca^{++} level, the complex dissociates and GRK1 becomes active.

In response to GPCR stimulation, GRK2 is rapidly phosphorylated by Src (Tyr13, Tyr86, and Tyr92) and MAPK (Ser670) kinases and then degraded by the proteasome [820]. Cell cycle progression requires changes in the activity or concentration of signaling proteins. The GRK2 kinase is phosphorylated (Ser670) by cyclin-dependent kinase CDK2 during the G2–M transition and subsequently binds to peptidyl-prolyl isomerase Pin1 to be degraded [820].

7.10 G-Protein-Coupled Receptor Phosphatases

G-protein-coupled receptors experience cycles of phosphorylation and dephosphorylation. Membrane-associated G-protein-coupled receptor phosphatase (GRP) aims at dephosphorylating GPCRs that have been phosphorylated by G-protein-

coupled receptor kinases to cause agonist-dependent receptor deactivation, hence restoring the ground state of the receptor. [31]

A family of receptor phosphatases contribute to the regulation of G-protein-coupled receptors. G-protein-coupled receptor phosphatase is an unsual form of protein phosphatase-2 with peculiar subcellular distribution and substrate specificity [822].

Desensitization of GPCRs is initiated by phosphorylation of Ser and Thr residues of intracellular loops and C-terminus by second messenger-activated kinases, such as protein kinase-A and GPCR kinases. Once phosphorylated, GPCRs generally bind to arrestin that prevents subsequent coupling between GPCR and G-protein and elicits GPCR integration into clathrin-coated pits for endocytosis. Moreover, when arrestins bind to GPCRs with high affinity, they preclude phosphatase action on receptors.

On the other hand, endocytosis can be a prerequisite for resensitization of G-protein-coupled receptors, such as for β2-adrenoceptors, when dephosphorylation occurs in endosomes, but not at the plasma membrane. Once internalized, low-affinity agonists such as adrenaline dissociate rapidly from β2-adrenoceptors. [32] This event is followed by arrestin dissociation, because the tethering of high-affinity arrestin needs agonist binding, in addition to GRK site phosphorylation. The rates of endocytosis and recycling control the level of β2-adrenoceptors in the plasma membrane.

Class-A GPCRs have higher affinity for arrestin-3 (or β-arrestin-2) than arrestin-2 (or β-arrestin-1). They internalize without arrestin and recycle rapidly to the plasma membrane. On the other hand, class-B GPCRs co-internalize with arrestin-2 or -3 and recycle slowly, often undergoing intracellular degradation. Once liganded, thyrotropin-releasing hormone receptor, neither class-A nor -B GPCR, is rapidly

31. Phosphorylation of GPCRs provokes a slight decrease in receptor activity. Above all, it also enhances GPCR affinity for arrestin. Arrestin binding can inactivate receptors by preventing their coupling to heterotrimeric G proteins triggered by agonist binding. In Drosophila, retinal degeneration-C (RDgC) is an unusual Ser–Thr phosphatase required for rhodopsin dephosphorylation [821]. Unlike vertebrate opsin, most invertebrate photopigments are not bleached after light activation, but can be photoconverted between the rhodopsin form and a thermally stable, active metarhodopsin form.

32. Agonists may be removed quickly from β2-adrenoceptors, especially in synapses, and GPCRs must be rapidly resensitized for appropriate functioning. β2-Adrenergic receptors contain phosphorylation sites for both GRK (Ser355 and Ser356) and PKA (Ser262) [823]. The concentration of agonist and rate constants of phosphorylation and dephosphorylation define the level of PKA- and GRK site phosphorylation. Dephosphorylation of the PKA site does not necessitate endocytosis, as it happens at the plasma membrane. Dephosphorylation of GRK sites occurs with a phase lag with respect to that of PKA site (possibly due to time necessary for phosphatase recruitment) and proceeds slowly. The rapid phase of resensitization (0–5 min) does not depend on dephosphorylation at the plasma membrane, but β-arrestin dissociation upon agonist stimulation of adenylate cyclase [823]. Rapid restoration of adenylate cyclase activity after removal of agonist can explain the fast dissociation of β-arrestin from membrane-bound β2ARs in association with recycling of the receptor to the plasma membrane. Moreover, the pattern of phosphatase inhibitor effects is much more consistent with PP1, rather than PP2 in PKA and GRK site dephosphorylation [823].

phosphorylated at numerous sites by GRKs, but not PKA enzyme. It then internalizes with arrestins-2 and -3. It can be dephosphorylated at the plasma membrane or in endosomes, once its hormonal agonist is removed.

β2-Adrenergic receptor is slowly phosphorylated by both PKA and GRK kinases. The latter recruits arrestin, but internalizes without it. On the other hand, thyrotropin-releasing hormone receptor co-internalizes with arrestin. Both receptors are dephosphorylated following agonist removal, but the latter is dephosphorylated much more rapidly at the plasma membrane.

Resensitization of most GPCRs comprises arrestin dissociation, receptor dephosphorylation, and recycling to the plasma membrane. Endocytosis often enables action of protein kinase-A, whereas GRKs mostly intervene at the plasma membrane. The GPCR cytoplasmic tails support arrestin linkage, but GPCR phosphorylation and dephosphorylation by GRKs and GRPs, respectively, depend on regions outside the cytoplasmic tail, but not their C-termini [824].

7.11 Arrestins

G-protein-coupled receptors stimulate heterotrimeric guanine nucleotide-binding protein and/or β-arrestins to regulate the intracellular concentration of various second messengers. Signaling is terminated by receptor desensitization and degradation of second messengers.

Four arrestin isoforms exist: arrestins-1 and -4 that regulate opsins (GPCRs of photoreceptor cells of the retina) and arrestins-2 and -3 (or β-arrestins-1 and -2, respectively. [33] Ubiquitous β-arrestins control the activity of most G-protein-coupled receptors. [34]

β-Arrestins were originally defined as terminators of GPCR signaling. They actually mediate desensitization and endocytosis of GPCRs and other types of receptors and molecules. [35]

However, β-arrestins also act as signal transducers. They indeed serve as signaling adaptors for GPCRs and other types of receptors. β-Arrestin-biased agonism is related to GPCR signaling after ligand binding via β-arrestins rather than subunits of heterotrimeric G proteins.

33. Some members of the arrestin family of adaptor proteins are called β-arrestins because they were discovered as β2-adrenoceptor-binding proteins.

34. β-Arrestins associate with β2-adrenergic receptor, angiotensin-2 type-1A receptor (AT_{1A}), arginine vasopressin receptor (V_2 [AVPR2]), peptidase-activated receptor-2, and parathyroid hormone receptor, to activate extracellular signal-regulated protein kinases (Vol. 4 – Chap. 5. Mitogen-Activated Protein Kinase Modules). G-Protein-coupled receptor kinases are required, at least, for both AT_{1A} and V_2 receptors.

35. β-Arrestins act on receptor Tyr kinases, such as insulin-like growth factor-1 receptor. In addition, β-arrestins are implicated in endocytosis of VE-cadherin. β-Arrestin-2 also binds to low-density lipoprotein receptor and enhances LDLR endocytosis [825]. β-Arrestin-2 links to type-3 transforming growth factor-β receptor (TβR3; a.k.a. β-glycan) and triggers the receptor phosphorylation by type-2 TGFβ receptor kinase (TβR2), thereby downregulating TGFβ signaling [826].

Mechanical stretch induces β-arrestin-biased signaling from angiotensin-2 receptors AT_1 in the absence of ligand or G-protein activation to promote cell survival [827]. In cardiomyocytes, acute variations in mechanical stress trigger an AT_1-mediated conformational change of β-arrestin similar to that caused by a ligand that primes a β-arrestin-associated process.

7.11.1 Post-Translational Modifications of Arrestins

Phosphorylated β-arrestins pertain to 2 classes: (1) class-A β-arrestin binders that transiently link GPCRs only at the plasma membrane, as they are deubiquitinated, and (2) class-B β-arrestin binders that form stable β-arrestin–GPCR complexes connected to endocytic vesicles.

Ligand-stimulated ubiquitination of β-arrestins governs the stability of receptor-β-arrestin interactions. Ubiquitination of β-arrestins tunes signal strength and localization, as ubiquitination serves to further modify the active conformation of β-arrestin to recruit endocytic and signaling mediators among members of the β-arrestin interactome.

β-Arrestin ubiquitination by DM2 ubiquitin ligase causes rapid endocytosis of β2-adrenergic receptor. Deubiquitinase ubiquitin-specific peptidase USP33 binds β-arrestin-2. Enzymes USP33 and DM2 favor the stability or lability of GPCR–β-arrestin complex, respectively, thereby controlling the longevity and subcellular localization of signalosomes. β2-Adrenoceptors promote a conformational change of β-arrestin for deubiquitinase binding, hence destabilizing β2AR–β-arrestin binding and lowering ERK activation [828]. Conversely, ubiquitination of β-arrestin by DM2 promotes β-arrestin-dependent signaling of activated β2-adrenoceptors. On the other hand, V_2 vasopressin receptor elicits a distinct β-arrestin conformation that favors the dissociation of deubiquitinase, thereby stabilizating β-arrestin–receptor complexes and increasing ERK activation. In summary, DM2 ligase directs endocytosis and signaling, whereas deubiquitinase USP33 causes signal termination.

7.11.2 Receptor Desensitization

β-Arrestins help to restore G-protein-coupled receptors to an inactive state after stimulation, although they can also have positive signaling roles. β-Arrestin-1 and -2 desensitize certain plasmalemmal receptors in conjunction with G-protein-coupled receptor kinases (GRK). [36]

Agonist-promoted desensitization indeed comprises phosphorylation of the receptor by a GRK that promotes the binding of β-arrestin that partially uncouple GPCR from its effectors. This event can lead to GPCR endocytosis, with either a recycling to the cell surface or degradation.

36. β-Arrestins bind to activated G-protein-coupled receptors after receptor phosphorylation by G-protein-coupled receptor kinases.

β-Arrestins recruit phosphodiesterases to activated Gs-coupled β2-adrenergic receptors to limit signaling. β-Arrestins also stop Gq-protein-coupled receptor signaling. In particular, β-arrestins regulate signaling primed by Gq-coupled M_1 muscarinic receptors by limiting the production of diacylglycerol and enhancing the degradation rate of this effector [829]. β-Arrestins indeed favor conversion of diacylglycerol into phosphatidic acid and interact with diacylglycerol kinases that degrade diacylglycerol.

7.11.3 Scaffolding of Intracellular Signaling Complexes

β-Arrestins also serve as endocytic and signaling adaptors. β-Arrestins indeed intervene as scaffolds for intracellular assembly of signaling complexes. Dissociation of β-arrestins allows recycling of G-protein-coupled receptors via Rab-dependent mechanisms. β-Arrestins target active kinases to specific locations within the cell and can regulate the lifetime of G-protein-coupled receptors in endosomes [830].

7.11.4 Examples of β-Arrestins–GPCR Linkages

7.11.4.1 β-Arrestins and Angiotensin-2 Receptors

G-protein-coupled receptor kinases and β-arrestins switch off G-protein-dependent signaling, but transduce another kind of signaling from receptors, such as AT_{1A} angiotensin-2 receptor.[37] Such a signaling causes positive inotropy and lusitropy (Vols. 5 – Chap. 6. Heart Wall and 6 – Chap. 3. Cardiovascular Physiology), possibly either by regulating the cytosolic Ca^{++} concentration or Ca^{++} sensitivity of troponin and myosin-binding protein-C [831].

7.11.4.2 β-Arrestins and Adrenoceptors

β2-Adrenergic receptors cooperate with Gi protein and β-arrestin in the cardiomyocyte, in addition to major β1-adrenergic signaling, to stimulate $Ca_V1.2$ channels and increase the intracellular Ca^{++} concentration. On the other hand, β2-adrenergic receptor stimulation via the Gs–PKA signaling inhibits TNFα-induced NFκB activation by stabilizing IκBα via β-arrestin-2. β-Arrestin-2 thus has an opposite role on NFκB signaling.

7.11.4.3 β-Arrestins and Smoothened GPCR

The association of the GPCR Smoothened of the morphogen sonic Hedgehog (Sect. 10.2) with β-arrestin-2 and its phosphorylation by G-protein-coupled receptor kinase-2 promote Smoothened endocytosis via clathrin-coated pits [832].

37. This signaling is thus carried out independently from the $G\alpha_q$–PKC pathway. β-Arrestin interacts with the receptor and prevents the coupling between the receptor and G proteins.

7.11.4.4 β-Arrestins and Frizzled GPCR

After binding of Wnt morphogens to Frizzled receptors (Sects. 7.13.22 and 10.3), cytoplasmic Disheveled is recruited to the plasma membrane and phosphorylated. The adaptor β-arrestin-2, also recruited by Frizzled-4, binds to phosphorylated Disheveled and allows Frizzled-4 endocytosis [833].

7.11.4.5 β-Arrestins and Lysophosphatidic Acid Receptors

The bioactive phospholipid lysophosphatidic acid binds to cognate G-protein-coupled receptors (Sect. 7.13.35) to prime the signaling cascades and activate several transcription factors such as nuclear factor-κB (Vol. 4 – Chap. 9. Other Major Signaling Mediators). β-Arrestin-2 (but not β-arrestin-1) links to caspase-recruiting domain and membrane-associated guanylate kinase homolog domain-containing protein CARMA3 that is recruited to LPA receptors for NFκB activation and interleukin-6 production [834]. Scaffold protein CARMA3 associates with BCL10 and MALT1 to activate IκB kinase that target inhibitor of NFκB IκBα. Receptors of LPA trigger the Gq–PKC signaling with different post-translational modification of targets that can then serves as activators or inhibitors.

7.12 Other Partners of G-Protein-Coupled Receptors

Some GPCR-interacting proteins connect to specific GPCRs upon agonist binding to GPCRs to prime particular receptor signaling as mediators, whereas other interactors associate with receptors independently of agonists, thus acting as modulators, but not mediators. Transfer of and signaling from GPCRs depend on the context.

7.12.1 Regulation of GPCR Activity

Activity of G-protein-coupled receptors can be regulated by interactions between GPCRs and not only kinases, phosphatases, arrestins, and other receptor types, but also receptor-selective, cytoplasmic or transmembrane, proteic partners (Tables 7.23 and 7.24).

G-Protein-coupled receptor-interacting proteins, the production of which differs according to the cell type, contribute to fine-tuning of GPCR function. Partners of GPCRs can: (1) regulate GPCR transport, as they can be required for exocytosis of their associated GPCRs down to the plasma membrane or sort endocytosis routes between recycling back to the plasma membrane or lysosomal degradation; (2) anchor GPCRs in suitable subcellular regions; (3) organize receptor signaling via G proteins; (4) act as scaffolds to modulate G-protein signaling, as they enhance signaling by tethering effectors in the vicinity of activated receptors or lower signaling intensity and/or duration either by impeding receptor–G-protein interactions or by

Table 7.23. G-Protein-coupled receptor interactors (modulators of GPCR signaling), excluding G proteins, GPCR kinases, arrestins, and regulators of G-protein signaling (**Part 1**; Source: [835]; AKAP: A-kinase anchor protein [AKAP5 and AKAP12 are also denoted AKAP79 and AKAP250, respectively]; AR: adrenergic receptor; AT_1: angiotensin-2 receptor; D_2: dopamine receptor; DOR, KOR, MOR: δ-, κ-, μ opioid receptor; Fz: Frizzled; GABA: gamma-aminobutyric acid; JaK: Janus kinase; LPAR: lysophosphatidic acid receptor; MAGI: membrane-associated guanylate kinase; mGluR: metabotropic glutamate receptor; MT: melatonin receptor; MuPP: multiple PDZ domain-containing protein; PKA/C: protein kinase-A/C; PAFR: platelet-activating factor receptor; PTH1R: parathyroid hormone-1 receptor; STAT: signal transducers and activators of transcription; V_2R: type-2 vomeronasal receptor).

Interactor	GPCR	Action
AKAP5	β1/2AR	PKA tethering
AKAP12	β1/2AR	PKA tethering
Calmodulin	$5HT_{1A}$	Competition with PKC for GPCR phosphorylation
	$5HT_{2A}$	Impairment of G-protein coupling
	$5HT_{2C}$	Promotion of arrestin-dependent ERK activation
	D_2	Modulation of G-protein signaling
	mGluR7	Regulation of GPCR phosphorylation
	PTH1R	GPCR inhibition
	V_2R	Enhancement of GPCR-induced Ca^{++} signaling
	MOR	Inhibition of G-protein coupling
Homer	mGluR1/5	Regulation of GPCR signaling and location
JaK2	AT_1	Promotion of JaK–STAT signaling
	PAFR	Promotion of JaK–STAT signaling
MAGI3	Fz4, LPAR2	Potentiation of GPCR-mediated ERK activation
MuPP1	$GABA_B$	Enhancement of GPCR-mediated Gi signaling
	MT_1	Enhancement of GPCR-mediated Gi signaling

recruiting inhibitors of G-protein signaling to the receptor; (5) directly mediate receptor signal transmission; and (6) influence binding of ligands, such as hormones, neurotransmitters, or sensory stimuli to GPCRs [835].

Interactors of GPCRs can initiate additional signal transduction cascades. Several GPCRs can initiate signaling via interactions with members of the Janus kinase family (Vol. 4 – Chap. 3. Cytosolic Protein Tyr Kinases) as well as non-receptor protein tyrosine phosphatase such as PTPn11 (or SHP2; Vol. 4 – Chap. 7. Cytosolic Protein Phosphatases) that complement G-protein-mediated signaling. On the other hand, agonist stimulation can dissociate an interacting protein from a GPCR, thereby altering the corresponding intracellular signaling pathway.

Among GPCR-associated proteins that enhance the efficiency of some G-protein-mediated signaling axes, members of the *A-kinase anchoring protein* family (AKAP) connect to β-adrenergic receptors and protein kinase-A to raise PKA-mediated phosphorylation of signaling mediators, such as β2AR and its effectors.

On the other hand, GPCR interactors can reduce G-protein-mediated signaling. In addition to arrestins, spinophilin interacts with some GPCRs, such as dopamine, adrenergic, and muscarinic acetylcholine receptors and several regulators of G-

Table 7.24. G-Protein-coupled receptor interactors, excluding G proteins, GPCR kinases, arrestins, and regulators of G-protein signaling (**Part 2**; Source: [835]; AR: adrenergic receptor; D_2: dopamine receptor; DOR, KOR, MOR: δ-, κ-, μ-opioid receptor; LPAR: lysophosphatidic acid receptor; mAChR: muscarinic acetylcholine receptor; MCHR: melanin-concentrating hormone receptor; mGluR: metabotropic glutamate receptor; MT: melatonin receptor; Ncdn: neurochondrin; NHERF: Na^+–H^+ exchange regulatory factor; P2Y: nucleotide receptor; PKA/C: protein kinase-A/C; PI3KR1/2: PI3K regulatory subunit (P85α/β); Ppl: periplakin [a.k.a. cornified envelope precursor protein]; PPP1R9b: protein phosphatase-1 regulatory (inhibitory) subunit-9B [a.k.a. PPP1R6, PPP1R9, neurabin-2, and spinophilin]; PTH1R: parathyroid hormone-1 receptor; RhoGEF*i*: Rho guanine nucleotide-exchange factor [$i = 11, 12$; a.k.a. ArhGEF*i*]; SSTR: somatostatin receptor).

Interactor	GPCR	Action
Ncdn	MCHR1	Disruption of G-protein signaling
NHERF1	PTH1R	Enhancement of GPCR-mediated Gq signaling
	β2AR	Activation of Na^+–H^+ exchange
	KOR	Activation of Na^+–H^+ exchange
NHERF2	LPAR2	Enhancement of GPCR-mediated Gq signaling
	mGluR5	Prolongation of GPCR-caused Ca^{++} mobilization
	$P2Y_1$	Prolongation of GPCR-caused Ca^{++} signaling
	PTH1R	Enhancement of GPCR-mediated Gq signaling
PI3KR1/2	SSTR2	Mediation of survival
RhoGEF11	LPAR2	Facilitation of GPCR-mediated Rho activation
RhoGEF12	LPAR2	Facilitation of GPCR-mediated Rho activation
Ppl	MCHR1,	
	MOR	Impairment of G-protein signaling
PPP1R9b	D_2	Reduction of G- and arrestin-mediated signaling
	α2AR	Reduction of GPCR-mediated Ca^{++} signaling
	mAChR	Reduction of GPCR-mediated Ca^{++} signaling

protein signaling that act as GαGAP and can also link to some GPCRs to reduce the intensity and duration of GPCR-stimulated G-protein signaling.

Calmodulin associates with Ca^{++} and dopamine, metabotropic glutamate, and serotonin, as well as other receptors to lower G-protein coupling, hence directing a inhibitory feedback to restrain GPCR-initiated G-protein signaling. Nonetheless, G-protein-independent signaling can be potentiated by GPCR–calmodulin interactions. For example, the calmodulin–$5HT_{2C}$ complex promotes arrestin-mediated signal transduction. Periplakin and neurochondrin, the former tethering to μ-type opioid receptor (MOR) and both to melanin-concentrating hormone receptor MCHR1 as well as few other GPCRs, attenuate G-protein-mediated signaling.

7.12.2 Regulation of Intracellular GPCR Transfer and Plasmalemmal Anchoring

Once synthesized, GPCRs take the exocytosis route to localize into the plasma membrane. After agonist stimulation, most GPCRs are internalized into endosomes

Table 7.25. Regulators of GPCR trafficking and/or ligand binding, excluding GPCR kinases, arrestins, and regulators of G protein signaling proteins (**Part 1**; Source: [835]; AR: adrenergic receptor; AT: angiotensin-2 receptor; ATBP50: 50-kDa AT_2 receptor binding protein; CNR: cannabinoid receptor; $D_{1(2)}$: D1 (or D2) dopamine receptor; DOR, KOR, MOR: δ-, κ-, μ opioid receptor; DRIP78: 78-kDa dopamine receptor-interacting protein; EP: prostaglandin receptor; GABA: gamma-aminobutyric acid; GASP: GPCR-associated sorting protein; GEC: glandular epithelial cell protein; M10: M10 family members of the major histocompatibility complex class 1b; MAGI: membrane-associated guanylate kinase, WW and PDZ domain-containing protein; mGluR: metabotropic glutamate receptor; MPP: MAGUK p55 subfamily member; MC2R: melanocortin receptor-2; MRAP: MC2R-accessory protein; MuPP1: multiple PDZ domain protein; NHERF: Na^+–H^+ exchange regulatory factor; NINA: neither inactivation nor afterpotential protein; OdR: odorant response abnormal protein; PICK1: protein interacting with C kinase; PSD: postsynaptic density protein; SSTR: somatostatin receptor; V_2R: type-2 vomeronasal receptor).

Partner	GPCR	Effect
ATBP50	AT_2	Promotes GPCR surface density
DRIP78	AT_1, D_1	Promotes GPCR surface density
GASP1	CNR1, D_2, DOR	Favors GPCR lysosomal degradation
GEC1	EP_3, KOR	Promotes GPCR surface density
Homer	mGluR1/5	Regulates GPCR signaling
M10	V_2R	Promotes GPCR surface density
MAGI2	β1AR	Promotes β1AR endocytosis
MPP3	$5HT_{2C}$	Impedes $5HT_{2C}$ endocytosis
MRAP1/2	MC2R	Promotes GPCR exocytosis
MuPP1	$5HT_{2C}$	Promotes GPCR clustering
	$GABA_B$	Promotes GPCR stability
	SSTR3	Targets GPCR to tight junctions
NHERF1	β2AR, KOR	Promotes GPCR recycling
NINAa	Rhodopsin	Promotes GPCR genesis and trafficking
OdR4	OdR10	Promotes GPCR surface density
PICK1	mGluR7a	Promotes GPCR clustering
PSD95	$5HT_{2A}$	Impedes GPCR endocytosis
	β1AR	Reduces GPCR endocytosis

and take one path among 2 possible routes: lysosomes for degradation or recycling endosomes for reinsertion into the plasma membrane. Interactors of GPCRs can influence exocytosis and post-endocytic sorting of GPCRs [835] (Tables 7.25 and 7.26).

Certain GPCR-interacting proteins regulate GPCR folding after translation before exocytosis as well as transport to and insertion into the plasma membrane. [38]

38. Ran-binding protein RanBP2, dynein light-chain component T-complex testis-specific protein DynLT1 (a.k.a. TCTex1), glandular epithelial cell protein GEC1 (a.k.a. $GABA_A$ receptor-associated protein-like protein GABARAPL1), receptor of activated protein kinase RACK1 (a.k.a. guanine nucleotide-binding-like protein GNB2L1), dopamine receptor-interacting protein DRIP78 (a.k.a. heat shock protein HSP40 [DnaJ] molecular chaperone

Table 7.26. Regulators of GPCR trafficking and/or ligand binding, excluding GPCR kinases, arrestins, and regulators of G protein signaling proteins (**Part 2**; Source: [835]; AR: adrenergic receptor; CalcR: calcitonin receptor; CGRP, calcitonin gene-related peptide; CRLR: calcitonin receptor-like receptor; DOR, KOR, MOR: δ-, κ-, μ opioid receptor; DynLT: dynein light chain Tctex (T-complex testis-specific) protein; mGluR: metabotropic glutamate receptor; OR: olfactory receptor; PAR: proteinase-activated receptor; RACK1: receptor of activated protein kinase-C1; RAMP: receptor activity-modifying protein; RanBP: Ran-binding protein; REEP: receptor expression-enhancing protein; RTP: receptor transporting protein; Shank: SH3- and multiple ankyrin repeat domain-containing protein; SNx: sorting nexin; SSTR: somatostatin receptor; T_2R: taste receptor-2; TXA2R: thromboxane-A2 receptor; USP: ubiquitin-specific-processing peptidase 4).

Partner	GPCR	Effect
RACK1	TXA2R	Promotes GPCR exocytosis
RAMP1	CRLR	Forms functional CGRP receptors
	CalcR	Forms functional amylin receptors
RAMP2	CRLR	Forms functional adrenomedullin receptors
RAMP3	CRLR	Forms functional adrenomedullin receptors
	CalcR	Forms functional amylin receptors
RanBP2	Opsin	Promotes GPCR exocytosis
REEP	OR	Promotes GPCR exocytosis
	T_2R	Promotes GPCR surface density
RTP	OR	Promotes GPCR exocytosis
	T_2R	Promotes GPCR surface density
RTP4	DOR, MOR	Promotes GPCR surface density
Shank	Lphn1	Promotes GPCR clustering
	mGluR1/5	Anchors GPCR in mature dendritic spines
SNx1	PAR1 (F2R)	Facilitates GPCR degradation
Syntrophin	α1dAR	Enhances GPCR stability
DynLT1	Rhodopsin	Promotes apical GPCR delivery
USP4	α2aAR	Promotes GPCR surface density

Other GPCR interactors influence post-endocytic transfer of GPCRs in response to agonist stimulation in a more receptor-selective manner than GRKs and arrestins. [39] Some GPCR interactors, such as Homer proteins and Disc large homologs, control GPCR anchoring to appropriate regions of the plasma membrane.

DnaJC14), AT_2-binding protein ATBP50, and ubiquitin specific-processing peptidase USP4 link to their corresponding GPCRs to enhance their transfer [835].

39. GPCR-associated sorting protein GASP1 promotes endocytosis toward lysosomes of δ-type opioid receptors (DOR), D_2 dopamine receptor, and CB_1 cannabinoid receptor [835]. On the other hand, sodium–hydrogen exchanger-regulatory factor NHERF1 promotes the recycling of β2-adrenoceptors and κ opioid receptors (KOR).

Table 7.27. Metabotropic acetylcholine muscarinic receptors (mAChR; M_1–M_5 or M1R–M5R) and their main targeted G proteins (Source: [736]). Ionotropic nicotinic acetylcholine receptors (nAChR) belong to the superfamily of ligand-gated ion channels (Sect. 2.5.2). Muscarinic M_1, M_3, and M_5 receptors couple preferentially to Gq subunit and activate phospholipase-Cβ. Muscarinic M_2 and M_4 receptors couple preferentially to Gi subunit and inhibit adenylate cyclase.

Type	Main transducer
ACh Muscarinic receptors	
M_1, M_3, M_5	Gq/11
M_2, M_4	Gi/o
ACh Nicotinic receptors	
α1–α7	Ligand-gated ion channels

7.12.3 Regulation of Ligand Binding

Some GPCR interactors influence agonist selectivity of their corresponding GPCRs. Transmembrane receptor activity-modifying proteins RAMP1 to RAMP3 tether to calcitonin receptor-like receptor to construct functional receptor for calcitonin gene-related peptide (RAMP1–CRLR) and adrenomedullin (RAMP2–CRLR and RAMP3–CRLR) as well as calcitonin receptor to form amylin receptors [835].

7.13 Types of G-Protein-Coupled Receptors

G-protein-coupled receptors represent drug targets. Handling of GPCR activation then allows design of agonist or antagonist ligands, in addition to signaling modeling.

7.13.1 Acetylcholine Muscarinic Receptors

Muscarinic acetylcholine receptors (M_1–M_5 or M1R–M5R) pertain to class-A GPCR receptors. Selective enhancers of acetylcholine binding and action exist for various receptor subtypes [5]. Main guanine nucleotide-binding (G) proteins targeted by muscarinic receptors are given in Table 7.27.

Muscarinic receptors M_1 and M_2 couple voltage sensing to acetylcholine binding. Conformational changes are associated with charge motions. [40] Ligand binding of GPCRs can then be modulated by voltage, amplifying or attenuating signals. Effects of muscarinic receptor subtypes are given in Table 7.28.

40. Voltage is sensed not only by voltage-gated ion channels, but also ion transporters, such as sodium–glucose cotransporter, voltage-dependent phosphoinositide phosphatase, and glutamate G-protein-coupled receptors $mGlu_1$ and $mGluR_3$.

Table 7.28. Types of muscarinic acetylcholine receptors and their functions.

Type	Effects
M_1	Cognition and memory (prefrontal cortex), secretion of salivary glands and stomach
M_2	Cardiac frequency reduction, inhibition of β-adrenergic-mediated SMC relaxation
M_3	Smooth muscle contraction (blood vessels and airways), modulation by stimulated NO release from endothelium, endocrine and exocrine gland secretion, learning and memory (hippocampus)
M_4	Locomotion control
M_5	Vasodilation in the cerebral vasculature, behavioral cognition (hippocampus)

7.13.1.1 Muscarinic Receptors in Cardiomyocytes

The activation of muscarinic receptors in cardiomyocytes depends on transsarcolemmal potential. Depolarization reversibly induces changes in affinity state of the receptor [838]. Depolarization raises and reduces the affinity of M_1 and M_2 receptors, respectively [839].

7.13.1.2 Muscarinic Receptors in Nervous Synapses

The actin depolymerizing factor–cofilin complex regulates actin-dependent vesicular transport of acetylcholine receptors to the postsynaptic membrane and contributes to membrane insertion, formation, and maintenance of muscarinic receptor clusters [840].

Scaffold 14-3-3 that sequesters the ADF–cofilin complex attenuates the synaptic insertion of muscarinic receptors and clustering. On the other hand, 2 counteracting nerve-derived factors, agrin and acetylcholine, regulate the redistribution of muscarinic receptors on the sarcolemma at neuromuscular junctions.

Agrin activates muscle-specific Tyr kinase (MuSK) that favors muscarinic receptor clustering on the postsynaptic membrane, whereas acetylcholine disperses extrasynaptic clusters of muscarinic receptors. The agrin–MuSK signaling involves P21-activated kinase, an activator of LIMK kinases that phosphorylates (inactivates) the ADF–cofilin complex. Protein 14-3-3γ colocalizes and interacts with MuSK at neuromuscular junctions.

7.13.1.3 M_1 Receptor

Central Nervous System

The M_1 subtype of muscarinic (acetylcholine) receptors is selectively distributed in the forebrain, where it intervenes in cognition and memory. It can mediate learning and memory via an indirect mechanism, i.e., stimulation of the prefrontal cortex. It also supports the synaptic transmission and excitatory postsynaptic potential in postganglionic nerves of autonomic ganglia.

The M_1 receptor is randomly distributed over the plasma membrane [841]. At any time, about 30% of the receptors are dimers. (Oligomers M_1 are not detected.) Interconversion between M_1 monomers and dimers occurs on a time scale of seconds.

Other Effects

Receptor M_1 transmits signal from the vagus nerves and cause a bronchoconstriction. In the digestive tract, M_1 provokes the secretion of salivary glands and gastric acid in the stomach.

7.13.1.4 M_2 Receptor

Nodal Cells and Cardiomyocytes

In the heart, M_2 receptor: (1) diminishes the speed of depolarization in the sinoatrial node (the natural pacemaker), thereby slowing the cardiac frequency, and (2) reduces the contractile force of atriomyocytes, but have no effect on that of ventriculomyocytes.

Smooth Muscle Cells

Muscarinic M_2 and M_3 receptors are coexpressed in many types of smooth muscle cells, such as those in walls of the gastrointestinal tract, bladder, blood vessels, and airways. The density of M_2 receptors is greater than that of M_3 receptors (relative density $\geq$4:1) [842].

In airway smooth muscle cells, activated Gi-coupled M_2 receptor inhibits β-adrenergic-mediated relaxation; activated Gq-coupled M_3 receptor initiates their contraction.

In smooth muscle cells, M_2 receptor inhibits AC5 and AC6 adenylate cyclases via $G\alpha_{i3}$, whereas M_3 activates AC5 and AC6 via $G\beta\gamma$ [843].

7.13.1.5 M_3 Receptor

Smooth Muscle and Endothelial Cells

Muscarinic M_3 receptor localizes to smooth muscle cells of the wall of blood vessels and respiratory conduits. This Gq-coupled receptor generates an increase in intracellular calcium concentration, thereby priming a contraction of smooth muscle cells (vaso- and bronchoconstriction). [41]

However, in the vasculature, activated M_3 on vascular endothelial cells augment the synthesis of nitric oxide that targets adjacent vascular smooth muscle cells and causes their relaxation. Therefore, nitric oxide modulates the direct action of acetylcholine on smooth muscle cells.

Pancreatic β Cell

Functioning of pancreatic β cell is regulated by many hormones and neurotransmitters. Most of them act on specific G-protein-coupled receptors. Muscarinic receptor M_3 (or M3R) is linked to parasympathetic nerves that innervate the endocrine pancreas.

In pancreatic β cells, M_3 receptor leads to the stimulation of G-protein subunits of the $G\alpha_q$ family and promotes glucose-stimulated insulin secretion. Subunit Gq are controlled by regulators of G-protein signaling. Protein RGS4, the most abundant RGS subtype synthesized in β cells, is a potent inhibitor of M_3 receptors [844].

Similarly to all GPCRs after agonist stimulation, the activity of M_3 is regulated via hyperphosphorylation by diverse protein kinases (GPCR kinases [GRK] and casein kinases CK1α and CK2), hence phosphorylation-dependent receptor endocytosis and β-arrestin recruitment. Additional arrestin-primed phosphorylation of M_3^P by protein kinase-D1 enables the sustained phase of glucose-dependent insulin release, independently of heterotrimeric G proteins [845]. [42]

Central Nervous System

In the hippocampus, M_3 participates in learning and memory [846]. These cognition processes rely on M_3 phosphorylation, receptor endocytosis, and arrestin recruitment.

7.13.1.6 M_4 Receptor

Muscarinic acetylcholine receptors regulate dopaminergic neurotransmission. Receptor M_4 is coexpressed with D_1 dopamine receptor in a specific subset of striatal projection neurons. Activated M_4 receptor in the striatum inhibits D_1-induced locomotor stimulation (in mice) [847].

41. Activation by M_3 of Gq subunit increases phosphoinositide hydrolysis and releases Ca^{++} ions from the sarcoplasmic reticulum.

42. Two phases of insulin release — transient and sustained — result from feeding. The early phase of insulin release depends on activation by Gq of calcium influx and protein kinase-C.

7.13.1.7 M_5 Receptor

Receptor M_5 is the sole mediator of ACh-induced vasodilation in the cerebral vasculature [848]. It is also involved in long-term potentiation at the hippocampal mossy fiber-CA3 synapse and hippocampal-dependent behavioral cognition.

7.13.2 Adenosine Receptors

Adenosine is an ubiquitous, multifunctional purine nucleoside that targets numerous enzymes, such as protein kinases and adenylate cyclases.[43] Intracellular adenosine can be formed by either dephosphorylation of AMP by 5′-nucleotidase or by hydrolysis of ^{S}adenosylhomocysteine. Extracellular adenosine can be produced from released adenine nucleotides by a cascade of ectonucleotidases such as ecto-5′-nucleotidase (NT5E or CD73), which is highly expressed in microglial cells, and ectonucleoside triphosphate diphosphohydrolase ENTPD1 (or CD39) [849].

In the central nervous system, the extracellular concentration of adenosine is controlled by astrocytes. Glial cells and neurons release ATP, a neurotransmitter that activates its P2X and P2Y receptors. Vesicular ATP release from astrocytes (but not from neurons) by exocytosis or through equilibrative nucleoside transporters, possibly pannexin-1 hemichannels, and Maxi anion channels, is a source of extracellular synaptic adenosine via ectonucleotidase action on ATP [849]. Adenosine thus influences synaptic transmission. Endocrine cells also secrete ATP, even without releasing stored hormone.

Adenosine receptors constitute a family of nucleoside receptors (P1 receptors; Table 7.29).[44] Adenosine activates 4 receptor types (A_1, A_{2A}, A_{2B}, and A_3). Gi-Coupled receptors A_1 and A_3 can also be activated by inosine. These adenosine receptors inhibit many intracellular ATP-using enzymes such as adenylate cyclases [5].

Adenosine is a cerebral vasodilator. Smooth muscle cells of cerebral arteries synthesize A_1, A_{2A}, A_{2B}, and A_3 receptors [850]. Adenosine mainly binds to A_{2A} and A_{2B} and generates superoxide using NADPH oxidase and mitochondria.

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43. Purines adenine and guanine are synthesized in vivo via inosine monophosphate (IMP), which is laso named inosinic acid and inosinate. The latter is also converted into nucleotides, i.e., bases attached to ribose 5-phosphate: (1) adenosine monophosphate (AMP; a.k.a. adenylic acid and adenylate) by adenylsuccinate synthetase and adenylosuccinate lyase and (2) guanosine monophosphate (GMP; a.k.a. guanidylic acid, guanylic acid, and guanylate) by IMP dehydrogenase and GMP synthase. Conversely, AMP can be converted into IMP by myoadenylate deaminase. Adenosine can be transformed into inosine via adenosine deaminase and AMP via adenosine kinase. De novo synthesis of adenosine does not exist.

44. The P1 receptor class is associated with a nucleoside. The term "nucleoside receptor" to define a P1 family member is more appropriate then purinergic receptor. Members of the family of nucleotide receptors (P2) link to both purines and pyrimidines. As both purine and pyrimidine species are nucleotides, the P2 family is better defined as the class of "nucleotide receptor." Nevertheless, the noun "purinergic" should be kept in mind to explain the aliases P1 and P2.

Table 7.29. Classes of the receptor family for nucleosides and nucleotides. Adenosine targets nucleoside receptors of the P1 (purinergic) class. Nucleotides are ubiquitous intercellular messengers that act via specific receptors: ionotropic P2X and metabotropic P2Y nucleotide receptors.

Set	Activator	Molecule type
P1 receptors	Adenosine	G-protein-coupled receptor
P2X receptors	ATP	Ligand-gated ion channel
P2Y receptors	Nucleotides	G-protein-coupled receptor

Adenosine receptors A_1 and A_3 preferentially interact with G-protein subunits of the Gi/o family; A_{2A} and A_{2B} receptors with members of the Gs family [851] (Tables 7.30 and 7.31).

Adenosine receptors A_1 and A_3 stimulate K^+ channels, reduce transient activity of voltage-dependent Ca^{++} channels, and inhibit cAMP formation. Whereas A_{2A} receptor couples to members of the Gs family (e.g., $G\alpha_{olf}$ in striatal neurons), A_{2B} receptor associates with many G-protein subunit types, such as Gs, Gq, and G12 [849]. Adenosine is approximately equipotent on A_1, A_{2A}, and A_3 receptors. The A_{2B} receptor may require higher agonist concentrations.

7.13.2.1 A_1 Receptor

Adenosine A_1 receptor is ubiquitous (Table 7.30). The A_1 receptor attenuates stimulatory actions of catecholamines on β-adrenergic receptors, reduces lipolysis in adipose tissue and urine production, and impedes neuronal activity [852]. Activated A_1 receptor not only decreases cAMP synthesis, but also stimulates phospholipase-C.

Central Nervous System

Adenosine receptor A_1 contributes to sleep regulation, as it is upregulated in cortical and subcortical brain regions, especially in the orbitofrontal cortex after prolonged wakefulness [853]. It hence promotes sleep and inhibits wakefulness via cholinergic neurons, in opposition with xanthine, an adenosine antagonist in coffee and tea. The activity of basal forebrain (substantia innominata) and mesopontine (laterodorsal tegmental nucleus) cholinergic neurons actually bear an inhibitory control by endogenous adenosine in cats [854].

The A_1 receptor in the nucleus of the solitary tract is involved in the baroreflex (Vols. 2 – Chap. 1. Remote Control Cells and 6 – Chap. 3. Cardiovascular Physiology). Its activity inhibits glutamatergic transmission in the nucleus of the solitary tract [855]. This receptor differentially inhibits and resets the baroreflex response of preganglionic adrenal, renal, and lumbar sympathetic nerve.

Table 7.30. Adenosine receptors A_1 and A_3, G mediators, expression loci, and tissue functions (↓: reduction; −: inhibition; +: stimulation; DAG: diacylglycerol; IP_3: inositol trisphosphate; PL(A/C/D): phospholipase-A/C/D; Source: [851]).

A_1	A_3
Gi/o (Gi1/2/3; Go) PLA2, PLC, PLD IP_3/DAG, cAMP −	Gi/o (Gi2/3) Gq/11 PLC IP_3/DAG, cAMP −
High expression	
Brain cortex, cerebellum, hippocampus, dorsal horn of spinal cord, eye, adrenal gland, atria	Mastocytes
Intermediate expression	
Other brain regions, skeletal muscle, adipose tissue, liver, kidney, salivary glands, esophagus, colon, antrum, testis	Cerebellum, hippocampus, pineal gland, lung
Low expression	
Lung, pancreas	Brain, adrenal, thyroid, testis, intestine, spleen, liver
Vasoconstriction Bradycardia Lipolysis − Glomerular filtration ↓ Ischemic preconditioning Sympathetic and parasympathetic activity ↓	Vasodilation Preconditioning Mastocyte degranulation

Glial cells that interact with neurons and blood vessels control adenosine that has a neuroprotective role in the central nervous system. Microglial cells, astrocytes, endothelial cells, oligodendrocytes, and neurons produce signals to manage inflammation, hence entry of cells of the innate and adaptive immune system in the brain. These cells release arachidonic acid metabolites, nitric oxide, cytokines, and chemokines as well as ATP and adenosine. The A_1 receptor on microglial cells reduce excessive immune activation of microglial cells [849]. It also stimulates oligodendrocyte migration.

The A_1 receptor on neurons, especially at nerve terminals, contributes to the dampening of neuronal activity mediated by adenosine generated from ATP released from astrocytes [849]. In nerve endings, it may preferentially signal via Go proteins to inhibit calcium channels. In neuron bodies and dendrites, it may preferentially regulate potassium channels via Gi proteins.

Table 7.31. Adenosine receptors A_{2A} and A_{2B}, G mediators, expression loci, and tissue functions (↓: reduction; −: inhibition; +: stimulation; DAG: diacylglycerol; IP_3: inositol trisphosphate; PL(A/C/D): phospholipase-A/C/D; Source: [851]).

A_{2A}	A_{2B}
Gs	Gs
G_{olf}, G15/16	Gq/11
PLA2, PLC, PLD	PLC
cAMP, IP_3/DAG	cAMP, IP_3/DAG
High expression	
Caudate-putamen, nucleus accumbens, tuberculum olfactorium, olfactory bulb spleen, thymus Leukocytes, platelets	Colon, bladder
Intermediate expression	
Heart, blood vessels, lungs	Median eminence, blood vessels, lungs, eyes Mastocyte
Low expression	
Other brain regions, heart, kidney	Brain, pituitary adrenal, ovary, kidney, liver, adipose tissue
Vasodilation	Vasodilation
Platelet aggregation −	Intestinal SMC relaxation
Neutrophil activation end	Monocyte/macrophage −
Regulation of sensorimotor	Mastocyte degranulation
Integration in basal ganglia	
Sensory nerve activity +	

7.13.2.2 A_2 Receptor

High-affinity A_{2A} and low-affinity A_{2B} receptors are coupled to stimulatory subunit $G\alpha_s$ of heterotrimeric G protein, thereby raising intracellular cAMP concentration (Table 7.31). Activated A_{2A} can protect against tissue injury and act as an anti-inflammatory agent [856].

Human A_{2A} receptor has a binding site that differ in position and orientation with respect to other GPCRs [857]. Extracellular loops in collaboration with the helical core allows ligand recognition.

Central Nervous System

In the brain, A_{2A} receptor is synthesized at high levels in striatal neurons and at low levels in other neurons and glial cells [849]. The A_{2A} production in glial cells

rises after brain insult. In astrocytes, activated A_{2A} by extracellular adenosine increases cell proliferation, but impedes NOS2 synthesis. In microglial cells, activated A_{2A} facilitates the release of cytokines and prostaglandin-E2, as it boosts the activity of cyclooxygenase-2, as well as that of nitric oxide synthase and production of nerve growth factor [849].

Activated A_{2A} in the nucleus of the solitary tract in the brainstem increases activity of preganglionic adrenal sympathetic nerves, but decreases that of renal sympathetic nerves [858].

The A_{2B} receptor can couple with Gq and Gs on astrocytes. It is responsible for the adenosine-induced stimulation of interleukin-6 from astrocytes.

Increased neural activity is associated with cerebral vasodilation. Neural activation-associated pial arteriolar dilation involves interactions between A_2 and inward rectifier K_{IR} channels [859]. Large-conductance, Ca^{++}-activated BK channel ($K_{Ca}1$) participates in the process within the glia limitans. The A_2 receptor provokes the synthesis by adenylate cyclase of cAMP that primes the phosphorylation of K^+ channels by protein kinase A.

Skin Microvasculature

In human dermal microvascular endothelial cells, activation of A_{2B}, but not A_{2A}, promotes angiogenesis. On the other hand, activated A_{2A}, but not A_{2B}, promotes angiogenesis in endothelial cells of human umbilical veins and lung microvasculature. Endothelial cells express A_{2A}, A_{2B}, or both.

Airways

In the respiratory tract, A_{2B} receptors regulate chloride channels via cyclic adenosine monophosphate.

7.13.2.3 A_3 Receptor

Like the adenosine receptor A_1, A_3 is coupled to inhibitory subunit $G\alpha_i$ of heterotrimeric G protein, thus inhibiting adenylate cyclase (Table 7.30). It has a protective function during tissue ischemia.

Neutrophil

Adenosine receptor A_3 contributes to inhibition of neutrophil degranulation in tissue injury. In neutrophils, ATP acts on P2Y receptors in synergy with adenosine that binds to A_3 receptors to stimulate migration.

Central Nervous System

Astrocytic A_3 receptor regulates chemokine release [849]. Activation of A_3 by adenosine protects astrocytes from death caused by hypoxia. Adenosine contributes to microglial migration caused by ATP predominantly via $P2Y_{12}$ receptors.

Table 7.32. Kinetics of A_{2A} adenosine receptor activation (Sources: [792, 860]).

Event	Time (ms)
GPCR activation and G-protein binding	∼50
Gs activation	∼500
Deactivation	∼2000

7.13.2.4 Activation and Deactivation Kinetics of Adenosine Receptors

Fluorescence resonance energy transfer-based assays can be used to assess the receptor-specific activation and deactivation kinetics of the different GPCR signaling steps, especially the activation kinetics of initial stages (GPCR–G-protein interaction that depends on the amount of available receptors and determines the activation G-protein and other effectors [860].

Both A_{2A} and β1-adrenergic receptors couple to Gs protein, thereby stimulating adenylate cyclase that produces cAMP messenger. Times of A_{2A} receptor activation, A_{2A}–Gs interaction, and Gs activation following stimulation by A_{2A} or β1-adrenoceptors are smaller than 40, 60, and 550 ms, respectively. Only a fraction of Gs proteins is activated when GPCR–Gs interaction is maximal. The rate of Gs activation limits the signaling initiation via Gs-coupled receptors. Activation time of Gs subunit is also similar to that of Gi stimulation after excitation by β1-adrenergic receptors.

Three main events occur between GPCR–G-protein interaction and G-protein activation: GDP release from Gα, GTP binding, and conformational change. Release of GDP is rate-limiting in G-protein activation. Half-time of cAMP activation is about equal to 35 s, but activation quickly appears (a few ssconds) due to the existence of Gs–ACase complexes.

In addition, although the activation kinetics via A_{2A} and β1-adrenergic receptors are similar, the deactivation kinetics differ. Termination of A_{2A}–Gs interaction and Gs deactivation (following A_{2A} deactivation with a time ∼2 s, which is similar to that of α2a-adrenoceptors) are significantly slower than that of β1-adrenoceptor signaling (termination of β1-adrenoceptor–Gi interaction ∼13 s). Termination of A_{2A}–Gs interaction is faster than Gs deactivation, which is linked to the activity of accessory proteins (activators and regulators of G-protein signaling) involved in GPCR signaling. Ligand washout is hence not limiting. Kinetics of A_{2A} recptor are given in Table 7.32.

7.13.2.5 Adenosine Receptors in the Cardiovascular Apparatus

Vascular Smooth Muscle Cells

In the vasculature, A_1 receptors as well as A_{2A}, A_{2B}, and A_3 are synthesized by smooth muscle cells [861]. The A_1 receptor causes contraction of vascular smooth

muscle via phospholipase-C. It hinders vascular relaxation mediated by other adenosine receptor subtypes.

The A_1 receptor upregulates PKCα, PKCβ1, PKCβ2, PKCγ, PKCε, and PKCζ isoforms (but not PKCδ and PKCμ) in a dose-dependent manner [862]. Activated A_1 tethers to Gi subtypes Gi1 to Gi3 or Go subunit, thereby inhibiting adenylate cyclase and decreasing cAMP concentration.

Gs-Coupled A_{2A} and/or A_{2B} receptors are involved in adenosine-mediated vascular relaxation of coronary and aortic beds in mice [861]. Gi-Coupled A_3 receptor does not cause a vasoconstriction, as it is inhibited by A_{2A} receptor. The A_3 receptor mediates the cardioprotective effect of adenosine.

In the central nervous system, adenosine, a potent vasodilator, intervenes in vasodilation during hypotension within the autoregulatory range of vascular autoregulation via both A_{2A} and A_{2B} receptors [863].

Vascular Endothelial Cells

Hypoxia stimulates hypoxia-inducible HIF1α and HIF2α transcription factors that promote angiogenesis.[45] The HIF2α factor, but not HIF1α, regulates A_{2A} receptor in pulmonary endothelial cells to increase cell proliferation and migration as well as elicit tube formation [856].

Cardiomyocytes

Cardiomyocytes, in particular ventriculoyocytes, express the 4 adenosine receptor subtypes. Receptors A_1 and A_{2A} regulate myocardial oxygen consumption and coronary blood flow.

Adenosine is released from the heart subjected to hypoxia or adrenergic stimulation. The A_1 receptor has a strong anti-adrenergic effect. Therefore, adenosine antagonizes adrenergic stimulation to avoid overstimulation. Stimulation of A_1 activates Gi subunit, releases the G$\beta\gamma$ dimer, and excites phospholipase-C and protein kinase-Cϵ [864]. In addition, activated Gi subunit counteracts effect of Gs subunits stimulated by catecholamines via adrenoceptors.

The A_1 receptor is involved in the protection acquired during ischemic preconditioning against myocardial ischemia–reperfusion injury [865]. Stimulated A_1 re-

45. In almost all cell types, HIF1α targets various genes, such as those that encode vascular endothelial growth factor and its receptors, glucose transporter-1, carbonic anhydrase-9 and -12, hexokinase-2, glucose phosphate isomerase, phosphofructokinase, aldolase-A and -C, glyceraldehyde 3-phosphate dehydrogenase, among others. On the other hand, HIF2α regulates a few genes in specific cell lines, such as those that encode erythropoietin, superoxide dismutase-1 and -2, glutathione peroxidase, catalase, frataxin, Octamer-4, insulin-like growth factor-binding protein IGFBP3, SRY-related HMG-box gene product Sox9, CBP and P300-interacting transactivator with Glu/Asp-rich C-terminal domain CITED2. In most cell types, the gene set regulated by HIF2α overlaps with that of HIF1α [856].

presses the pacemaker cell function and decreases action potential conduction, thus reducing the heart rate [852]. It also lowers the atrial contractility. [46]

Whereas the A_1 receptor reduces β1-adrenergic receptor-induced increase in contractility in rat ventriculomyocytes, the A_{2A} receptor enhances cardiac contractility via Gs and Ca^{++} transient [868]. Cardiac A_1 and A_{2A} receptors use PKCϵ and the cAMP–PKA pathway, respectively. Cardiac A_{2A} receptor acts like β1-adrenoceptor, as it also primes the cAMP–PKA pathway, but with smaller Ca^{++} transient amplitude.

The A_{2A} receptor, but not the A_{2B} receptor, counteracts the A_1 receptor. It indirectly enhances heart contractility, as it modulates the A_1 anti-adrenergic effect [869]. On the other hand, the A_{2B} receptor exerts a direct contractile effect, but does not alter β-adrenergic or A_1 anti-adrenergic effects.

Activated A_{2A} receptor before reperfusion following coronary artery occlusion reduces infarct size and improves cardiac function, but activation must occur before or less than 1 h after reperfusion [870].

7.13.2.6 Inflammation

The A_2 receptor acts as a sensor of tissue damage. The A_{2A} receptor attenuates inflammation and tissue damage. The A_{2B} receptor abounds in vascular smooth muscle cells. It regulates the activity of the G-protein-coupled CXCR4 receptor that attracts vascular progenitor cells from bone marrow during tissue regeneration, avoiding excessive tissue growth after injury [871]. Chemokine CXCR4 receptor controls migration of inflammatory cells and platelet aggregation. It is thus involved in vascular repair and remodeling after injury. [47] Upregulation of CXCR4 in injured vessels is limited by A_{2B} receptors. In addition, adenosine inhibits smooth muscle cell proliferation via activated A_{2B} receptor.

46. Overexpression of A_1 causes supraventricular arrhythmias [866]. A strong A_1 overexpression also induces dilated cardiomyopathy [867].

47. Chemokine CXCR4 receptor is expressed on the surface of macrophages, lymphocytes, hematopoietic stem cells, bone marrow stromal cells, megakaryocytes, and platelets. It is involved in the migration of hematopoietic progenitors and stem cells during cardio- and vasculogenesis and hematopoiesis. It is regulated by TNFα secreted especially by several bone marrow cell types.

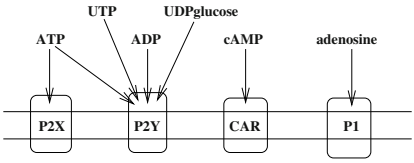

Figure 7.4. Nucleotide receptor types and their ligands.

7.13.3 Nucleotide P2Y Receptors

Nucleotide receptors of the P2Y family bind to both purine and pyrimidine nucleotides.[48] Purines include nucleic acid bases adenine and guanine[49] as well as hypoxanthine, xanthine, theobromine, caffeine, uric acid, and isoguanine.[50]

Ubiquitous nucleoside and nucleotide receptors (or purine and pyrimidine receptors) include P1 receptors (A_1–A_3) and P2 receptors (P2X and P2Y; Table 7.33). P1 Receptors bind adenosine, P2 receptors extracellular adenosine and purine nucleosides and nucleotides [872] (Fig. 7.4).

Ionotropic P2X receptors that encompass 7 known types ($P2X_1$–$P2X_7$) are ion channels (Sect. 2.5.7).[51] Most P2X channels are cation-selective pores that discriminate more or less efficiently different cations, particularly Ca^{++} ions.

Many identified metabotropic, G-protein-coupled P2Y receptors ($P2Y_1$–$P2Y_{14}$) are activated by ATP, ADP, UTP, UDP, and ^{UDP}glucose. The P2Y receptors differ according to their ligands (adenine vs. uracil-derived nucleotides as well as tri- vs. diphosphonucleotides), signaling mode, and cell expression (Tables 7.34 and 7.35). Calcium fluxes generated by P2Y receptors are larger for a given depolarization than equivalent amplitude hyperpolarization [873].

48. The term "purinergic receptors" used to designate receptors of the P2Y family is inappropriate. In addition, receptors of the P1 family connect to purine nucleoside adenosine. The term "nucleotide receptors" to denote both members of the P1 and P2Y family of the GPCR superclass should be likewise avoided.

49. Double-stranded DNA relies on base pairing between nucleotide constituents. Purines adenine and guanine couple with pyrimidines thymine and cytosine, respectively. Whereas each DNA strand is composed of 4 bases — adenine, cytosine, guanine, and thymine —, single-stranded RNA contains adenine, cytosine, guanine, and uracil.

50. Purines are components of numerous molecules, such as: (1) purine nucleosides adenosine and guanosine; (2) adenylate (ATP, ADP, AMP, and cAMP) and guanylate (GTP, GDP, GMP, and cGMP) nucleotides; and (3) nucleotides constituents of enzymatic cofactors (coenzyme-A, flavin adenine dinucleotide [FAD], flavin mononucleotide [FMN], and nicotinamide adenine dinucleotide phosphate [NADH]). Pyrimidines include nucleobases cytosine, thymine, and uracil. Uridine is a pyrimidine nucleoside. Pyrimidine nucleotides encompass uridine mono- (or 5′-uridylic acid [UMP]), di- (UDP), and triphosphate (UTP).

51. Nucleotide receptors P2X are fast-acting, ligand-gated channels that are selective for calcium. When ATP binds to plasmalemmal P2X receptors, extracellular Ca^{++} enters and activates signal transduction pathways. These homo- and hetero-oligomeric channels contain 2 transmembrane domains.

Table 7.33. Receptors for purines and pyrimidines (Source: [106]; CNS: central nervous system; NST: nucleus of the solitary tract).

Receptor	Main location
	Receptors of the P1 family
A_1	Brain, spinal cord, autonomic nerve terminals, heart, testis
A_{2A}	Brain, heart, lungs, spleen
A_{2B}	Colon, bladder
A_3	Brain, heart, lung, liver, testis
	Receptors of the P2X family
$P2X_1$	Cerebellum, dorsal horn spinal neurons, smooth muscle cell, platelet
$P2X_2$	CNS, autonomic and sensory ganglia, retina, chromaffin and smooth muscle cells
$P2X_3$	Sensory neurons, NST, some sympathetic neurons
$P2X_4$	CNS, testis, colon
$P2X_5$	Proliferating cells
$P2X_6$	CNS, motor neurons in spinal cord
$P2X_7$	Apoptotic cells
	Receptors of the P2Y family
$P2Y_1$	Epithelial and endothelial cells, platelets, immune cells, osteoclasts
$P2Y_2$	Epithelial (kidney tubule) and endothelial cells, immune cells, osteoblasts
$P2Y_4$	Endothelial cells
$P2Y_6$	Some epithelial cells, T cells
$P2Y_{11}$	Granulocytes
$P2Y_{12}$	Platelets, glial cells
$P2Y_{13}$	Brain, lymph nodes, bone marrow, spleen
$P2Y_{14}$	Brain regions, adipose tissue, digestive tract

Nucleotides can be stored and secreted upon adequate cell excitation to serve as messengers that bind to P2Y receptors. In the chromaffin granules of the adrenal medulla, ATP concentration reaches 100 mmol (cytoplasmic concentration 3–5 mmol) [874]. Dense granules of platelets contain about 500 mmol of ADP and ATP, as well as lower amounts of other nucleotides (adenine dinucleotides, GTP, and UTP). Following secretion and/or degranulation upon appropriate stimulation, [52] concentration of released nucleotides transiently reaches high levels. Nucleotide concentration is much higher near producing cell surface than in bulk extracellular fluid.

52. Change in blood flow pattern induces ATP release from endothelial cells. Release of ATP from human nasal epithelial cells increases upon mechanical stimulation or rising intracellular concentration of calcium ions. Corelease of ATP and nucleotidases from sympathetic nerves terminates the action of a neurotransmitter.

Table 7.34. Adenosine P1 and nucleotide P2Y receptors, their main targeted G proteins, and order of ligand potency (Sources: [736, 874]). The P2X receptors are ATP-gated ion channels for Na^+, K^+, and Ca^{++} ions. Some P2Y receptors couple exclusively to a single Gα-protein subunit, whereas others are dually coupled to different Gα-protein subunits (e.g., Gq/11 and Gi). Four subtypes of P1 receptors (A_1, A_{2A}, A_{2B}, and A_3) exist. The P1/A_2 receptors are coupled to Gs subunit and stimulates adenylate cyclase, whereas P1/A_1 and P1/A_3 receptors couple to subunits of the Gi/o family and inhibit adenylate cyclase. The $P2Y_5$ receptor corresponds to LPA_6 receptor that primes the G13–Rho pathway. The $P2Y_9$ receptor (or LPA_4) is most closely related to $P2Y_5$ (both act as lipid and nucleotide receptors).

Type	Main transducer	Potency order
Adenosine receptors (preferentially)		
A_1, A_3	Gi/o	
A_1	Gi1–Gi3	
A_3	Gi2, Gi3, Gq/11	
A_{2A}, A_{2B}	Gs	
	Nucleotide receptors	
$P2Y_1$	Gq/11	ADP > ATP
$P2Y_2$	Gi/o, Gq/11	UTP ∼ ATP
$P2Y_3$	Gq/11	UDP
$P2Y_4$	Gi/o, Gq/11	UTP > ATP
$P2Y_6$	Gq/11	UDP ≫ UTP > ATP
$P2Y_{11}$	Gs, Gq/11	ATP > UTP
$P2Y_{12}$	Gi/o	ADP ≫ ATP
$P2Y_{13}$	Gs, Gi/o	ADP ≫ ATP
$P2Y_{14}$	Gi/o, Gq/11	^{UDP}glucose (= ^{UDP}galactose)

Table 7.35. Effectors of some nucleotide P2Y receptors (Source: [874]; ACase: adenylate cyclase; Ca_V: voltage-gated Ca^{++} channel; K_{IR}: inwardly rectifying K^+ channel; PLC: phospholipase-C; RoCK: Rho-associated, coiled-coil-containing protein kinase). Voltage-gated K^+ channels of the K_V7 family cause the neuronal M current that regulates action potential firing.

Type	Effectors
$P2Y_1$	PLC, RhoA–RoCK
$P2Y_2$	PLC, K_{IR} channel, $Ca_V2.2$ channel (N-type)
$P2Y_4$	PLC, ACase
$P2Y_6$	PLC, K_V7 channels (M-type)
$P2Y_{11}$	PLC, ACase

Extracellular nucleotides operate as auto- or paracrine mediators (Tables 7.36 and 7.37). Afterward, extracellular nucleotides are rapidly degraded by ubiquitous ectonucleotidases on the cell surface. [53]

53. Two families of integral membrane ectonucleotidases exist: ectonucleotide diphosphohydrolases (ENPDH or CD39) and ecto-phosphodiesterase–nucleotide pyrophosphatase

Table 7.36. Autocrine and paracrine effects of extracellular ATP under various stimulation contexts (Source: [103]; **Part 1**; ↑: increase; IL: interleukin; JNK: Jun N-terminal kinase; NO: nitric oxide; Lφ: lymphoid lineage; MKC: megakaryocytes; RBC: red blood cell; SMC: smooth muscle cell). In some cases, the specific receptor type is unknown (P2).

Cell Type	P2 Type	Effect
		Cardiovascular apparatus
Endothelial	$P2Y_2$	Ca^{++} influx, ↑ NO production
	$P2X_{4/5}$	Ca^{++} influx
	P2	↑ NO production
	$P2X_7$	↑ IL1α release
VSMC	P2	↑ JNK activity
		Blood cells
RBC	$P2Y_1$	↑ Osmolyte permeability
Lφ	$P2X_7$	Proliferation
Macrophage	$P2X_7$	Apoptosis
MKC	$P2Y_1$	Ca^{++} influx
Monocyte	$P2X_7$	Interleukin (IL1β, IL18) secretion
Neutrophil	$P2Y_2$	Cell polarization
Platelet	$P2Y_1$	Ca^{++} influx, inhibition of platelet aggregation
	$P2Y_{12}$	Inhibition of platelet aggregation

Nucleotides exert their effects mainly via binding P2 receptors, although phosphorylation of plasmalemmal proteins by ectoprotein kinases constitutes an additional mechanism by which ATP can modulate cell function. [54]

Nucleotide P2Y receptors can be partly characterized by order of potency of ligand binding. These receptors are activated by a set of nucleotides: adenosine diphosphate (ADP), adenosine triphosphate (ATP), uridine triphosphate (UTP), and uridine diphosphate–glucose (^{UDP}glucose; Table 7.34). [55] The P2Y receptors can possess a main ligand, whereas other nucleotides behave as partial agonists. Nonetheless,

(EPDNP). The latter catalyze the cleavage of ATP into AMP and pyrophosphate, the conversion of cAMP into AMP, as well as several ATP–ADP, ADP–AMP, and AMP–adenosine reactions. Agent CD39 expressed by vascular endothelial cells hydrolyzes both ATP and ADP released from platelets.

54. Various plasmalemmal proteins are phosphorylated in their ectodomains by cell-surface protein kinase, the ectoprotein kinase. Protein CD36 (a.k.a. glycoprotein-3b and -4) is a class-B scavenger receptor that binds many ligands, such as collagen, thrombospondin, lipoproteins, oxidized low-density lipoproteins and phospholipids, as well as long-chain fatty acids. In platelets, cAMP-dependent ectoprotein kinase phosphorylates CD36 and causes platelet reactivity and adhesion to collagen, but reduces thrombospondin binding. Conversely, CD36 dephosphorylation transforms CD36 into a thrombospondin receptor [874].

55. Nucleotide UDP is phosphorylated into UTP by uridine diphosphokinase.

Table 7.37. Autocrine and paracrine effects of extracellular ATP under various stimulation contexts (Source: [103]; **Part 2**; ↑: increase; AA: arachidonic acid; NFAT: nuclear factor of activated T cells; PP: protein phosphatase). In some cases, the specific receptor type is unknown (P2).

Cell Type	P2 Type	Effect
		Respiratory tract
Epithelial	$P2Y_2$	Cl^- channel stimulation, inositol phosphate synthesis, Ca^{++} influx
	$P2Y_1$	Ca^{++} mobilization
	$P2Y_6$	Ca^{++} influx
		Kidney
Epithelial	P2	ENaC activation, Ca^{++} influx, contraction, ↓ AA Release, cAMP production
		Connective tissue
Fibroblast	$P2Y_2$	Ca^{++} influx
	$P2X_7$	Fibronectin ↑, IL6 release, apoptosis
Stromal	P2, $P2Y_1$	Ca^{++} influx, PP3 activation, NFAT translocation, proliferation

some nucleotide P2Y receptors can be activated almost equipotently by 2 nucleotide species. Others can be ligand-selective receptors, such as *purinoceptors* (e.g., $P2Y_1$) and *pyrimidinoceptors* (e.g., $P2Y_6$), for which ADP and ATP are either strong or only active ligands, or very weak stimulators or fully inactive, respectively.

In addition, effect of a given nucleotide on a given nucleotide receptors P2Y can depend on mammalian species, as P2Y can require either full or partial agonist activation. Moreover, several types of P2Y receptors can colocalize in a given cell type.

The P2Y receptors are coupled to distinct G proteins (Gq/11, Gi/o, and Gs; Table 7.34). They are then associated to phospholipase-C (Table 7.35). They can stimulate adenylate cyclase via $G\alpha_s$, although other pathways can be used (activation of some adenylate cyclase isoforms by $G\beta\gamma$ subunits, of other isoforms by Ca^{++}–calmodulin or protein kinase-C, and mediation by prostaglandins). Conversely, they can inhibit adenylate cyclase via $G\alpha_i$.

7.13.3.1 $P2Y_1$ and $P2Y_2$

The $P2Y_1$ receptor is linked to the RhoA-RoCK pathway (Vol. 4 – Chaps. 8. Guanosine Triphosphatases and Their Regulators and 4. Cytosolic Protein Ser/Thr Kinases) in addition to phospholipase-C.

The $P2Y_2$ receptor is linked to PLCβ1 via $G\alpha_{q/11}$ and to PLCβ3 via $G\beta\gamma\alpha_{i3}$. In vascular endothelial and smooth muscle cells, activation of $P2Y_2$ receptors by ATP and UTP induces phosphorylation (activation) of extracellular signal-regulated protein kinases ERK1 and ERK2 downstream from phospholipase-C [874].

In rat cardiac fibroblasts as well as pressure-overloaded mouse cardiomyocytes, ATP stimulates $P2Y_2$ receptor and activates nuclear factors of activated T cells NFAT1 and NFAT3, thereby increasing the concentration of inducible NO synthase (NOS2) [875]. Enzyme NOS2 interacts with the P65 subunit (or RelA) of NFκB and causes its S-nitrosylation. [56] Subunit $P65^{SNO}_{NF\kappa B}$ has a reduced transcriptional activity. Consequently, the density of type-1 angiotensin receptor (AT_1) falls.

7.13.3.2 Effects on Ion Channels

The P2Y receptors can also partner with ion channels. The $P2Y_2$ receptor links to Ca^{++}-dependent Cl^-, inward-rectifier K^+, and $Ca_V2.2$ channels [874].

Receptor $P2Y_6$ can associate with Cl^- channels. Via UDP, it can hamper the activity of $K_V7.2$ and $K_V7.3$ channels.

In neurons and other excitable cells, GPCRs can modulate the activity of voltage-gated ion channels, by mainly closing, or in certain circumstances opening or potentiating, various types of K^+ channels and voltage-gated Ca^{++} and Na^+ channels, via G-protein subunits and protein kinases. Specific couplings between P2Y receptors and certain Ca^{++} and K^+ channels occur in short time scales (O[100 ms]; Table 7.38) [876]. Cells of the superior cervical ganglion (SGC) are endowed with 2 main types of voltage-gated channels: (1) $K_V7.2$ and $K_V7.3$ (M-type K^+ channel) and (2) $Ca_V2.2$ (N-type Ca^{++}) channels. In SCG neurons, Gi/o-coupled $P2Y_{12}$ and $P2Y_{13}$ receptors close $Ca_V2.2$ channel. The $P2Y_1$ and $P2Y_2$ receptors act similarly to Gq/11-linked M_1 receptor. Both UDP and UTP nucleotides are strong agonists of $P2Y_6$ that provoke $Ca_V2.2$ channel closure [876].

7.13.3.3 P2 Receptors in Immunocytes

Immunocytes release the auto- and paracrine controller ATP that activates P2X and P2Y receptors. Stimulated T-cell receptors provoke Ca^{++} influx in the cytosol and then mitochondria, where it promotes ATP synthesis. Messenger ATP, a co-stimulatory signal to T lymphocytes, released from activated T lymphocytes through pannexin-1 hemichannels excites P2X receptors for a sustained mitogen-activated protein kinase signaling [877]. On the other hand, oxidized ATP, a P2X antagonist, impedes MAPK activation in stimulated T cells.

Nucleotide signaling is also required for neutrophil activation. Activated neutrophils release ATP in response to exogenous stimuli such as formylated bacterial peptides and inflammatory mediators that stimulate Fcγ(Fc receptors for IgG), interleukin-8, C5a complement component, and leukotriene-B4 receptors. Stimulated formyl peptide receptors that colocalize with nucleotide $P2Y_2$ receptors

56. β-Arrestins enable the formation of the quaternary arrestin–P65–IκB–NOS2 complex.

Table 7.38. Interactions between P2Y receptors and ion channels and involved G-protein subunits (Source: [876]; Gβγ (Gi/o, Gq/11): Gβγ dimer associated with Gi/o and Gq/11 family subunits of heterotrimeric G proteins; GIRK: G-protein-activated inwardly rectifying K^+ channel).

Type	$Ca_V2.2$ inactivation	K_V7 inactivation	GIRK activation
$P2Y_1$	Gq/11, Gβγ (Go, Gq)	Gq/11	Gβγ
$P2Y_2$	Gq/11, Gβγ (Go, Gq)	Gq/11	Gβγ
$P2Y_4$	Gβγ (Go)	Gq/11	Unobserved
$P2Y_6$	Gq/11, Gβγ	Gq/11	Unobserved
	Gβγ (Gi/o)	Unobserved	Gβγ

causes ATP release through pannexin-1 hemichannels. Nucleotide signaling that uses pannexin-1 and $P2Y_2$ receptors facilitates neutrophil activation [878].

7.13.3.4 P2 Receptors in the Ventilatory Apparatus

Nucleotides increase the apical permeability to Cl^- in airway epithelial cells and enhance mucin secretion by goblet cells and submucosal gland secretion [874]. They then promote mucociliary clearance. [57] Nucleotides ATP and UTP not only augment formation of inositol phosphates and intracellular calcium concentration in human airway epithelial cells via $P2Y_1$ and $P2Y_2$, but also regulate outwardly rectifying chloride channels via $P2Y_2$ receptors. Like muscarinic receptors, the activity of $P2Y_1$ receptors depends on the plasmalemmal potential.

7.13.3.5 P2 Receptors in the Cardiovascular Apparatus

Nucleotide receptors participate in the regulation of the cardiac function, as P2Y receptors (especially $P2Y_6$, $P2Y_7$, $P2Y_9$, and $P2Y_{14}$) reside in heart (Fig. 7.5). In blood vessel walls, nucleotide signaling controls the vascular tone, smooth muscle cell proliferation, and platelet aggregation. Different types of cells in the cardiovascular system selectively express nucleotide receptor types (Fig. 7.6).

Vascular Smooth Muscle Cells

Contraction of vascular smooth muscle cells results from activated $P2X_1$ and several P2Y subtypes ($P2Y_2$, $P2Y_4$, and $P2Y_6$). [58] The $P2X_1$ receptor causes a rapid

57. In cystic fibrosis, CFTR mutations limit Cl^- secretion and cause formation of underhydrated, viscous mucus that obstructs airways. Aerosolized UTP may be used to treat cystic fibrosis.

58. Contractile type of smooth muscle cells possess larger amounts of $P2Y_4$ and $P2Y_6$, but a lower quantity of $P2Y_2$, than synthetic type of smooth muscle cells.

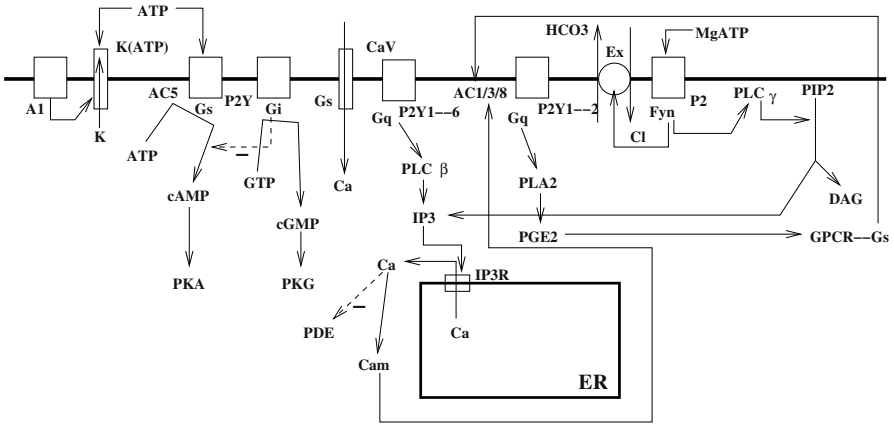

Figure 7.5. Nucleotide receptors in cardiomyocyte, their associated G proteins, and effectors.

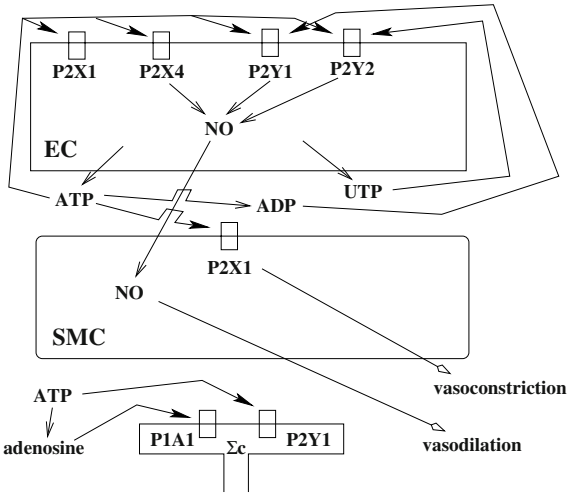

Figure 7.6. Nucleotide receptors in the vascular wall (Source: [879]). Messenger ATP, a cotransmitter of noradrenaline and neuropeptide-Y, released from perivascular sympathetic nerves binds receptors $P2X_1$, $P2X_2$, and $P2X_4$ of smooth muscle cells and induces vasoconstriction. Adenosine produced from ATP degradation binds to receptors A_1 (P1) of sympathetic nerves and inhibits transmitter release. Stressed endothelial cells release: (1) ATP, which binds receptors $P2Y_1$ (with a selective agonist action from ADP) and $P2X_4$, and (2) UTP, a ligand for $P2Y_2$. Both ATP and UTP trigger nitric oxide delivery to smooth muscle cells with subsequent vasodilation.

influx of Ca^{++} to initiate smooth muscle contraction. Gq/11-Coupled P2Y receptors activate phospholipase-C, thereby generating IP_3, which mobilizes Ca^{++} from its intracellular stores.

Prolonged P2Y signaling can cause vasoconstriction, hypertension, vascular smooth muscle hypertrophy, and hyperplasia. However, GPCR-desensitizing GRKs and arrestins prevent prolonged and inappropriate GPCR-initiated signaling.[59] In mesenteric arteries, both GRK2 (but not GRK3, GRK5, or GRK6) and arrestin-2 (but not arrestin-3) regulate UTP-stimulated $P2Y_2$ on smooth muscle cells [880].

Endothelial Cells

Endothelial cells express nucleotide receptors $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$,[60] as well as $P2X_1$,[61] to $P2X_7$.[62] The $P2X_4$ receptor is the most abundant P2X receptor subtype in vascular endothelial cells. It contributes to ATP- and flow-induced Ca^{++} influx in endothelial cells. The P2 receptors (particularly $P2X_4$) on endothelial cells bind ATP that triggers secretion of nitric oxide and causes vasodilation. The P2X receptors are implicated in autocrine loops in endothelial cells. Vascular smooth muscle cells abundantly produce $P2X_1$, $P2Y_2$, and $P2Y_6$ receptors. The P2 receptors also localize to the vessel wall adventitia. Perivascular adrenergic and cholinergic nerves have nucleotide receptors.

Endothelial cells that ensure hemostasis release nucleotides, but ectonucleoside triphosphate diphosphohydrolase ENTPD1[63] on the surface of endothelial cells can degrade both anti-aggregatory ATP and pro-aggregatory ADP [874]. Further cleavage by nucleotidase generates adenosine that inhibits platelet aggregation via A_{2A} receptors and cAMP messenger. Moreover, ATP and ADP prime the release of anti-aggregatory mediators prostacyclin and nitric oxide from endothelial cells, as they activate endothelial $P2Y_1$ and $P2Y_2$ receptors.

Erythrocytes and Coronary Endothelial Cells

Erythrocytes release ATP when the blood partial pressure of oxygen decreases. During exercise, when oxygen extraction by the myocardium is high,[64] the coronary blood flow rises. This augmentation is correlated with ATP concentration, as ATP

59. Among 7 GPCR kinases (GRK1–GRK7) and 4 arrestin (arrestin-1–arrestin-4) subtypes, GRK2, GRK3, GRK5, and GRK6, and arrestin-2 and -3 (i.e., β-arrestins) are ubiquitous. The GRK2 kinase is an important subtype for the contraction of vascular smooth muscle cells triggered by AT_1, α1d-adrenergic, and ET_A receptors.

60. The $P2Y_4$ receptor is slightly expressed in endothelial cells, whereas $P2X_4$, $P2Y_1$, $P2Y_2$, and $P2Y_{11}$ are the most abundant among endothelial P2 receptors [872].

61. The $P2X_1$ receptor abounds in endothelial cells of rat mesenteric arteries [881].

62. Receptors $P2X_1$ to $P2X_3$ and $P2X_7$ have similar levels in the endothelium of internal mammary and radial arteries and saphenous veins, whereas levels of $P2X_5$ and $P2X_6$ are lower [882]. Synthesis of $P2X_4$ differs between arteries and veins. Both radial and internal mammary arteries have very low $P2X_4$ levels.

63. A.k.a. ecto-apyrase CD39 and ectoATP diphosphohydrolase EATPDase1.

64. In dogs, the myocardial oxygen consumption increases approximately 3.2 fold, the coronary blood flow about 2.7 fold, and the partial pressure of oxygen in the coronary venous sinus decreases from 2.5 to 1.7 kPa [883].

level increases in blood of the coronary venous sinus (from 31.1 to 51.2 nmol) [883]. Adenine nucleotide ATP is one of the factors that control the coronary blood flow during exercise. It acts on microvascular endothelial cells to produce a vasodilation of upstream arterioles to match the oxygen delivery to myocardial oxygen consumption during exercise.

Adenosine triphosphate released from erythrocytes is catabolized into ADP and AMP in coronary capillaries, where these products target nucleotide receptors such as $P2Y_1$ on the surface of endothelial cells [884]. Activated nucleotide receptors initiate a retrograde vasodilatory signal to upstream arterioles presumably via gap junctions to control coronary blood flow resistance using a feedback loop.

Leukocytes

The $P2Y_{13}$ receptor is synthesized in blood leukocytes. The $P2Y_{11}$ receptor participates in granulocytic differentiation. Agent ATP can trigger hematopoiesis via the $P2Y_{11}$–Gs–ACase–cAMP–PKA pathway [874].

Thrombocytes

Major platelet activators include ADP, an auto- and paracrine regulator secreted by platelets, and thrombin, a peptidase of the coagulation cascade that triggers the release of ADP by platelets. Platelet activators interact with their respective GPCRs to prime and amplify platelet activation and aggregation. Binding of von Willebrand factor, collagen, and thrombin to their corresponding receptors on thrombocyte surface enhances the release of ADP and thromboxane-A2.

Sustained platelet aggregation by ADP requires activation of both $P2Y_1$ and $P2Y_{12}$ receptors. Ligand ADP binds to: (1) Gq-coupled $P2Y_1$ receptor to cause cell shape change for optimal affinity for fibrinogen and von Willebrand factor, calcium mobilization, and initiation of reversible aggregation;[65] and (2) Gi-coupled $P2Y_{12}$ receptor to amplify the platelet aggregation via the ACase–cAMP pathway [885]. Metabotropic receptor $P2Y_1$ also mediates the release of nitric oxide caused by adenine nucleotides.

In platelets, nucleotide (ADP) $P2Y_{12}$ receptor functions downstream from peptidase (thrombin)-activated PAR_1 and PAR_4 receptors to amplify the platelet response to thrombin, but can also operate in collagen-induced platelet activation and thrombin–PAR-independent pathways [886]. Furthermore, $P2Y_{12}$ signaling is more important than that based on PAR receptors in hemostasis, but not in arterial thrombosis for which PAR signaling is predominant.

In addition, ionotropic receptor $P2X_1$ abounds in platelets. Agent ATP, a potent agonist of $P2X_1$, triggers a rapid and quickly desensitized Ca^{++} influx.

65. The resulting platelet activation triggers a conformational change in $\alpha_{2B}\beta_3$-integrins (or glycoproteic complex GP2b–GP3a) that increases their affinity for fibrinogen and von Willenbrand factor. These ligands then bind to receptors to bridge adjacent platelets.

Table 7.39. Nucleotide receptors in the nephron (PCT: proximal convoluted tubule, tDL: thin descending limb, TDL: thick descending limb of the Henle loop, tAL: thin ascending limb, TAL: thick ascending limb of the Henle loop, DCT: distal convoluted tubule, CD: collecting duct, Source: [888]).

Type	Location
$P2X_1$	Afferent arteriole, glomerulus,
$P2X_2$	Afferent arteriole
$P2X_4$	PCT, tDL, tAL, TAL, DCT, CD
$P2X_5$	TAL, DCT, CD
$P2X_6$	PCT, TDL, tDL, tAL, TAL, DCT, CD
$P2Y_1$	Afferent and efferent arterioles, glomerulus, TDL
$P2Y_2$	Glomerulus, TAL, CD
$P2Y_4$	PCT, TDL

Table 7.40. Adiponectin receptors (Source: [5]; Apn_{FL}: full-length adiponectin; ^{G}Apn: globular adiponectin).

AdipoR1	AdipoR2
$^{G}Apn > Apn_{FL}$	$^{G}Apn = Apn_{FL}$

Cardiomyocytes, Neurons, and Nephron Epithelial Cells

The $P2X_4$ receptor improves survival of cardiomyocytes in a mouse model of cardiomyopathy [887]. Nucleotides intervene in synaptic transmission. In neurons of the central and peripheral nervous system, ATP colocalizes and is cosecreted with neurotransmitters, such as catecholamines and acetylcholine. The P2 receptors localize in every part of the nephron that regulates water (blood) volume and electrolyte concentration (Table 7.39).

7.13.4 Adiponectin Receptors

Adiponectin receptors (Table 7.40; Sect. 6.9.1) respond to adiponectin synthesized in adipose tissues (Vol. 2 – Chap. 3. Growth Factors). Unlike typical GPCRs, its C- and N-termini are extra- and intracellular, respectively [5]. Receptor AdipoR1 abounds in the skeletal muscle and liver, whereas AdipoR2 is predominantly produced in the liver.

These receptors are produced by endothelial cells and cardiomyocytes. T-cadherin (Vol. 1 – Chap. 7. Plasma Membrane) may act as a coreceptor for mid- (Apn_m) and high-molecular-weight (Apn_h) adiponectin on endothelial and smooth muscle cells, but not low-molecular-weight trimeric (Apn_l) and globular (^{G}Apn) forms [889, 890].

Signaling via adiponectin receptors avoids G proteins and instead stimulates protein phosphorylation via AMP-activated protein kinase (Vol. 4 – Chap. 4. Cytosolic Protein Ser/Thr Kinases) and the mitogen-activated protein kinase module, possibly

using Adaptor phosphoTyr interaction, PH and Leu zipper domain-containing protein APPL1. Both Ca^{++}–calmodulin-dependent protein kinase kinase CaMKKβ and protein Ser/Thr kinase STK11 (or LKB1) are required for adiponectin-induced full AMPK activation.

Adiponectin is an antidiabetic and anti-atherogenic adipokine.[66] It induces influx of extracellular Ca^{++} via adiponectin receptor AdipoR1. Subsequent activation of Ca^{++}–calmodulin-dependent protein kinase kinase-β, AMPK, and sirtuin-1. Both AMPK and SIRT1 are required for elevated expression and attenuated acetylation of peroxisome proliferator-activated receptor-γ coactivator PGC1α primed by the Apn–AdipoR1 axis. This pathway raises mitochondrial content and activity in myocytes [891]. It also heightens oxidative type-1 myofibers and oxidative stress-detoxifying enzymes in the skeletal muscle, thereby promoting insulin sensitization and exercise endurance.

7.13.5 Adrenergic Receptors (Adrenoceptors)

Two main groups of ubiquitous adrenergic receptors (or adrenoceptors) include α- and β-adrenergic receptors (AR; Table 7.41). Both groups bind both adrenaline and noradrenaline. These catecholamine messengers regulate diverse physiological systems.

Adrenergic receptors are composed of 3 major types, each of which is further subdivided into 3 subtypes: (1) α1-adrenoceptors with α1a (or α1c), α1b, and α1d subtypes; (2) α2-adrenoceptors with α2a (or α2d), α2b, and α2c subtypes; and (3) β-adrenoceptors with β1, β2, and β3 subtypes. Each adrenoceptor type is characterized by its target pathways, receptor distribution, and effects (Table 7.42).

7.13.5.1 α-Adrenergic Receptors

α1-Adrenergic Receptors

α1-Adrenergic receptors are activated by adrenaline and noradrenaline with about equal potency. Numerous splice variants of α1-adrenoceptors exist. α1d-Adrenoceptors form heterodimers with α1b- or α2-adrenoceptors [5]. Signaling is carried out predominantly via Gq/11, but also Gi/o, Gs, G12/13, and Gh subunit.[67] α1-Adrenoceptor subtypes show differences in regulation and coupling efficiency to Ca^{++} signaling (α1a > α1b > α1d) and mitogen-activated protein kinase modules (α1d > α1a > α1b) [5].

α1-Adrenergic receptors of vascular smooth muscle cells as well as those of prostate cause contraction and favor cell growth and proliferation [896]. In heart, Gq-coupled α1-adrenoceptor has positive inotropic effect [897]. Activated Gq excites phospholipase-Cβ that hydrolyzes phosphatidylinositol (4,5)-bisphosphate to

66. Plasma adiponectin concentration lowers in obesity, insulin resistance, and type-2 diabetes.

67. Subunit Gh consists of the 74-kDa Gα subunit and a 50-kDa β subunit. Gh-protein-coupled α1-adrenoceptor stimulates membrane-bound phospholipase-C [895].

Table 7.41. Adrenoceptors, their main targeted G proteins, and order of ligand potency (α1c-adrenoceptor is derived from α1a-adrenoceptor; Source: [736]). α1d-Adrenoceptors are able to couple to Gi, at least in human skin fibroblast [892]. All combinations of $G\alpha_{i1}1$, $G\alpha_{i2}$, or $G\alpha_{i3}$ with Gβ1, Gβ2, or Gβ4 are activated by endogenous α2-adrenoceptors [893]. α2-Adrenoceptors couple not only to Gi but also to Gs [894].

Type	Main transducer	Ligand potency order
α1a	Gq/11	Ad ∼ NAd
α1b	Gq/11	Ad ∼ NAd
α1d	Gq/11	Ad ∼ NAd
α1	Gi	
α2a	Gi/o	Ad > NAd
α2b	Gi/o	Ad > NAd
α2c	Gi/o	Ad > NAd
α2	Gs	
β1	Gs	Ad < NAd
β2	Gs, Gi	Ad > NAd
β3	Gs, Gi/o	Ad ∼ NAd

inositol (1,4,5)-trisphosphate and diacylglycerol. Increased diacylglycerol level stimulates protein kinase-C, but the latter is not significantly involved in cardiac inotropy. Positive inotropy indeed relies mainly on myosin light-chain kinase and RoCK kinase.

α1a-Adrenoceptors

α1a-Adrenoceptor, or α1c-adrenoceptor,[68] undergoes alternative splicing that generates transcript variants and isoforms with distinct C-termini, but similar ligand-binding properties. α1a-Adrenoceptors do not form heterodimers. α1a-Adrenoceptor mediates G1–S cell cycle arrest that is associated with reduced activities of cyclin-E and cyclin-dependent kinase CDK6 and elevated action of cyclin-dependent kinase inhibitor CKI1b [898].

α1b-Adrenoceptors

α1b-Adrenoceptor (encoded by the ADRA1B gene) contributes to the cell cycle progression of many cell types [898]. It can hence acts as a proto-oncogene product. It interacts with subunit-μ2 (AP2M2: adaptor-related protein complex-2 subunit-μ2 or clathrin-associated adaptor protein ClAPM2; but not μ1, μ3, or μ4 subunits of other adaptor complexes) of the clathrin adaptor complex-2 for clathrin-mediated receptor endocytosis [900]. α1b-Adrenoceptor heterodimerizes.

68. A.k.a. AdRa1a, AdRa1c, and AdRa1L.

Table 7.42. Adrenergic receptors are coupled to: (1) Gs and the ACase–cAMP–PKA pathway; (2) Gi/o and the Gβγ–PI3K–PKB, Gβγ–PLC–PKC, and Gβγ–NOS–PKG pathways; and (3) Gq/11 and the Gβγ–PI3K–PKB pathway (vSMC: vascular smooth muscle cells; bSMC: bronchial smooth muscle cells; CMC: cardiomyocytes; NC: nodal cells; AC: adipocyte; HC: hepatocyte; TC: thrombocyte; PNE: presynaptic nerve ending; A: artery; V: vein; B, C, D, I, and L: bathmo- (nodal excitability [excitation threshold]), chrono- (heart rate), dromo- (nodal conduction velocity), ino- (strength of contraction), and lusitropic (relaxation) effects in cardiac electromechanical coupling; main sources: [736], Wikipedia). In addition to cardiovascular effects, α1-adrenoceptors also cause smooth muscle contraction of ureter, urethral sphincter, vas deferens, uterus at end of pregnancy, arrector pili muscles, and bronchioles. They induce glycogenolysis and gluconeogenesis in adipose tissue and liver, secretion from sweat glands, and sodium reabsorption from kidney. α2-Adrenoceptors inhibit insulin release and provoke glucagon release in pancreas, and induce contraction of sphincters of the gastrointestinal tract. β1-Adrenoceptors elicit renin release from juxtaglomerular cells and lipolysis in adipose tissue. β2-Adrenoceptors relax urinary sphincter, detrusor urinae muscle of bladder wall, and pregnant uterus, but contract sphincters of the gastrointestinal tract, thicken salivary secretions, induce glycogenolysis and gluconeogenesis, inhibit histamine-release from mastocytes, and increase renin secretion from kidney.

Receptor	Pathways	Sites	Effects
α1a	Gq/11	vSMC (A, V)	Vasoconstriction
		CMC	I+
α1b	Gq/11	vSMC	Vasoconstriction
		CMC	I+
		HC	Glycogenolysis and gluconeogenesis
α1d	Gq/11	vSMC	Vasoconstriction
α2a	Gi/o	PNE	Neurotransmitter inhibition (reduction in sympathetic output)
		TC	Platelet aggregation
		AC	Glycogenolysis and gluconeogenesis
		AC	Lipolysis inhibition
α2b	Gi/o	PNE	α2a Counteraction
α2c	Gi/o	vSMC (V)	Vasoconstriction
β1	Gs	NC, CMC	B+, C+, D+, I+, L+
β2	Gs, Gi, Gq	CMC	I+, L+
		vSMC	Vasodilation
		bSMC	Bronchorelaxation
β3	Gs, Gi/o	CMC	I−
		AC	Lipolysis

α1d-Adrenoceptors

α1d-Adrenoceptor (encoded by the ADRA1D gene), like α1a-adrenergic receptor, mediates cell cycle arrest during the G1–S transition [898]. Whereas α1a- and

α1b-adrenoceptors localize to the plasma membrane, α1d-adrenergic receptors are intracellular. α1b-Adrenoceptor, but not α1a-adrenoceptor, forms heterodimers with α1d-adrenoceptors to cause their translocation to the cell surface [899].

In the developing fetus, cerebral arteries differ from those in adults. Maturational differences in adrenergic-mediated contractility of cerebral arteries result from distinct expression and/or activities of α1-adrenergic receptor subtypes. Once stimulated, they increase the intracellular inositol (1,4,5)-trisphosphate concentration, hence Ca^{++} influx, as well as activation of protein kinase-C and extracellular signal-regulated kinases ERK1 and ERK2. In sheep fetuses, α1d-adrenoceptors are expressed at a significantly greater level than α1a- and α1b-adrenoceptors [901]. However, α1AR subtype expression is approximately 20% in fetal cerebral arteries compared with that in adults. In fetal cerebral arteries, α1b and α1dAR subtypes contribute to vasocontriction as well as activation of ERK1 and ERK2.

α2-Adrenergic Receptors

α2-Adrenergic receptors are activated by adrenaline and noradrenaline with a potency order (adrenaline > noradrenaline) [5]. α2-Adrenoceptors activate the $G\alpha_{i/o}$ family of guanine nucleotide-binding regulatory (G) proteins that, in turn, regulates several effectors, such as adenylate cyclase (inhibition), Ca^{++} channels (stimulation or inhibition), K^+ channels and H^+–Na^+ antiporter (stimulation) [902].

α2a-Adrenoceptor mediates the central baroreflex control (hypotension and bradycardia) as well as influence platelet aggregation [5]. α2b Subtype is involved in neurotransmission in the spinal cord. α2c-Adrenoceptor regulates the catecholamine release from adrenal chromaffin cells.

α2a-Adrenoceptors

α2a-Adrenoceptor is widespread in the body's tissues (brain, vasculature, submandibular gland, and kidney, as well as platelets). Its desensitization after acute stimulation via that phosphorylation by GRKs depends on tissue and species types. It is constitutively palmitoylated. It can interact with α-subunit of eukaryotic initiation factor-2B and 14-3-3ζ protein, as well as spinophilin [902]. α2a-Adrenoceptor is able to form homo- and heterodimer with α2c subtype as well as β1-adrenoceptor.

Gi-Coupled α2a-adrenergic receptor (encoded by the ADRA2A gene) impedes insulin secretion, as it causes a reduced exocytosis of pancreatic β cells and insulin-granule docking at the plasma membrane [903].

α2b-Adrenoceptors

α2b-Adrenoceptor has a very limited expression in humans. It can promote mitogen-activated protein kinase activation. Desensitization relies on phosphorylation by GRK2 or GRK3 and, then, interaction with β-arrestin. On the other hand, scaffold spinophilin (or neurabin-2) tethers the receptor at the cell surface of dendritic cells. In addition, it can form complexes with α subunit of eukaryotic initiation factor-2B and 14-3-3ζ protein [902].

α2c-Adrenoceptors

α2c-Adrenoceptor is mainly expressed in the brain. It forms homo- or heterodimer with α2a subtype that can impair GRK phosphorylation and β-arrestin recruitment. It can also interact with β2-adrenergic receptors. In addition, it can build complexes with α subunit of eukaryotic initiation factor-2B and 14-3-3ζ protein [902].

7.13.5.2 β-Adrenergic Receptors

Three βAR subtypes (β1AR–β3AR) can bear distinct regulation and triggers different signaling cascades. Specificity of βARs for G-protein coupling is particularly dictated by the third intracellular loop and C-terminal tail. Intracellular domains of βARs are important for binding of βAR regulatory proteins such as G-protein-coupled receptor kinases and β-arrestins. [69]

β-Adrenoceptors possess a set of contacts between helices 3 and 6, the so-called ionic lock that forms a molecular switch for receptor activation. Molecular dynamics simulations of β2-adrenergic receptor show that the ionic lock forms reproducibly [904]. Inactive β2AR conformations vary from formed lock to broken lock, the latter conformation being observed in crystal structures.

β1-Adrenergic receptors activate adenylate cyclase and strongly elevate the cAMP concentration, whereas β2 and β3-adrenergic receptors can couple to Gi. They also stimulate many other signaling pathways, particularly mitogen-activated protein kinases. In rat ventriculomyocytes, β2-adrenoceptors couple to Gs (preferentially) and Gi protein, activating either Gs or both Gs and Gi protein [905]. Adenylate cyclase activation by Gi2-coupled β2-adrenoceptors can be done by preventing Gi2 to interact with the inhibitory $G\alpha_i$ site of adenylate cyclase [906].

The control of cAMP transients via degradation by phosphodiesterases (Vol. 4 – Chap. 10. Signaling Pathways) corresponds to a specific procedure for GPCR–cAMP signaling [907] (Table 7.43). Liganded β2AR recruits a preformed β-arrestin–PDE4d5 complex. Inactivated β1AR forms a signaling complex with another cAMP-specific PDE, PDE4d8, in cardiomyocytes. Ligand-induced dissociation of PDE4d from the β1AR–PDE4d8 complex produces a localized cAMP transient.

β1-Adrenoceptor

Gs-Coupled β-adrenergic receptors activate adenylate cyclase. β1-Adrenoceptor is the predominant subtype in heart. It is also a major mediator of lipolysis in adipocytes and renin release from kidney cells. Lastly, it is the principle subtype in certain brain regions, such as cerebral cortex and hippocampus, where it modulates long-term potentiation and, thereby, synaptic remodeling and memory [908].

69. Upon agonist stimulation, GRKs are recruited to the plasma membrane and phosphorylate activated βARs. β-Arrestins then bind to phosphorylated βARs to prime a rapid desensitization and receptor internalization.

Table 7.43. Difference in signaling complex composition (transducer, scaffold, and effector) and production between β-adrenoceptors (Sources: [907]). Different modes of interaction with phosphodiesterases (PDE) generate distinct cAMP signals for β-adrenoceptor signaling specificity.

	β1-Adrenoceptor	β2-Adrenoceptor
Signaling complex	PDE4d8	β-Arrestin–PDE4d5
Agonist binding	Complex dissociation	Complex formation

Human β1AR is glycosylated (Asn15). It heterodimerizes with α2aAR that has a lower potency for agonist stimulation of cAMP production. It interacts with Gs, but, unlike β2AR, not with Gi. Its desensitization results from phosphorylation by protein kinase-A and -C. Its phosphorylation by GRK is much less than that of β2AR; hence arrestin binding and desensitization is weaker [908].

Many class-1 PDZ domain-containing proteins link to β1AR, influencing either β1AR endocytosis or signaling. β1-Adrenoceptor indeed connects to Disc large homologs DLg1[70] and DLg4,[71] members of the membrane-associated guanylate kinase (MAGuK) family, as well as MAGuK-like proteins with an inverted domain structure (WW and PDZ domain-containing proteins) MAGI2 and MAGI3 [909]. Protein DLg4 inhibits and MAGI2 stimulates β1AR endocytosis, respectively. The association of MAGI3 with β1AR impairs activation of ERK1 and ERK2 without affecting cAMP generation or internalization. Cystic fibrosis transmembrane conductance regulator-associated ligand (CAL)[72] also connects to the β1AR C-terminus. β1-Adrenoceptor also interacts with GAIP-interacting protein C-terminus product GIPC and RapGEF2. The former regulates β1AR-mediated activation of ERK. The latter promotes the activation of Ras by β1AR.

Appropriate β1AR transfer after agonist-promoted endocytosis is needed for resensitization of its signaling pathway. Efficient β1AR recycling requires binding of protein kinase-A-anchoring protein AKAP79 to β1AR C-terminus. Moreover, AKAP79 forms a complex with DLg1 and PKA, the so-called β1AR *receptosome* [910]. This scaffold allows β1AR recycling and PKA-mediated β1AR phosphorylation (Ser312).

In addition, endophilins enhance β1AR internalization [911].[73] They are involved in clathrin-mediated endocytosis. Endophilins colocalize with endocytic reg-

70. A.k.a. synapse-associated protein SAP97.

71. A.k.a. postsynaptic density protein PSD95.

72. A.k.a. GOPC and FIG.

73. Endophilins are encoded by the genes of the EEN family (EEN, EENB1, and EENB2). Endophilins are also identified as Src homology domain-3-containing proteins (SH3P) and SH3-domain GRB2-like proteins (SH3GL). Endophilin-1 (a.k.a. Een, SH3GL1, and SH3P8), -2 (a.k.a. EenB1, SH3GL2, and SH3P4), and -3 (a.k.a. EenB2, SH3GL3, and SH3P13) bind to β1-adrenoceptors.

ulators, such as GTPase dynamin, phosphatase synaptojanin, and amphiphysin, an adaptor of the cytoplasmic surface of synaptic vesicles in nerve terminals.

β2-Adrenoceptor

β2-Adrenergic receptors are synthesized in most cell types. However, β2-adrenoceptor is the predominant type in the lung, smooth muscle, and liver. In lungs, β2AR resides on airway smooth muscle and epithelial cells. It is also detected in cardiomyocytes and inflammatory cells. It targets MAPK and PI3K kinases. In cardiomyocytes, β2AR-mediated activation of these kinases can counter apoptosis caused by chronic β1AR stimulation [912]. Phosphorylation by GRK followed by β-arrestin binding leads to β2AR endocytosis.

MicroRNA Let7f represses the translation of the ADRB2 mRNA, thereby attenuating the production of β2-adrenergic receptor under stable exposure in a given time range of agonists [913]. [74] On the other hand, during agonist activation as well as long-term, chronic agonist exposure, the Let7f level decays (positive feedback loop that relieves the Let7f-mediated repression of ADRB2 translation).

β2-Adrenergic receptor is activated by the hormone and neurotransmitter adrenaline that is strongly involved in cardiovascular and pulmonary physiology. Ligand-binding site is accessible by conformational changes in the extracellular loop [914]. The β2AR basal (activator-independent) activity (and structural instability) involves interactions (hydrogen bonds and charge interactions, the so-called ionic lock) between cytoplasmic ends of transmembrane helices TM3 and TM6 [915].

β2-Adrenergic receptor phosphorylation by G-protein-coupled receptor kinases and protein kinase-A rapidly desensitizes these receptors, hence attenuating airway smooth muscle cell relaxation and bronchodilation. G-protein-coupled receptor kinases GRK2 and GRK5 with subsequent binding of β-arrestins reduce liganded β2AR–Gs coupling. Protein kinase-A also lowers β2AR–Gs coupling, but promotes β2AR–Gi coupling [916]. Protein kinase-A primes the major desensitization pathway in airway smooth muscle cells, especially at low agonist concentrations.

β2-Adrenergic receptor interacts with the Na^+–H^+-exchanger regulatory factors NHERF1 (or SLC9a3r1) and NHERF2 (or SLC9a3r2) to regulate Na^+–H^+ exchanger as well as β2AR postendocytic sorting following agonist-promoted internalization [909]. The recruitment of NHERF1 to β2AR precludes inhibition by NHERF1 on Na^+–H^+ exchanger NHE3 (or SLC9a3) in renal cells. Interactions between NHERF1 with β2AR are regulated by agonists.

Endocytosis of β2-adrenoceptors is regulated by agonist concentration and mediated by pre-existing clathrin-coated pits. β2-Adrenoceptors regulate the duration

74. The so-called "agonist-promoted downregulation" corresponds to the loss of the receptor response under prolonged exposure to an endo- or exogenous agonist. The miR–mRNA interaction prevents mRNA translation into a protein. The Let7f microRNA thus contributes to set the baseline of expression of β2-adrenergic receptors. The 3′ untranslated region of ADRB1 and ADRB2 mRNAs does not contain Let7f-binding sites for all subtypes (βAR-specific mechanism) [913]. Two other microRNAs, miR15 and miR30, have predicted binding sites in the 3′ UTR of ADRB2 mRNAs.

between clathrin coat deposition and membrane scission by a mechanism that involves receptor scaffolding to cortical actin [11]. Activation of protein kinase-A by β2ARs and subsequent receptor phosphorylation regulates Rab4-dependent, rapid recycling, but not slower recycling.

In rodent cardiomyocytes, Gs-coupled β2AR are confined to transverse tubules, whereas β1ARs are distributed across the entire cell surface. Both respond to the same ligands and couple to the same effectors, but they elicit different cellular responses.[75] In cardiomyocytes of a rat model of chronic heart failure, β2ARs redistribute from the transverse tubules (sites of coupling between the plasma membrane depolarization to calcium entry) to the plasma membrane [917]. A diffuse receptor-mediated cAMP signaling then results in contrast to a spatially confined β2AR-induced cAMP signals in normal condition. Loss of proper PKA localization is also observed in human heart failure.

The classical receptor theory postulates that receptors switch between inactivation and activation. Full agonists stimulate all of the signaling pathways of a given receptor to the same extent. The concept of "functional selectivity", or "biased signaling", states that a natural ligand can have distinct performance on different triggered signaling pathways. The endogenous ligands of β2-adrenergic receptor are adrenaline and noradrenaline. β2-Adrenergic receptor has 2 primary modes of activation of adenylate cyclase either via Gs subunit or, upon endocytosis, via β-arrestins and mitogen-activated protein kinases. The type of conformational change differs according to the type of agonists [918]. Both ligand types activate with an almost similar efficiency Gs and adenylate cyclase. However, noradrenaline is less efficient in signaling to β-arrestin. Therefore, noradrenaline is more tightly coupled to the Gs–ACase–cAMP pathway than signaling initiated during receptor endocytosis.

β3-Adrenoceptor

In humans, whereas β1AR and β2AR are ubiquitous, β3AR is essentially produced in the digestive tract and adipose tissue. In humans, β3AR abounds in brown, but not white adipocytes. β3-Adrenergic receptor regulates metabolic and endocrine functions, using the Gs–cAMP–PKA and PKC–P38MAPK pathways [919]. Sustained action of insulin reduces the density of β3AR, the major βAR subtype of adipocyte, without effect on β1AR (β2AR is undetectable) [920]. Activated β3AR stimulates lipolysis and thermogenesis in human adipocytes.

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75. Upon stimulation, β1ARs generate positive ino- and chronotropic effects via phosphorylation by PKA of regulators of the intracellular concentration of calcium. β1-Adrenoceptors produce cytosolic cAMP that diffuses toward sarcomeres to enhance contractility and frequency. In heart failure, β1AR causes cell apoptosis. β2-Adrenoceptors are also coupled to Gs subunit and adenylate cyclase, but not calcium regulators and myofilaments that control contraction. In addition, β2AR activity opposes β1AR signaling because they switch sequentially from Gs to Gi, hence producing different cAMP pools in the cardiomyocyte and subcellular compartmentation of signals.

Table 7.44. Distinct action of β1- and β2-adrenergic receptors in cardiac physiology and pathology (Sources: [922, 923]; ACase: adenylate cyclase; cAMP: cyclic adenosine monophosphate; PI3K: phosphoinositide 3-kinase; PKA: protein kinase-A). β1- and β2-adrenoceptors are the predominant subtypes expressed in hearts. Both β1- and β2-adrenoceptors trigger the Gs–ACase–cAMP–PKA pathway. β2-Adrenoceptor also activates Gi protein that prime the $G\alpha_i G\beta\gamma$–PI3K–PKB pathway. The latter competes with the former for protein phosphorylation (calcium channels Ca_V1, phospholamban, and troponin) via PI3K during acute stimulation: β2AR–Gi coupling partially inhibits β2AR–Gs complex activity, i.e., positive inotropy (I+) and lusitropy (L+). Persistent β1AR stimulation induces cardiomyocyte hypertrophy and apoptosis via protein kinase-A-independent activation of calmodulin-dependent protein kinase-2 (CamK2). On the other hand, persistent β2AR activation exerts a survival effect in cardiomyocytes via protein kinase-B (PKB).

β1-Adrenoceptor	β2-Adrenoceptor
Gs–ACase–cAMP–PKA pathway	Gs–ACase–cAMP–PKA pathway Gi–Gβγ–PI3K–PKB pathway
I+, L+ Cardiomyocyte apoptosis Cardiomyocyte hypertrophy	Reduced I+, L+ effect Cardiomyocyte survival

In human ventricles, β3AR inhibits cardiac contractility. On the other hand, β3AR activation increases human atrial contractility via the cAMP–PKA axis and Ca^{++} channel Ca_V1 [921].

Cardiomyocyte β-Adrenoceptors

The sympathetic nervous system stimulates blood circulation in response to stress or exercise by activating β1- and β2-adrenoceptors, which activate G-protein subunit stimulatory for adenylate cyclase. Yet, a sustained β-adrenoceptor stimulation can yield pathological cardiac remodeling.

Cardiac β1-adrenoceptors are coupled to Gs and β2-adrenoceptors to both Gs and Gi (Table 7.44). The duration and magnitude of receptor expression determine the cardiomyocyte fate. β2-Adrenoceptors, like most GPCRs, exist at very low concentrations in the sarcolemma.

In heart, α1a- and α1b-adrenoceptors have distinct roles [924]. Overexpression of α1bARs depresses heart contractile response to βAR activation via Gi and predisposes heart to hypertrophy, whereas α1aAR overexpression increases cardiac contractility and improves outcomes after pressure overload or myocardial infarction. Both subtypes hinder the generation of inositol trisphosphate and protect from arrhythmogenesis in early postischemic reperfusion.

In cardiomyocytes, β1- and β2-adrenergic receptors activate mitogen-activated protein kinases P38MAPK and ERK, respectively. α1-Adrenergic receptors can activate the PLC–PKC pathway, stress-activated protein kinases (P38MAPK and Jun N-terminal kinase), Rho and Rac GTPases, and Ca^{++}–calmodulin-dependent protein kinase [925].

A single or several (1–7) ryanodine receptors of a given couplon release Ca^{++} sparks from the sarcoplasmic reticulum in response to its natural trigger, the Ca^{++} sparklet generated by a single Ca_V1 channel. β-Adrenergic receptors regulate the pace and strength of heart contraction, as they cause both prolonged Ca_V1 channel opening duration and accelerated channel coupling. They raise the coordination between Ca_V1 channels and ryanodine receptors via the PKA pathway. The enhanced synchronization of these 2 types of Ca^{++} channels generates higher and quicker Ca^{++} transients to support stronger and faster heart contraction. Channel synchronization relies on: (1) synchronized Ca_V1 channel activity (trigger); (2) synchronized initiation of ryanodine receptor activity (accelerated activation kinetics); and (3) synchronized ryanodine receptor activity (recruitment) [926]. Protein kinase-A phosphorylates Ca_V1 channels, thereby reducing their activation latency and enhancing Ca_V1-mediated Ca^{++} influx that, in turn, by improving connection between the 2 channel types elicits greater Ca^{++} release from the sarcoplasmic reticulum via ryanodine receptors, which are also phosphorylated. Because the Ca^{++} conductance of ryanodine receptors is not sensitive to phosphorylation, β-adrenoceptors increase the kinetics and number of ryanodine receptors that respond nearly simultaneously to the Ca^{++} flux delivered by Ca_V1 channels.

Acute β1-adrenoceptor stimulation triggers the cAMP–PKA pathway and modulates the cardiac excitation–contraction coupling [922]. On the other hand, sustained β1-adrenoceptor stimulation elicits cardiomyocyte hypertrophy and apoptosis via increased intracellular calcium concentration and Ca^{++}–CamK2 activity independently of PKA. However, a persistent β2AR activation can exert a survival effect in cardiomyocytes via protein kinase-B. Activation of ERK1, ERK2, and P38MAPK by the Gi subunit also favors β2AR-mediated anti-apoptotic effect in adult rat cardiomyocytes.

Several transcriptional targets of α1-adrenergic receptors have been identified in cardiomyocytes, particularly α-actin, β-myosin heavy chain, atrial natriuretic factor. [76]

Adipocyte β-adrenoceptors

Four adrenoceptor types are involved in the regulation by catecholamines of lipolysis in adipocytes. The control of adenylate cyclase activity involves stimulatory β1-, β2-, and β3-adrenoceptors. On the other hand, α2-adrenoceptors reduce intracellular cAMP content, thereby reducing lipolysis. Adipocytes, like platelets, possess α2a- rather than α2d-adrenoceptors, at least in rabbits [928]. In humans,

76. α1ARs target transcription factors, such as Jun (Jun stands for avian sarcoma virus-17 oncogene; Japanese: 10 [Ju] + 7 [Nana]), Fos (cellular Finkel Biskis Jinkins murine osteosarcoma virus sarcoma oncogene), and EGR1 (early growth response factor-1), as well as transcriptional corepressor CARP. The zinc finger-only protein Zfp260 of the Krüppel family of transcriptional regulators, highly expressed in the embryonic heart, is downregulated during postnatal development. Protein Zfp260 is a nuclear effector of α1-adrenergic receptors. It is also a transcriptional activator of atrial natriuretic factor and a cofactor for cardiac regulator GATA4, used in MAPK signaling [927].

adrenaline stimulates and inhibits lipolysis in visceral (omental) and subcutaneous adipocytes that have a high and low β- to α2-adrenoceptor ratio, respectively.

In white adipose tissues, types of adrenoceptors on the cell surface depends on mammal species. Adipocytes produce mainly α2- and few β3-adrenoceptors in humans, and conversely in rodent adipocytes [929]. Adrenaline controls lipid mobilization, as it activates antilipolytic α2ARs in human subcutaneous adipocytes during exercise [930]. Estrogens promote and maintain the female type of adipose tissue distribution. They attenuate the lipolytic response by increasing the number of antilipolytic α2a-adrenoceptors only in subcutaneous fat depots, thereby shifting the assimilation of lipids from omental depots to subcutaneous adipose tissues [931]. In addition, increased $[Ca^{++}]_i$ inhibits lipolysis induced by β-adrenoceptor activation via phosphodiesterase inhibition of cAMP signaling. In adipocytes, insulin attenuates β1-adrenoceptor-mediated lipolysis via activation of protein kinase-C. The PKCβ1 isoform interacts with β1AR signaling to decay lipolysis [932].

β3-Adrenoceptors are major mediators of the lipolytic and thermogenic effects of catecholamines. β3-Adrenoceptor activates extracellular signal-regulated kinases ERK1 and ERK2 via epidermal growth factor receptor and Src kinase (Vol. 4 – Chap. 3. Cytosolic Protein Tyr Kinases). This pathway in association with protein kinase-A contributes to maximal β3AR-stimulated lipolysis. Vimentin of intermediate filaments interacts with the β3AR–ERK pathway, whereas actin microfilaments and microtubules do not affect lipolysis and ERK activation [933].

Hepatocyte β-adrenoceptors

Hepatocytes express adrenergic receptors that modulate liver regeneration, hepatocyte proliferation, glycogenolysis, gluconeogenesis, urea synthesis, and fatty acid metabolism. Under basal conditions, the liver supplies glucose only via gluconeogenesis to spare glycogen stores, whereas in situations of enhanced demands primed by glucagon or sympathetic signal, additional glucose release occurs via glycogenolysis.

Glucagon is the main factor that increases cAMP level, which is correlated with the rate of glycogenolysis and exercise intensity [934]. Both α- and β-adrenergic receptors exist in liver. However, α-adrenergic stimulation is much more important than that of β-adrenoceptor to prime formation of cAMP via adenylate cyclase for glycogenolysis, gluconeogenesis, and ureogenesis. Adrenergic control of the hepatic function is mainly due to α1-adrenoceptors. Transactivation from α1AR of epidermal growth factor receptor involves Gq/11, Src kinase, signal transducer and activator of transcription STAT3, and the Src–STAT3 complex (pathway crosstalk) [935]. Normal and regenerating hepatocytes express α1b-, but not α1a-adrenoceptors [936]. Liver regeneration after partial hepatectomy involves β-adrenoceptors in the initial stage and afterward α1-adrenoceptors [937].

G-protein-coupled receptors can signal without G proteins. Stimulated β2-adrenoceptor recruits β-arrestin that forms a complex with phosphodiesterase PDE4 to regulate plasmalemmal PKA activity, which phosphorylates β2-adrenoceptor [938].

Table 7.45. Angiotensin receptors and their main targeted transducers, either G proteins or protein phosphatases (Source: [736]; PSTP: protein Ser/Thr phosphatase; PTP: protein Tyr phosphatase). s Different types of angiotensin receptors exist: AT_{1A}, AT_{1B}, and AT_2 to AT_4. Receptor AT_4 is activated by angiotensin-4.

Type	Main transducer
AT_1	Gq/11, G12/13, Gi/o
AT_2	Gi2/3
	PTP, PSTP

Arrestin-mediated uncoupling of G-protein-mediated signaling hampers Gs activation and switches to Gi stimulation that activates extracellular signal-regulated kinase ERK2.

7.13.6 Angiotensin Receptors

Angiotensins (ATn1–ATn4) act mainly via angiotensin type-1 (AT_1) and type-2 (AT_2) receptors (Table 7.45). Endogenous ligands are mostly angiotensin-2 and -3, whereas angiotensin-1 is weakly active in some systems [5]. In fact, different types of angiotensin receptors exist: AT_{1A}, AT_{1B}, and AT_2 to AT_4. The AT_4 receptor is activated by angiotensin-4, an angiotensin-2 metabolite. This specific receptor of angiotensin-4 resides in the brain and kidney.

Specific receptors for angiotensin-2 in adrenal zona glomerulosa,[77] vascular smooth muscle, kidney, brain, and anterior pituitary gland[78] exhibit generally similar binding properties. Angiotensin receptors of the anterior pituitary are not affected by changes in sodium balance in opposition to adrenal and vascular receptors [939]. In the brain, angiotensin receptors localize to several regions, although they are particularly concentrated in the circumventricular organs.[79] The angiotensin receptors of the renal cortex are localized in glomeruli, whereas in the renal medulla they are distributed diffusely.

Initiated signaling pathways include, in particular, receptor Tyr kinases (e.g., PDGFR and EGFR), IRS1, and cytosolic Tyr kinases, such as Src, Janus, and focal adhesion kinases, as well as mitogen-activated protein kinase.

77. Zona glomerulosa is the superficial layer of the adrenal cortex.

78. Among endocrine cell types - corticotrophic, gonadotrophic, lactotrophic, somatotrophic, and thyrotrophic cells - of anterior pituitary gland, angiotensin-2 receptors are located in corticotrophs and lactotrophs.

79. Circumventricular organs surround the brain ventricular network, primarily the third and fourth ventricles. They correspond to the few cerebral sites that have an incomplete blood–brain barrier, so that neurons can sense various blood compounds, particularly hormones. These organs secrete different neurotransmitters, hormones, and cytokines. Circumventricular organs include pineal gland (circadian rhythm), subfornical organ and organum vasculosum of the lamina terminalis (body fluid regulation), choroid plexi, area postrema (toxin detection), median eminence (regulation of anterior pituitary activity), subcommissural organ (somatostatin secretion), and posterior pituitary (oxytocin and vasopressin sensing).

7.13.6.1 AT_1

Type-1 receptor AT_1 mediates vasoconstriction, secretion of aldosterone and vasopressin, cardiac contractility and hypertrophy, augmented peripheral noradrenergic activity, vascular smooth muscle cells proliferation, decreased renal blood flow, renin inhibition and sodium reuptake in kidneys, modulation of central sympathetic nervous system activity, central osmocontrol, and extracellular matrix formation.

Angiotensin-2 receptor AT_1 activates: (1) $G\alpha_{q/11}$, $G\alpha_{i/o}$, and $G\alpha_{12/13}$ subunits of guanine nucleotide-binding (G) proteins; (2) tyrosine [80] (Vol. 4 – Chap. 3. Cytosolic Protein Tyr Kinases) or serine/threonine kinases (Vol. 4 – Chap. 4. Cytosolic Protein Ser/Thr Kinases); (3) phospholipases PLA2, PLCβ and PLCγ, and PLD (Vol. 4 – Chap. 1. Signaling Lipids); [81] (4) small monomeric GTPases of the Ras superfamily; and (5) ion channels (Chap. 3).

Angiotensin-2 receptor AT_1 predominantly and robustly couples to Gq/11 subunit family. Once bound to AT_1 receptor, angiotensin-2 stimulates vascular cells. In vascular smooth muscle cells, upon AT_1 binding, angiotensin-2 activates phospholipases as well as kinases and various oxidases. G12-Coupled AT_1 can stimulate monomeric GTPase Rho and inhibit PDGF-activated Rac GTPase. The G$\beta\gamma$ subunit associated with G12 and/or Gq/11 family subunits mediates PLC activation by angiotensin-2. Associated with G12 subunit, it also intervenes in angiotensin-2-induced PLD1 activation, possibly via Src- and RhoA-dependent mechanisms. [82] In cultured rat vascular smooth muscle cells, angiotensin-2-mediated activation of phospholipase-D1 via G$\beta\gamma$ provides a major source of sustained generation of second messengers. The G12 subunit contributes to PLD activation, whereas Gi and Gq/11 have no effect [941]. Small GTPase RhoA participates in this signaling pathways. Hydrolysis of phosphatidylcholine by PLD causes a strong production of phosphatidic acid and subsequent generation of diacylglycerol by phosphatidic acid phosphatase. Diacylglycerol contributes to continuous PKC activation. Phosphatidic acid contributes to the activation of NADH/NADPH oxidase that mediates the hypertrophic effects of angiotensin-2.

Angiotensin-2 promotes the association of scaffold proteins (paxillin and talin), leading to focal adhesion. It also stimulates NADPH oxidase via AT_1 receptor. Resulting reactive oxygen species are components of ATn2-mediated signal transduc-

80. Once bound to agonist, AT_1 can recruit a complex of non-receptor protein tyrosine kinase and phosphatase, i.e., Janus kinase JaK2 and PTPn11 that facilitates JaK2 phosphorylation (activation). This additional G-protein-independent AT_1–JaK2 pathway complements signaling primed by G-protein subunit Gq that can further potentiate the JaK–STAT signaling via Ca^{++} import in the cytosol [835].

81. Phospholipase-Cβ1, then PLCγ1, are activated by corresponding stimulated G proteins within 5 s [940]. Diacylglycerol and inositol trisphosphate are thus generated, the production ending within minutes. The transient PLC activation is followed by a sustained activation of phospholipase-D via G proteins. Concomitant stimulation of phospholipase-A2 initiates arachidonic acid signaling.

82. The G$\beta\gamma$ subunit can activate PLD by binding to Rho or ARF GTPases.

tion in vascular smooth muscle cells. In addition, AT_1 and bradykinin B2 receptors can heterodimerize [5].

7.13.6.2 AT_2

The AT_2 receptor is much less abundant than AT_1 in adult tissues. It is upregulated in pathological conditions. [83] Receptor AT_2 counteracts several growth responses initiated by AT_1. Receptors AT_1 and AT_2 stimulates and inhibits ERK1 and ERK2, respectively.

Type-2 receptor AT_2 has a more restricted expression pattern than the AT_1 receptor. It intervenes in cell growth and differentiation and tissue development. It is highly expressed in fetuses, whereas in adults, it is confined to heart, vascular smooth muscle, brain, adrenal cortex, uterus, and ovarian follicles. Activated AT_2 dilates blood vessels, inhibits growth, and causes apoptosis. It provokes a rapid activation of Gi and Go (but not of Gq and Gs) [943].

7.13.6.3 Agonists

Angiotensin-2 causes cell proliferation, hypertrophy, vasoconstriction, and fibrosis [944]. On the other hand, angiotensin$_{(1--7)}$, an active heptapeptide formed from angiotensin-1 and-2, antagonizes angiotensin-2. It provokes vasodilation, as it causes the release of nitric oxide and vasodilatory prostaglandins, and can amplify bradykinin effect [944]. In addition, it has a potent natriuretic and antifibrotic action. It also inhibits the growth of vascular smooth muscle cells and cardiomyocytes.

Angiotensin$_{(1--7)}$ is preferentially formed from angiotensin-2 by Angiotensin-converting enzyme-2 (ACE2). Angiotensin-2 reduces ACE2 synthesis and activity in cardiac myocytes and fibroblasts. Endothelin-1 also attenuates ACE2 synthesis in cardiomyocytes. Decrease in ACE2 production by angiotensin-2 and endothelin-1 requires mitogen-activated protein kinase kinase MAP2K1 and extracellular signal-regulated kinases ERK1 and ERK2. Atrial natriuretic peptide reverses ATn2- and ET1-mediated ACE2 downregulation, when it acts simultaneously with these factors (but not alone). [84]

Angiotensin$_{(1--7)}$ impedes ATn2- and ET1-mediated ACE2 downregulation (but not its synthesis) via a specific $AT_{(1--7)}$ receptor [945]. [85] Stimulated $AT_{(1--7)}$ activates the PI3K–PKB signaling cascade and endothelial nitric oxide synthase (NOS3)

83. The AT_1 receptor is involved in the evolution of aortic aneurysm in Marfan syndrome, an autosomal dominant connective tissue disorder characterized by heterozygous mutations in the FBN1 gene that cause a deficiency of fibrillin-1. Transforming growth factor-β signaling augments. Manifestations include aortic aneurysm, emphysema, degeneration of the atrioventricular valves, and myopathy. On the other hand, AT_2 confers protection [942].

84. Atrial natriuretic peptide, a peptide hormone released mainly from the atrium, activates the guanylate cyclase-A receptor (NPR1 or NPRa; Sect. 6.4.1). Activated NPR1 produces cGMP from GTP. Second messenger cGMP causes natriuresis and hypovolemia, and impedes the proliferation of vascular smooth muscle cells.

85. A..k.a. Mas.

in endothelial cells and cardiomyocytes [944]. Moreover, angiotensin$_{(1--7)}$ primes the secretion of atrial natriuretic peptide [944]. In atriomyocytes, angiotensin$_{(1--7)}$ increases ANP release at high atrial pacing via the PI3K–PKB pathway. Conversely, Na^+–H^+ exchanger-1 and Ca^{++}–calmodulin-dependent kinase CamK2 prevent the augmentation of high atrial pacing-induced ANP secretion by ATn$_{(1--7)}$.

Angiotensin$_{(1--12)}$ can trigger a vasoconstriction via AT_1 receptor. It can serve as a substrate for the formation of angiotensin-2 and angiotensin$_{(1--7)}$, according to the available, local type of angiotensin-converting enzyme. In the brain, its concentration is higher than those of angiotensin-1 and -2. It is produced in the nucleus tractus solitarius (baroreceptor reflex center), but do not affect the autonomic control of the cardiac frequency in normal conditions. However, angiotensin$_{(1--12)}$ may be active in hypertension [946]. Exogenous angiotensin$_{(1--12)}$ is converted into angiotensin-2 within the nucleus tractus solitarius. It can cause a transient depressor response [946].

7.13.6.4 Angiotensin Receptors in the Cardiovascular System

The vasculature is characterized by a low density of AT_1 in endothelial cells ($ecAT_1$) and its relative abundance in vascular smooth muscle cells ($smcAT_1$). Endothelial cell activation by angiotensin-2 via $ecAT_1$ reduces the tone of vascular smooth muscle cells, as it elevates expression of endothelial nitric oxide synthase [947]. Therefore, angiotensin-2 stimulation of $ecAT_1$ reduces $smcAT_1$-mediated vasoconstriction.

The AT_1 receptor contributes to vascular relaxation in cerebral resistance arteries via transactivation of epidermal growth factor receptor kinase and extracellular signal-regulated protein kinases ERK1 and ERK2 [948].

In hearts, pathways leading to ERK activation differ according to cell types. In cardiac fibroblasts, angiotensin-2 activates ERKs via $G\beta\gamma$ subunit of Gi, Src kinase, adaptors SHC and GRB2, and Ras GTPase.

In cardiomyocytes, mechanical stretch-induced ERK activation that can occur even in the absence of angiotensin-2 involves Gq and protein kinase-C [949]. Cardiomyocyte stretch indeed leads to conformational change and activation of AT_1 independently of angiotensin-2 [950, 951]. The AT_1 receptor is able to detect mechanical stress and transduces it into biochemical signals. Mechanical stretch induces association of AT_1 receptor with Janus kinase-2 and translocation of G proteins into the cytosol.

Angiotensin-2 operates as a potent hypertrophic agent for cardiomyocytes that promotes production of TGFβ1 and atrial natriuretic factor [952].[86] The AT_2 receptor is involved in maladaptive cardiac hypertrophy. On the other hand, cardiac

86. Angiotensin-2 can, hence, indirectly cause cell division, fibrosis, cytokine infiltration, α- to β-myosin heavy chain switch, and stimulation of the renin–angiotensin system. Interactions among cardiomyocytes, fibroblasts, and vascular cells are sources of secreted signals. Locally produced factors, such as growth factors FGF2, IGF1, TNFα, cardiotrophin-1, and endothelin, can also trigger cardiomyocyte hypertrophy. In addition, MAP3K7 can provoke heart failure.

AT_{1A} receptor causes cardiac fibrosis, but not hypertrophy [953]. Transforming growth factor-β1 isoform couples work load and angiotensin-2 to cardiac adaptative hypertrophy

Angiotensin-2 produces reactive oxygen species in vascular smooth muscle cells and cardiomyocytes. In rat neonatal cardiomyocytes, angiotensin-2 binds to $G\alpha_{12/13}$-coupled AT_1 that primes a cascade with the following mediators, which operate successively: (1) small GTPase Rho and RoCK kinase; (2) small GTPase Rac; (3) NADPH oxidase; (4) reactive oxygen species; and (5) JNK and P38MAPK [954].

7.13.6.5 Angiotensin Receptors in the Liver

Angiotensin-2 acts as a pro-inflammatory agent and growth factor in the liver. After injury to the liver, it assists in tissue repair by stimulating: (1) hepatocytes and hepatic stellate cells to synthesize extracellular matrix proteins and secrete cytokines and (2) myofibroblasts to proliferate.

Angiotensin-2 activates nuclear factor NFκB via AT_1 and caspase recruitment domain-containing proteins CaRD10 and B-cell lymphoma/leukemia protein BCL10 as well as paracaspase mucosa-associated lymphoid tissue lymphoma translocation protein MALT1 [955]. [87]

7.13.7 Apelin Receptors

Apelin APJ receptor [88] (Sect. 6.9.2) responds to apelins [741], mainly long $apelin_{36}$ and short $apelin_{13}$ [89] (potency order $apelin_{13} > apelin_{36}$ [5]). Main transducers are subunits of the $G\alpha_{i/o}$ family of guanine nucleotide-binding (G) proteins. The hydrophobic region of APJ is similar to that of the angiotensin receptor [956].

The APJ receptor is particularly produced in the cardiovascular and central nervous systems, and in the latter by neurons, oligodendrocytes, and astrocytes [958]. [90] Apelin is also observed in adipocytes as well as gastric mucosa and Kupffer cells in the liver [961].

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87. Caspase recruitment domain-containing proteins (CaRD9 to -11 and -14, as well as BCL10) operate as upstream regulators in NFκB signaling. Proteins CaRDs interact with BCL10 to act as NFκB signaling complexes. Protein BCL10 forms a complex with MALT1 to synergize NFκB activation.

88. The APJ receptor is encoded by chromosome 11.

89. Apelin derives from a precursor, preproapelin (77 amino acids). Apelins include long types (e.g., 36 amino acids; $apelin_{(42--77)}$) expressed in lungs, testes, and uterus, and short types (e.g., 13 amino acids; $apelin_{(65--77)}$) produced in mammary glands, which also synthesize the long apelin type [957]. Long and short forms of apelin differently interact with APJ receptor.

90. Apelin serves a neuropeptide in the hypothalamus, especially in the supraoptic, paraventricular, and arcuate nuclei. Apelin-containing cell bodies of the supraoptic and paraventricular nuclei, such as arginine vasopressin- and oxytocin-containing magnocellular neurons, project toward the internal layer of the median eminence and posterior pituitary (neurohypophysis).

In the central nervous system, the apelin–APJ complex helps maintain the fluid homeostasis and regulates vasopressin release from the hypothalamus [959]. In response to osmotic stimulation, the release of arginine vasopressin, leucine enkephalin (^{Leu}enkephalin), and dynorphin from neurons of the supraoptic and paraventricular nuclei is augmented. [91] Apelin targets vasopressinergic neurons and inhibits their activity, thus impeding arginine vasopressin release. Apelin and arginine vasopressin that coexist in magnocellular neurons have opposite effects (autocrine somatodendritic feedback loop) to maintain body fluid homeostasis.

In the cardiovascular system, apelin is primarily expressed in vascular and endocardial endothelial cells (at least in recesses of the right atrium) [962]. [92] Cardiomyocytes can also express apelin. Apelin receptors on vascular smooth muscle and endothelial cells as well as cardiomyocytes are activated by the family of apelin peptides [963]. They operate in both auto- and paracrine signalings.

Apelin, a direct vasoconstrictor, [93] can mediate vasodilation via nitric oxide [961]. In fact, the endothelium-dependent vasodilation results from a prostanoid-dependent mechanism [963]. In mammary artery, the 3 main forms of apelin — N-terminal pyroglutamylate ^{pGlu}apelin$_{13}$, apelin$_{13}$, and apelin$_{36}$ — cause vasodilation, when the endothelium is functional. These regulators act with comparable potency in a concentration-dependent manner.

In human hearts, apelin peptides are among the most potent endogenous positive inotropic agents [963]. The N-terminal pyroglutaminated apelin$_{13}$ is the predominant isoform in hearts from patients with coronary artery disease. Apelin acts on neighboring cardiomyocytes and exerts positive inotropic effect via Na^+–H^+ exchanger NHE1 (or SLC9a1), Na^+–Ca^{++} exchanger (reverse mode), phospholipase-C, and protein kinase-C [739]. [94] Autocrine signaling by apelin and APJ receptor in mouse left ventriculomyocytes plays a modest role in basal conditions, but becomes significant under stress, as they both improve sarcomeric shortening and velocity of contraction and relaxation without change in calcium transient [964].

Apelin is also involved in angiogenesis, as it enlarges developing blood vessels through which the flow rate is higher upon stimulation by the Ang1–TIE2 complex [965]. [95] Angiopoietin-1 synthesized by mural cells and migrated hematopoietic stem cells activates its receptor TIE2 to generate apelin production in sprout endothelial cells.

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91. Hypothalamic magnocellular neurons of the paraventricular and supraoptic nuclei synthesize not only vasopressin and oxytocin, but also tyrosine hydroxylase (enzyme of catecholamine synthesis), as well as galanin, dynorphin, and cholecystokinin. High plasma osmolality induces increased mRNA levels for vasopressin, oxytocin, Tyr hydroxylase, galanin, dynorphin, and cholecystokinin [960].

92. Apelin is produced by endothelial cells of, at least, large blood vessels and vessels of heart, kidneys, adrenal glands, and lungs.

93. Apelins are potent vasoconstrictors in endothelium-denuded saphenous veins and mammary arteries.

94. Apelin is downregulated in chronic ventricular pressure overload.

95. Apelin binds to APJ to enlarge blood vessels. Expression of APJ is enhanced by VEGF.

The APJ receptor is coupled to $G\alpha_{i1}$ or $G\alpha_{i2}$, but not $G\alpha_{i3}$ [966]. Apelin inhibits adenylate cyclase and increases the phosphorylation of ERK or PKB. Apelin efficiency in inhibiting adenylate cyclase depends on: (1) activation and deactivation kinetics of the apelin type and (2) cell type. Activation of apelin receptors by short fragments is transient as APJ is quickly internalized. The duration of desensitization depends on the rate of receptor recycling to the plasmalemma ($\sim$1 h). Long subtypes produces a sustained activation.

7.13.8 Bile Acid Receptor

Bile acid receptor GPBAR1 [96] responds to bile acids produced in the liver with the following potency order [5]:

lithocholic acid > deoxycholic acid > chenodeoxycholic acid, cholic acid.

It activates deiodinases that convert prohormone thyroxine (T4) into active hormone tri-iodothyronine (T3). It signals via Gs and cAMP as well as mitogen-activated protein kinase.

7.13.9 Bombesin Receptors

Bombesin receptors (BB_1–BB_3) are activated by bombesin homologs [97] gastrin-releasing peptide (GRP) and neuromedin-B and -C [5]. [98] These receptors couple primarily to Gq/11 subunit family of G proteins. Activated BB_1 and BB_2 receptors stimulate tissue growth, smooth muscle cell contraction, and secretion. The BB_3 receptor may intervene in energy balance and control of body weight [5].

7.13.10 Bradykinin Receptors

Bradykinin receptors (B_1–B_2 or B1R–B2R) are activated by the nonapeptide bradykinin and its derived peptides kinin and kallidin (Table 7.46). [99] Kinins are polypeptides formed locally after tissue damage and inflammation. They provoke smooth muscle contraction as well as vasodilation via the release of nitric oxide and prostaglandins by endothelial cells, increase the vascular permeability, and exert a mitogenic effect using the mitogen-activated protein kinase modules.

Kinins and their derivatives generated from kininogens by kallikreins and other serine peptidases compose the *kallikrein-kinin system*. Kallidin is a bioactive kinin that possesses an additional lysine residue at the N-terminal end, hence its name ^{Lys}bradykinin. This substrate of carboxypeptidase-M and -N signals through the kinin receptor. It is formed in response to injury from kininogen precursors by

96. A.k.a. M-BAR, GPCR19, GPR131, BG37, and TGR5.
97. Bombesin is a tetradecapeptide originally isolated from skins of amphibians.
98. A.k.a. GRP_{18--27}.
99. The B_1 receptor is expressed at a much lower level than B_2 in mice kidneys, but it is induced upon stimulation by endotoxins or cytokines.

Table 7.46. Receptors of the bradykinin/kinin family with their main targeted G proteins (Source: [736]).

Type	Main transducer
B_1, B_2	Gq/11 Gi ($G\beta\gamma$)

kallikreins. Kallidin can be converted to bradykinin by aminopeptidase. Bradykinin and kallidin are produced by the proteolysis of high- or low-molecular-weight kininogen by plasma and tissue kallikrein, respectively.

Bradykinin binds and stimulates at least the 2 bradykinin receptors: B_1 and B_2. The B_2 receptor is constitutively expressed in various cell types. It relays the majority of the vascular actions of bradykinin and kallidin. On the other hand, the B_1 receptor is scarce in normal tissue and expressed during tissue damage (e.g., inflammation and ischemia), by exposure to pro-inflammatory cytokines, growth factors, or oxidative stress, in various cell types, such as vascular smooth muscle and endothelial cells, cardiomyocytes, fibroblasts, and neurons. Bradykinin receptors are coupled to Gq and/or Gi subunits depending on the cell type.

Carboxypeptidases remove the C-terminal arginine of bradykinin and kallidin to generate desArg9BK (or $BK_{(1-8)}$) and desArg10kallidin (also denoted as $^{Lys}BK_{(1-8)}$ or ^{Lys}desArg9BK), respectively. Receptor B_1 is preferentially activated by the metabolites $BK_{(1-8)}$ and $^{Lys}BK_{(1-8)}$ peptides.

Angiotensin-2 and endothelin-1 increase the production of B_1 receptor in cardiovascular diseases. These pro-oxidative peptides cooperate via ROS, PI3K, and NFκB on rat vascular smooth muscle cells using AT_1 and ET_A receptors [967]. Newly synthesized B_1 receptors can activate the MAPK module. Once the AT_1 receptor is activated, the effect of angiotensin-2 can be mediated by the subsequent ET1 release and ET_A stimulation.

Regulator of G-protein signaling RGS4 can serve as GTPase-activating proteins for both Gi and Gq (but not for Gs and G12). The RGS4 protein attenuates Gi-mediated inhibition of cAMP synthesis and impedes Gq-mediated activation of phospholipase-Cβ [969]. Both Gq and Gi subunits are able to activate phospholipase-C, the latter via $G\beta\gamma$.

Bradykinin GPCRs can signal without G proteins. In renal cells, the B_2 receptor interacts with protein Tyr phosphatase PTPn11 to prevent cell proliferation [970].

7.13.10.1 Bradykinin Receptors and Angiotensin-Converting Enzyme

Angiotensin-1-converting enzyme (ACE) is a dipeptidyl carboxypeptidase that removes 2 amino acids from the C-terminus of inactive angiotensin-1 to convert it into active angiotensin-2. Moreover, ACE is also a kininase that converts active vasodilatory kinins into inactive metabolites, as it also deletes 2 amino acids from their C-termini.

In humans, the polymorphism of the Ace gene due to the presence or absence of an Alu retrotransposon in intron 16 is associated with up to a 2-fold difference in relative plasma ACE concentration. [100] The kallikrein-kinin system that partly mediates the effects of the polymorphism has a protective role [968]. Kinins indeed bind to Gq-coupled bradykinin B_1 and B_2 receptors to reduce the risk of diabetic complications.

7.13.10.2 Bradykinin Receptors in the Cardiovascular Apparatus

In adult rat cardiomyocytes, bradykinin activates P21 activated kinase PAK1 downstream from monomeric GTPases. Kinase PAK1 stimulates protein phosphatase-2 that dephosphorylates both cardiac troponin-I and phospholamban [971]. Protein phosphatase-2 is able to complex with both PAK1 and phospholamban.

Vasodilation and Vessel Remodeling

Bradykinin is a potent vasodilator of arteries that irrigate muscle, kidney, and heart, among other viscera. Tissue kallikrein does not contribute to uterine artery remodeling during and after pregnancy [972].

Bradykinin synthesized by tissue kallikrein from kininogen stimulates endothelial β2-adrenergic receptors, subsequently releasing nitric oxide [101] and prostacyclin to control the vascular tone.

Both receptors are coupled with Gq proteins. Their stimulation activates phosphatidylinositol-specific phospholipases that augment intracellular Ca^{++} concentration and leads to activation of endothelial nitric oxide synthase (NOS3). Kinins that act via B_2 also support the expression of inducible NOS (NOS2). Nitric oxide reversibly suppresses mitochondrial oxidation, at least partly by inhibition of cytochrome-C oxidase of the electron transport chain. Kinins also facilitate the synthesis of prostanoids, such as prostaglandins PGE2 and PGI2, which heighten intracellular cAMP concentration [968]. Messenger cAMP also decreases mitochondrial respiration, as it activates the NADH-ubiquinone oxidoreductase activity of complex I and inhibits cytochrome-C oxidase. Therefore, nitric oxide and kinins reduce oxidative stress. In particular, bradykinin reduces mitochondrial superoxide generation in human vascular endothelial cells [968].

Sympathoexcitatory Reflex

Bradykinin primes afferent reflex in the heart owing to cardiac sympathetic afferent fibers. Bradykinin causes a sympathoexcitatory reflex [102] characterized by

100. This polymorphism does not significantly affect blood pressure and angiotensin-2 and aldosterone concentration. Nevertheless, it is associated with different risks of diabetic complications.

101. Endothelial nitric oxide synthase expression and, consequently, nitric oxide production, increase during pregnancy.

102. The sympathoexcitation reflex happens when all other cardiovascular reflexes (baroreceptor reflex, cardiopulmonary reflex with vagal afferents) are not efficient enough.

increased arterial blood pressure and renal sympathetic nerve activity via epicardial B_2 receptors and sympathetic cardiac afferent fibers [973]. Intrapericardial bradykinin generates a blood pressure rise, whereas circulating bradykinin acts as a vasodilator, thereby lowering blood pressure.

Cardioprotection

In the heart, bradykinin is released during cardiac ischemia and myocardial infarction. Both bradykinin receptors B_1 and B_2 contribute to the cardioprotective effect of ACE inhibition mediated by bradykinin [974]. The B_2 receptor is the main kinin receptor involved in the cardioprotection yielded by ischemic preconditioning [975]. It forms a complex with angiotensin-converting enzyme that sequesters the latter. This process determines a crosstalk between the renin–angiotensin pathway and the kinin–kallikrein axis.

7.13.11 Calcitonin, Amylin, CGRP, and Adrenomedullin Receptors

Calcitonin (Ct) [103] is a hormone that results from the proteolytic cleavage of a prepropeptide that is encoded by the CALCA gene (or CALC1) primarily in parafollicular cells [104] of the thyroid. Procalcitonin (PCt) belongs to a family of related hormone precursors that include islet amyloid precursor, calcitonin gene-related peptide, and adrenomedullin precursor. It decreases bone resorption.

Amylin (Amy) [105] is a hormone secreted by pancreatic β cells. Amylin reduces nutrient intake and contributes to the control of glycemia, as it optimizes glucose metabolism.

Calcitonin gene related peptide-2 (CGRP2) [106] is a member of the calcitonin family of peptides. It is produced in both peripheral and central neurons. It is a potent vasodilator.

Adrenomedullin-1 (AM1) is ubiquitously synthesized from a precursor (proadrenomedullin) encoded by the AM gene. It abounds in vascular endothelial and smooth muscle cells. It is also a potent vasodilator. Furthermore, it regulates body fluids, as it precludes drinking and sodium intake and causes natri- and kaliuresis [976]. [107] Different excisions form various peptides, such as an inactive adrenomedullin, ePAMP, adrenotensin, and $AM_{95--146}$. In humans, mature adrenomedullin is rapidly catabolized. Circulating adrenomedullin comprises both mature, amidated and inactive, glycated form. The related peptide adrenomedullin-2 (AM2), or intermedin, participates in the regulation of blood circulation. It raises the concentrations

103. A.k.a. calcitonin-related polypeptide-α, CalcA, and Calc1, as well as calcitonin gene-related peptide CGRP1 and α-CGRP (Vol. 2 – Chap. 1. Remote Control Cells – Sect. Endocrine System and Hormones).

104. A.k.a. C cells.

105. A.k.a. (pancreatic) islet amyloid polypeptide (IAPP).

106. A.k.a. calcitonin-related polypeptide-β, β-CGRP, CalcB, and Calc2.

107. Angiotensin-2 is a dipsogenic hormone. Atrial natriuretic peptide also attenuates the drinking rate [977].

Table 7.47. Receptor family of the calcitonin–calcitonin gene-related peptide family of peptide hormones (Source: [5]; AM: adrenomedullin; Amy: amylin; CGRP: calcitonin gene-related peptide; Ct: calcitonin).

Receptor	Composition	Main transducer	Potency order
CT	CT	Gs, Gq	Ct $\geq$ Amy, CGRP $>$ AM1, AM2
Amy_1	CT–RAMP1	Gs	Amy $\geq$ CGRP $>$ AM2 $>$ Ct$>$AM1
Amy_2	CT–RAMP2	Gs	
Amy_3	CT–RAMP3	Gs	Amy $>$ CGRP $>$ AM2 $>$ Ct $>$ AM1
CGRPR	CL–RAMP1	Gs, Gq	CGRP $>$ AM1 $\geq$ AM2 $>$ Amy
AM_1	CL–RAMP2	Gs	AM1 $\gg$ CGRP, AM2 $>$ Amy
AM_2	CL–RAMP3	Gs	AM1 $\geq$ CGRP, AM2 $>$ Amy

of atrial and brain natriuretic peptides and renin in plasma. Adrenomedullin-5 that also derives from a prohormone decreases arterial pressure.

Calcitonin, amylin, calcitonin gene-related peptide, and adrenomedullin receptors are generated by the genes CALCR and CALCRL that encodes the calcitonin receptor (CalcR, CTR, and CTR1) and calcitonin receptor-like receptor (CalcRL and CLR). Calcitonin receptors that activate G-protein subunits Gs and Gq are involved in the maintenance of calcium homeostasis. Their function changes according to their interaction with receptor activity-modifying proteins (RAMP1–RAMP3) that form multimeric amylin receptors Amy_1 (CalcR–RAMP1), Amy_2 (CalcR–RAMP2), and Amy_3 (CalcR–RAMP3; Table 7.47). Splice variants of the CalcR receptor produce variants of Amy receptors.

Calcitonin receptor-like receptors also connect to receptor activity-modifying proteins. Receptor of CGRP2 (CGRPR) is a heteromer composed of the G-protein-coupled receptor CalcRL and receptor activity-modifying protein RAMP1. The CalcRL subunit also produces the adrenomedullin receptor AM_1 and AM_2 with RAMP2 and RAMP3, respectively.

Adrenomedullin-1 targets the CalcRL–RAMP2 or CalcRL–RAMP3 complexes, whereas adrenomedullin-2 interacts non-selectively with all 3 CalcRL–RAMP heteromers [978]. Adrenomedullin-5 tethers to AM_5 receptors (other than CalcRL–RAMP or CalcR–RAMP) to exert its cardiovascular actions in mammals [979].

7.13.12 Calcium-Sensing Receptors

Calcium-sensing receptors (CaR, CaS, or CaSR) are G-protein-coupled receptors (Table 7.48) that sense the concentration of extracellular Ca^{++} ions. Calcium-sensing receptor responds to extracellular concentrations of calcium and magnesium in the millimolar range and of gadolinium and some polycations in the micromolar range [5]. The extracellular Ca^{++}-sensing receptor responds also to many other ligands. In particular, its activity is allosterically regulated by amino acids and H^+ ions.

Table 7.48. Calcium-sensing receptor, targeted G proteins, and order of potency between extracellular concentrations of Ca^{++} and Mg^{++} (Source: [736]).

G-protein transducers	Potency order
Gq/11, Gi/o, G12/13	$[Ca^{++}]_e > [Mg^{++}]_e$

This GPCR resides especially on hematopoietic stem cells. It retains hematopoietic stem cells close to the endosteal surface of the bone marrow. It interacts with extracellular matrix components, particularly collagen-1.

Calcium-sensing receptor also lodges in parathyroids, where it participates in parathyroid hormone response to extracellular Ca^{++} concentration ($[Ca^{++}]_e$). In addition, CaRs are produced by cells of the kidney, liver, and thyroid gland.

In the liver, calcium-sensing receptor is specifically expressed in hepatocytes (not in stellate, endothelial, and Kupffer cells). It mobilizes calcium ions from IP_3-sensitive stores to stimulate bile flow [980].

This major sensor and regulator of $[Ca^{++}]_e$ also localizes to the stomach and intestinal tract, especially on gastrin-secreting G cells in the stomach. Luminal nutrients, particularly Ca^{++} and amino acids are potent stimulators of gastrin and acid secretion [981].

In cardiomyocytes, CaSR predominantly localizes to caveolae. It ensures a cardioprotective role in ischemic preconditioning [982].

7.13.13 Cannabinoid Receptors

Cannabinoid receptors are targeted by endo- and exogenous cannabinoids. Endocannabinoids comprise anandamide, or ^{N}arachidonoyl ethanolamine (AEA), as well as ^{N}homo γ-linolenoyl ethanolamine, ^{N}docosatetra (7,10,13,16)-enoyl ethanolamine, and 2-arachidonyl glycerol (2AG). [108] Anandamide and 2-arachidonoyl glycerol are 2 major endocannabinoids. Endocannabinoids 2-arachidonyl glycerol and anandamide are released from the cell membrane by activated phospholipases.

Cyclooxygenase-2 transforms 2-arachidonyl glycerol and anandamide into prostaglandin glycerol esters (^{PGH2}G) and ethanolamides (^{PGH2}EA). [109] In cells, glycerol esters and ethanolamides of prostaglandins PGE2, PGD2, and PGF2α are endocannabinoid-derived COx2 products (^{PGH2}G and ^{PGH2}EA). The sequential action of COx2 and thromboxane-A synthase (TxaS) on AEA and 2AG yields thromboxane-A2 ethanolamide and glycerol ester, respectively [983]. Similarly, prostaglandin synthases PGdS, PGeS, and PGiS (i.e., prostaglandin-D, -E, -H2, and prosta-

108. 2-Arachidonyl glycerol is also termed 2-arachidonoyl-glycerol, (5,8,11,14)-eicosatetraenoic acid, and 2-hydroxy 1-(hydroxymethyl)ethyl ester.

109. Activated phospholipase releases arachidonic acid from phospholipids. Afterward, cyclooxygenase oxygenates liberated fatty acid to generate the hydroxy-endoperoxide prostaglandin-H2. The latter can be converted into one among various derivatives: prostaglandins, thromboxane, and prostacyclin. Thromboxane-B2 (TxB2) is an inactive TxA2 metabolite. Cyclooxygenase-1 generates thromboxane-A2 in platelets.

cyclin synthases) contribute to the formation of prostaglandin and prostacyclin glycerol esters and ethanolamides.

The endocannabinoid system is involved in signal transduction not only in the central nervous system, but also in other organs. Endocannabinoids are lipid mediators that are released and rapidly degraded by numerous enzymes. The most abundant endocannabinoid in the central nervous system, 2-arachidonoyl glycerol, is synthesized from diacylglycerol by diacylglycerol DAGLα (mainly) and DAGLβ lipases. These enzymes may also produce arachidonic acid. 2-Arachidonoyl glycerol functions as a retrograde signaling molecule that suppresses synaptic transmission in the central nervous system and regulates axonal growth and guidance. [110]

Two known cannabinoid isoreceptors are: (1) type-1 (CB_1) expressed mainly in the brain, but also lung, liver, and kidneys, as well as vascular smooth muscle and endothelial cells, monocytes, and macrophages; and (2) type-2 (CB_2) cannabinoid receptor chiefly produced in hematopoietic cells (B and T lymphocytes as well as macrophages), and also keratinocytes and vascular smooth muscle and endothelial cells. Novel cannabinoid receptors exist in endothelial cells as well as in the central nervous system. Cannabinoid-like receptors encompass GPR18 for ^{N}arachidonoylglycine, GPR55 with a wide spectrum of cannabinoid agonists and antagonists, GPR119 for fatty acid ethanolamides [5]. Anandamide also targets TRPV1 channel.

Cannabinoid receptors participate in the regulation of adult neurogenesis [984]. Receptors CB_1 and CB_2 participate in the genesis of atherosclerosis. Monocytes undergo a significant change in CB_1 and CB_2 expression profile during differentiation into macrophages [985]. The CB_1:CB_2 ratio on monocytes and macrophages, indeed, regulates their inflammatory activity and ability to produce reactive oxygen species [985]. These receptors have opposing influences on ROS production. In macrophages, CB_1, but not CB_2, causes phosphorylation of P38MAPK that causes ROS production and synthesis of pro-inflammatory cytokines such as tumor-necrosis factor-α and CCL2 chemokine. [111] On the other hand, the CB_2 receptor activates small GTPase Rap1 that counteracts CB_1-stimulated ROS production. In endothelial cells, the CB_2 receptor also lowers TNFα-induced proliferation and migration of smooth muscle cells and expression of adhesion molecules and chemokines.

The type of cannabinoid receptor ligand influences Gα-subunit response. A given ligand of cannabinoid receptor CB_1 can act as an agonist for Gi1 and Gi2 and as an antagonist for Gi3. Another type of ligand can behave as an inverse agonist of Gi1 and Gi2 and as an agonist for Gi3.

Cannabinoid receptors target many effectors, such as adenylate cyclase, inwardly rectifying potassium channels (K_{IR}) and other K^+ channels, calcium channels, protein kinase-A and -C, kinases Raf1, ERK, JNK, and P38MAPK, and transcription factors Fos and Jun.

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110. Endocannabinoids are released from postsynaptic neurons and cause retrograde suppression of synaptic transmission. In the hippocampus, the postsynaptic release of an endocannabinoid transiently suppresses GABA-mediated transmission at inhibitory synapses [984].

111. A.k.a. monocyte chemoattractant protein-1).

Endocannabinoids released from a neuron that bears endocannabinoid action bind to CB_1 receptor in presynaptic neuron and reduces GABA liberation, hence GABA-mediated neurotransmission. Cannabinoid receptor CB_1 targeted by glucocorticoids in the basolateral nucleus of the amygdala intervenes in the consolidation of emotional memory, although glucocorticoids usually regulate gene transcription by either binding homo- or heterodimers of intracellular glucocorticoid receptors to nuclear DNA or by interacting with transcription factors [986]. Endogenous cannabinoid binding to CB_1 stimulates appetite.

Activated presynaptic CB_1 receptors also repress sympathetic innervation of blood vessels. Anandamide and 2-arachidonyl glycerol produced by macrophages and platelets, respectively, can cause hypotension.

In the liver, activated CB_1 increases fatty acid synthesis via lipogenic transcription factor SREBP1c and acetyl coenzyme-A carboxylase-1 and fatty acid synthase [987].

Cannabinoid CB_2 receptor abounds in mature B lymphocytes. It also exists in myeloid and natural killer cells, and various other cell types. Ligand for CB_2, 2-arachidonoyl glycerol, is generated from arachidonic acid-containing phospholipids. Expression of CB_2 in peripheral B lymphocytes is correlated to the production level of immunoglobulin λ-chain [988].

Production and function of CB_2 are upregulated in immature B lymphocytes. Migration in bone marrow sinusoids of immature B cells depends on Gi-protein-coupled cannabinoid receptor CB_2 [988]. Immunoglobulin-M+ immature B cells can be retained in bone marrow sinusoids owing to integrin-$\alpha_4\beta_1$ and its endothelial ligand VCAM1.

G-protein-coupled receptor GPR119 can also serve as a cannabinoid receptor. It is synthesized predominantly in pancreas and gastrointestinal tract. It regulates the secretion of incretin and insulin. It is activated by endogenous fatty acid ethanolamines, particularly ^{N}oleoylethanolamine and ^{N}palmitoylethanolamine [5]. It is mainly coupled to Gs subunit.

7.13.14 Chemokine Receptors

Chemokine receptors constitute a large class of GPCRs that are activated by at least one chemokine.[112] Chemokines can be subdivided according to their structure into 4 subclasses: 28 known CC,[113] 16 identified CXC,[114] 2 detected C, and 1 CX_3C chemokines. Chemokines can also be classified according to their function into homeostatic and inflammatory groups.

Most chemokine receptors can bind with a high affinity to multiple chemokines (Table 7.49, 7.50, and 7.51). Most chemokines tether to several receptor subtypes. However, chemokines bind almost always to the same structural receptor subclass.

Chemokine receptor CCR7 expressed on mature, naive T lymphocytes intervenes in immune responses that require the coordinated interaction of various cell types

112. I.e., cytokine with chemotactic activity for leukocytes.
113. A.k.a. β-chemokines.
114. A.k.a. α-chemokines.

Table 7.49. Chemokine receptors CCR and their ligands (Source: [5]; ALP: CC chemokine with an ALP (Ala–Leu–Pro) N-terminal sequence; BCA: B-cell attracting chemokine; CTACK (CTAK): cutaneous T-cell-attracting chemokine; ELC: EBV induced gene-1 ligand chemokine; HCC: human C–C motif chemokine; ILC: IL11Rα-locus chemokine; IP: interferon-γ-induced protein; LARC: liver- and activation-regulated chemokine; MCP: monocyte chemoattractant protein; MDC: macrophage-derived chemokine; MEC: mucosa-associated epithelial chemokine; MIP: macrophage inflammatory protein; MPIF: myeloid progenitor inhibitory factor; RANTES: regulated upon activation, normal T-cell expressed, and secreted product; SLC: secondary lymphoid tissue chemokine; STCP: stimulated T-cell chemotactic protein; TARC: thymus and activation-regulated chemokine; TECK: thymus-expressed chemokine).

Type	Main transducer	Agonists
CCR1	Gi/o	CCL3 (MIP1α), CCL5 (RANTES), CCL7 (MCP3), CCL8 (MCP2), CCL13 (MCP4, BCA1), CCL14a (HCC1), CCL15 (HCC2), CCL23 (MIP3, MPIF1)
CCR2	Gi/o	CCL2 (MCP1), CCL7, CCL8, CCL13, CCL16 (HCC4)
CCR3	Gi/o	CCL11 (eotaxin), CCL5, CCL7, CCL8, CCL13, CCL15, CCL24 (eotaxin-2, MPIF2), CCL26 (eotaxin-3), CCL28 (MEC)
CCR4	Gi/o	CCL22 (MDC, STCP1), CCL17 (TARC)
CCR5	Gi/o	CCL3, CCL4 (MIP1β), CCL5, CCL8, CCL11, CCL14a, CCL16
CCR6	Gi/o	CCL20 (LARC)
CCR7	Gi/o	CCL19 (ELC, MIP3β), CCL21 (SLC)
CCR8	Gi/o	CCL1 (I309), CCL4, CCL16, CCL17
CCR9	Gi/o	CCL25 (TECK)
CCR10	Gi/o	CCL27 (eskine, skinkine, ALP, CTACK, ILC), CCL28

within lymphoid tissues. Agonists CCL19 and CCL21 target CCR7 to initiate chemotaxis. Ligand CCL19 leads to CCR7 phosphorylation by both GRK3 and GRK6 and β-arrestin-2 recruitment, whereas CCL21 activates only GRK6. Only CCL19 leads to receptor desensitization, internalization, and degradation, whereas both agonists launch extracellular signal-regulated protein kinase via GRK6 [989].

The Gi-coupled chemokine receptor CXCR4 binds to CXCL12 produced by bone marrow stromal cells. It enables the retention of neutrophils and hematopoietic stem and progenitor cells in the bone marrow. [115] Conversely, disruption of CXCL12-mediated chemoattraction of CXCR4+ cells mobilize neutrophils and hematopoietic stem and progenitor cells into the blood circulation. A pepducin [116] mobilizes bone

115. The CXCR4 receptor resides on hematopoietic stem cells, myeloid progenitors, and immature neutrophils.

116. A pepducin is composed of a peptide derived from the amino acid sequence of one of the intracellular loops of a target GPCR coupled to a lipid. Synthetic pepducins are thus cell-

Table 7.50. Chemokine receptors CXCR and their ligands (Source: [5]; BCA: B-cell-attracting chemokine; BLC: B-lymphocyte chemoattractant; ENA: epithelial-derived neutrophil-activating protein; GCP: granulocyte chemoattractant (chemotactic) protein; GRO: growth-regulated oncogene; IL: interleukin; IP: interferon γ-inducible protein; ITAC: interferon-inducible T-cell alpha chemoattractant; MIG: monokine induced by interferon-γ; NAP: neutrophil-activating peptide; SDF: stroma cell-derived factor; SRPSOx: scavenger receptor for phosphatidylserine and oxidized low-density lipoprotein).

Type	Main transducer	Agonists
CXCR1	Gi/o	CXCL6 (GCP2), CXCL8 (IL8)
CXCR2	Gi/o	CXCL1 (GROα), CXCL2 (GROβ), CXCL3 (GROγ), CXCL5 (ENA78), CXCL6, CXCL7 (NAP2), CXCL8
CXCR3	Gi/o	CXCL9 (MIG), CXCL10 (IP10), CXCL11 (ITAC)
CXCR4	Gi/o	CXCL12α (SDF1α), CXCL12β (SDF1β)
CXCR5	Gi/o	CXCL13 (BLC, BCA1)
CXCR6	Gi/o	CXCL16 (SRPSOx)

Table 7.51. Chemokine receptors CXCR and their ligands (Source: [5]).

Type	Main transducer	Agonists
CX_3CR1	Gi/o	CX_3CL1 (fractalkine)
XCR1	Gi/o	XCL1α and β (lymphotactin-α and -β)

marrow hematopoietic cells [990]. It can be thus used to more easily collect hematopoietic stem and progenitor cells before autologous bone marrow transplantation.

7.13.15 Complement (Anaphylatoxin) and Formyl Peptide Receptors

Anaphylatoxins, or anaphylotoxins, are fragments (C3a, C4a, and C5a) produced once the complement cascade is activated. Anaphylatoxins are able to trigger degranulation of endothelial and mastocytes and phagocytes to produce a local inflammation. Anaphylatoxins indirectly cause smooth muscle cell contraction, increase blood capillary permeability, and provoke chemotaxis of leukocytes. Anaphylatoxin and formyl peptide receptors are activated by anaphylatoxins C3a and C5a (Table 7.52).

penetrating lipopeptides. Any subtype of pepducins inhibits signal transmission from GPCRs to G proteins,

Table 7.52. Receptors of the complement system and anaphylatoxin and formyl peptide receptors, targeted G protein subunits, and order of potency (Source: [5]; fMLP: formyl-Met-Leu-Phe).

Receptor type	G-Subunit transducers	Potency order
C3aR	Gi/o, Gi/z	C3a > C5a
C5aR	Gi/o, Gi/z, Gq/16	C5a > C3a
FPR1	Gi/o, Gz	fMLP > cathepsin-G > annexin-1

Table 7.53. Cholecystokinin receptors, main G-protein subunit transducers, and ligands (Source: [5]).

Type	Transducer	Potency order
CCK_1	Gs, Gq, G11	Cck8 $\gg$ gastrin > Cck4
CCK_2	Gs, Gq	Cck8 $\geq$ gastrin, Cck4

Formyl peptide receptors (FPR) [117] are expressed at high levels on phagocytes. They are involved in chemotaxis, production of reactive oxygen species, and release of peptidases. Activated formyl peptide receptors trigger cytoskeleton rearrangement that, in turn, facilitates cell migration. They lead to activation of: (1) phospholipase-C and the IP_3–Ca^{++} and DAG–PKC cascades; (2) small GTPase Ras and the MAPK module; and (3) CD38 ectoenzyme that converts NAD^+ into cyclic ^{ADP}ribose, which interacts with ryanodine receptors for sustained Ca^{++} influx. In addition, formyl peptide receptor-like proteins of vomeronasal sensory neurons have an olfactory function associated with pathogens.

7.13.16 Cholecystokinin Receptors

Cholecystokinin receptors (CCK_1–CCK_2 or CCKa–CCKb) belong to the class A of G-protein-coupled receptors. They are activated by cholecystokinins Cck4, Cck8, and Cck33, as well as gastrin (Table 7.53).

The CCK_2 receptor is the dominant receptor in the central nervous system [991]. It is highly synthesized in the gastric mucosa, where it mediates gastrin-stimulated gastric acid secretion. It also contributes to gastric epithelial renewal. It resides in exo- and endocrine pancreas, enteric smooth muscle, liver, kidney, and adrenal gland, as well as circulating monocytes. The CCK_2 receptor recognizes each of the mature forms of cholecystokinins and gastrin with similar affinities and responds to them with similar potencies. It binds to progastrin and glycine-extended gastrin with lower affinity. It can couple to Gq G-protein subunit to signal predominantly via phospholipase-C and calcium import. It can also couple to Gs subunit. It

117. Formyl peptide receptors have been originally identified by their ability to bind ^{N}formyl peptides, such as exogenous bacterial product, the formylated tripeptide ^{N}formyl-methionyl-leucyl-phenylalanine (fMLP). The fMLP receptor is now called FPR1.

Table 7.54. Corticotropin-releasing factor receptors, main G-protein subunit transducers, and ligands (Source: [5]). Ligand CRF preferentially activates CRF_1, urocortin-1 can activate both CRF receptors, and urocortin-2 and -3 are selective CRF_2 agonists.

Type	Main transducer	Ligands
CRF_1	Gs	Urocortin-1, CRF (preferentially)
CRF_2	Gs	Urocortin-1–urocortin-3

can stimulate several signaling pathways, such as mitogen-activated protein kinase modules (ERK, JNK, and P38MAPK) as well as the PI3K, JAK2–STAT, and Src pathways [991]. It is phosphorylated by GRKs and protein kinase-C. It can interact with arrestins, regulator of G-protein signaling RGS2, and protein Tyr phosphatase PTPn11.

Besides adipose leptin, gastric leptin can, like cholecystokinin, inhibit splanchnic sympathetic nerve discharge and decreases activity of a subset of presympathetic vasomotor neurons in the rostroventrolateral medulla [992]. Its acute nervous and cardiovascular effects (reduced arterial pressure and heart rate) are exerted via vagal transmission and cholecystokinin receptor CCK_1 activation.

7.13.17 Corticotropin-Releasing Factor Receptors

Corticotropin-releasing factor receptors CRF_1 (or CRFR1) and CRF_2 (or CRFR2) are activated by peptides of the CRF family, i.e., CRF [118] and urocortins (Ucn1–Ucn3; Table 7.54). [119] Urocortin-1 connects to both CRF_1 and CRF_2, whereas Ucn3 is a high-affinity ligand for CRF_2 receptor. Activation of CRF_2 on afferent terminals in the medial nucleus tractus solitarius by Ucn1 and Ucn3 releases glutamate that, in turn, causes a decrease in mean arterial pressure and cardiac frequency via activation of ionotropic glutamate receptors [993] (Sect. 2.5.4).

Corticotropin-releasing factor is a major neuroregulator of the hypothalamus–pituitary–adrenal axis that has many central and peripheral actions. The CRF_1 receptor is expressed in anterior pituitary corticotropes. In response to hypothalamic CRF hormone, CRF_1 triggers the release of adrenocorticotropic hormone. This release causes the secretion of glucocorticoids from the adrenal cortex that, in particular, stimulates liver gluconeogenesis to increase glycemia.

Activity of CRF_1 primed by CRF complements the action of CRF_2 receptor activated by urocortin-3 that is responsible for auto- and paracrine glucose-mediated stimulation of insulin secretion. In pancreatic β cells that produce urocortin-3, activation of CRF_1 promotes insulin secretion [994]. Therefore, the insulinotropic action of pancreatic CRF_1 antagonizes the effect of activated CRF_1 on anterior pituitary corticotropes, as the release of glucocorticoids counteracts the action of insulin.

118. A.k.a. corticotropin-releasing hormone (CRH).

119. Urocortin-2 and -3 are also called stresscopin-related peptide and stresscopin, respectively. Urocortin-1 and -3 are members of the corticotrophin-releasing factor (CRF) peptide family.

Table 7.55. Dopamine receptors, main G-protein subunit transducers, and ligands (Source: [5]).

Type	Main transducer
D_1-like group	
D_1 (D_{1A})	$G\alpha_s$, $G\alpha_{olf}$Golf
D_5 (D_{1B})	$G\alpha_s$
D_2-like group	
D_2	$G\alpha_{i/o}$
D_3	$G\alpha_{i/o}$
D_4	$G\alpha_{i/o}$

The CRF_1 receptor stimulates insulin secretion only when glucose concentration is intermediate to high. The higher the glycemia, the greater the CRF_1-dependent phosphorylation of extracellular signal-regulated protein kinases ERK1 and ERK2 and subsequent cAMP response element-binding phosphorylation. Phosphorylation of ERK1 and ERK2 caused by CRF is similar to glucose-dependent actions of incretins, glucagon-like peptide GLP1 and glucose-dependent insulinotropic peptide (GIP), via incretin receptors GLP1R and GIPR that pertain to the class-B G-protein-coupled receptors.

7.13.18 Dopamine Receptors

Dopamine is the predominant catecholamine neurotransmitter in the central nervous system as well as a neurohormone released by the hypothalamus to inhibit the release of prolactin from the adenohypophysis (anterior lobe of the pituitary gland). [120]

Dopamine receptors include 5 proteins (D_1-D_5) distributed in 2 groups: (1) the D_1-*like* (D_1 and D_5) and (2) D_2-*like* (D_2–D_4) groups (Table 7.55). Two D_1-like receptor subtypes couple to $G\alpha_s$ subunit, thereby activating adenylate cyclase. The other receptor subtypes of the D_2-like group inhibit adenylate cyclase via $G\alpha_{i/o}$ and activate K^+ channels.

The D_1 and D_5 receptor genes are intronless. Pseudogenes of D_5 exist. Alternative splicing yields different species- and tissue-dependent types of D_2 and D_3 receptors. In humans, the D_4 receptor gene exhibits extensive polymorphic variation [995].

In the central nervous system, dopamine receptors are involved in the control of locomotion, cognition, emotion, affect, food intake, and neuroendocrine secretion. In peripheral organs, dopamine receptors are prominent in the vasculature, kidney, and pituitary gland, where they influence mainly sodium homeostasis, vascular tone, renal function, gastrointestinal motility, and hormone secretion [995] (Table 7.56).

120. The adenohypophysis regulates stress response, growth, and reproduction.

Table 7.56. Distribution and function of peripheral dopamine receptors (Source: [995]; ALLH: ascending limb of loop of Henle; CCD: cortical collecting duct; JGA: juxtaglomerular apparatus; PT: proximal tubule).

Tissue	Receptor type	Effect
Blood vessels		
Adventitia	D_2–D_4	Inhibition of noradrenaline release
Media	D_1, D_5	Vasodilation
Intima	D_2–D_4	
Heart	D_4	
Sympathetic ganglia and endings	D_2–D_4	Inhibition of noradrenaline release
Adrenal gland		
Glomerulosa	D_1, D_5	
	D_2–D_4	Inhibition of aldosterone secretion
Medulla	D_1, D_5	Stimulation of adrenaline and noradrenaline release
	D_2–D_4	Inhibition of adrenaline and noradrenaline release
Kidney		
Glomerulus	D_1, D_5	Increase of filtration rate
JGA	D_1, D_5	Stimulation of renin secretion
PT	D_1, D_5	Inhibition of Na^+ reabsorption
ALLH	D_1, D_5	Inhibition of Na^+ reabsorption
CCD	D_1, D_5	Inhibition of Na^+ reabsorption
	D_2–D_4	Inhibition of vasopressin action

The Fos transcription factor, a product of the immediate-early FOS gene required for long-lasting modifications of gene expression in response to acute stimuli, is a final targets in the signaling initiated by dopamine receptors. The D_1 and D_2 receptors operate synergistically on FOS expression [995].

7.13.18.1 D_1 Receptor

Receptor D_1 stimulates phospholipase-C (PLC), thereby leading to the production of inositol trisphosphate that causes Ca^{++} influx by mobilization of calcium from intracellular stores on the one hand, and activating PKC enzyme.

Receptor D_1 can stimulate the activity of Ca_V1 channel via the cAMP–PKA pathway [995]. On the other hand, D_1 reduces Ca^{++} flux through $Ca_V2.1$ and $Ca_V2.2$ channels due to channel dephosphorylation by a phosphatase stimulated by PKA enzyme.

Receptor D_1 precludes the activity of Na^+–H^+ exchanger that regulates intracellular pH and cell volume using both cAMP-dependent and -independent mechanisms [995]. Moreover, D_1 inhibits Na^+–K^+ ATPase that maintains the electrochemical gradient for appropriate excitability of neurons and myocytes and enables

the transport of fluid and solutes across epithelial membranes [995]. However, in the kidney, dopamine receptor activates Na^+–K^+ ATPase via phosphorylation by PKA and PKC kinases.

In the kidney, dopamine is produced in renal nerves and epithelial cells of certain nephron segments. Dopamine synthesized in the renal tubular epithelium acts as a para- and autocrine factor that regulates sodium reabsorption in the nephron. In addition, dopaminergic nerve endings lodge near the juxtaglomerular apparatus. Receptor D_1 stimulate the secretion of renin [995]. Dopamine impedes Na^+–K^+ ATPase via both D1-like and D2-like receptors (D_1–D_2 synergism).

Both D_1 and D_2 receptors in the hippocampus mediate the effect of dopamine on learning and memory [995]. Both D_1 and D_2 receptors are involved in mesolimbocortical effect (reward and reinforcement) of dopamine.

Striatal efferent neurons undergo the influence of dopamine. Two major types of neurons are identified by their primary sites of axonal projections and neuropeptide synthesis [995]. A first striatonigral population projects to the entopeduncular nucleus and substantia nigra pars reticulata and synthesizes substance-P and dynorphin, an opioid peptide stored in large (size 80–120 nm), dense-core vesicles. Striatonigral gabaergic neurons preferentially express D_1 receptor that fosters the production of substance-P and dynorphin. The second striatopallidal pool projects to the external segment of the globus pallidus and contains enkephalin. Striatopallidal gabaergic neurons prominently synthesize D_2 receptor that prevents the production of preproenkephalin. A similar organization of dopamine receptors is observed in the nucleus accumbens: D_1 receptor is mostly expressed in substance-P+ neurons, D_2 receptor in enkephalin+, neurotensin+ neurons, and D_3 receptor in substance-P+, neurotensin+ neurons [995]. However, in most of the neuronal populations, D_1 and D_2 are coexpressed rather than segregated.

In the cardiovascular system, more precisely in blood vessels, postjunctional D_1 receptor provokes vasodilation of the renal artery and prejunctional D_2 receptor on postganglionic sympathetic nerve terminals prevents noradrenaline release, thereby indirectly causing vasodilation in the femoral artery [995].

7.13.18.2 D_2 Receptor

Two alternatively spliced D_2 isoforms exist (D_{2_L} and D_{2S}) [995]. Both isoforms may prime a phosphatidylinositol-mediated mobilization of intracellular calcium. However, in many other cell types, D_2 receptor do not launch this pathway. It can also inhibit inward calcium currents. In addition, D_2 receptor increase outward potassium currents, thareby provoking a cell hyperpolarization.

Receptor D_2 potentiates the release of arachidonic acid via Gi subunit [995]. Unlike D_1, D_2 receptor activates Na^+–H^+ exchanger in many cells.

The D_2 dimer binds to a single heterotrimeric G protein [996]. The maximal activation of the minimal signaling unit, i.e., a receptor dimer coupled to a single heterotrimeric G protein, is achieved by agonist binding to a single subunit of the dimeric D_2 receptor. The asymmetrically activated dimer can be modulated by the

activity state of the second monomer. Ligand-independent constitutive activation of and inverse agonist binding to the second monomer enhances signaling.

In the ventral striatum, activated D_2 autoreceptor reduces dopamine release, hence locomotor activity [995]. On the other hand, activated postsynaptic D_2 receptor slightly increases locomotion.

Receptors of the D_2-like group may promote some aspects of cell differentiation [995].

Prejunctional D_2 receptor on postganglionic sympathetic nerve terminals attenuates the cardiac contractility [995].

Dopamine represses aldosterone secretion by adrenal glomerulosa cells via D_2 receptor [995]. During sodium depletion, dopamine release decays and circulating aldosterone level and plasma aldosterone responsiveness to angiotensin-2 augments.

Dopaminergic neurons of sympathetic ganglia inhibit release of adrenaline and noradrenaline by neuroendocrine chromaffin cells of the medulla of adrenal glands via D_2 receptor [995]. Adrenal chromaffin cells possess also D_1 receptor. The latter causes a rapid catecholamine secretion in response to stress.

7.13.18.3 D_3 Receptor

Receptor D_3 localizes mainly postsynaptically to the nucleus accumbens, where it impedes locomotion, hence antagonizing effect of D_2 receptors [995]. Receptor D_3 have an opposite action w.r.t. that of D_2 on neurotensin gene expression in the nucleus accumbens. [121]

Receptor D_3 stimulates thymidine incorporation in some cell types [995]. It may activate the mitogen-activated protein kinase module.

7.13.18.4 D_4 Receptor

Different forms of the D_4 receptor exist with different number of repeats in the third intracellular loop. The four-repeat form (D_{4_4}) is the predominant in the human population (60%) [995]. [122]

7.13.18.5 D_5 Receptor

Receptor D_5 is highly expressed in the hippocampus, where it may mediate the effects of dopamine on learning and memory [995]. It is coexpressed with D_2 in cholinergic interneurons of the striatum.

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121. In the ventral shell of the nucleus accumbens, D_3 receptor activates neurotensin synthesis. In the septal pole of the nucleus accumbens, D_2 receptor hinders neurotensin production.
122. The D_{4_7} variant is observed in 14% of the population and the D_{4_2} in 10%.

Table 7.57. Endothelin receptors, their main targeted G proteins, and order of ligand potency (Source: [736]). Endothelin-1 (ET1) is a very strong vasoconstrictor and potent mitogen.

Type	Main transducer	Potency order
ET_A	Gq/11, Gs	ET1 ∼ ET2 > ET3
ET_B	Gq/11, Gi/o	ET1 ∼ ET2 ∼ ET3

7.13.19 Endothelin Receptors

Endothelin is secreted by endothelial cells and targets neighboring smooth muscle cells to regulate the vasomotor tone (Table 7.57; Vol. 5 – Chaps. 8. Smooth Muscle Cells and 9. Endothelium). Among 4 identified isoforms (ET1–ET4), endothelin ET1 is the most commonly found subtype in the cardiovascular system, including blood. Endothelins are synthesized by vascular endothelial cells of both vessel (intima) and heart (endocardium) walls (especially endothelial cells of right atrium and left ventricle), vascular smooth muscle cells, and cardiomyocytes, as well as by extravascular cells of the lung, pancreas, spleen, and nervous system [5].

Vasoconstrictor endothelin-1, the major type in the vasculature, intervenes as an auto- and paracrine regulator. Endothelin-1 indeed either counteracts (paracrine action on vascular smooth muscle cells) or cooperates with vasodilator nitric oxide (via an autocrine loop on endothelial cells). Physical factors such as wall shear stress and chemical agents, such as thrombin, adrenaline, angiotensin-2, growth factors, cytokines, and free radicals trigger ET1 secretion. Several substances, such as nitric oxide, cGMP, atrial natriuretic peptide, and prostacyclin reduce ET1 release from the vascular endothelium.

Two endothelin-1 receptors (ET_A and ET_B) [123] bind ET1 with equal affinity to regulate the gene expression devoted to cell contraction, proliferation, and survival. Receptor subtypes ET_A and ET_B include ET_{A1} and ET_{A2} and and ET_{B1} and ET_{B2}.

The vasoconstrictor activity of endothelins is mediated by Na^+–H^+ and Na^+–Ca^{++} exchangers. An initial activation of Na^+–H^+ exchangers causes an increase in Na^+ concentration inside the smooth muscle cell [997]. Subsequently, Na^+–Ca^{++} exchangers export Na^+ and import Ca^{++} into the smooth muscle cell that provokes contraction. The Na^+–H^+ exchanger also reduces the intracellular H^+ concentration.

Messengers ET1 to ET3 potentiate the mitogenic activity of platelet-derived growth factor via ET_A receptors. Endothelin-1 is a potent mitogen for both vascular endothelial and smooth muscle cells. Ligand ET1 activates extracellular signal-regulated kinase ERK1 and ERK2 by GPCR-induced activation of the receptor Tyr kinase EGFR (Sect. 8.2.5.2). [124]

123. A third receptor subtype specific for ET3 isoform — ET_C — exist in Xenopus laevis, but not in mammals.

124. G-protein-coupled receptors can indeed activate Src kinases with subsequent RTK phosphorylation. Once activated, Src and/or RTK phosphorylate SHC adaptor. Both phosphorylated SHC and activated RTK then bind growth factor receptor-bound protein GRB2 and

7.13.19.1 ET_A Receptor

Type-A endothelin-1 receptor phosphorylates the docking growth factor receptor-bound protein-2 (GRB2)-associated binder-1 (GAB1) and activates ERK1 [998]. [125] Phosphorylation of GAB1 induced by ET1 is stimulated by phosphoinositide 3-kinase and Src kinases and inhibited by PP1 phosphatase. Protein Tyr phosphatase PTPn11 potentiates ET1-induced ERK1 activation.

7.13.19.2 ET_B Receptor

Receptor ET_B localizes to the nervous system, heart, vasculature, lung, endocrine system, liver, gastrointestinal tract, pancreas, and placenta [999]. In the cardiovascular apparatus, ET_B is mainly produced in endothelial cells; it is synthesized at low levels in vascular smooth muscle cells and cardiomyocytes. It can lodge in caveolae. igands ET1, ET2, and ET3 activate ET_B with equal affinity and potency.

Among 3 splice variants, variant-1 is the canonical isoform. Variant-1 and -3 have functional characteristics; variant-2 that has similar binding properties, but lacks the functional coupling to phosphoinositide [999].

Both ET_B and ET_A can homo- and heterodimerize. In addition, ET_B can heterodimerize with dopamine D_3 receptor and angiotensin AT_1 receptor in rat renal proximal tubule cells [999].

Most ET_B effects result from coupling to Gq and Gi, but ET_B can also couple to other G-protein subunits, such as Go, Gs and G12/13. Receptor ET_B is thus involved in the inhibition or activation of adenylate cyclase, activation of phospholipase-A2, -C, and -D, guanylate cyclase, protein kinases such as mitogen-activated protein kinases [999]. In vascular smooth muscle cells, liganded ET_B causes a biphasic activation of ERK1 and ERK2 kinases. The delayed phase of ERK activation follows ET_B proteolysis by metallopeptidase and EGFR transactivation.

Two ET_B subtypes may reside on endothelial and vascular smooth muscle cells to cause vasodilation and vasoconstriction, respectively. In any case, activated ET_B counteracts the potent vasoconstrictor effect of ET_A stimulation, as it provokes vasodilation, natriuresis, and clearance of ET1 from the circulation by endothelial cells of the lung, kidney, and liver. However, ET_B is produced at low concentrations in vascular smooth muscle cells, in which it causes contraction [999].

In the lung, ET_B on airway smooth muscle cells launches bronchoconstriction and cell proliferation [999].

Receptor ET_B can be phosphorylated (deactivated) by G-protein coupled receptor kinase GRK2 [999]. The agonist–ET_B complex, like liganded ET_A, dissociates very slowly. Following ligand binding, ET_B receptor is internalized using a dynamin–clathrin-dependent pathway for lysosomal degradation. Endocytosis of the

its associated Ras guanine nucleotide-exchange factor Son-of-sevenless to trigger the Ras–MAPK signaling.

125. The docking protein GAB1 can be phosphorylated by receptor Tyr kinases and cytokine receptors to regulate cell survival and proliferation aa well as from activated G-protein-coupled receptors.

Table 7.58. Endothelin receptor distribution in the rat coronary bed (Source: [1000]). Endothelin receptors on endothelial smooth muscle cells mediate vasodilation and -constriction, respectively. Endothelial ETRs thus modulate the activity of smooth muscle ETRs. In other words, endothelin activity depends on a balance between smooth muscle and endothelial endothelin receptors.

ET_A	ET_B
Vascular endothelia	
Capillary	Artery, capillary, vein
Vascular smooth muscles	
Artery	Artery, vein

$ET1–ET_B$ complex and subsequent lysosomal degradation enable clearance of ET1 from plasma.

7.13.19.3 Endothelin Receptor Distribution in the Cardiovascular System

In the coronary bed of rat heart, ET_A is detected in arterial smooth muscle and capillary endothelial cells, whereas ET_B resides in arterial, venous, and capillary endothelial cells as well as arterial and venous smooth muscle cells [1000] (Table 7.58). Receptors ET_A and ET_B control arterial vasoconstriction. Postcapillary vascular resistance is exclusively regulated by ET_B. The presence of ET_A in capillary endothelium attenuates increase in vasomotor tone, especially in cardiac microvascular permeability during ischemia–reperfusion events.

Cultures of human coronary and internal mammary arteries, as well as rat omental, mesenteric, and cerebral arteries during 24 to 48 h modify endothelin receptor expression on endothelial and smooth muscle cells. Both endothelin receptor types are upregulated in vascular smooth muscle cells in vessel culture. Conversely, ET_Bs are downregulated in endothelial cells in vessel culture [1001]. Ligand administration during 5 d in dogs induces a heterogeneous distribution of endothelin receptors in coronary arteries [1002]. Right coronary artery is enriched in ET_B 3 times more than left coronary artery (in vascular smooth muscle cells).

Endothelin-1 increases the myocardial contractility (positive inotropic effect) with a greater potency than endothelin-3. Receptor ET_A is involved in ET-induced increase in myocardial contractility. Activated ET_B receptor has an opposite effect [997]. However, ET_B receptor can lead to positive inotropic effect via coronary vasodilation that increases myocardial contraction and oxygen consumption (*Gregg's phenomenon*). [126]

126. Coronary perfusion pressure affects coronary arterial resistance (autoregulation) as well as myocardial oxygen consumption (Gregg's phenomenon). Gregg's phenomenon results from increased contractility.

Table 7.59. Endothelin receptors ET_A and ET_B have opposite effects.

ET_A	ET_B
Vasoconstriction (NO inhibition)	Vasodilation (NOS coupling; NO release)
ROS production (vSMC)	ROS decrease (EC)
Extravasation	Extravasation inhibition

7.13.19.4 Endothelin Receptor Desensitization

Signal transduction relies on 2 intertwined processes: (1) signal transmission by stimulating appropriate mediators and (2) signaling termination to avoid prolonged reaction and prepare cells for forthcoming stimulation. G-Protein-coupled receptor kinases (GRK1–GRK7) enable a rapid GPCR desensitization after activation. In vascular smooth muscle cells, the predominant ET1 receptor ET_A is desensitized by GRK2 [1003].

7.13.19.5 Endothelin–NO Interactions

Endothelin receptors ET_A and ET_B have opposite roles (Table 7.59). The ET_B receptor mediates vasodilation [127] as well as endothelial cell survival and proliferation. [128] On the other hand, the ET_A receptor favors vasoconstriction.

Responses to ET_B signaling depends on cell types. Ablation of ET_B exclusively from endothelial cells decreases NO release and increases plasma endothelin-1 [1006]. Endothelial ET_B receptor mediates vasodilation without affecting blood pressure in response to a high-salt diet, whereas non-endothelial ET_B controls blood pressure.

The amount of circulating endothelin-1 and endothelial nitric oxide synthase increases and decreases in persistent pulmonary hypertension, respectively. Endothelin-1 increases ROS production in pulmonary arterial smooth muscle cells in culture. Reactive oxygen species downregulate NOS3 in smooth muscle cells [1007]. Endothelin-1 increases H_2O_2 level in fetal pulmonary arterial smooth muscle cells via ET_A in vitro. On the other hand, ET1 decreases H_2O_2 level in fetal pulmonary arterial endothelial cells in monoculture via ET_B receptor. Furthermore, H_2O_2 at low concentration (12 μmol) increases NOS3 level without affecting NOS3 promoter activity, whereas at higher concentration (100 μmol) it reduces both NOS3 promoter activity and NOS3 level in fetal pulmonary arterial endothelial cells in monoculture.

127. The ET_B receptor is functionally coupled to NOS to produce NO via a Tyr kinase- and calcium–calmodulin-dependent pathway [1004].

128. Endothelin-1 promotes the proliferation and migration of human umbilical vein endothelial cells in a dose-dependent manner, by activating ET_B more effectively than ET_A [1005]. In addition, ET1 stimulates matrix metallopeptidase-2 production. It also enhances VEGF angiogenic effects on endothelial cells in vitro.

In addition, ET1 decreases NOS3 promoter activity in these cells cultured with fetal pulmonary arterial smooth muscle cells.

7.13.19.6 Opposite effects of ET_{B1} and ET_{B2} Receptors

Endothelin is able to exert: (1) a persistent constrictor effect by increasing the intracellular calcium concentration resulting from an influx through activated Na^+–H^+ and Na^+–Ca^{++} exchangers and from cellular stores due to the stimulated PLC–IP_3 pathway in vascular smooth muscle cell and (2) a transient dilator effect via the activation of endothelial nitric oxide synthase mediated by protein kinase-B and receptor ET_{B1} [997]. Receptor subtypes ET_{B1} (endothelial) and ET_{B2} (muscular) mediate opposite effects on vascular tone. Receptors ET_A and ET_{B2} indeed prime vasoconstriction, whereas receptor ET_{B1} triggers transient vasodilation.

Endothelin-1 intervenes during birth when pulmonary vascular resistance falls with the initiation of ventilation and the ductus arteriosus constricts, as well as postnatally. At birth, in both term and preterm, oxygen triggers long-lasting vasoconstriction and closure of ductus arteriosus by activating a specific, cytochrome-P450-mediated reaction that leads to ET1 synthesis; ET1 then operates via predominant constrictor ET_A as well as ET_{B2} [1009]. The number of ET_{B1} dilator receptors is reduced in pulmonary hypertension.

7.13.19.7 Cardiac Endothelin Receptors

Receptor ET_A is able to increase myocardial contractility. This inotropic effect results from an increase in intracellular Ca^{++} concentration induced by Na^+–H^+ and Na^+–Ca^{++} exchangers stimulated by protein kinase-C, which is activated by the PLC–DAG axis initiated by endothelin. Receptors ET_{B1} and ET_{B2} may mediate negative and positive inotropic effects, respectively [1008].

Endothelin-1 is a potent cardiomyocyte survival factor. It causes nuclear translocation of PP3-regulated nuclear factors of activated T cells NFAT1 and production of anti-apoptotic BCL2 protein. Factor P300 potentiates NFAT1 binding to the Bcl2 gene promoter in cardiomyocytes [1010]. Transcription factor NFAT also regulates transcription of several genes, such as those that encode adenylosuccinate synthase AdsS1, heart fatty acid-binding protein hFABP, pyruvate decarboxylase, cytochrome-C oxidase, and succinate dehydrogenase.

α1-Adrenoceptor stimulation of cardiomyocytes causes synthesis of angiotensin-1 that acts as an endothelin-releasing factor on smooth muscle cells in coronary arterioles, as it provokes endothelin release via NADPH oxidase and subsequent vasoconstriction [1011].

7.13.19.8 Diapedesis

The ET_B receptor impedes T-cell adhesion to endothelium, especially in cancers, characterizd by an overexpression of endothelial ET_B receptor. [129] The ET_A receptor

129. Endothelin-1 is produced at high concentrations by ovarian cancer cells. Endothelin-1 inhibits in vitro T-lymphocyte adhesion to endothelial cells, hence extravasation into the

elicits T-cell homing on endothelium of lung vessels during allergy and inflammation. Increased release of NO mediates effects of ET_B receptors with reduced expression of ICAM1 intercellular adhesion molecule.

7.13.19.9 Axonal Growth and Guidance

The sympathetic compartment of the autonomic nervous system is composed of preganglionic sympathetic neurons that have synapses with cell bodies of postganglionic sympathetic neurons in para- and prevertebral sympathetic ganglia. Postganglionic sympathetic neurons innervate smooth muscle layers of blood vessels and intestine, myocardium and nodal tissue, exocrine and endocrine glands and ducts, etc.

During development, sympathetic neurons extend axons along paths, often jointly with artery trajectories, to innervate target tissues. Endothelin-3 released from the neural crest-derived smooth muscle layer of the external carotid arteries acts via ET_A to direct extension of axons of a subset of sympathetic neurons from the superior cervical ganglion (located near the bifurcation of the common carotid artery) along the external carotid artery, which serves with its branches as gateway to appropriate organs [1013]. On the other hand, ET3–ET_A signaling does not participate in projections to the internal carotid artery. Other factors expressed by the mesoderm-derived smooth muscle layer of the internal carotid artery are used for navigating projections from the superior cervical ganglion toward the internal carotid artery and its branches.

7.13.20 Estrogen G-Protein-Coupled Receptor

Estrogens signal via 2 cytoplasmic and 1 plasmalemmal receptor. Nuclear receptors include estrogen receptor-α and -β (NR3a1–NR3a2; Sect. 6.3.5.1). Estrogen receptors ERα and ERβ are able to associate with plasmalemmal components. On the other hand, estrogens bind with high affinity to G-protein-coupled estrogen receptor (GPER or GPR30). Liganded G-protein-coupled receptor GPR30 promotes rapid estrogen signaling.

G-protein-coupled estrogen receptor targets cAMP signaling, as it couples to Gs and Gi/o subunits of guanine nucleotide-binding proteins [5]. Plasmalemmal GPER not only regulates adenylate cyclase, but also primes extracellular release of

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tumor. The ET_B receptor on the wetted surface of tumor endothelium prevents transendothelial migration from the blood stream of T lymphocytes, which then cannot infiltrate tumors [1012]. Suppression of ET_B activity is required for tumor immunotherapy by increasing homing of tumor-specific T lymphocytes.

proheparin-binding EGF-like growth factor (Sect. 8.2.5.2). [130] In addition, G protein subunits, such as $G\alpha_i$ and $G\beta\gamma$, interact with ERα. [131]

The GPER receptor activates phosphatidylinositol 3-kinase and protein kinase-B, as well as members of the mitogen-activated protein kinase modules, such as extracellular signal-regulated protein kinases ERK1 and ERK2. The activation of the PI3K pathway by GPER occurs via GPER-mediated transactivation of the epidermal growth factor receptor (Sect. 7.7).

In arterial smooth muscle cells, activated GPER increases intracellular calcium concentration. In cardiomyocytes, GPER activation ensures cardioprotection during myocardial ischemia–reperfusion events, as it reduces postischemic dysfunction and infarct size via the PI3K–PKB and ERK pathways [1015]. Agent PI3K is required, but not MAPK kinases that phosphorylate (activate) extracellular signal-regulated protein kinases. The GPER receptor inhibits mitochondrial permeability transition pore opening via the ERK pathway, hence protecting the heart against ischemia–reperfusion injury [1016].

7.13.21 Free Fatty Acid Receptors

Fed mammals use glucose as the main metabolic fuel. However, short-chain fatty acids produced by the fermentation of dietary fibers by the intestinal colonic bacterial flora [132] also contribute to a significant proportion of daily energy requirement. Under starvation and ketogenic conditions, ketone bodies produced in the liver from fatty acids are used as the main energy sources. Feeding and fasting regulate chemical energy availability via the sympathetic nervous system. Feeding enables con-

130. Heparin-binding epidermal growth factor-like growth factor (HBEGF) of the EGF family is synthesized as a type-1 transmembrane precursor (proHBEGF). At the cell surface, proHBEGF is a juxtacrine growth factor that signals to neighboring cells via an intercellular contact. The ectodomain of proHBEGF is shed and can serve as an N-terminal soluble ligand of EGFR ($_N$HBEGF or simply HBEGF) that can indirectly transactivate HER2, HER3, and HER4 heterodimerized with EGFR (HER1).

131. Plasmalemmal estrogen receptor-α is a palmitoylated protein. 17β-Estradiol reduces ERα palmitoylation and its interaction with caveolin-1 in a time- and dose-dependent manner [1014]. Palmitoylation of ERα enables rapid E_2 signaling via the ERK and PI3K–PKB pathways. Upon 17β-estradiol binding, ERα undergoes depalmitoylation and dissociates from caveolin-1 (inactivation–activation cycle).

132. Commensal bacterial communities are closely associated with the human skin, oral cavity, gastrointestinal tract, and the female genital tract. Gut flora consists of microorganisms (bacteria, fungi, and protozoa). Most bacteria belong to the species Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, and Bifidobacterium, as well as, but to a lesser extent, Escherichia and Lactobacillus. The major species are Bacteroides fragilis, Bifidobacterium adoloscentis, and Eubacterium areofaciens [1017]. The remainder is mainly Escherichia coli, Streptococcus viridans, Streptococcus salivaris, and lactobacilli. Anaerobic species outnumber aerobes (at least 10-fold). The Bacteroides-Prevotella group (Gram− anaerobes) and Clostridium species (Gram+ anaerobes) predominate [1018]. Fungi of the gut flora pertain to the genera Candida, Saccharomyces, Aspergillus, and Penicillium.

Table 7.60. Free fatty acid receptors and their main G-protein subunit transducers (Sources: [5, 1019]; O3FAR: omega-3 fatty acid receptor). Long-chain saturated and unsaturated fatty acids (lcFFA) activate free fatty acid FFA_1 receptor, whereas short-chain fatty acids (scFFA) activate FFA_2 and FFA_3 receptors. The GPR84 receptor is stimulated by medium-chain free fatty acids (mcFFA). The GPR120 receptor mediates the anti-inflammatory and insulin-sensitizing effects of omega-3 fatty acids. Four genes — Ffar1 to Ffar3, and Gpr42 — form a cluster on human chromosome 19q13.

Type	Other alias	Main transducer	Ligand
FFA_1	GPR40	Gq/11	lcFFA
FFA_2	GPR43	Gq/11, Gi/o	scFFA
FFA_3	GPR41	Gi/o	scFFA
GPR42 (gene/pseudogene)	FFAR1L, FFAR3L, GPR41L, GPR42P		No
GPR84	GPCR4, EX33	Gi/o	mcFFA
GPR120	O3FAR1, PGR4 GT01, GPR129	Gq/11	mc/lcFFA

sumption of chemical energy into thermal energy. During fasting, chemical energy is saved by the reduction of the sympathetic activity.

Free fatty acids (FFA) serve not only as nutrients, but also as signaling molecules. Free fatty acid receptors (FFAR) are G-protein-coupled receptors. Four subtypes encoded by different genes exist: FFAR1, or GPR40, FFAR2, or GPR43, FFAR3, or GPR41, and GPR42 (Table 7.60). Receptors GPR84 and GPR120 can also be activated by FFAs. Receptors FFAR1 and GPR120 are activated by medium- and long-chain free fatty acids [1019]. [133] The GPR84 receptor is stimulated by medium-, but not long-chain, free fatty acids. Both FFAR2 and FFAR3 are activated by short-chain free fatty acids.

The FFAR1 receptor is mainly expressed in pancreatic β cells, where it fosters insulin secretion [1020]. Receptors FFA_2 and FFA_3 are synthesized in adipocytes; FFA_3 also in enteroendocrine cells. The GPR84 receptor is manufactured in the spleen. The GPR120 receptor abounds in the intestine, where it promotes the secretion of glucagon-like peptide-1.

7.13.21.1 FFAR1 (FFA_1)

Mid- and long-chain saturated and unsaturated fatty acids are ligands for FFAR1 receptor. The latter is coupled to the formation of inositol (1,4,5)-trisphosphate, Ca^{++} influx, and activation of extracellular signal-regulated kinases ERK1 and ERK2. Linolenic acid targets FFA_1 on rat pancreatic β cells and reduces the voltage-gated K^+ current via the cAMP–PKA axis to enhance β-cell excitability and insulin secretion [1019].

133. Eicosatrienoic acid is the most potent agonist of FFAR1 receptor.

In mice, FFAR1 is also expressed in endocrine cells of the gastrointestinal tract, such as those that synthesize the incretin hormones glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide (GIP) [1019]. It is also observed on splenocytes and human peripheral blood mononuclear cells.

7.13.21.2 FFAR2 and FFAR3 (FFA_2 and FFA_3)

Both FFAR2 and FFAR3 are activated by short-chain fatty acids, such as formate, acetate, propionate, butyrate, and pentanonate [1019]. Whereas FFAR3 is activated equally by propionate, butyrate, and pentanonate, FFAR2 prefers propionate.

The FFAR2 receptor is synthesized predominantly in immunocytes. Its expression is also induced during the differentiation of leukocyte progenitors into monocytes or neutrophils. It can be found in enteroendocrine cells that produce peptide-YY and mucosal mastocytes.

The FFAR2 receptor abounds in adipocytes (but not FFAR3), where it may be implicated in the production of leptin, a potent anorexigenic hormone [1019].

Short-chain fatty acids such as propionate and ketone bodies control the amount of chemical energy, as they regulate the activity of sympathetic ganglia via the Gi/o-coupled receptor FFAR3 that triggers the $G\beta\gamma$–PLCβ–MAPK pathway [1020]. A major short-chain fatty acid, propionate, supports the GPR41-mediated activation of the sympathetic nervous system. On the other hand, a ketone body — β-hydroxybutyrate — produced during starvation or diabetes antagonizes FFAR3 receptor.

7.13.21.3 GPR84 and GPR120

Among medium-chain FFAs, capric, undecanoic, and lauric acids are the most potent agonists of the GPR84 receptor. In humans, GPR84 is expressed in the brain, heart, muscle, lung, kidney, liver, intestine, colon, thymus, spleen, and placenta, as well as leucocytes. In activated T lymphocytes, GPR84 participates in the regulation of early production of interleukin-4 [1019].

The GPR120 receptor, first identified as an orphan G-protein-coupled receptor, is bound by mid- and long-chain, saturated and unsaturated fatty acids, It is highly expressed in the human intestinal tract. Endocrine cells that produce glucagon-like peptide GLP1 in the large intestine synthesize GPR120. In addition, K cells that release glucose-dependent insulinotropic polypeptide in the duodenal and jejunal epithelia strongly express FFAR1, GPR119, and GPR120 [1019]. In mice, enteroendocrine STC1 cells that secrete GLP1 and cholecystokinin upon stimulation by FFAs are endowed with GPR120.

The GPR120 receptor is also synthesized in adipocytes, type-2 taste cells of rat taste buds and Clara cells in lungs [1019]. In adipose tissue, the expression of GPR120 is higher in adipocytes than in stromal-vascular cells.

Table 7.61. Frizzled receptors (Source: [5]).

Type	Alias and other name
Frizzled-1	Fz1
Frizzled-2	Fz2
Frizzled-3	Fz3
Frizzled-4	Fz4, CD344
Frizzled-5	Fz5
Frizzled-6	Fz6
Frizzled-7	Fz7
Frizzled-8	Fz8
Frizzled-9	Fz9, CD349
Frizzled-10	Fz10, CD350

7.13.22 Frizzled Receptors

Frizzled receptors (Fz; Sect. 10.3) that are activated by Wnt ligands trigger β-catenin-dependent (canonical) and -independent (non-canonical) signaling. The Wnt–Fz axis can raise intracellular calcium concentration, signal via Disheveled phosphoproteins to monomeric small GTPase Rac1 and Jun N-terminal kinase, as well as Rho and RoCK kinases, activate cGMP-specific phosphodiesterase PDE6, and heighten cAMP via heterotrimeric G proteins. Like other GPCRs, members of the Frizzled family (Table 7.61) depend on β-arrestin scaffold protein for internalization and signaling. The Wnt–Fz signaling is controlled by: (1) additional ligands that can enhance (extracellular Norrin or R-spondins-1 to -4) or inhibit Fz (secreted Frizzled-related protein-1 to -5, extracellular Wnt inhibitory factor WIF1, and Dickkopf-1) as well as (2) modulatory proteins with positive (RYK and ROR1 and ROR2 kinases) and negative (extracellular Kremen-1 and -2) regulatory effects [5].

7.13.23 γ-Aminobutyric Acid Receptor

γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system. Ionotropic γ-aminobutyric acid receptors ($GABA_A$; Sect. 2.5.3) are pentameric, ligand-gated, Cl^- channels.

Metabotropic γ-aminobutyric acid receptors ($GABA_B$: Table 7.64) are expressed in almost all neurons of the brain. They regulate synaptic transmission and signal propagation. They indeed control the activity of voltage-gated calcium (Ca_V) and inward rectifier potassium channels (K_{IR}).

The $GABA_B$ receptor can indeed associate with $Ca_V2.1$ and $Ca_V2.2$ and $K_{IR}3$ channels [5]. Its main transducer is a subunit of the Gi/o family. Selective agonists of $GABA_B$ include 3-aminopropyl ᴘmethylphosphinic acid (3APMPA) and 3-aminopropylphosphinic acid (3APPA).

Metabotropic $GABA_B$ receptor is a heterodimer formed by 2 similar subunits $GABA_{B1}$ and $GABA_{B2}$. The $GABA_{B1}$ subunit alone binds cognate ligands (antagonists and agonists), but with much lower affinity (10–100 fold less) [5]. Calcium

Table 7.62. Galanin receptors, main G-protein subunit transducers, and ligands (Source: [5]; GaLP: galanin-like peptide).

Type	Main transducer	Potency order
Gal_1	Gi/o	Galanin > GaLP
Gal_2	Gi/o, Gq/11	GaLP ≥ galanin
Gal_3	Gi/o	GaLP > galanin

ion binds to $GABA_{B1}$ to act as a positive modulator. Coexpression of $GABA_{B1}$ and $GABA_{B2}$ enables the transport of $GABA_{B1}$ to the plasma membrane and generates a functional receptor. [134] The $GABA_{B2}$ subunit mediates G-protein-coupled signaling.

The $GABA_{B2}$ subunit increases $GABA_{B1}$ affinity for agonists [5]. Reciprocally, $GABA_{B1}$ facilitates coupling of $GABA_{B2}$ to G proteins. Therefore, the $GABA_{B2}$ subunit does not signal by itself in response to γ-aminobutyric acid, but is essential for cell surface expression of the binding $GABA_{B1}$ subunit and for signaling of the heterodimer, as $GABA_{B1}$ transactivates $GABA_{B2}$.

Several $GABA_{B1}$ isoforms exist. The $GABA_{B1a}$ and $GABA_{B1b}$ isoforms are predominant subtypes in neonatal and adult central nervous system, respectively [5]. [135]

In the central nervous system, the $GABA_B$ receptor is constituted by not only $GABA_{B1}$ and $GABA_{B2}$, but also members of a subfamily of auxiliary K^+ channel tetramerization domain-containing proteins (KCTD) [1021]. These KCTD proteins increase the potency of agonists and accelerate receptor–effector coupling, hence G-protein signaling.

7.13.24 Galanin Receptors

Galanin receptors (Gal_1–Gal_3 or GalR1–GalR3) are activated by galanin and galanin-like peptide (Table 7.62). They localize to the peripheral and central nervous systems and endocrine system.

The galanin family includes galanin and galanin message-associated peptide (GMAP) that are generated from the same peptide precursor on the one hand, and galanin-like peptide (GalP) encoded by a different gene and alarin, a GALP splice variant, on the other. Several neuropeptides are involved in the pathophysiology of depression, in addition to noradrenaline and serotonin. Galanin mainly inhibits both the noradrenergic and serotonergic networks via Gal_1 and Gal_3 receptors. It also impedes neurotransmission via Gal_2 and Gi/o subunits, but can stimulate neurotransmitter release via Gal_2 and Gq/11 subunits. Galanin hampers neurotransmission in memory acquisition, modulates food intake and sexual behavior, and participates in the control of pain. Galanin is also a neuroendocrine peptide of the gastrointestinal tract. Galanin precludes insulin release.

134. Subunit $GABA_{B1}$ alone is not transported to the cell surface and remains non-functional.
135. The $GABA_{B1a}$ heterodimers on distal axons impede glutamate release in CA1–CA3 nerve terminals and GABA release onto the layer 5 pyramidal neurons. The $GABA_{B1b}$ heterodimers on dendritic spines mediate slow postsynaptic inhibition.

Table 7.63. Main G-protein subunit transducers and ligands of glucagon receptors (Source: [5]; GHRH: growth hormone-releasing hormone; GIP: glucose-dependent insulinotropic polypeptide; GLP: glucagon-like peptide).

Selective ligands	Main transducer
Glucagon	Gs
GLP1, exendins-3/4	Gs
GLP2	Gs
GIP	Gs
GHRH	Gs
Secretin	Gs

7.13.25 Ghrelin Receptor

Ghrelin is cleaved from its precursor preproghrelin. Alternative splicing generates a second peptide, des-Gln14-ghrelin with equipotent activity [5]. Both peptides undergo a post-translational octanoylation (Ser3) for full activity on binding to Gq/11-coupled ghrelin receptor (a.k.a. growth hormone secretagog receptor type-1 and growth hormone-releasing peptide receptor) in the hypothalamus and pituitary gland and release of growth hormone from the pituitary gland.

7.13.26 Glucagon Receptors

Glucagon receptors are activated by glucagon, glucagon-like peptides GLP1 and GLP2,[136] glucose-dependent insulinotropic polypeptide,[137] growth hormone-releasing hormone (GHRH), and secretin.

Growth hormone-releasing peptide stimulates growth hormone release via the GH secretagog-receptor GHSR1a, the ghrelin G-protein-coupled receptor. In addition, growth hormone-releasing peptide protects the cardiovascular apparatus independently of growth hormone. Growth hormone releasing-peptide is a ligand for leukocyte differentiation antigen cluster of differentiation CD36, or ScaRb3 scavenger receptor.[138] Transmembrane protein CD36 binds to collagen, thrombospondin, anionic phospholipids, long chain fatty acids, and oxidized LDLs. It is involved in cell adhesion and transport. Growth hormone-releasing peptides enhance the synthesis of ATP-binding cassette transporters ABCa1 and ABCg1 that are responsible for cholesterol efflux from macrophages, hence having a potent anti-atherosclerotic activity (Sect. 6.3.6.4).

136. Glucagon as well as GLP1 and GLP2 have a common precursor.
137. A.k.a. gastric inhibitory polypeptide.
138. A.k.a. collagen-1 and thrombospondin receptor, coronary heart disease susceptibility protein CHDS7, fatty acid translocase (FAT), periodic acid-Schiff staining platelet-agglutinating substance PAS4, and glycoproteins GP3b, GP4, and GP88.

Table 7.64. Metabotropic glutamate and GABA receptors, and their main transducers (Source: [736]). Glutamate ionotropic receptors include members of ^{N}methyl ^{D}aspartate (NMDA; GluN1, GluN2a–GluN2d, and GluN3a–GluN3b) and α-amino 3-hydroxy 5-methyl 4-isoxasole propionic acid (AMPA; GluR1–GluR4), and kainate (GluK1–GluK5) receptor families. The $GABA_A$ receptors are ligand-gated ion channels. The $GABA_B$ receptors can couple to $Ca_V2.1$ and $Ca_V2.2$ as well as $K_{IR}3$ channels.

Type	Main transducer
Glutamate metabotropic receptors	
$mGlu_1/_5$	Gq/11
$mGlu_{2-4}$, $mGlu_{6-8}$	Gi/o
γ-Aminobutyric acid receptors	
$GABA_B$	Gi/o

7.13.27 Glutamate Receptors

Ionotropic glutamate receptors include members of NMDA- and AMPA-type glutamate receptor channel family [139] that have a high relative permeability to calcium ions and are blocked by magnesium at rest potential. Hydrogen and zinc ions also inhibit NMDA receptor channel.

Metabotropic glutamate receptors ($mGlu_1$–$mGlu_8$ or mGluR1–mGluR8) are (Table 7.64) activated by ^{L}glutamate as well as ^{L}aspartate, ^{L}serine^{O_P}, [140] ^{N}acetylaspartylglutamate, and ^{L}cysteine sulfonic acid.

Three groups of receptors have been defined according to their sequence, G-protein coupling, and ligands: group 1 ($mGlu_1$ and $mGlu_5$), 2 ($mGlu_2$ and $mGlu_3$), and 3 ($mGlu_4$ and $mGlu_6$ to $mGlu_8$). Positive (potentiators) and negative allosteric modulators operate in the presence of agonists.

^{L}glutamate is the main neurotransmitter in the central nervous system that mediates fast excitatory neurotransmission by activating ionotropic glutamate receptors. Metabotropic glutamate receptors that are not located in the synaptic cleft prime slower effects. Type-1 mGluRs that operate in synaptic transmission are associated with slow synaptic excitatory currents and long-term remodeling. Kinetics of mGluR activation and deactivation determine features of synaptic transmission. Metabotropic glutamate receptors can be characterized by fast activation and slow deactivation. Activation time course can actually reach a minimum of approximately 10 ms, whereas deactivation time course nearly equals to 50 ms [1022]. Sensitization can develop over about 400 ms.

Neuronal stimulation of astrocytic metabotropic glutamate receptors induces an astrocytic calcium wave that propagates to astrocytic endfeet close to brain arterioles.

139. NMDA: ^{N}methyl ^{D}aspartate; AMPA: α-amino 3-hydroxy 5-methyl 4-isoxasole propionic acid.

140. This ^{L}serine precursor (^{L}SOP) in the serine synthesis pathway is produced by phosphoserine (Ser^P) aminotransferase (PSAT) and metabolized to ^{L}serine by phosphoserine phosphatase (PSP). It is an agonist at group-3 metabotropic glutamate receptors.

Table 7.65. Glycoprotein hormone receptors and their main G-protein subunit transducers (Source: [5]; FSHR: follicle-stimulating hormone receptor; LHR: luteinizing hormone receptor; TSHR: thyroid-stimulating hormone receptor).

Type	Main transducer
FSHR	Gs
LHR	Gs, Gi, Gq/11
TSHR	Gs, Gi, Gq/11, G12/13

Active neurons then rapidly (latency < 2 s) trigger dilation of intracerebral arterioles. Astrocytic calcium signaling activates astrocytic large-conductance, Ca^{++}-sensitive K^+ channels (BK) for local release of potassium into the perivascular space. A modest rise in extracellular potassium activates inward rectifier K^+ channels ($K_{IR}2.1$) of brain arteriolar smooth muscle cells, inducing membrane potential hyperpolarization and relaxation [1023]. [141] Augmented neuronal activity thus increases the local cerebral blood flow to supply sufficient amounts of glucose and oxygen (functional hyperemia).

Homer proteins associate with metabotropic glutamate receptors mGluR1 and mGluR5 as well as inositol trisphosphate receptors to increase mGluR-stimulated Ca^{++} signaling. In addition, Homer proteins facilitate anchoring and clustering of mGluR1 and mGluR5 in postsynaptic dendritic spines. Scaffolds of the Shank family of SH3- and multiple ankyrin repeat domain-containing proteins interact with both Homers and mGluRs to strengthen mGluR anchoring [835].

7.13.28 Glycoprotein Hormone Receptors

Glycoprotein hormone receptors are activated by heterodimeric glycoproteins made up of a common αand a β chain that confers the specificity of these glycoproteic heterodimers. These glycoprotein hormones include: (1) follicle-stimulating hormone (FSH, or follitropin); (2) luteinizing hormone (LH, a.k.a. lutropin and, in males, interstitial cell-stimulating hormone [ICSH]); (3) choriogonadotropin (or chorionic gonadotropin); and (4) thyroid-stimulating hormone (TSH, or thyrotropin; Table 7.65).

Follicle-stimulating hormone receptor (FSHR) in gonads is targeted by pituitary FSH glycoprotein for gonadal development, maturation at puberty, and sustained gamete production during the reproductive phase of life. Follicle-stimulating and luteinizing hormones that are both synthesized and secreted by the anterior pituitary gland act synergistically in reproduction. At the moment of menstruation, FSH initiates follicular growth. Stimulated FSHR not only prevents atresia of early antral follicles, but also promotes granulosa cell proliferation, estrogen synthesis, and LHR expression [1024]. An acute release of LH (LH surge) triggers ovulation and corpus

141. Elevation of the concentration of external K^+ that depolarizes vascular smooth muscle cells induces vasoconstriction of cerebral arteries and arterioles.

Table 7.66. Gonadotropin-releasing hormone receptors, main G-protein subunit transducers, and ligands (Source: [5]).

Type	Main transducer	Potency order
$GnRH_1$	Gq/11	GnRH1 > GnRH2
$GnRH_2$	Gq/11	GnRH2 > GnRH1

luteum development. In the ovary, the number of FSHRs increases during follicular maturation under low levels of FSH and decays after ovulatory LH surge.

In both males and females, FSH stimulates the maturation of germ cells. In males, it stimulates Leydig cell production of testosterone, enables persistance of Sertoli cells during gonadal development, and causes inhibin secretion in these cells. Unlike LHR, gonadal FSHR expression is specific to cell type [1024]. In the ovary and testis, FSHRs are expressed on granulosa cells of developing follicles and Sertoli cells, respectively.

Follicle stimulating hormone receptor interacts with arrestins and G-protein-coupled receptor kinases [1024]. Immature FSHR also associates with chaperone proteins calnexin, calreticulin, and disulfide isomerase as well as 14-3-3 adaptor protein, APPL (adaptor protein, phosphoTyr interaction, pleckstrin homology domain- and leucine zipper-containing protein), and FOXO1a.

Signaling mediated by the thyroid-stimulating hormone receptor and the G-protein subunit Gs can be enhanced by receptor endocytosis [11].

7.13.29 Gonadotropin-Releasing Hormone Receptors

Gonadotropin-releasing hormone (GnRH) is a hypothalamic decapeptide (Vol. 2 – Chap. 1. Remote Control Cells – Sect. Endocrine System and Hormones). Isoforms GnRH1 and GnRH2 as well as their cognate receptors $GnRH_1$ and $GnRH_2$ exist in mammals (Table 7.66). The $GnRH_1$ receptor is produced primarily in pituitary gonadotrophs and contribute to the central control of reproduction. Receptors $GnRH_1$ and $GnRH_2$ couple primarily to Gq/11, but also to Gs and Gi subunits. The $GnRH_2$ receptor can also target protein kinases [5].

7.13.30 Histamine Receptors

Histamine [142] is a neurotransmitter of the nervous system [143] and a signaling molecule in the immune system, skin, and gut. [144] Endothelial cells do not synthesize histamine and histamine receptors, but they can take up histamine.

Histamine binds to 4 known metabotropic histamine receptors (H_1–H_4 or H1R–H4R). These receptor subtypes associate with different types of G proteins to prime various effects (Table 7.67). Receptor H_3 abounds in the brain; H_4 receptor lodges mainly in peripheral tissues. Receptors H_1 and H_2 are mostly excitatory; H_3 homo- and heterodimers are inhibitory [1025].

Bordetella pertussis that can cause encephalomyelitis and disrupts the blood–brain barrier. On the other hand, H_1 on endothelial cells counteracts Bordetella pertussis effect. It actually reduces the permeability of the blood–brain barrier [1029].

Histamine mediates the stress-induced surges of adrenocorticotropic hormone, β-endorphin, and vasopressin from the pituitary gland (hypophysis) [1025]. It controls stress-related activity of aminergic networks (serotonin-, noradrenaline-, dopamine-, and acetylcholine-containing neurons).

The preferential site of histamine-induced suppression of food intake is Histamine stimulates neurons in the supraoptic nucleus that release the antidiuretic hor-

142. I.e., amine in biological tissues. This organic nitrogen compound s also called imidazolethylamine or imidazolethanamine. Histamine is synthesized from histidine taken up into the cerebrospinal fluid and neurons by amino acid transporter via oxidative decarboxylation by histidine decarboxylase. It is stored and transported into a vesicle using the vesicular monoamine transporter VMAT2, and, upon appropriate cues, released and methylated (inactivated) by neuronal histamine ^{N}methyltransferase using ^{S}adenosyl methionine. The latter enzyme also resides in blood vessel walls and processes circulating and mastocyte-released histamine. The main histamine-degrading enzyme in peripheral organs is diamine oxidase that converts histamine into imidazoleacetic acid [1025].

143. In the mammalian brain, histaminergic neurons localize exclusively in the tuberomamillary nucleus of the posterior hypothalamus and send their axons to the entire central nervous system, like other amine networks [1025]. All amine networks rely on autoreceptors that yield a negative feedback on excitability and amine neurotransmitter release and synthesis. Histaminergic neurons are active only during waking; they maintain wakefulness and attention. Mutual interactions with other transmitter networks are involved in higher brain functions (sleep–wake regulation, circadian and feeding rhythms, immunity, learning, and memory. Although histamine is the main neurotransmitter in the tuberomamillary nucleus, cotransmitters include GABA, galanin, enkephalins, thyrotropin-releasing hormone, and substance-P [1025]. Glutamatergic fibers from the cortex and hypothalamus secrete glutamate that excites neurons of the tuberomamillary nucleus. On the other hand, glycine inhibits a subpopulation of histaminergic neurons. In addition, gabaergic cues from mostly hypothalamic origin suppress the firing of histaminergic neurons. Aminergic (noradrenergic via gabaregic and serotoninregic) and cholinergic neurons also send projections to histaminergic neurons. Nucleotides excite histaminergic neurons using ionotropic and metabotropic receptors.

144. In neuroepithelial and hematopoietic cells, histamine participates in contraction of airway and intestinal smooth muscles, but vasodilation, as well as gastric acid secretion, mastocyte-based innate and acquired immunity, allergy, and inflammation, T-cell-associated immunity modulation, and control of epi- and endothelial barriers [1025].

Table 7.67. Histamine receptors and their effects (Sources: [1026–1028]; CNS: central nervous system; PN: peripheral nerves; AM: adrenal medulla; SMC: smooth muscle cell [v: vascular]; EC: endothelial cell; TL: T lymphocyte; GC: gastric cells; EcC: enterochromaffin cell; NKC: natural killer cell; MC: monocyte; DC: dendritic cells; Nϕ: neutrophil; C: chronotropy; I: inotropy [−: negative; + positive]; VGCaC: voltage-dependent Ca^{++} current).

Type	Gα	Site	Effects
H_1	Gq/11	CNS AM, heart, SMC, EC	Vasodilation, I−, bronchoconstriction, ileum contraction, modulation of the circadian cycle,
H_2	Gs	CNS, heart, vSMC GC TL	Smooth muscle cell relaxation, C+, I+, stimulation of gastric acid secretion, inhibition of T-cell function, facilitation of signal transduction in CNS
H_3	Gi/o	CNS, PN, EC, EcC	Inhibition of neurotransmitter release, increase VGCaC in smooth muscle cells, firing inhibition in tuberomammilary (histaminergic) neurons
H_4	Gi/o	NKL MC, DC, Nϕ	Innate immune cell chemotaxis, neutrophil release from bone marrow mastocyte chemotaxis

mone [1025]. Histamine and vasopressin-containing neurons exert reciprocal interactions.

likely the satiety center of the ventromedial hypothalamus. Histamine effects on food intake are associated with other facors, such as neuropeptide-Y, peptide-YY, and bombesin. In addition, orexigenic action of orexins and anorexigenic effects of leptin and glucagon-like peptide-1, which depend on corticotropin-releasing hormone released by neurons of the paraventricular nucleus rely on H_1 receptor [1025].

The brain histamine network controls thermogenesis, mostly via feeding and motor activity [1025]. The body's autonomic response that regulates heat conservation and production relies on the paraventricular nucleus and dorsomedial hypothalamus, and the nucleus raphe pallidus, respectively. Most of the structures implicated in thermoregulation are targets of histaminergic innervation.

Histaminergic activity exhibits a circadian rhythm with high levels during the day and low levels during the night [1025]. Histamine can entrain molecular clockworks outside the suprachiasmatic nucleus.

7.13.30.1 H_1

The Gq/11-coupled H_1 receptor activates phospholipase-C, thereby supporting IP_3-dependent release of Ca^{++} from its intracellular stores and diacylglycerol-primed activation of protein kinase-C. The latter forsters Ca^{++} influx through voltage-dependent calcium channels, transient receptor potential channels of the TRPC family, and Na^{+}–Ca^{++} exchanger. Other effects include production of arachidonic acid, nitric oxide, and cGMP using Gi/o-mediated activation of phospholipase-A2, Ca^{++}-dependent NOS, and NO-dependent guanylate cyclases, respectively. Moreover, H_1 activate AMPK kinase and nuclear factor-κB [1025].

Glia cells produce H_1 and H_2 receptors. Histamine promotes release of neurotrophins and cytokines from astrocytesas well as ATP in the hypothalamus [1025]. Histamine causes the opening of the blood–brain barrier, mainly via H_2 receptor.

7.13.30.2 H_2

Gs-coupled H_2 receptor stimulates adenylate cyclase, thereby elevating intracellular cAMP concentration, then activating protein kinase-A and CREB transcription factor [1025]. In addition, cAMP can interact with hyperpolarization-activated cation HCN2 channel. Upon PKA-mediated phosphorylation, H_2 represses Ca^{++}-activated, small conductance K^{+} channel (SK), which enables the long-lasting (seconds) after-hyperpolarization after action potentials in pyramidal cells, as well as $K_V3.2$ channel in interneurons involved in fast spiking [1025].

7.13.30.3 H_3

Gi/o-coupled H_3 receptor links to $Ca_V2.1$ and $Ca_V2.2$ channels [1025]. Due to crosstalk with other GPCRs, H_3 can initiate Gq/11 signaling and activate phospholipase-A2, the PKB–GSK3 axis, and MAPK module to control axonal and synaptic remodeling.

7.13.30.4 H_4

Gi/o-coupled H_4 receptor is expressed mainly in peripheral cells of blood, lung, liver, spleen, and gut [1025]. It may also reside in some regions of the brain.

7.13.31 Kiss1, NPff, PRP, and QRFP Receptors

Endogenous peptides with an arginine-phenylalanine-amide motif: (1) chemotaxis-inhibitory kisspeptin-54 (KP54 or metastin) cleaved from its precursor, a Kiss1 gene product; (2) pain-modulatory neuropeptide-FF (NPff); (3) prolactin-releasing peptide (PRP); and (4) pyroglutamylated arginine-phenylalanine (QRF)-amide peptide (QRFP) bind to their cognate receptors [5] (Table 7.68).

Table 7.68. Receptors for kisspeptin (KP), neuropeptide-AF and -FF (NPaf and NPff), prolactin-releasing peptide (PRP), and pyroglutamylated arginine-phenylalanine-amide peptide (QRFP), main G-protein subunit transducers, and ligands (Source: [5]).

Type	Other names	Main transducer	Potency order
GPR10	PrlHR, PrRPR	Gq/11	PRP
GPR54	Kiss1R, AXOR12	Gq/11	KP
GPR74	NPffR2	Gq/11	NPaf, NPff > PRP > QRFP
GPR103	QRFPR, AQ27	Gq/11, Gi/o	QRFP
GPR147	NPffR1	Gi/o	NPff > NPaf > QRFP, PRP

7.13.32 Latrophilin Receptors

Latrophilin receptors (Lphn1–Lphn3; a.k.a. calcium-independent receptors for latrotoxin [$CIRL_1$–$CIRL_3$]) constitute a group of GPCRs of the class-B secretin family [835]. They operate in both cell adhesion and signal transduction. They are located in pre- and postsynaptical membranes in the central nervous system. They connect to postsynaptic proteins, such as SH3- and multiple ankyrin repeat domain-containing proteins Shank1 to Shank3, a.k.a. somatostatin receptor-interacting protein (SstRIP) and proline-rich synapse-associated proteins ProSAP1 and ProSAP2, respectively [835].

7.13.33 Leukotriene Receptors

Leukotrienes comprise 5-lipoxygenase-derived family of short-lived eicosanoid lipid mediators that modulate vascular function and inflammatory cell reactivity. Leukotrienes as well as lipoxins are active metabolites derived from arachidonic acid. They are mainly involved in auto- and paracrine signaling.

5-Lipoxygenase (5LOx) in conjunction with arachidonate 5-lipoxygenase-activang protein (ALOx5AP), [145] an integral membrane protein of the nucleus membrane, converts arachidonic acid into 5-hydroperoxy eicosatetraenoic acid (5HPETE) that spontaneously reduces to 5-hydroxy eicosatetraenoic acid (5HETE). 5-Lipoxygenase converts 5HETE into leukotriene-A4, an unstable epoxide. In cells equipped with LTa4 hydrolase, such as neutrophils and monocytes, LTa4 is converted to leukotriene-B4. In cells that possess LTc4 synthase, such as mastocytes and eosinophils, LTa4 gives rise to leukotriene-C4. Hydrolysis of LTc4 produces leukotriene-D4 and -E4.

Leukotrienes include *cysteinyl-leukotrienes* (cysLTs: LTc4, LTd4, and LTe4) and *dihydroxy-leukotriene* (LTb4). Cysteinyl leukotrienes secreted by basophils and mastocytes together constitute the slow-reacting substance of anaphylaxis, as they trigger a prolonged, slow contraction of smooth muscle cells, especially vasoconstriction and bronchoconstriction.

Leukotriene receptors are activated by leukotrienes LTb4, LTc4, LTd4, LTe4, 12^{S}HETE, and 12^{R}HETE (Table 7.69). In addition to their receptors and ALX

145. A.k.a. five-LOx-activation protein (FLAP).

Table 7.69. Leukotriene receptors, their main targeted G proteins, and order of ligand potency (Sources: [5, 736]; EPA: eicosapentaenoic acid; fMLP: formyl methionyl-leucyl-phenylalanine; HETE: hydroxyeicosatetraenoic acid; HPETE: hydroperoxyeicosatetraenoic acid; 5oxoETE: 5-oxo-eicosatetraenoic acid; RARRes2CTP [ChCTP]: retinoic acid receptor [RAR] responder-2 C-terminal peptide [chemerin C-terminal peptide; chemoattractant chemerin is also called tazarotene-induced gene-2 protein (TIG2)]; RvE1: resolvin-E1 [EPA-derived (in the presence of aspirin), non-classic eicosanoid; inflammation reducer]). Receptors $CysLT_1$ and $CysLT_2$ are coexpressed by most myeloid cells. The $CysLT_2$ receptor suppresses $CysLT_1$ expression. The RS nomenclature (Latin rectus [R]: right behavior, straight, in a straight line, upright, direct path [used in the sense of dexter: right, on the right side]; sinister [S]: left, at the left side) for an enantiomer (Greek εναντιος: opposite) defines the chirality (Greek χειρ: strong-handed) of a subtype of amino acid or carbohydrate (X) among 2 stereoisomers (^{R}X and ^{S}X).

Type	Main transducer	Potency order
BLT_1	Gq/11, Gi/o (G16, Gi2)	LTb4 > 12^{R}HETE
BLT_2	Gq/11, Gi/o (Gz-like)	LTb4 > 12^{S}HETE ∼ 12^{S}HPETE > 12^{R}HETE
$CysLT_1$	Gq/11	LTd4 > LTc4 > LTe4
$CysLT_2$	Gq/11	LTc4 ∼ LTd4 ≫ LTe4
$CysLT_E$		LTe4 > LTc4, LTd4
ALX	Gi, Gq/16	LXA4 > LTc4 ∼ LTd4 ≫ fMLP
OxER1	Gi/o	5oxoETE ≫ 5^{S}HPETE ≫ 5^{S}HETE
RvE1R		RvE1 > RARRes2CTP > 18^{R}HEPE > EPA

lipoxin receptor, leukotrienes connect to enzymes of their metabolism (glutathione S-transferase-2, or LTc4 synthase, γ-glutamyl transpeptidase, and several aminopeptidases) and peroxisome proliferator-activated PPARα (nuclear receptor NR1c1).

Lipoxins are short-lived, endogenous, non-classical eicosanoids generated by lipoxygenases that act as anti-inflammatory mediators. Leukotriene-B4 and related hydroxyacids target BLT receptors BLT_1 and BLT_2. Cysteinyl-leukotrienes activate CysLT receptors $CysLT_1$ and $CysLT_2$, as well as LTe4-specific $CysLT_E$ receptor. These leukotriene GPCRs are classified as either chemoattractants (BLT_1 and BLT_2) or nucleotide receptors ($CysLT_1$ and $CysLT_2$). Both BLT and CysLT receptors via Gq and Gi elicit a large increase in intracellular calcium concentration and a decrease in cAMP level, as well as kinase activation (i.e., extracellular signal-regulated protein kinases ERK1 and ERK2) to promote cell differentiation and chemotaxis. Main effects of leukotrienes and lipoxins are given in Table 7.70.

Lipoxins LXA4 and LXB4 [146] activate lipoxin receptor ALX, a.k.a. formyl peptide receptor FPR2, formyl peptide receptor-like protein FPRL1, and formyl peptide

146. Lipoxins are eicosanoids that are generated within the vascular lumen by leukocytes. Lipoxins-A4 and -B4, as well as some peptides, are high-affinity ligands for LXA4 receptor (LXA4R), also called formyl peptide receptor-like receptor FPRL1. Similarly to leukotrienes, LXA4 can form cysteinyl-lipoxins LXC4, LXD4, and LXE4. At subnanomolar concentra-

Table 7.70. Main effects of leukotrienes and lipoxins (Source: [1032]).

LTb4	Leukocyte activation, cytokine secretion, IgE synthesis
LTc4, LTd4, LTe4	Vasoconstriction (via LTc4/D4), bronchospasm, vasodilation (pulmonary artery and vein via LTd4) cardiodepression, eosinophil recruitment, smooth muscle cell proliferation, plasma exudation, mucus secretion
Lipoxin-A4	Anti-angiogenesis, inhibition of cell proliferation, resolution of pulmonary edema, chemokine and cytokine expression, enhancement of macrophage phagocytosis of leukocytes

receptor homolog FPRH1, that is also included in the chemoattractant receptor class with formyl peptide receptors. The ALX receptor is synthesized mainly by leukocytes (granulocytes, monocytes, macrophages, and dendritic cells). It is also detected in microglia cells and astrocytes, enterocytes, and synovial fibroblasts [1030]. Most peptide ligands activate signaling pathways similar to those primed by FPR1 receptor. All ALX peptide ligands, such as formyl peptides and host-derived (annexin-A1 and its related N-terminal peptides, serum amyloid-A, soluble cleaved form of urokinase-type plasminogen activator, humanin, pituitary adenylate cyclase activating polypeptide-27 [a vasoactive intestinal peptide homolog], vasoactive intestinal peptide, amyloid $A\beta_{(1--42)}$, neutrophil and epithelial cell-derived cathelicidin LL37, and truncated splice variant of chemokine CCL23) [147] and pathogen-derived non-formyl peptides, mobilize Ca^{++} in neutrophils [1030]. They then stimulate neutrophil functions, hence causing chemotaxis, degranulation, and superoxide production. On the other hand, the only lipid ligand LXa4 of ALX receptor binds ALX at nanomolar concentrations to exert anti-inflammatory function [1030]. The ALX receptor thus has both pro- and anti-inhibitory effects. In human neutrophils,

tions, LXA4 and LXB4 rapidly inhibit leukotriene-stimulated interactions of human neutrophils and endothelial cells [1031].

147. Annexin-A1 and its related N-terminal peptides target ALX to prime their anti-inflammatory effects, in particular, neutrophil transmigration [1030]. Serum amyloid-A binds to ALX receptors of monocytes. Several uPA fragments connect to ALX to cause chemotaxis of monocytes and basophils [1030]. Neuroprotective peptide non-formylated humanin (as well as much more potent N-formyl humanin) tethers ALX and prevents $A\beta$(1-42) activity [1030]. Peptide PACAP27 that also activates G-protein-coupled vasoactive intestinal peptide receptor $VIPR_1$ ($VPAC_1$) stimulates α_M-integrin (CD11b) in human neutrophils [1030]. Vasoactive intestinal peptide also links to Gs-coupled vasoactive intestinal peptide receptors $VIPR_1$ and $VIPR_2$ ($VPAC_2$) to provoke smooth muscle relaxation, exocrine and endocrine secretion, and water and ion flux in lung and intestinal epithelia. It stimulates ALX to activate phosphatidylinositol 3-kinase and extracellular signal-regulated kinase and cause α_M-integrin synthesis [1030].

Table 7.71. Leukotriene-producing leukocytes (Source: [1032]). Leukotriene-C4 is metabolized to LTd4 and LTe4 by LTc4-synthesizing cells.

LTb4	Eosinophils, neutrophils, monocytes, macrophages
LTc4	Eosinophils, neutrophils, monocytes, macrophages
LTd4	Eosinophils, neutrophils, monocytes, macrophages
LTe4	Eosinophils, neutrophils, monocytes, macrophages

Table 7.72. Major cells that express leukotriene receptors (Source: [1032]). Signaling from LTb4 in granulocytes is mediated by Gi as granulocytes abundantly express Gi, mainly Gi2, whereas nervous cells mainly produce Gi1 and Go. LTb4-induced calcium mobilization results from Gq activation. BLT1-mediated phospholipase-C activation is mediated by Gi6 and $G\beta\gamma$. BLT2 is less efficient for calcium mobilization than BLT1. BLT2 induces chemotaxis via Gi and the Rac–ERK–ROS cascade. LTd4 activates CysLT1 to produce second messengers diacylglycerol, inositol 3-phosphate, and Ca^{++}. Subsequently, protein kinase-C and phospholipase-A2 are activated, the latter generating arachidonic acid. LTd4 primes constriction of smooth muscle cells in bronchi and intestine via protein kinase-Cϵ.

Type	Location
BLT_1	Thymus, spleen
	Leukocytes
BLT_2	Ovary, liver
	Leukocytes (ubiquitous)
$CysLT_1$	Spleen
	Smooth muscle cells (lung, intestine), blood leukocytes
$CysLT_2$	Heart, spleen, adrenal medulla, brain
	Blood leukocytes
ALX	Lung, spleen
	Blood leukocytes

ALX is involved in serum amyloid-A-stimulated interleukin-8 production and nuclear factor-κB activation. Although ALX activity regulation is similar to that of FPR1, FPR2/ALX differs from FPR1 receptor. Gelsolin-derived peptide PBP10 selectively blocks ALX-mediated mobilization of granules and production of oxygen radicals [1030].

Oxoeicosanoid receptor OxER1 (or GPR170) is activated by endogenous chemotactic eicosanoids oxidized at the C5 position [5]. 5-oxo-Eicosatetraenoic acid (5oxoETE) is its most potent agonist. *Resolvin receptors* are activated by anti-inflammatory resolvin-E1 (RvE1) that derives from ω-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) by aspirin-modified cyclooxygenase and lipoxygenase [5].

Leukotrienes are synthesized not only by multiple cell types, but also via transcellular metabolism involving different cells, such as neutrophils, platelets, and vascular cells (Table 7.71). Primary expression of leukotriene receptors is given in Table 7.72.

Leukotriene-B4, a chemotactic and immune-modulating agent implicated in allergic and inflammatory reactions, acts via G-protein-coupled receptors BLT_1 and BLT_2. Receptors BLT_1 and BLT_2 signal via 3 classes of G protein subunits, Gi, Gq-like, and Gz. [148] Leukotriene-B4 [149] is a chemoattractant produced by neutrophils, macrophages, and mastocytes for polymorphonuclear leukocytes and T lymphocytes. Leukotrienes are involved in arteriosclerosis.

In mature endothelial cells, LTb4–BLT signaling enables angiogenesis. Endothelial cell migration depends on CysLT receptors. Moreover, 12LOx-dependent pathway via 12HETE and BLT_2 is involved in VEGFa-induced angiogenesis. Embryoid bodies derived from human embryonic pluripotent cells differentiate into endothelial cells as well as different hematopoietic lineages (monocytes and macrophages, T lymphocytes, NK cells, and dendritic cells). Leukotrienes LTb4 and, to a lesser extent, LTd4 concomitantly with their cognate receptors BLT and CysLT are expressed in embryonic stem cells during embryogenesis, particularly endothelial plexus during the later phase of vasculogenesis and angiogenesis, at least in rodents. [150] Myeloid-like cells that synthesize 5LOx and its cofactor FLAP produce LTb4 that operates as a paracrine agent to stimulate endothelial progenitors via BLT receptors to initiate vasculogenesis [1034]. Leukotriene action in differentiating embryonic stem cells is mediated via extracellular signal-regulated kinase ERK1 and ERK2.

7.13.34 Lysophospholipid Receptors

Lysophospholipids are built on a glycerol backbone and are defined by the nature of the phosphate groups linked to the carbon atom at position 3 (stereospecific nomenclature sn3). They contain a single fatty acid side chain. On the other hand, phospholipids typically contain saturated and unsaturated fatty acids at the sn1 and sn2 positions, respectively.

Lysophospholipids can be generated from phospholipids by phospholipases that cleave the sn1 or sn2 acyl fatty acids. The sn2 acyl side chain of lysophosphatidic acid (LPA) is unstable and moves to the sn1 position, generating the predominant form of LPA: 1-acyl 2-hydroxy sn-glycero 3-phosphate (Table 7.73). The sequential actions of phospholipases PLD and PLA2 can generate lysophosphatidic acid from phosphatidic acid (PA). Structural and functional diversity arises from differences in fatty acid moieties esterified to sn1 that may influence LPA binding to its receptors. The synthesis of LPA involves either the sequential action of secretory phospholipase-A and lysophospholipase-D, or phospholipase-D and cytosolic

148. The expression of BLT receptors can be enhanced in endothelial cells by lipopolysacharides, cytokines, such as tumor-necrosis factor-α and interleukin-1β, and LTb4 [1033].

149. LTb4 Leukotriene is synthesized from arachidonic acid by the concerted action of LTa4 hydrolase and 5-lipoxygenase, which is assisted by 5-lipoxygenase-activating protein.

150. Myeloid-like, CD11b+ (α_M-integrin) embryonic stem cells are also 5LOx+ and FLAP+, whereas endothelial-like, VEGFR2+, CD105+ (endoglin of the TGFβ receptor complex) embryonic stem cells are non-synthesizing leukotriene cells.

Table 7.73. Main axes of lysophosphatidic acid (LPA) synthesis, either from phosphatidic acid (PA) using phospholipases (PL), or from lysophosphatidylcholine (lysoPC) using autotaxin, or lysophospholipase-D (lysoPLD). Autotaxin is produced in high endothelial venules, where it can support transendothelial migration and lymphocyte entry into lymph nodes and secondary lymphoid organs, as well as lymphocyte migration into inflammatory sites.

Axis	Enzymes
PC–LysoPC–LPA	sPLA2 and LysoPLD, or PLD and cPLA2
PA–LPA	PLD and PLA2

Table 7.74. Endothelial differentiation gene (EDG) family of GPCRs.

Gene	Name	Ligand
EDG1	$S1P_1$	S1P
EDG2	LPA_1	LPA
EDG3	$S1P_3$	S1P
EDG4	LPA_2	LPA
EDG5	$S1P_2$	S1P
EDG6	$S1P_4$	S1P
EDG7	LPA_3	LPA
EDG8	$S1P_5$	S1P

PLA. The second major axis of LPA synthesis uses lysophospholipase-D (lysoPLD), or autotaxin, that cleaves the choline group from lysophosphatidylcholine. In addition, membrane-bound phosphatidic acid-selective phospholipase-1α (MPAPLA1), or lipase-H (LipH), is a 2-acylLPA-producer. The generation of LPA can also be achieved by phosphorylation of monoacylglycerol (MAG) by MAG kinases.

The most prominent source of LPA in the blood circulation (at micromolar concentrations) arises from autotaxin. Lysophosphatidic acid circulates bound to serum proteins, especially albumin and gelsolin, a member of the extracellular actin scavenger pool that depolymerises and removes from the blood actin released in the extracellular medium to avoid thrombus creation. In response to inflammation, LPA diffuses into the extravascular space, where it supports cell chemotaxis and proliferation, cytokine and chemokine secretion, platelet aggregation, and smooth muscle cell contraction (vasoconstriction).

Glycerol- and sphingosine-based phospholipids are abundant components of cellular membranes. They are metabolized into eicosanoids and lysophospholipids, such as lysophosphatidic acid, lysophosphatidylcholine, sphingosylphosphoryl choline, and sphingosine 1-phosphate. G-protein-coupled receptors for lysophospholipids include 12 identified members. The lysophospholipid GPCR family, i.e., the endothelial differentiation gene (EDG) family of GPCRs, encompasses receptors for sphingosine 1-phosphate and lysophosphatidic acid, as well as lysophosphatidylcholine and sphingosylphosphoryl choline psychosine groups [1035] (Table 7.74).

Upon receptor activation, plasmalemmal phospholipids are metabolized into lysophospholipid mediators, such as sphingosine 1-phosphate and lysophosphatidic acid. These messengers regulate angiogenesis, cardiac development, neuronal survival, and immunity [1035].

7.13.35 Lysophosphatidic Acid Receptors

Lysophosphatidic acid (LPA), the simplest natural phospholipid,[151] is produced by: (1) hydrolysis of phosphatidic acid that localizes mainly at the inner leaflet of the plasma membrane by phospholipase-A1 or -A2 and (2) conversion of lysophospholipids, such as lysophosphatidylcholine (LPC),[152] lysophosphatidylethanolamine (LPE), and lysophosphatidylserine (LPS), which are also generated from membrane phospholipids by phospholipase-A1 or -A2, to LPA by lysophospholipase-D, or autotaxin.[153]

This bioactive lipid mediator involved in stress fiber formation using the Rho–RoCK axis[154] and cell proliferation, differentiation, survival, and motility. Lysophosphatidic acid is dephosphorylated (inactived) by lipid phosphate phosphohydrolases (LPP) into monoacylglycerol. The LPP phosphatase has its catalytic site oriented toward the external face of the plama membrane.

Lysophosphatidic acid can be synthesized outside the cell by membrane or secreted enzymes, such as secreted phospholipase-A2 (sPLA2) and lysophospholipase-D (sLysoPLD), especially after membrane lipid reorganization during inflammation.

Extracellular LPA that serves as lipid mediator is water soluble. It resides in many biological fluids, where it is maintained in solution owing to its connection to

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151. Lysophosphatidic acid is composed of a single fatty acyl chain, a glycerol backbone, and a phosphate group. In fact, many LPA species exist. These LPA species possess an alkyl or alkenyl ether-linked fatty acyl chain of various lengths and unsaturation degrees. A saturated or unsaturated fatty acid chain is esterified either at the sn1 (1-acylLPA; e.g., 1-palmitoylLPA) or sn2 (2-acylLPA; e.g., 2-arachidonoylLPA) position (sn: stereospecific nomenclature) of the glycerol backbone of LPA phospholipid. Ether-linked LPAs carry an alkyl- (alkylLPA; e.g., hexadecylLPA) or an alkenyl-linkage (alkenylLPA) at the sn1 position [1036]. Activity of LPA depends on: (1) the length and the unsaturation level of the carbon chain attached to the glycerol backbone and (2) the type of linkage and its position. Lysophosphatidic acid is indeed synthesized via numerous routes. In particular, autotaxin, a lysophospholipase-D, removes the choline group from lysophosphatidylcholine.

152. Lysophosphatidylcholine abounds in biological fluids linked to albumin or lipoproteins. It is synthesized from phosphatidylcholine by PLA2 or lecithin cholesterol acyltransferase (LCAT).

153. Autotaxin (Atx; a.k.a. lysophospholipase-D and type-2 ecto-nucleotide pyrophosphatase phosphodiesterase) is a transmembrane ecto-enzyme. It is upregulated by certain peptide growth factors. Soluble autotaxin derives from a membrane-bound form by proteolytic cleavage.

154. In 1960, Vogt observed that LPA causes the contraction of isolated rabbit duodenum. Afterward, the contraction of smooth muscle cells generated by several LPA species was demonstrated on vascular cell types.

Table 7.75. Lysophosphatidic acid (LPA) receptors, main G-protein subunit transducers, and ligands (Sources: [5, 1036]; EDG: endothelial differentiation gene product). Lysophosphatidic acid also binds PPARγ receptors. In addition to the 3 G-protein-coupled receptors of the endothelial differentiation gene (EDG) family (LPA_1–LPA_3), lysophosphatidic acid operates via 3 other homologous receptors (LPA_4–LPA_6) that are structurally distinct from the EDG family LPA receptors. The latter are involved, in particular, in angiogenesis and platelet activation. The LPA receptors of the EDG family that preferntially link to acylLPA than alkylLPA provoke intracellular Ca^{++} influx, inositol phosphate production, adenylate cyclase inhibition, and mitogen-activated protein kinase activation. Receptors LPA_6, or $P2Y_5$, and LPA_8, or $P2Y_{10}$, are activated by LPA and LPA and S1P, respectively.

Type	Other names	Main transducers
LPA_1	EDG2	Gi/o, Gq/11, G12/13
LPA_2	EDG4	Gi/o, Gq/11, G12/13
LPA_3	EDG7	Gs, Gi/o, Gq/11
LPA_4	GPR23, $P2Y_9$	Gs, Gi/o, Gq/11, G12/13
LPA_5	GPR92, GPR93	Gq, G12/13
LPA_6	GPR87, $P2Y_5$	G12/13, Gs, Gi/o
	LPA_7	
LPA_8	$P2Y_{10}$	Gq/11

its carrier albumin. In addition to albumin, other proteins, such as fatty acid-binding proteins and gelsolin, link to LPA.

Almost all cells, except in the liver, coexpress several types of LPA receptors (LPA_1–LPA_6; Table 7.75). Receptors LPA_4 and LPA_6 correspond to $P2Y_9$ and $P2Y_5$, respectively [1037, 1038]. The $P2Y_9$ receptor is most closely related to $P2Y_5$ that is coupled to the G13–Rho pathway. It is relatively close to nucleotide receptors $P2Y_1$, $P2Y_4$, and $P2Y_6$ as well as lipid receptors of the group of *proton-sensing GPCRs* [155] and other lipid receptors, such as platelet-activating factor [156] receptor and leukotriene receptors $CysLT_1$ and $CysLT_2$.

Lysophosphatidic acid receptors are coupled to G-protein subunits Gs, Gq, Gi, and/or G12/13 (Tables 7.75 and 7.76). Activated Gq stimulates phospholipase-C that activates protein kinase-C for calcium influx. Activated Gi targets 3 pathways, as it: (1) inhibits adenylate cyclase; (2) stimulates Ras–mitogen-activated protein ki-

155. Lipid ligands modulate action of protons that activate proton-sensing G-protein-coupled receptors. Proton-sensing GPCRs include: (1) Gs-coupled GPR4 that is targeted by sphingosylphosphorylcholine and lysophosphatidylcholine; (2) psychosine receptor GPR65 (or T-cell death-associated gene product TDAG8) that activates adenylate cyclase; (3) GPR68 (or ovarian cancer GPCR OGR1); and (4) GPR132 (or G2A), a high-affinity receptor for lysophosphatidylcholine. Sphingosyl-phosphorylcholine, lysophosphatidylcholine and psychosine are high- and low-affinity ligands for GPR4 and GPR65, respectively. Lipids favor effects of protons as activators of GPR4. Psychosine and its related lysosphingolipids behave as antagonists of protein-sensing receptors [1039].

156. Platelet-activating factor is a phospholipid (acetyl-glyceryl-ether phosphorylcholine) produced by neutrophils, basophils, platelets, and endothelial cells.

nase cascade; and (3) activates phosphatidylinositol 3-kinase, guanosine nucleotide-exchange factor TIAM1 (RacGEF), hence small GTPase Rac, as well as protein kinase-B. Hence, LPA has mitogenic and anti-apoptotic effects.

Activated LPA_1 and LPA_2 coupled to G12/13 stimulates small GTPase RhoA that causes cytoskeletal contraction and favors cell motility and adhesion.

Lysophosphatidic acid receptor LPA_2 interacts with scaffold membrane-associated guanylate kinase, WW and PDZ domain-containing protein MAGI3 to enhance receptor-mediated activation of extracellular signal-regulated kinase. On the other hand, LPA_2 connects to 2 other PDZ domain-containing scaffolds, RhoGEF11 and RhoGEF12 to potentiate LPAR2-induced stimulation of Rho signaling on cytoskeleton dynamics [835]. Therefore, LPA_2 can activate PLCβ, ERK, or Rho GTPase according to the synthesis level of PDZ domain-containing scaffold, hence cell type.

Lysophosphatidic acid receptor LPA_2 (or LPAR2), like purinergic receptor $P2Y_1$, parathyroid hormone receptor PTH1R (or PTH_1), and metabotropic glutamate receptor mGluR5 can link to Na^+–H^+ exchanger regulatory factors NHERF1 and NHERF2 independently of agonists to enhance Gq-protein- and PLCβ-mediated signaling. [157] Protein NHERF2 can also bridge LPA_2 to cystic fibrosis transmembrane regulator Cl^- channel. On the other hand, β2AR and KOR opioid receptors also connect to NHERF proteins, but in an agonist-dependent manner [835].

Due to its unique C-terminal binding domain, LPA_2 bind to several PDZ motif-containing proteins (NHERFs, RhoGEF11, RhoGEF12, and membrane-associated guanylate kinase with an inverted domain structure protein MAGI3) [1040]. These scaffolds modulate LPA-induced activation of ERK and/or RhoA GTPase. Both NHERF2 and MAGI3 can recruit phosphatase and tensin homolog to repress the PI3K–PKB signaling. In addition, NHERF2 can also serve as a scaffold for 3-phosphoinositide-dependent protein kinase PDK1 that activates AGC family kinases such as PKB. Therefore, NHERF2 restricts or promotes the PI3K–PKB pathway according to the relative synthesis rate of PTen and PDK1 enzymes.

In addition to NHERF2 scaffold, LPA_2 interacts with thyroid hormone receptor interactor TRIP6 and apoptosis-inducing factor Siva-1. The LPA_2–TRIP6–Siva-1 complex and NHERF2 enable LPA_2-mediated protection against apoptosis [1040].

Whereas LPA_1 and LPA_2 can be coupled to Gi, thus inhibiting adenylate cyclase, LPA_3 can be tethered to Gs, thereby activating adenylate cyclase.

Liganded LPA_4 coupled to Gs and Gq subunits increases intracellular concentrations of Ca^{++} and cAMP messengers. Receptor LPA_4 coupled to G12/13 proteins activates Rho GTPase, thereby causing cytoskeleton remodeling. Therefore, LPA_4 can counteract the effect of LPA_1, as Rho and Rac exert a mutual inhibition.

Receptor LPA_5 can activate adenylate cyclase independently of $G\alpha_s$ [1036]. It also mobilizes Ca^{++} ions. In mice, LPA_5 is highly produced in the small intestine and stomach as well as dorsal root ganglion. In mouse small intestine, LPA_5 is synthesized not only in epithelial cells, but also in intraepithelial lymphocytes.

157. Many NHERF-binding partners, such as TRPC channels, various isoforms of PLCβ, protein kinases PKC and PKD, are components of Gq–PLCβ pathways.

Plasmalemmal protein LPA_5 is the most prevalent LPA receptor in human mastocytes [1042]. [158] Cell proliferation caused by LPA stimulation of mastocytes results from the activity of LPA_1 and LPA_3, whereas cytokine generation is induced by LPA_2 receptor. Receptor LPA_5 is responsible for release of CCL4 chemoattractant launched by LPA [1042].

Lysophosphatidic acid is a potent pro-aggregating factor for platelets. In humans, platelets synthesize LPA_1 to LPA_6 transcript; the most abundant are LPA_4 and LPA_5 transcripts [1041]. At the protein level, among LPA receptors, platelets produce mainly LPA_5 receptor. In humans, platelets aggregate more potently in response to alkylLPA than to corresponding acylLPA [1036].

Lysophosphatidic acid induces not only smooth muscle contraction, platelet aggregation, and increase in endothelial permeability [1043], [159] but also cell survival, proliferation, and migration, as well as attachment of myeloid cells to endothelium (Table 7.76). In addition it causes gap-junction closure and tight-junction opening, favors wound healing, and elicits the production of endothelin, angiogenic factors (vascular endothelial growth factor and interleukin-6 and -8), urokinase-type plasminogen activator, [160] metallopeptidases such as MMP2 and ADAM17 [1044]. Lysophosphatidic acid is a ligand for the transcription factor perixosome proliferating activating receptor-γ. Five G12/13-coupled LPA receptors can contribute to angio- and lymphangiogenesis.

Transcription of LPA is activated by growth factors. Lysophosphatidic acid can indirectly regulate cell functions by stimulating G-protein-coupled receptor-regulated transmembrane metallopeptidase that cleaves precursor heparin-binding EGF-like growth factor (HBEGF) at the plasma membrane and activating EGF receptor (Sect. 8.2.5.2).

7.13.36 Mas1-Related G-Protein-Coupled Receptors

Signaling molecules expressed in nociceptive sensory neurons include vanilloid receptor VR1, purinergic receptors such as $P2X_3$, and tetrodotoxin-insensitive voltage-gated sodium channels that convey most of inward flux during upstroke and shoulder during the falling phase of nociceptor action potentials, with significant contribution from tetrodotoxin-sensitive sodium channels and voltage-dependent calcium channels, respectively, and regulate duration of action potentials. Several GPCRs are expressed in nociceptive sensory neurons, such as bradykinin,

158. Mastocytes lodge particularly around the microvasculature and in the skin and respiratory and gastrointestinal tracts, ready to respond to damages and infections.

159. LPA causes a rapid, reversible, dose-dependent decrease in paracellular flux resistance in brain endothelial cells. LPA modulates tight junction permeability without relocalization of adherens junction- or tight junction-associated proteins.

160. In some types of migrating cells, urokinase-type plasminogen activator receptor (uPAR) associates urokinase-type plasminogen activator (uPA) to the leading edges and controls the activation of small GTPase Rac. As uPAR is not a transmembrane receptor, it interacts with plasmalemmal and cortical proteins such as integrins as well as matrix components such as vitronectin to transmit signals to the cytoskeleton.

Table 7.76. Lysophosphatidic acid activity (Source: [1044]). Upon LPA receptor stimulation, G12/13 proteins activate Rho GTPase and RoCK and LIMK1 kinases that phosphorylate cytoskeletal proteins, such as myosin light chain, moesin, and cofilin. (Calcium influx and Rac activation are not involved in LPA-induced cell shape change.)

Action	Pathway
Cell survival	Gi–PI3K–PKB
Cell growth	Gi–Ras–ERK1/2
Cell migration	Gi–PI3K–TIAM1–Rac, RhoA, Cdc42
Cell shape (rounding)	G12/13–RhoGEF–RhoA
Cell differentiation (inhibition or reversal)	G12/13–RhoGEF–RhoA ERK, P38MAPK
Endothelial permeability	G12/13–RhoGEF–RhoA
Gap junction communication	Gq–PLC
SMC contraction	G12/13–RhoGEF–RhoA
Platelet aggregation	G12/13–RhoGEF–RhoA

Table 7.77. Melanin-concentrating hormone receptors and their main G-protein subunit transducers (Source: [5]).

Type	Other names	Main transducer
MCH_1	MCHR1, GPR24, SLC1	Gi/o, Gq/11
MCH_2	MCHR2, GPR145, SLT	Gq/11

neuropeptide-Y, opioid, peptidase-activated, and prostaglandin receptors, as well as Mas1-related G-protein-coupled receptors (MRGPRd–MRGPRg; MRGPRx1–MRGPRx4) [1045].

7.13.37 Melanin-Concentrating Hormone Receptors

Melanin-concentrating hormone receptors (Table 7.77) bind to melanin-concentrating hormone (MCH) that is a nonadecameric cyclic orexinogenic hypothalamic peptide. The latter regulates the feeding behavior, hence energy balance. Melanin-concentrating hormone is generated from a precursor, proMCH, that also produces neuropeptide-EI and -GE. It interacts with neuropeptide-Y [5]. [161]

7.13.38 Melanocortin Receptors

Melanocortin has a protective effect in the cardiovascular system. It attenuates inflammation during myocardial infarction and stroke. The melanocortin receptor fam-

161. Melanin-concentrating hormone and α-melanocyte-stimulating hormone (αMSH) have mostly antagonistic, but also agonistic effects. Neuropeptide-EI is a MCH-antagonist and MSH-agonist. Neuropeptide-GE derived from proMCH can mimic MSH signaling via MCHR1 receptor.

Table 7.78. Melanocortin receptors, main G-protein subunit transducers, and ligands (Source: [5]).

Type	Main transducer	Potency order
MC_1	Gs	αMSH > βMSH $\geq$ ACTH, γMSH
MC_2	Gs	ACTH
MC_3	Gs	γMSH, βMSH $\geq$ ACTH, αMSH
MC_4	Gs	βMSH $\geq$ αMSH, ACTH > γMSH
MC_5	Gs	αMSH $\geq$ βMSH $\geq$ ACTH > γMSH

ily includes 5 GPCRs (MC_1–MC_5 or MC1R–MC5R; Table 7.78). The MC_2 receptor is a component of the hypothalamic–pituitary–adrenal axis, whereas MC_3 and MC_4 intervene in energy homeostasis. They are activated by members of the melanocortin family of peptide hormones that include different forms of melanocyte-stimulating hormone (MSHα–MSHγ) and adrenocorticotrophin (ACTH).

Endogenous antagonists include Agouti and agouti-related protein. Melanocortin receptor accessory proteins MRAP1 and MRAP2 heighten the plasmalemmal density of MC_2 as well as their responsiveness to melanocyte-stimulating hormone, but lower those of MC_1 and MC_3 to MC_5 [1046]. Melanocortin MC_2 receptor on the plasma membrane requires the presence of MRAP1 for its activation. On the other hand, MRAP2 is an endogenous inhibitor, as it competes with MRAP1 for MC_2 binding and decreases the action potency of adrenocorticotropic hormone [1047]. The ACTH hormone indeed binds with high affinity to MC_2 in the presence of MRAP1, but not MRAP2. The influence of MRAP1 and MRAP2 on ligand-binding affinity is specific to MC_2. The balance between stimulatory and inhibitory accessory proteins controls MC_2 sensitivity to its agonist. These proteins actually have little effect on the binding of α-melanocyte-stimulating hormone to the MC_4 receptor.

Thyroid hormones regulate metabolism and appetite. Active triiodothyronine (T_3) stimulates the basal metabolic rate. [162] Increased T_3 level prevents transcription of the thyrotropin-releasing hormone (Trh) gene in TRH+ neurons in the paraventricular nucleus of the hypothalamus. These neurons produce both nuclear thyroid hormone receptors [163] and MC_4 melanocortin receptor. Triiodothyronine exerts a negative feedback on MC_4 in hypothalamic paraventricular thyrotropin-releasing hormone neurons [1048].

The MC_4 receptor intervenes in signaling by leptin, a satiety hormone, and links the central energy control to the status of peripheral metabolic reserve. The leptin–melanocortin pathway operates in the hypothalamo–pituitary–thyroid axis to adapt energy expenditure to metabolic reserves. Leptin stimulates TRH production via MC_4 receptor. Activation of MC_4 reduces food intake and increases energy expenditure. Both thyroid hormone receptors ThRα (NR1a1) and ThRβ (NR1a2) con-

162. Hyper- and hypothyroidism causes weight loss and gain, respectively.

163. Functional thyroid hormone nuclear receptors (ThRα1, or NR1a1-1, as well as ThRβ1 and ThRβ2, or NR1a2-1 and NR1a2-2) colocalize in many hypothalamic nuclei. Each receptor isoform exerts specific transcriptional activities on the Trh promoter.

Table 7.79. Melatonin receptors and their main G-protein subunit transducers (Source: [5]). Melatonin-related receptor MT_3, or Mel_{1C}, is also called GPR50 in humans [608].

Type	Other names	Main transducer
MT_1	Mel_{1A}	Gi/o
MT_2	Mel_{1B}	Gi/o
MT_3	Mel_{1C}, GPR50	

tribute to MC_4 regulation. The T_3 hormone represses Mc4r gene transcription together with that of Trh gene. It prevents Mc4 expression not only via hypothalamic neurons, but also those of other energy-related brain regions, such as Agouti-related protein-containing neurons [1048]. The T_3 hormone dampens cerebral responsiveness to melanocortin anorectic signaling, but stimulates orexigenic pathways via neuropeptide-Y.

7.13.39 Melatonin Receptors

Melatonin receptors (MT_1–MT_3) are activated by the endogenous ligands melatonin secreted by the pineal gland to set the circadian rhythm and ^{N}acetylserotonin [5] (Table 7.79). They lodge in the brain and some other organs.

7.13.40 Motilin Receptors

Gq/11-coupled motilin receptor (MlnR, MtlR1, or GPR38) is activated by the polypeptide motilin that derives from a precursor that also generates motilin-associated peptide. Motilin is secreted by endocrine M cells in crypts of the small intestine to stimulate gastric activity.

7.13.41 G-Protein-Coupled Natriuretic Peptide Receptor

Natriuretic peptide receptors include 2 main sets of receptors. Receptors NPR1 and NPR2 are linked to guanylate cyclases (Sect. 6.4.1), whereas NPR3 is a G-protein-coupled receptor. The NPR3 receptor — the *clearance receptor* — binds to and removes natriuretic peptides from the blood circulation. It has an extracellular binding domain homologous to that of NPR1 and NPR2, but possesses a truncated intracellular domain that couples via Gi/o proteins to phospholipase-C and inwardly rectifying potassium channels, besides its inhibition of adenylate cyclase.

7.13.42 Receptors of Neuromedin-U and Neuromedin-S

Neuromedin-U receptors (NMU_1–NMU_2;[164] Table 7.80) are activated by neuromedin-U (NmU). Neuromedin-U is produced at its highest levels in the central ner-

164. The NMU_1 receptor was designated as the orphan GPR66 or FM3 and NMU_2 as FM4 or TGR1.

Table 7.80. Neuromedin-U receptors and their main G-protein subunit transducers (Source: [5]).

Type	Other names	Main transducer
NMU_1	NMUR1, FM3, GPR66, SNORF62	Gq/11
NMU_2	NMUR2, FM4, TGR1, SNORF72	Gq/11

vous system, bone marrow, upper gastrointestinal tract, and fetal liver. The structurally related peptide neuromedin-S is also an endogenous agonist of NMU receptors with equivalent potency to that of neuromedin-U [5].

Receptors NMU_1 and NMU_2 couple mainly to Gq/11, but also Gi/o. Receptor NMU_1 is predominantly expressed in peripheral tissues, particularly the gastrointestinal tract, whereas NMU_2 abounds within the central nervous system (brain and spinal cord) [1049]. Neuromedin-U causes vasoconstriction predominantly via NMU_1 and nociception and bone remodeling via NMU_2. It also prevents obesity. Neuromedin-S is more potent when it binds to NMU_2 receptors in vivo. Like neuromedin-U, it suppresses feeding and contributes to the regulation of the circadian rhythm [1049].

7.13.43 Receptors of Neuropeptide-B and Neuropeptide-W

Neuropeptide-B/W receptors ($NPBW_1$–$NPBW_2$; Table 7.81) are activated not only by neuropeptide-B (23 amino acid peptides NPB23) and -W (NPW23), but also their C-terminally extended forms (NPB29–NPW30) [5]. These receptors are predominately produced in the central nervous system.

7.13.44 Neuropeptide-S Receptor

Neuropeptide-S (NPS) is synthesized in the central nervous system. It suppresses anxiety. It targets neuropeptide-S receptor (NPSR; Table 7.81).

7.13.45 Neuropeptide-Y Receptors

Neuropeptide-Y (NPY) receptors (Table 7.81) are activated by neuropeptide-Y (NPY) and its fragment NPY_{3--36}, peptide-YY (PYY) and its fragment PYY_{3--36}, and pancreatic polypeptide (PP) [5]. Neuropeptide-Y is a sympathetic neurotransmitter involved in the behavior (control of the circadian rhythm and appetite, integration of emotional behavior, and cerebrocortical excitability). Under strong stress, NPY is released with noradrenaline from the peripheral sympathetic nerve terminals to possibly enhance permeability of vascular walls to large molecules, proliferation of vascular smooth muscles, and formation of vascular sprouts in ischemic tissues.

Neuropeptide-Y receptors are connected to Gi subunit of heterotrimeric G protein. Five cloned mammalian neuropeptide-Y receptors exist (Y_1–Y_2 and Y_4–Y_6;

Table 7.81. Receptors for neuropeptide-B (NPB), -W (NPW), -S (NPS) and -Y (NPY), their main G-protein subunit transducers, and ligands (Source: [5]; GPRA: G protein-coupled receptor for asthma susceptibility; PP: pancreatic polypeptide; PYY: peptide-YY; VRR: vasopressin receptor-related receptor). The Y_3 receptor is the CXCR4 chemokine receptor.

Type	Other names	Main transducer	Potency order
$NPBW_1$	NPBWR1, GPR7	Gi/o	NPB29>NPB23>NPW23>NPW30
$NPBW_2$	NPBWR2, GPR8	Gi/o	NPW23>NPW30>NPB29>NPB23
NPSR1	GPR154, GPRA, VRR1, PGR14, ASRT2	Gs, Gq/11	
Y_1		Gi/o	NPY $\geq$ PYY $\gg$ PP
Y_2		Gi/o	NPY $\geq$ PYY » PP
Y_4		Gi/o	PP > NPY $\sim$ PYY
Y_5		Gi/o	NPY $\geq$ PYY $\geq$ PP
Y_6		Gi/o	NPY $\sim$ PYY > PP

Table 7.81). [165] Neuropeptide-Y heightens the permeability of endothelial monolayers to large molecules via Gi-coupled Y_3 receptor [1050]. Noradrenaline enhances neuropeptide-Y effect via Y_3 receptor subtype on lung vascular permeability [1051]. The PLC–PKC and PI3K pathways may be activated for both permeability and cell proliferation primed by NPY under hypoxia [1050].

7.13.46 Neurotensin Receptors

Neurotensin receptors (NTS_1–NTS_2; Table 7.82) are activated by tridecapeptide neurotensin as well as neuromedin-N. Both ligands of neurotensin receptors derive from a common precursor. Receptors NTS_1 and NTS_2 are high- and low-affinity neurotensin receptors, respectively.

7.13.47 Nicotinic Acid Receptors

Receptors of the nicotinic acid family (GPR81 and niacin receptors NiacR1 [or GPR109a] and NiacR2 [or GPR109b]) are targeted by organic acids, such as

165. Receptors Y_1, Y_2, Y_4, and Y_5 are also designated as NPY1R, NPY2R, PPYR1, and NPY5R, respectively. The Y_3 receptor has not been cloned. However, Y_3 receptor is characterized biologically by its inability to be activated by peptide-YY and an ability to be inhibited by NPY_{3-36} [1050].

Table 7.82. Neurotensin receptors, main G-protein subunit transducers, and ligands (Source: [5]).

Type	Other names	Main transducer	Potency order
NTS_1	NTRH, NTR1	Gq/11	Neurotensin > neuromedin-N
NTS_2	NTRL, NTR2	Gq/11	Neurotensin ∼ neuromedin-N

Table 7.83. Nicotinic acid receptors and their main G-protein subunit transducers (Source: [5]; PUMaG: protein upregulated in macrophages by interferon-γ). Receptors GPR109a and GPR109b have a high and low affinity for nicotinic acid. The organic compound and essential nutrients — nicotinic acid — is also called niacin (hence the alias NiacR), vitamin-B3, and vitamin–PP.

Type	Other names	Main transducer
GPR81	GPR104	Gi/o
GPR109a	NiacR1, HM74a, HM74b, PUMaG	Gi/o
GPR109b	NiacR2, HM74, PUMaG	Gi/o

lipid-lowering nicotinic acid (niacin or vitamin-B3). Niacin is converted to nicotinamide and then nicotinamide adenine dinucleotide (NAD) and dinucleotide phosphate (NADP). Therefore, niacin is a precursor to NAD^+ involved in redox reactions, NADH used as a reducing agent (electron donor), and coenzyme $NADP^+$ and its reduced form NADPH. [166] Receptors GPR109a and GPR109b are activated by submicromolar and millimolar concentrations of nicotinic acid, respectively, whereas GPR81 does not respond to nicotinic acid [5] (Table 7.83).

7.13.48 Opioid and Opioid-like Receptors

Opioid and opioid-like receptors are activated by various peptides, such as endogenous opioid peptide neurotransmitters ^{Met}enkaphalin, ^{Leu}enkephalin [167] and β-endorphin, [168] as well as active peptides released from prodynorphin cleavage by pro-

166. Coenzyme NAD^+ is reduced to NADH in glycolysis and tricarboxylic acid cycle. $NADP^+$ Enzyme cofactor serves as a reducing agent in fatty acid and nucleic acid synthesis.

167. Enkephalins are pentapeptides that contribute to the regulation of nociception. Three different preprohormones contain the enkephalin sequence: preproopiomelanocortin (prePOMC or PPOMC) and preproenkephalin-A and -B (PPEnkA and PPEnkB).

168. β-Endorphin results from the cleavage of proopiomelanocortin that is also the precursor for adrenocorticotrophic hormone. Endorphins (endogenous morphine) are opioid peptides produced by the pituitary gland and the hypothalamus that function as neurotransmitters. The anterior pituitary gland produces the prohormone proopiomelanocortin (POMC). The latter is cleaved into adrenocorticotropin (ACTH) and β-lipotropin. Lipotropin-β is a POMC C-terminal fragment. Lipotropin-β is also involved in lipolysis and steroidogenesis. In particular, hypothalamic histaminergic neurons mediate the ACTH and β-endorphin response to lipopolysaccharides [1052].

protein convertase-2 (α-neodynorphin and dynorphin-A and -B) and prodynorphin partial processing, i.e., big dynorphin, [169] in addition to nociceptin, and tetrapeptides endomorphin-1 and -2 [5]. [170] Endogenous opioids act on plasmalemmal receptors to modulate synaptic transmission.

Precursors of endogenous opioid peptides encompass preprodynorphin (PPDyn, PDyn, or PPEnkB), preproenkephalin (PPEnk or PPEnkA; also PEnk or PEnkA), prepronociceptin (PPNoc or PNoc), and preproopiomelanocortin (PPOMC). They are encoded by different genes (PPDYN/PDYN, PPENK/PENK, PPNOC/PNOC, and PPOMC). [171] Prodynorphin (proDyn or PDyn; a.k.a. neoendorphin–dynorphin and preprodynorphin, and proenkephalin-B) is cleaved by proprotein convertase-2 to generate dynorphin-A and -B and α (β)-neoendorphin [1054]. Proenkephalin (proEnk or PEnk), or proenkephalin-A is cleaved into non-opioid synenkephalin (or proenkephalin$_{(1--70)}$), $^{\text{Met}}$enkaphalin, $^{\text{Leu}}$enkaphalin, $^{\text{Met}}$-enkephalin$^{\text{Arg--Gly--Leu}}$, $^{\text{Met}}$-enkephalin$^{\text{Arg--Phe}}$, proenkephalin$_{(114--133)}$, proenkephalin$_{(143--183)}$, and proenkephalin$_{(237--258)}$. The proenkephalin gene is expressed in neurons, hematopoietic (granulocytes, or polymorphonuclear leukocytes, lymphocytes, mastocytes, monocytes, and macrophages) and reproductive cells. Prepronociceptin gives rise to nociceptin (Noc), [172] as well as nocistatin, prepronociceptin$_{(154--181)}$, prepronociceptin$_{(169--176)}$, Noc2, [173] a nociceptin antagonist in pain transmission, and biologically inactive Noc3 protein. [174] Proopiomelanocortin (POMC) [175] is processed into adrenocorticotropin (ACTH) and β-lipotropin. [176] Lipotropin-β can also be cleaved into smaller peptides, such as γ-lipotropin, β-melanocyte-stimulating hormones (MSH), α- to γ-endorphin, and $^{\text{Met}}$enkephalin (Table 7.84).

Four major types of opioid receptors exist: δ- (or Op_1), κ- (or Op_2), μ- (or Op_3), and nociceptin receptors (or Op_4; Table 7.85). δ-Opioid receptors (δ1–δ2 or DOR1–DOR2) are bound by enkephalins. Dynorphin is the primary endogenous ligand

169. Dynorphin inhibits neurotransmitter release at presynaptic terminals.

170. ενδον: within [ενδογενης: endogenous, born in the house]; Μορφευς: god of sleep and dream. Endomorphins are 2 endogenous opioid peptides of the nucleus of the solitary tract and the periventricular and dorsomedial hypothalamus, especially in hypothalamic histaminergic neurons.

171. Increase of preproopiomelanocortin mRNA in arcuate nucleus and decrease of preprodynorphin mRNA in dentate gyrus of spontaneously hypertensive rats may be associated with the genesis of spontaneous hypertension [1053].

172. Nociceptin is a heptadecapeptide (content 17 amino acid; επτα: 7; δεκα: 10; επτακαιδεκα: 17) that modulates ion channel activity (hence synaptic transmission) and analgesia.

173. The other heptadecapeptide Noc2 corresponds to the immediately downstream segment from nociceptin in the PNoc precursor.

174. Protein Noc3 corresponds to Noc2 with an added motif of 3 arginine residues (Noc2–Arg–Arg–Arg).

175. Proopiomelanocortin (POMC) is a precursor polypeptide that contains 241 amino acid residues. It is synthesized from a precursor, preproopiomelanocortin (prePOMC), that possesses 285-amino acid residues.

176. Hence its other name corticotropin–lipotropin.

Table 7.84. Opioid precursors and products (ACTH: adrenocorticotropic hormone; CLIP: corticotropin-like intermediate peptide; LPH: lipotropin hormones; MSH: melanocyte-stimulating hormones [a.k.a. melanotropins and intermedins]). Endogenous opioid peptides include dynorphins, endomorphins, endorphins, and enkephalins. They share the common N-terminal sequence (opioid motif Tyr–Gly–Gly–Phe–[Met or Leu]). Dynorphins are produced in the hypothalamus, hippocampus, midbrain, medulla, pons, and spinal cord. Endomorphin-1 is widely distributed in the brain and upper brainstem, whereas endomorphin-2 is more prevalent in the spinal cord and lower brainstem. Endorphins (αEnd–γEnd and σEnd) are produced by the hypophysis and hypothalamus. The set of melanocortins includes ACTH, αMSH, βMSH, and γMSH. Proopiomelanocortin, β-lipotropin, corticotropin (ACTH), and corticotropin-like intermediate peptide are secreted by corticotrope cells of the adenohypophysis, or anterior pituitary gland.

Precursor	Opioid peptides
Prodynorphin	Dynorphin-A, dynorphin-B, neoendorphin
Proenkephalin	$^{\text{Met}}$enkaphalin, $^{\text{Leu}}$enkaphalin, $^{\text{Met}}$-enkephalin$^{\text{Arg}--\text{Phe}}$, proenkephalin$_{(114--133)}$, proenkephalin$_{(143--183)}$, proenkephalin$_{(237--258)}$, synenkephalin
Pronociceptin	Nociceptin, nocistatin, Noc2, Noc3, prepronociceptin$_{(154--181)}$, prepronociceptin$_{(169--176)}$
Proopiomelanocortin	γMSH, ACTH, βLPH, αMSH, CLIP, γLPH, βMSH, β-endorphin, $^{\text{Met}}$enkaphalin
Corticotropin	
β-Lipotropin	
	Endomorphin-1, endomorphin-2

of κ-opioid receptors (κ1–κ3 or KOR1–KOR3). κ-Opioid receptors can associate with $Ca_V2.2$ channels [1055] and G-protein-linked inward rectifier potassium channel GIRK1 ($K_{IR}3.1$) [1056]. Pre- and postsynaptic μ-opioid receptors (μ1–μ3 or MOR1–MOR3) typically inhibit neurotransmitter release.

β-Endorphin has its highest affinity for μ1-, slightly lower affinity for μ2- and δ-, and low affinity for κ1-opioid receptors. Endomorphin-1 and -2 have the highest known affinity for μ-opioid receptors. [177]

Nociceptin, or orphanin-FQ (OFQ), is an opioid-related peptide that derives from prepronociceptin (PPNoc). Nociceptin binds to nociceptin receptor (a.k.a. κ3-related

177. In the mouse pons and medulla, G-protein activation by endomorphin-1 and -2 is mediated by both MOR1 and MOR2 receptors and that by β-endorphin via MOR2, but not by MOR1 receptors [1058].

Table 7.85. Opioid and opioid-like receptors, main G-protein subunit transducers, and ligands (Sources: [5, 1057]; DOR, KOR, MOR: δ-, κ-, μ-opioid receptor; NOP: nociceptin receptors; TyrMIF: Tyr-Pro-Leu-Gly-NH_2; TyrWMIF: Tyr-Pro-Trp-Gly-NH_2). Endomorphin-1 and -2 are endogenous opioid peptides highly selective for Op_3 receptor, whereas β-endorphin, another endogenous opioid peptide, targets Op_1 and Op_3 receptors. In general, endogenous opioid receptor ligands modulate effects of various neurotransmitters (acetylcholine, dopamine, noradrenaline, and serotonin) and neurohormones (oxytocin and vasopressin). The majority of opioid peptides undergo a rapid extracellular degradation mainly by integral membrane exo- and endopeptidases. Enkephalin is a pentapeptide derived from preproopiomelanocortin and preproenkephalin- and -B (^{Met}enkaphalin: Tyr-Gly-Gly-Phe-Met [Met in position 5]; ^{Leu}Met-enkephalin: Tyr-Gly-Gly-Phe-Leu [Leu in position 5]).

Type	Other Aliases	Main Transducer	Ligands (endogenous opioids)
Op_1	OpRδ, DOR	Gi/o	[Met^5]/[Leu^5]enkephalins, Deltorphin-1/2
Op_2	OpRκ, KOR	Gi/o	Dynorphin-A/B, dynorphin-$A_{(1-8)}$,
Op_3	OpRμ, MOR	Gi/o	Endomorphin-1/2, β-endorphin, β-Casomorphins-5/7, hemorphins-4/7 morphiceptin, Tyr-MIF-1, Tyr-W-MIF-1
Op_4	NOP	Gi/o	Nociceptin (orphanin-FQ)

opioid receptor KORL3, ORL1, OpRL1, OOR, NOCIR, and NOP). This receptor is considered as opioid-related rather than opioid receptor, because, despite its structural homology with conventional opioid receptors, it exhibits a distinct pharmacology. Nociceptin and its receptor NOP lodge in the central and peripheral nervous system, where they modulate nociception [1057]. Nociceptin is, indeed, a potent anti-analgesic.

Exogenous nociceptin decreases blood pressure and heart rate via OP_4 receptor in rodents. In the cardiovascular system, OP_4 receptor localizes to sensory afferent fibres, the nucleus tractus solitarius, and rostral ventrolateral medulla, on preganglionic and/or postganglionic sympathetic and parasympathetic nerve fibers innervating blood vessels and heart, as well as in these target organs [1059].

Opioid receptor-δ that belongs to the B-group of rhodopsin subfamily of GPCRs is widely distributed throughout the central nervous system. Opioid Op_1 receptor has also been detected in peripheral tissues (cochlea, cornea, eyelid, and lip, as well as skin fibroblast-like cells, monocytes and T and B lymphocytes [1060]. Endorphins and enkephalins have the highest affinity for Op_1 receptor. The Op_1 receptor can form homodimers as well as heterodimers with other opioid receptors and GPCRs (α2a- and β2-adrenergic receptors, CCR5, CXCR2, and CXCR4 chemokine receptors, and sensory neuron-specific receptor SNSR4) [1060]. Agonist-induced monomerization process may be required for receptor endocytosis. It is not only coupled to

Table 7.86. Orexin receptors, main G-protein subunit transducers, and ligands (Source: [5]).

Type	Main transducer	Potency order
OX_1	Gq/11	Orexin-A > orexin-B
OX_2	Gq/11	Orexin-A ∼ orexin-B

Table 7.87. Parathyroid hormone receptors, main G-protein subunit transducers, and ligands (Source: [5]).

Type	Main transducer	Potency order
PTH_1	Gs, Gq/11	PTH ∼ PTHRP
PTH_2	Gs, Gq/11	PTH ≫ PTHRP

Gi, but also Gz and G16 subunits [1060]. Coupled to Gi, it inhibits adenylate cyclase and Ca^{++} channels. Coupled to Gz, it also inhibit adenylate cyclase, but stimulates phospholipase-C and activates the extracellular signal-regulated kinases ERK1 and ERK2. Coupled to G16, it also stimulates phospholipase-C. In addition, it can regulates P38MAPKs, phospholipase-D2, voltage-gated sodium $Na_V1.7$ channel, and potassium channels. Furthermore, it intervenes in signaling cascades stimulated by kinases PKB, Src, Raf1 (MAP3K), and receptor Tyr kinases as well as small GTPases CDC42, Rac, and Ras [1060]. The Op_1 receptor can be palmitoylated, glycosylated, phosphorylated (by GRKs and protein kinase-C), and ubiquitinated.

Opioid receptor-κcan regulate the Na^+–H^+ exchange via agonist-induced interactions with Na^+–H^+ exchanger regulatory factor NHERF1 that thus confers cell type-specific signaling to this GPCR. Stimulation of KOR, indeed, activates Na^+–H^+ exchanger NHE3 in cell lines that express high levels of NHERF1, but not other cell types [835].

7.13.49 Orexin Receptors

Orexin receptors are activated by orexin-A and -B [178] that derive from a common precursor, the preproorexin, by proteolytic cleavage [5] (Table 7.86).

7.13.50 Parathyroid Hormone Receptors

Receptors PTH_1 (or PTH1R) and PTH_2 (or PTH2R; Table 7.87) are activated by parathyroid hormone (PTH) and parathyroid hormone-related peptide (PTHRP) as well as related peptides ($PTHRP_{(1--36)}$, $PTHRP_{(38--94)}$, and osteostatin) [5]. Parathyroid hormone regulates calcium homeostasis and bone metabolism via type-1 PTH receptor.

Binding of PTH to PTH_1 (or PTH1R) activates protein kinase-A and -C. Signaling initiated by PTH terminates by PTH_1 endocytosis triggered by β-arrestins. The

178. A.k.a. hypocretin-1 and -2.

PTH_1 receptor interacts with Na^+–H^+ exchanger regulatory factor NHERF2 in cells that contain high NHERF levels, such as endothelial cells. In the latter, PTH_1 signals mainly via Gαq and PLCβ, whereas in osteoblasts that do not express detectable levels of NHERFs, PTH_1 transduces signals mainly via Gαs subunit and adenylate cyclase [835]. Scaffold NHERF not only binds to PTH_1, but also phospholipase-Cβ, protein kinase-C, and transient receptor potential channels that can then reside in close proximity to PTH_1 receptor.

The PTH_1 receptor also associates with type-2 transforming growth factor-β receptor TβR2 and forms an endocytic complex in response to PTH [1061]. The TβR2 kinase phosphorylates the PTH_1 cytoplasmic domain to promote PTH-induced endocytosis of the PTH_1–TβR2–Arrβ2 complex.

7.13.51 Platelet-Activating Factor Receptor

Platelet-activating factor (PAF, alkyl acetyl-glycerophosphocholine) is a phospholipid mediator that binds to a single G-protein-coupled receptor — PAFR — to activate multiple signaling pathways via Gq/11 and Gi/o family subunits of guanine nucleotide-binding proteins (Table 7.92). The PAFR receptor may also be activated by lysophosphatidylcholine, oxidized phosphatidylcholine, as well as bacterial lipopolysaccharide [5].

Platelet-activating factor receptor can associate with members of the Janus kinase family of cytosolic protein Tyr kinases such as the TyK2–JaK2 complex composed of tyrosine kinase-2 and Janus kinase-2.

7.13.52 Prokineticin Receptors

Two prokineticin receptors (PK_1–PK_2 or PKR1–PKR2) are activated by prokineticin-1 (PK1) [179] and -2 (PK2) [180] (Table 7.88). [181] Prokineticins are widespread. In the cardiovascular system, PK_1 is observed in cardiomyocytes, endothelial cells, and epicardial-derived progenitor cells, [182] but not in vascular smooth muscle cells.

Prokineticins potently contract gastrointestinal smooth muscle cells and favor angiogenesis, hence cardiomyocyte survival [1062], among other functions (Table 7.89). In mouse hearts, PK_1 receptor upregulates prokineticins that, in turn, acts

179. Prokineticin-1 was originally named endocrine gland-derived vascular endothelial growth factor (egVEGF). It is also termed mambakine.

180. A.k.a. Bv8.

181. Initially, the peptide hormones prokineticins (PK1–PK2) were identified in the gastrointestinal tract as potent constrictors. They were investigated mostly in the context of angiogenesis in the digestive and reproductive tracts. Subtype PK2 exerts an auto- and paracrine control via PK_1 in the heart and kidney.

182. The actin regulator thymosin-β4 that assists wound repair is able to activate epicardial-derived progenitor cells in adults that then differentiate into endothelial cells, smooth muscle cells, and fibroblasts. Prokineticin-2 is also capable of stimulating these progenitors that then differentiate into endothelial and smooth muscle cells, but not fibroblasts.

Table 7.88. Prokineticin receptors, main G-protein subunit transducers, and ligands (Source: [5]; ProK: prokineticin). Receptor PK_2 predominates in the adult brain, whereas PK_1 is widely distributed in peripheral organs.

Type	Other names	Main transducer	Potency order
PK_1	PKR1, PROKR1, GPR73a, ZAQ	Gq/11	ProK2 $\geq$ ProK1
PK_2	PKR2, PROKR2, GPR73b, GPR73L1, GPRg2, KAL3	Gq/11	ProK2 $\geq$ ProK1

Table 7.89. Examples of effects of prokineticins (PK1–PK2; Source: [1063]). Signaling from PK_1 receptor is required for cardiomyocyte survival and angiogenesis. This receptor is involved in postnatal cardiac and renal vascularization, as it activates organ-specific progenitor cells.

Role	Target cell types or framework
Cell survival	Cardiomyocytes, endothelial cells, hematopoietic cells, neurons
Cell motility	Hematopoiesis, vasculo-, angio-, neurogenesis
Cell excitability	Circadian rhythm
Behavior	Feeding, drinking

as paracrine factors to promote the differentiation of transcription factor TCF21+ [183] progenitor cells into a vasculogenic cell type [1063]. In isolated renal TCF21+ progenitors, PK2 binds to PK_1 and provokes the differentiation of these progenitors into endothelial and smooth muscle cells.

In the reproductive tract, PK1 production rises upon stimulation by estrogen, progesterone, and human chorionic gonadotrophin, as well as hypoxia-inducible factor-1α [1063]. In neurons of the olfactory bulb, PK2 synthesis is primed by basic helix–loop–helix (bHLH) transcription factors neurogenin-1 (NeuroG1 or bHLHa6) and achaete–scute complex-like factor ASCL1 (or bHLHa46) [184] and repressed by transcriptional factors distal-less homeobox DLx1 and DLx2.

Prokineticins can cause differentiation of human bone marrow cells into the monocyte–macrophage lineage, monocyte proliferation and differentiation, and macrophage migration. In monocytes and neutrophils, PK2 is upregulated in response to granulocyte colony-stimulating factor (CSF3).

183. Transcription factor TCF21 is also called epicardin, capsulin, class-A basic helix–loop–helix protein bHLHa23, and podocyte-expressed protein Pod1.

184. A.k.a. mammalian achaete–scute homolog mASH1. The achaete–scute complex is a set of 4 genes (achaete, scute, lethal of scute, and asense) in Drosophila melanogaster that encode basic helix–loop–helix transcription factors.

Prokineticin receptors activate many signaling pathways and mobilize calcium messenger. Activated PKRs stimulate PKB, MAPK modules, and the PP3–NFAT axis, as well as phosphoinositol turnover [1063]. In addition, they reduce the production of zonula occludens-1 of tight junctions and can activate protein kinase-C and subsequently transient receptor potential vanilloid TRPV1 channel.

Hepatic sinusoidal endothelial cells express only PK_2 receptor. In coronary endothelial cells, PK_1 predominates over PK_2 [1064]. Activated PK_1 that colocalizes with $G\alpha_{11}$ primes mitogen-activated protein kinases and protein kinase-B signaling to stimulate cell proliferation and migration for angiogenesis. Once stimulated by prokineticin-2, PK_1 undergoes endocytosis. On the other hand, prokineticins also cause fenestrations in endothelia [1064]. When PK_2 predominates over PK_1 in coronary endothelial cells, these cells contain a large number of multivesicular bodies and caveolar clusters. Prokineticin-2 activates PK_2 associated with $G\alpha_{12}$ subunit. The latter binds to tight junction protein zonula occludens-1 and trigger its degradation.

7.13.53 Prostanoid Receptors

Prostanoid receptors are activated by various types of prostaglandins, i.e., PGD2, PGE2, PGF2α, PGH2, PGI2 (prostacyclin), and thromboxane-A2 [185] (TxA2; Table 7.90). Inflammatory cytokines stimulate the production from arachidonic acid of lipid mediators, such as prostanoids. Rate-limiting enzymes in prostanoid production include cyclooxygenases, [186] in particular constitutively expressed COx1 and inducible COx2. Cyclooxygenase COx2 induced in macrophages and endothelial cells by inflammation stimuli, such as oxidized low-density lipoprotein and interleukin-1, produces eicosanoids that enhance vascular permeability and promote cell chemotaxis.

The effects of prostacyclin and prostaglandin-E2 are transduced by the I prostanoid receptor (IP) and E prostanoid receptors (EP), respectively (Table 7.90; Vol. 5 – Chap. 7. Vessel Wall). Receptors EP_2 and EP_4 are linked to Gs that activates adenylate cyclase, whereas EP_1 and EP_3 are coupled to Gq and/or Gi subunits. Receptors EP_1 and EP_3 mediate PGE2-induced vasoconstriction. Prostaglandin PGE2 can activate platelets by EP_3. Receptor EP_4 mediates anti-inflammatory effects and activation of MMP9 metallopeptidase.

Deletion of EP_2 and IP causes salt-sensitive hypertension. In cardiomyocytes, dominant COx2-derived products are prostacyclin and prostaglandin-E2 that target I prostanoid and E prostanoid EP_2 receptor, respectively. Both PGI2 and PGE2 serves as mediators of cardioprotection. In mice, COx2-deficient cardiomyocytes have impaired ventricular function subsequent to pressure overload [1066].

Although prostanoid receptors are expressed in many of the body's tissues, the distribution of prostanoid receptor types and cell identities that produce each receptor

185. Thromboxanes of the eicosanoid superfamily include thromboxane-A2 and -B2. Thromboxane-A synthase that in platelets converts prostaglandin-H2 to thromboxane. Thromboxane-B2 is an inactive metabolite of thromboxane-A2.

186. There are 3 isoforms of cyclooxygenases, COx1, COx2, and COx3.

Table 7.90. Prostanoid receptors, their main targeted G proteins, and order of ligand potency (Sources: [736, 1065]).

Type	Main transducer	Potency order
DP_1	Gs	PGD2 $\gg$ PGE2 > PGF2α > PGI2, TxA2
DP_2	Gi/o	PGD2 $\gg$ PGE2, PGF2α > PGI2, TxA2
EP_1	Gq/11	PGE2 > PGF2α, PGI2 > PGD2, TxA2
EP_2	Gs	PGE2 > PGF2α, PGI2 > PGD2, TxA2
EP_3	Gi/o	PGE2 > PGF2α, PGI2 > PGD2, TxA2
EP_{3A}	Gi	
EP_{3B}	Gs	
EP_{3C}	Gs	
EP_{3D}	Gi, Gs, Gq	
EP_4	Gs	PGE2 > PGF2α, PGI2 > PGD2, TxA2
FP	Gq/11	PGF2α > PGD2 > PGE2 > PGI2, TxA2
IP	Gs, Gq	PGI2 $\gg$ PGD2, PGE2, PGF2α > TxA2
TP	Gq/11	TxA2, PGH2 $\gg$ PGD2, PGE2, PGF2α, PGI2
TPα	Gq, Gi	
TPβ	Gq, Gs	

Table 7.91. Distribution of prostanoid receptors (Source: [1065]).

Type	Distribution
DP	Low levels in humans (least abundant among prostanoid receptors) Small intestine and leptomeninges
EP_1	Kidney, lung, stomach, thalamus
EP_2	Least abundant among EP receptors
EP_3	Widely distributed throughout the body
EP_4	Widely distributed throughout the body
FP	Corpus luteum, kidney, heart, lung, and stomach
IP	Neurons of dorsal root ganglion, megakaryocytes, Vascular smooth muscle cells, mature thymocytes, splenic lymphocyes
TP	Abundant in lung, kidney, and heart, Lymphoid organs (thymus and spleen) Platelets

type is difficult to determine, as prostanoid receptors are expressed at a relatively low level (Table 7.91). A given cell can synthesize several types of prostanoid receptors.

Prostaglandin-D2 is produced by cyclooxygenase-2 that is inducible by mitogens, cytokines, and tumor promoters in activated mast cells, macrophages, and TH2 cells. Prostaglandin-D2 targets receptor DP that is highly expressed by tumor endothelial cells to suppress vascular hyperpermeability via an increase in intracellular cAMP production in tumors [1067]. On the other hand, PGE2 promotes tumor growth and angiogenesis.

Prostaglandins PGI2 and TxA2 impede and cause platelet activation and aggregation, respectively. The balance between PGI2 and TxA2 prevents thrombosis and vasospasm, but maintains the body ready to perform efficient hemostasis.

7.13.53.1 Prostaglandins and the Vasomotor Tone

Prostaglandins PGI2 and TxA2 are a potent vasodilator and vasoconstrictor, respectively. They abound in vascular endothelial cells and platelets, respectively. However, PGI2 and its main *IP receptor* are less potent regulators of blood circulation than endothelium-derived vasorelaxant nitric oxide.

Prostaglandin-E2 produced by cyclooxygenase-2 can promote relaxation of vascular smooth muscle cells. Prostaglandin PGE2 is synthesized in response to a high-salt diet and works via relaxant EP_2 receptor. Its dysfunction elicits salt-sensitive hypertension. Prostaglandin-E2 EP_1 receptor impairs Na^{+}–Ca^{++} exchanges and leads to neurotoxicity [1068]. Membrane-associated proteins involved in eicosanoid and glutathione metabolism (MAPEG) affect the expression of endogenous PGE2 and PGI2. Microsomal prostaglandin-E synthase-1 (mPGES1), a member of the MAPEG family, is a major source of PGE2 formation. Deletion of mPGES1 does not increase blood pressure and retards atherogenesis, whereas mPGES1-derived PGE2 accelerates atherogenesis [1069]. On the other hand, prostacyclin protects the cardiovascular function.

In pulmonary arteries, chronic hypoxia increases cyclooxygenase COx2 expression and PGF2α release that activates thromboxane *TP receptors* [1070]. In pulmonary vascular smooth muscle cells, hypoxia upregulates COx2 expression, thereby increasing production of vasorelaxant (PGI2, PGE2, and PGD2) and vasoconstrictors (PGF2α). The COx1 enzyme, the major TxA2 source, is produced in all wall layers of pulmonary arteries. Its expression remains unaltered in chronic hypoxia. TxA2 synthase that is mainly located in the media of pulmonary arteries is markedly downregulated in chronic hypoxia. In addition, COx enzymes are potential sources of reactive oxygen species in pulmonary arteries, especially in pulmonary arterial hypertension associated with hypoxia. Prostaglandin-F2α activates TP receptors to generate vasocontriction of pulmonary arteries. Whereas cyclooxygenase-catalyzed metabolism of arachidonic acid leads to an endothelium- and COx1-dependent relaxation of pulmonary arteries in normoxia, it causes an endothelium-independent, COx2- and TP receptor-dependent vasocontriction in hypoxia [1070].

7.13.53.2 Prostaglandins in Heart

In cardiomyocytes, prostaglandins target prostanoid F-receptor (FP) that increases myofilament sensitivity to Ca^{++} by enhancing myosin light chain-2 phosphorylation. Prostanoid F-receptor is coupled to Gq/11 protein that leads to production of inositol trisphosphate and diacylglycerol. This second messenger activates PKC, but its effect extends beyond this classical pathway, possiby via G12/13 protein [1071]. Positive inotropic effect of Gq/11-protein-coupled receptors (e.g., α1-adrenoceptor, $5HT_{2A}$, and endothelin-1 receptors) results, at least partly, from myofilament Ca^{++} sensitization by increased MLC2 phosphorylation. The level of

Table 7.92. Peptidase-activated and platelet-activating factor receptors and their main targeted G proteins (Source: [736]).

Type	Main transducer
Peptidase-activated receptors	
PAR_1	Gq/11, Gi/o, G12/13
$PAR_{2/3/4}$	Gq/11, Gi/o
Platelet-activating factor receptor	
PAFR	Gq/11, Gi/o

MLC2 phosphorylation is regulated by both Ca^{++}–calmodulin-dependent activation of myosin light chain kinase and Rho kinase-mediated inhibition of myosin light chain phosphatase. On the other hand, slow positive inotropic effect of receptor FP results from increased phosphorylation of myosin phosphatase targeting subunit MyPT2 and myosin light chain MLC2 by stimulating the RhoA–RoCK pathway, hence decreasing MLCP activity, and activating MLCK, respectively.

7.13.54 Tissue Factor and Peptidase-Activated Receptors

Peptidase-activated receptors (PAR_1–PAR_4; Table 7.92) are highly expressed in platelets, but also in endothelial cells, myocytes, and neurons. Peptidase-activated receptors are activated by proteolytic cleavage of their N-terminal exodomains by serine peptidases.

Among serine peptidases, *thrombin* and *trypsin* can be agonist peptidases for certain peptidase-activated receptors in vivo.[187] Alternative endogenous peptidases or ligands to thrombin for PAR_1, PAR_3, and PAR_4 exist. Several peptidases, such as *cathepsin-G* and *chymotrypsin*, inhibit PAR_1 receptor, as they prevent activation by thrombin [5]. Activated *protein-C*,[188] an anticoagulant peptidase that elicits an anti-inflammatory response and protects against endothelial barrier dysfunction caused by thrombin, also activates PAR_1 receptor. Therefore, PAR_1 is equipped for peptidase-selective signaling. *Tissue factor* can also bind peptidase-activated receptor (Table 7.92). Peptidase-activated receptor PAR_1 is activated by both thrombin and PAR_1-*activating peptide* (PAR_1-AP). Like PAR_1, PAR_2 on smooth muscle and respiratory epithelial cells can be activated by tryptase, trypsin, and PAR_2-*activating peptide* (PAR_2-AP).

187. Thrombin is produced from prothrombin by factor Xa to mediate the formation of fibrin from fibrinogen that forms the fibrous matrix of blood clots. Thrombin is also a strong activator of platelet aggregation. Moreover, thrombin acts on various cell types, such as monocytes, smooth muscle cells, endothelial cells, fibroblasts, and lymphocytes, among others [1072].

188. Protein-C on the endothelial cell surface is activated by the thrombin–thrombomodulin complex. Activated protein-C binds to endothelial protein-C receptor (EPCR) and cleaves (inactivates) clotting factors Va and VIIa, thereby reducing thrombin generation. Moreover, it provokes cellular responses via PAR_1 receptor.

Table 7.93. Human peptidase-activated receptors and their activators and loci (Source: [1073]).

	Primary activator peptidase	Localization
PAR_1	Thrombin	Platelets, endothelial and epithelial cells, fibroblasts, myocytes, neurons, astrocytes
PAR_2	Trypsin Tryptase	Endothelial and epithelial cells, Fibroblasts, myocytes, neurons, astrocytes
PAR_3	Thrombin	Endothelial cells, myocytes, astrocytes
PAR_4	Thrombin, Trypsin	Platelets, endothelial cells, Myocytes, astrocytes

Four known peptidase-activated receptors are expressed in the entire body (Table 7.93). Thrombin targets mainly PAR_1, PAR_3, and PAR_4, and trypsin chiefly activates PAR_2 (Tables 7.94 and 7.95). Most PARs act via Gi, G12/13 (Ras–Raf pathway), and Gq (PLC–DAG–PKC and PLC–IP_3–Ca^{++} axes) signaling (Table 7.96). Peptidase-activated receptors participate in the regulation of the vasomotor tone and operate in inflammation, muscle growth, and bone cell differentiation and proliferation. Sorting nexin-1 links to peptidase-activated receptor PAR_1 to favor lysosomal path among endocytosis routes.

7.13.54.1 Thrombin and PAR Receptors

Thrombin facilitates production and release of growth factors, such as PDGF TGFβ, and ET1, as well as formation of VEGF receptors. Activated PAR_1 participates in synthesis of extracellular factors that contribute to wound healing. Thrombin stimulates procollagen synthesis by smooth muscle cells and pulmonary fibroblasts. It also regulates the release of matrix metallopeptidases, such as MMP1, MMP2, MMP3, and progelatinase-A.

PAR_1 Receptor and prothrombin are expressed in numerous regions of the central nervous system, such as thalamus, hypothalamus, cortex, and cerebellum. Thrombin causes increased synthesis of nerve growth factor and endothelin-1. However, in some circumstances, thrombin induces cell death.

Thrombin provokes endothelial-dependent relaxation of smooth muscle cells of aortic and coronary arteries via prostacyclin and by nitric oxide. However, in certain territories, it generates vasoconstriction via an increase in intracellular calcium concentration [1072]. Relative expression and function of PAR_1 in endothelial and smooth muscle cells in different vessels can explain the difference in effect on the vasomotor tone.

7.13.54.2 PAR Receptors and the Vasomotor Tone

Peptidase-activated receptors PAR_1 and PAR_2 modulate the endothelium-dependent regulation of the vasomotor tone in arteries and veins, such as internal mam-

Table 7.94. Cellular distribution and effects of PAR_1 (Source: [1072]; GEC: glomerular epithelial cells; GMC: glomerular mesangial cells; SMC: smooth muscle cell; IL: interleukin; TNFα: tumor-necrosis factor-α; gmCSF: granulocyte-macrophage colony-stimulating factor; IgCAM: immunoglobulin-like cell adhesion molecule).

Cell	Effects
Platelets	Degranulation; ↑ aggregation; ↑ TxA2
Vascular SMC	Vasorelaxation or contraction; mitogenesis
Endothelial cell	↑ Release of von Willebrand factor, NO, ↑ expression of selectins and IgCAMs, proliferation
Airway wall cells	↑ Release of procollagen, PDGF, prostanoids, relaxation or contraction
Osteoblasts	Proliferation
Synovial fibroblasts	↑ proliferation; ↑ IL6 and gmCSF
Keratinocytes	↑ proliferation; inhibition of differentiation
Monocytes, T cells	↑ IL1, IL6, TNFα, LProliferation
Mastocytes	Degranulation
Intestinal SMC	Relaxation or contraction
Stomachal SMC	Relaxation or contraction
Skeletal myocytes	↑ $[Ca^{++}]_i$
GEC, GMC	↑ Production of clusterin and TGFβ
neuron, glial cell	↑ Proliferation; neuronal apoptosis
astrocyte	Proliferation

mary artery and greater saphenous vein that are used in coronary artery bypass grafting. Amounts of PAR_1 and PAR_2 are similar in both vessel types [1074]. Although selective PAR_2-activating peptide (PAR_2-AP) fails to induce vasorelaxation, PAR_1-activating peptide (PAR_1-AP) generates vasodilation. The endothelium-dependent relaxation is greater in internal mammary artery than in greater saphenous vein. In addition, inflammatory stimuli such as TNFα enhance endothelium-dependent relaxation selectively via PAR_2-AP in internal mammary artery.

Trypsin and peptidase-activated receptor-2-activating peptides, via Ca^{++} mobilization, endothelin receptor ET_{B1}, and subsequent activation of endothelial NO synthase (NOS3), as well as via prostacyclin, are able to cause in vitro an endothelium-dependent (nitric oxide-mediated) relaxation of vascular smooth muscle cells. On the other hand, trypsin and high concentrations of PAR_2-activating peptides can also initiate endothelium-dependent contraction in both rat pulmonary artery and human umbilical vein [1072]. Whereas endothelium-dependent vasodilation initiated by PAR_2-APs is due to PAR2 activation, endothelium-independent contraction primed by PAR_2-APs is done via a receptor different than PAR_2 [1075].

Table 7.95. Cellular distribution and effects of PAR_2 (Source: [1072]; GEC: glomerular epithelial cells; GMC: glomerular mesangial cells; SMC: smooth muscle cell; IL: interleukin; TNFα: tumor-necrosis factor-α; gmCSF: granulocyte-macrophage colony-stimulating factor; IgCAM: immunoglobulin-like cell adhesion molecule).

Cell	Effects
Vascular SMC	Vasorelaxation or contraction; mitogenesis
Endothelial cell	↑ Release of von Willebrand factor, NO
Airway wall cells	↑ PGE2 release, relaxation or contraction, proliferation
Osteoblasts	↑ $[Ca^{++}]_i$
Keratinocytes	Inhibition of differentiation and proliferation, ↑ IL6 and gmCSF
Leukocytes, mastocytes	↑ Adhesion, rolling, and migration
Salivary gland cells	↑ Saliva production, ↑ amylase and mucin secretion
Gallbladder SMC	Contraction, prostanoid release
Intestinal SMC	Contraction, PGE2 release
Stomachal SMC	Contraction
Skeletal myocytes	↑ $[Ca^{++}]_i$
Kidney cortex cells	↑ Cl^- secretion
Ureter sphincters	↓ Beating
Neurons, glial cells, astrocytes	↑ $[Ca^{++}]_i$

7.13.54.3 PAR_1

PAR_1 Receptor and Heterotrimeric G Protein

The PAR_1 receptor interacts with Gi/o, Gq/11, and G12/13. The Gi subunit inhibits cAMP and triggers various effects via its activated partner Gβγ dimer. The Gq/11 subunit stimulates phospholipase-C that hydrolyzes phosphoinositides into IP_3 and DAG that increases the cytosolic calcium content and activates protein kinase-C, respectively. Subunits of the G12/13 family activate the RhoGEF–Rho–RoCK pathway for cell remodeling and migration, as well as JNK using small Ras or Rac GTPases.

Thrombin also stimulates hydrolysis of other phospholipids via PLA2, PLC, and PLD. The PAR_1 receptor activates Ras via Gi and Tyr kinases after recruitment of adaptors SHC and GRB2, SOS, and Raf1 to the cell cortex then extracellular-regulated kinase. The Gβγ subunit activates phosphatidylinositol 3-kinase.

PAR_1 Receptor and Peptidase-Selective Signaling

Both activated protein-C and thrombin target PAR_1, but have antagonistic effects, especially on blood coagulation, endothelial barrier permeability, and inflammation.

Table 7.96. Signaling pathways activated by PAR_1 in platelets (Source: [1073]; DAG: diacylglycerol; GEF: guanine nucleotide-exchange factor; GRK: G-protein-coupled receptor kinase; IP_3: inositol trisphosphate; NRTK: non-receptor tyrosine kinase; PI3K: phosphatidylinositol 3-kinase; PKC: prtoein kinase-C; PLC: phospholipase-C; RoCK: Rho-associated, coiled-coil-containing protein kinase).

G Protein subunit	Pathways (effects)
Gαi/z	RhoGEF–Rho–RoCK
Gαq	PLCβ–IP_3–Ca^{++}
	PLCβ–DAG–PKC
	(granule secretion)
	(activation of Ca^{++}-regulated kinases and phosphatases)
	(activation of RTKs and RasGEFs)
	(growth factor shedding)
Gα12/13	ACase Inhibition
Gβγ	PI3K
	PLCβ
	K^+ channels
	GRK
	NRTK
	(Recruitment to plasma membrane of kinases, GEFs, and scaffold proteins)

Thrombin excites small GTPase RhoA-associated signaling without Rac1 activation, whereas activated protein-C stimulates small GTPase Rac1, but not RhoA GTPase. Peptidase-selective signaling via PAR_1 relies on caveolin-1, hence PAR_1 compartmentation, as activated protein-C requires caveolin-1, but not thrombin [1076]. Activated protein-C cofactor endothelial protein-C receptor, PAR_1, Gq and Gi subunits are partitioned into caveolin-1-containing membrane rafts (caveolae). Signal transduction mediated by Gi/o family subunits protects endothelial barrier. In addition, activated protein-C causes PAR_1 phosphorylation. It also desensitizes endothelial cells to thrombin. Moreover, it limits receptor proteolysis, its endocytosis, and degradation, even after prolonged exposure to activated protein-C.

7.13.54.4 PAR_2

The PAR_2 receptor on both endothelial cells and leukocytes intervenes in inflammation. Trypsin and PAR2-activating peptides stimulate activation of T lymphocytes and neutrophils, and promote leukocyte recruitment (rolling, adhesion, and extravasation) by a mechanism dependent on platelet-activating factor release [1077].

7.13.54.5 PAR_3 and PAR_4

The PAR_3 receptor resides in heart, small intestine, and bone marrow, among other organs, especially on airway smooth muscle cells, vascular endothelial cells,

and astrocytes. In mouse platelets, PAR_3 expression is necessary for full activation by thrombin. Tissue distribution of PAR_4 differs from that of other PARs, with the highest levels in the lung, small intestine, pancreas, thyroid, and testis. The PAR_4 receptor operates as a low-affinity thrombin receptor.

7.13.54.6 Tissue Factor in Coagulation, Inflammation, and Angiogenesis

Tissue factor can have 2 distinct structural and functional modes, as it is an initiator of blood clotting, [189] or a cofactor for cell signaling that is unable to promote coagulation. [190] In other words, plasmalemmal tissue factor binds to: (1) serine peptidase factor VIIa to activate coagulation or (2) peptidase-activated receptor-2 to trigger inflammation and angiogenesis, as tissue factor belongs to the cytokine receptor family. [191] Tissue factor–PAR_2 complex formation can be inhibited without preventing the coagulation activity of tissue factor.

Protein disulfide isomerase (PDI) [192] stabilizes a distinct tissue factor–factor VIIa complex that does not bind factor X [1078]. PDI inhibits the coagulation activity of the tissue factor, and switches tissue factor to cell signaling. [193]

Inflammation and coagulation initiated by tissue factor are coupled by a crosstalk between peptidase-activated receptor-1 and sphingosine 1-phosphate receptor-3, at least in dendritic cells [1079]. Signaling from PAR_1 is also coupled to S1P in endothelial cells. Dendritic cells in the lymphatics can cause exacerbated inflammation and coagulation in deregulated innate immune responses such as disseminated intravascular coagulation. Block of PAR_1 is then sufficient to interrupt systemic dissemination of inflammation and coagulation.

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189. Tissue factor, an integral membrane protein, is normally excluded from the endothelial wetted surface. Tissue factor also circulates and becomes active only with a growing thrombus. Coagulation (Vol. 5 – Chap. 9. Endothelium) is triggered when tissue factor is exposed to zymogen coagulation peptidases in plasma after vessel wall damage. It binds and activates factor VII. The complex made of coagulant tissue factor and factor VIIa binds and activates factor X. Factor Xa acts with its cellular cofactor Va for thrombin production. Thrombin cleaves fibrinogen to generate fibrin and causes platelet aggregation.

190. Tissue factor is also activated in inflammation, vascular development, and cancer.

191. The non-coagulant form of tissue factor bound to factor VIIa (binary signaling complex) activates receptor PAR_2. Factor Xa signals via peptidase-activated receptors PAR_1 and PAR_2 either as a monomer or as a ternary complex with tissue factor and factor VIIa. Factor Xa is inhibited by antithrombin-3 and tissue factor pathway inhibitor. Factor VIIa is also inhibited by tissue factor pathway inhibitor bound to factor Xa. Thrombin is a potent activator of peptidase-activated receptors, except PAR_2 receptor. It activates PAR_1 and PAR_4 for platelet aggregation. Like Factor Xa, thrombin is inhibited by antithrombin-3. Thrombomodulin hampers the binding of thrombin with PAR_1 on the endothelial cell surface.

192. Protein disulfide isomerase cleaves disulfide bonds in the extracellular domains of certain receptors to regulate protein activity. Protein disulfide isomerase breaks a disulfide bond that is required to activate the coagulation. Nitric oxide regulates PDI activity, hence, suppresses the coagulant activity of tissue factor.

193. Disulfide/thiol exchange is required for the formation of Tissue factor–PAR_2 complex.

Table 7.97. Receptors of the relaxin family peptides, main G-protein subunit transducers, and ligands (Source: [5]; InsL*i*R: insulin-like peptide type-*i* receptor; LGR: leucine-rich repeat-containing G-protein-coupled receptor; Rln3R: relaxin-3 receptor; SALPR: somatostatin and angiotensin-like peptide receptor). The $G\alpha_{oB}$ subunit is a member of the $G\alpha_{i/o}$ family (with $G\alpha_{oA}$, $G\alpha_{i1}$, α_{i2}, α_{i3}, $G\alpha_{Tr}$, and $G\alpha_{z}$).

Type	Other names	Main transducer	Ligands Potency order
$RXFP_1$	RXFPR1, RX_1, LGR7	Gs, GoB, Gi3 (ACase, PI3K, PKA, PKC, ERK1/2)	Relaxins-1/2/3 Relaxin-2 > relaxin-3 ≫ InsL3
$RXFP_2$	RXFPR2, RX_2, LGR8, GREAT, InsL3R, GPR106	Gs, GoB (ACase)	Relaxins-1/2/3, InsL3 InsL3 > relaxin-2 ≫ relaxin-3
$RXFP_3$	RXFPR3, RX_3, Rln3R1, GPCR135, SALPR	Gi/o (ERK1/2)	Relaxin-3
$RXFP_4$	RXFPR4, RX_4, Rln3R2, InsL5R, GPR100, GPCR142	Gi/o	Relaxin-3, InsL3/5 InsL5 ∼ relaxin-3

7.13.55 Receptors of the Relaxin Family Peptides

The set of relaxin family peptide receptors are divided into 2 categories: group 1 ($RXFP_1$–$RXFP_2$) and 2 ($RXFP_3$–$RXFP_4$; Table 7.97). They are targeted by heterodimeric peptide hormones relaxin-1 to -3 and insulin-like peptide InsL3 and InsL5 [5]. Relaxin receptors reside in central and autonomous nervous system, heart, smooth muscle, and connective tissue. At least 2 binding sites, with high and low affinity, exist on $RXFP_1$ and $RXFP_2$ receptors [5].

The $RXFP_1$ receptor activates adenylate cyclase, protein kinase-A and -C, phosphatidylinositol 3-kinase, and extracellular signaling regulated kinases ERK1 and ERK2 [1080]. It also intervenes in nitric oxide signaling. Relaxin targets $RXFP_1$ for connective tissue remodeling.

Gs-Coupled $RXFP_2$ activates and Gi-coupled $RXFP_3$ and $RXFP_4$ and inhibit adenylate cyclase, respectively. The $RXFP_3$ receptor also activates ERK1 and ERK2 kinases.

7.13.56 Serotonin (5-Hydroxytryptamine) Receptors

Except ionotropic receptors of the $5HT_3$ class that are ligand-gated Na^+ and K^+ cation channels, all other serotonin (or 5-hydroxytryptamine) receptors ($5HT_1$–$5HT_2$ and $5HT_4$–$5HT_7$; Table 7.98) are G-protein-coupled receptors. The diversity of metabotropic 5HT receptors is augmented by alternatively spliced variants. Subtypes $5HT_{2A}$, $5HT_{2C}$, and $5HT_6$ are non-functional [5]. Furthermore, RNA editing produces $5HT_{2C}$ isoform that differ in efficiency and specificity of Gq/11 coupling.

Serotonin metabotropic receptors reside in the central and peripheral nervous system, where they activate an intracellular cascade to produce an excitatory or inhibitory response (Table 7.99), as well as other tissues, particularly vasculature. The bioamine serotonin is synthesized from tryptophan. Once released, serotonin increases the smooth muscle tone.

The central serotoninergic system innervates diverse brain regions. In the central nervous system, cell bodies that contain serotonin localize to nuclei raphé, near the midline of the brainstem, and project to the brainstem and spinal cord as well as forebrain [1081]. The activity of the raphé network is modulated by activators and inhibitors of the release of serotonin.

Various serotonin receptors modulate the synaptic transmission and postsynaptic excitability. Serotonin receptors indeed influence the release of many neurotransmitters, such as acetylcholine, adrenaline, dopamine, GABA, glutamate, and noradrenaline, as well as many hormones, such as corticosterone, corticotropin, cortisol, oxytocin, prolactin, substance-P, vasopressin, etc.

Disc large homolog DLg4 [194] connects to $5HT_{2A}$ and $5HT_{2C}$ to facilitate their anchoring and clustering to postsynaptic dendrites and agonist-dependent internalization, respectively [835].

Breathing is controlled by the nervous system. The serotoninergic apparatus belongs to the arousal system that controls the body's ventilation. Adjustment of excitability of the ventilatory command is also regulated by acetylcholine, adenosine, catecholamines, opioids, and other neuropeptides [1081].

Apneusis consists of a pause at inspiration and prolonged breath holding. Central apnea results from the loss of inspiratory motions. [195] Serotonin receptors can counteract both apneusis and opioid-induced apnea [1081]. In mammals, the respiratory rhythm is generated by the *pre-Bötzinger complex* (PBC), a cluster of interneurons in the ventrolateral medulla of the brainstem. Many neurons of the pre-Bötzinger complex produce adenosine (A_1 and A_{2A}), GABA, glutamate, neurokinin-1 (NK_1), and serotonin receptors, such as $5HT_{1A}$, $5HT_{2A}$, $5HT_{2B}$, $5HT_4$, and $5HT_7$. Serotonin receptors colocalize with opioid Op_3 receptor in respiratory neurons of the pre-Bötzinger complex to promote activity of neurotrophins. Gq-Coupled $5HT_{2A}$ and $5HT_{2B}$ receptor isoforms are coexpressed in these neurons. They does not excite breathing. Only $5HT_{1A}$ and $5HT_4$ isotypes are able to counteract opioid-induced respiratory depression [1081].

194. A.k.a. PSD95.

195. These respiratory disorders can be caused by brainstem tumors or degeneration such as olivopontocerebellar hypoplasia, as well as opioid or barbiturate administration.

Table 7.98. Subtypes of serotonin receptors and their direct functions in the circulatory and ventilatory systems as well as via the central (CNS) and peripheral (PNS) nervous system (Source: Wikipedia). Among the 7 categories of 5HT receptors ($5HT_1$–$5HT_7$), 1 ionotropic serotonin receptor ($5HT_3$ transmitter-gated Na^+–K^+ ion channel), and 15 different metabotropic isoforms have been identified.

Subtype	Distribution	Function
$5HT_{1A}$	Blood vessel CNS	Vasoconstriction, blood pressure, inhibitory nervous signal, ventilation depression
$5HT_{1B}$	Blood vessel CNS	Vasoconstriction
$5HT_{1C}$	see $5HT_{2C}$	
$5HT_{1D}$	Blood vessel CNS	Vasoconstriction
$5HT_{1E}$	Blood vessel CNS	
$5HT_{1F}$	Blood vessel CNS	Vasoconstriction
$5HT_{2A}$	Blood vessel CNS, PNS Platelet, SMC	Vasoconstriction
$5HT_{2B}$	Blood vessel CNS, PNS Platelet, SMC	Vasoconstriction, excitatory neural input
$5HT_{2C}$	Blood vessel CNS, PNS Platelet, SMC	Vasoconstriction
$5HT_3$	CNS, PNS	
$5HT_4$	CNS, PNS	Ventilation stimulation
$5HT_{5A}$ $5HT_{5B}$	CNS absent in humans	
$5HT_6$	CNS	
$5HT_7$	Blood vessel CNS	Vasoconstriction Ventilation stimulation

7.13.56.1 $5HT_{1A}$

In the central nervous system, Gi-coupled $5HT_{1A}$ is predominantly expressed in the hippocampus, lateral septum, and brain cortex, as well as in brainstem regions, such as nuclei raphé and hypoglossal nuclei. On presynaptic terminals, it precludes presynaptic transmitter release. At postsynaptic membranes, it activates via $G\beta\gamma$ subunit inwardly rectifying K^+ channels and inhibits Ca^{++} channels.

Table 7.99. Subfamilies of serotonin receptors in the nervous system (Sources: Wikipedia and [5]).

Type	Gαsubunit (preferential)	Mediator	Response
$5HT_1$	Gi/o	cAMP	Inhibition
$5HT_2$	Gq/11	IP_3, DAG	Excitation
$5HT_4$	Gs	cAMP	Excitation
$5HT_5$	Gi/o	cAMP	Inhibition
$5HT_6$	Gs	cAMP	Excitation
$5HT_7$	Gs	cAMP	Excitation

In the central respiratory network, serotonin lowers excitability of respiratory neurons [1081]. Activated $5HT_{1A}$ receptor enhances opioid-induced bradycardia, but partially compensates the decrease in vascular resistance and sympathetic activity, in addition to breathing restoration [1082].

7.13.56.2 $5HT_{1B}$

The Gi/o-coupled $5HT_{1B}$ receptor controls several signaling axes [1083]: (1) inhibition of adenylate cyclase; (2) inhibition of Rap1 guanine nucleotide-exchange factor (Rap1GAP); (3) activation of phospholipase-C and stimulation of Ca^{++} release; (4) phosphorylation of extracellular signal regulated kinase, phosphatidylinositol 3-kinase, P70 ribosomal S6 kinase (S6K), and protein kinase-B; (5) activation of Ca^{++}-dependent K^+ channels; and (6) stimulation of nitric oxide production.

It is primarily expressed in the central nervous system, both pre- and postsynaptically (on serotoninergic axon terminals), where it regulates the release of various neurotransmitters (glutamate, dopamine, GABA, acetylcholine, and ^{N}acetyl aspartyl glutamate), including serotonin itself (e.g., terminal autoreceptors of serotoninergic neurons) [1083]. It also lodges in sympathetic nerves of the gastrointestinal tract.

The $5HT_{1B}$ receptor is also synthesized in smooth muscle cells of cerebral arteries and other blood vessels. It impedes noradrenaline release in the vena cava as well as trigeminal ganglion-stimulated plasma extravasation in rodents [1083]. It mediates the vasoconstriction of rat caudal arteries.

The $5HT_{1B}$ receptor can homo- and heterodimerize with $5HT_{1A}$ and $5HT_{1D}$. It can interact with GSK3β, glutathione S-transferase-κ1, and non-metastatic cell subunit NME2 of nucleoside diphosphate kinase. It is internalized and interacts with various regulators.

7.13.56.3 $5HT_{2A}$

Most activated GPCRs associate with arrestin. A single GPCR can then trigger multiple signaling events according to the presence or absence of β-arrestin

and the type of binding agonist. Different patterns of signal transduction and functional selectivity of $5HT_{2A}$ receptor [196] is determined by β-arrestin. The absence of β-arrestin-2 abrogrates many effects of serotonin from $5HT_{2A}$ receptor, but has no or little effect on the same signaling pathways with other kinds of agonists.

7.13.56.4 $5HT_{2B}$

In the central nervous system, $5HT_{2B}$ receptor [197] participates in embryo- and fetogenesis. It is also involved in anxiety, migraine, schizophrenia, autism, and depression [1081].

7.13.56.5 $5HT_{2C}$

The $5HT_{2C}$ receptor (previously called $5HT_{1C}$) is coupled to $G\alpha_q$, hence to phospholipase-Cβ. It is exclusively expressed in the central nervous system. It is the single GPCR that undergoes adenosine-to-inosine RNA editing at 5 positions, hence generating multiple functional variants with different G-protein-coupling properties and transfer modes [1084]. [198]

The $5HT_{2C}$ receptor intervenes in various behavioral and physiological processes, such as regulation of mood, nociception, motor behavior, endocrine secretion, thermoregulation, modulation of appetite, cerebrospinal fluid production by choroid plexi, and control of exchanges between the central nervous system and cerebrospinal fluid [1084]. It contributes to the control of mesocorticolimbic and nigrostriatal dopaminergic systems, as it inhibits dopamine release.

The $5HT_{2C}$ receptor has both constitutive and inducible activity [1084]. Desensitization of its agonist-dependent activation results from receptor phosphorylation by G-protein-coupled receptor kinase GRK2 that is followed by recruitment of β-arrestins, which uncouples $5HT_{2C}$ from G protein and promotes its internalization into endosomes. Endocytosis not only ensures receptor desensitization, but also allows receptor dephosphorylation and recycling to the plasma membrane for resensitization.

Owing to transactivation of the small GTPase RhoA, $5HT_{2C}$ receptor can activate phospholipase-D. It can also stimulate phospholipase-A2 and extracellular signal-regulated kinases ERK1 and ERK2. It also interacts with phosphatase and tensin homolog.

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196. In the central nervous system, $5HT_{2A}$, preferentially coupled to Gq localizes to the neocortex, entorhinal and pyriform cortex, claustrum, caudate nucleus, nucleus accumbens, olfactory tubercle, hippocampus, and cerebellum [1081].

197. The $5HT_{2B}$ receptor resides in the neocortex, cerebellum, dorsal hypothalamus, and medial amygdala [1081].

198. Pre-mRNA editing generates multiple functional variants with decayed constitutive activity, agonist affinity, and PLC activation potency, as well as additional G13-coupling capacity, but modified mediator selectivity and trafficking.

7.13.56.6 $5HT_4$

In the central nervous system, Gs-coupled $5HT_4$ receptor[199] contributes to the control of transmitter secretion and cognition. It localizes also to the myocardium, adrenal glands, digestive tract (from esophagus to colon), and bladder.

Splice variants have distinct C-terminus ($5HT_{4A}$–$5HT_{4H}$) [1081]. Types $5HT_{4A}$ and $5HT_{4B}$ couple also to G13 and Gi/o family subunit, respectively. Unlike $5HT_{1A}$, $5HT_{4A}$ receptor further reduces the decrease in opioid-induced vascular resistance, heart rate, and sympathetic activity, but partially rescues breathing [1082].

7.13.56.7 $5HT_7$

In the central nervous system, $5HT_7$ receptor[200] intervenes in the circadian rhythm. It also resides in smooth muscle cells of blood vessels and digestive tract.

Alternate splicing of intron 2 generate 4 isoforms of the $5HT_7$ receptor ($5HT_{7A}$–$5HT_{7D}$) that differ in their C-termini, but neither in their tissue distribution, nor pharmacological and signaling features [1081]. Gs-Coupled $5HT_7$ receptor activates adenylate cyclase AC5 as well as Ca^{++}–calmodulin-regulated AC1 and AC8 isoforms. In addition, it can trigger the mitogen-activated protein kinase cascade to activate extracellular signal-regulated protein kinases ERK1 and ERK2 [1081].

7.13.57 Somatostatin Receptors

Somatostatin[201] regulates the endocrine system and influences neurotransmission and cell proliferation. Two active forms of somatostatin derive from alternative cleavage of a single preproprotein: $somatostatin_{14}$[202] and $somatostatin_{28}$ [5].[203] It acts on 5 subtypes of the somatostatin receptor (Sst_1–Sst_5) to inhibit the secretion of many hormones (Table 7.100).

Agonist stimulation can dissociate P85 regulatory subunit of phosphoinositide 3-kinase (PI3K) and somatostatin receptor Sst_2, thereby reducing the cell survival PI3K–PKB pathway [835]. In the absence of agonist, constitutive association of P85 subunit with Sst_2 enhances PI3K activity.

199. The HT_4 receptor lodges in basal ganglia, hippocampus, olfactory tubercle, and limbic structures [1081].
200. The $5HT_7$ receptor is detected in the thalamus, hypothalamus, hippocampus, and cerebral cortex [1081].
201. A.k.a. somatotropin release-inhibiting factor (SRIF) and growth hormone-inhibiting hormone (GHIH). It is produced by neuroendocrine neurons of the periventricular nucleus of the hypothalamus.
202. A.k.a. SRIF14 (with 14 amino acids).
203. A.k.a. SRIF28 (with 28 amino acids).

Table 7.100. Somatostatin receptors and their main G-protein subunit transducers (Source: [5]).

Type	Other name	Main transducer
Sst_1	SstR1	Gi
Sst_2	SstR2	Gi
Sst_3	SstR3	Gi
Sst_4	SstR4	Gi
Sst_5	SstR5	Gi

Table 7.101. Sphingosine 1-phosphate receptors and their main targeted G proteins (Source: [736]; SPC: sphingosylphosphorylcholine). Receptors $S1P_4$ and $S1P_5$ are observed in hematopoietic cells and neurons, whereas the others are ubiquitous.

Type	Main transducer	Potency order
$S1P_1$	Gi/o	S1P > SPC
$S1P_2$	Gs, Gq, G12/13	S1P > SPC
$S1P_3$	Gs, Gi/o, Gq	S1P > SPC
$S1P_4$	Gs, Gi/o, G12/13	S1P > SPC
$S1P_5$	Gi/o, G12/13	S1P > SPC

7.13.58 Sphingosine 1-Phosphate Receptors

Sphingosine 1-phosphate is a lipid growth factor (Vol. 2 – Chap. 3. Growth Factors) that acts via its specific G-protein-coupled receptors (Table 7.101). Sphingosylphosphorylcholine (SPC) also activates this receptor with equal, smaller, or greater potency than that of sphingosine 1-phosphate according to the receptor type. The S1P receptor family comprises 5 receptors ($S1P_1$–$S1P_5$) that regulate cell survival, proliferation, and migration.

Sphingosine 1-phosphate receptors are members of the *endothelial differentiation gene* (EDG) family with lysophosphatidic acid receptors. Receptors $S1P_1$ to $S1P_3$ are widespread, whereas $S1P_4$ and $S1P_5$ reside in cells of the nervous and immune systems. Endocytosis of S1P GPCRs can trigger Gi-mediated signaling similarly to that initiated from the plasma membrane [11].

7.13.58.1 $S1P_1$ Receptor

The major $S1P_1$ receptor is observed on vascular endothelial and smooth muscle cells. It couples to Gi protein. It regulates endothelium-dependent vasorelaxation, smooth muscle contraction, and blood vessel maturation during embryo- and fetogenesis [1085]. It also controls the transfer of immunocytes.

Lipidic S1P ligand is predominantly stored and released by erythrocytes. It is present at high concentrations (100 nmol–1 μmol) in the blood and lymph. A large S1P fraction is bound to plasma lipoproteins and albumin.

Table 7.102. Signaling from $S1P_2$ receptor (Source: [1085]; ACase: adenylate cyclase; ERK: extracellular signal-regulated protein kinase; JNK: Jun N-terminal kinase; MAPK: mitogen-activated protein kinase; PI3K: phosphatidylinositol 3-kinase; PKB: protein kinase-B; PLC(D): phospholipase-C(D); PTen: phosphatase and tensin homolog deleted on chromosome 10; RacGAp: Rac GTPase-activating protein).

Gα type	Pathways
Gi	ERK PI3K–PKB–Rac
Gq	PLC–Ca^{++}
G12/13	Rho–RacGAP–Rac (−), Rho–PKB (−) ACase–cAMP PTen
	PLD; JNK; P38MAPK

Upon S1P binding, $S1P_1$ receptor primes egress of lymphocytes from secondary lymphoid organs toward the blood (low S1P concentration) or lymph (high S1P concentration) [1086].

In endothelial cells, transient exposure of exogenous $S1P_1$ ligand[204] triggers ligand-bound $S1P_1$ endocytosis and long-lasting signaling with inhibition of adenylate cyclase and increased ERK phosphorylation that causes augmented cell migration, whereas calcium response is abrogated [1086]. On the other hand, S1P agonist does not provoke persistent signaling from internalized $S1P_1$ receptors.

7.13.58.2 $S1P_2$ Receptor

Widespread $S1P_2$ couples to Gi, Gq, and G12/13 types of Gα subunit of heterotrimeric G-proteins to regulate several mediators, such as Rho GTPase, PTen phosphatase, ERK kinase, and VE-cadherin (Table 7.102). However, it preferentially activates G12/13 family subunits.

Activated $S1P_2$ on endothelial cells provokes disruption of VE-cadherin-based adherens junctions, hence increasing endothelial paracellular permeability [1085]. Because $S1P_2$ activates RhoA, it impedes Rac activity. The $S1P_2$ receptor actually inhibits small GTPase Rac, as it stimulates a GTPase-activating protein for Rac GTPase, thereby impeding cell migration. In addition, $S1P_2$ stimulates cAMP synthesis via G13 (but not Gs). Once coupled to G12/13 family subunits, it can also activate monomeric Rho GTPase.

The $S1P_2$ receptor is able to activate the Gi–Ras–MAPK axis to regulate cell proliferation and survival. Phosphorylation of ERK kinases, in turn, stimulates Jun and Fos transcription factors. In addition, $S1P_2$ activates other members of the MAPK

204. E.g., immunomodulator drug fingolimod.

Table 7.103. Expression of S1P receptor types in various cell types of the cardiovascular apparatus (Source: [1087]). Five known receptors bind to S1P ligand. Receptors $S1P_1$ to $S1P_3$ are widespread; $S1P_4$ and $S1P_5$ reside in blood cells. In cells of the cardiovascular apparatus, $S1P_1$ couples exclusively to Gi protein; $S1P_2$ and $S1P_3$ to subunits of the Gi, Gq, and G12/13 families; and $S1P_4$ and $S1P_5$ to those of the Gi and G12/13 families. The Gi subunit inhibits adenylate cyclase, whereas $G\beta\gamma$ can activate ion channels and kinases; Gq subunit targets phospholipase-C; and G12/13 subunit connects to RhoA guanine nucleotide-exchange factor. Because Gi protein prevents cAMP formation, it precludes PKA-mediated activation of Ca_V1 channel. The $G\beta\gamma$ dimer associated with Gi acts on Gi-regulated inward rectifier $K_{IR}3$ channel (K_{ACh} current) that contributes to resting membrane potential in human atriomyocytes.

Tissue	S1P Receptor expression
Cardiomyocytes	$S1P_1 \gg S1P_3 > S1P_2$
Cardiac fibroblasts	$S1P_3 \gg S1P_1 > S1P_2$
Aortic smooth muscle cells	$S1P_2 > S1P_3 \gg S1P_1$
Vascular endothelial cells	$S1P_1 > S1P_3 \gg S1P_2$

family — JNK and P38MAPK — that operate in response to cell stress, in particular for cytokine production. It also activates the Gq–PLC–Ca^{++} pathway as well as phospholipase-D.

7.13.58.3 S1P Receptors in the Cardiovascular System

Vascular endothelial and smooth muscle cells as well as cardiomyocytes and cardiac fibroblasts express $S1P_1$ to $S1P_3$ receptors, but with different expression patterns (Table 7.103). Because $S1P_1$ is the most abundant type in endothelial cells and cardiomyocytes, most S1P-primed responses occur via Gi-coupled $S1P_1$ receptor alone or in combination with Gi-, Gq-, or G12/13-coupled $S1P_3$ receptor. In vascular smooth muscle cells, $S1P_2$ has the highest density. Stimulated $S1P_1$ or $S1P_3$ activates Rac GTPase, whereas stimulated $S1P_2$ inhibits Rac GTPase. Cardiac fibroblasts express predominantly $S1P_3$ receptor. In addition, sphingosine kinase activity is higher in cardiac fibroblasts than in cardiomyocytes [1087].

Cardiomyocytes

In cardiomyocytes, $S1P_1$ receptor inhibits cAMP formation and antagonizes adrenergic receptor-mediated inotropy [1087]. The $S1P_1$ receptor localizes to caveolae in ventriculomyocytes. The $S1P_3$ receptor induces bradycardia. Both $S1P_2$ and $S1P_3$ receptors favor cardioprotection in ischemia–reperfusion injury. Receptors of S1P also participate in remodeling, proliferation, and differentiation of cardiac fibroblasts.

Endothelial Cells

In endothelial cells, caveolar domains of the plasma membrane are enriched in NOS3 and contain $S1P_1$ receptors. Lipid S1P activates NOS3 via: (1) Ca^{++}–calmodulin that relieves caveolin inhibition and (2) protein kinase-B that phosphorylates NOS3.

Sphingosine 1-phosphate enhances the endothelial barrier. In particular, it prevents the permeability increase caused by platelet-activating factor or bradykinin. Among $S1P_1$ to $S1P_3$ located on endothelial cells, only $S1P_1$ protects the endothelial barrier against the effect of platelet-activating factor and bradykinin in rat venules [1088].

Sphingosine 1-phosphate stabilizes newly formed vessels and antagonizes thrombin that disrupts the endothelial barrier via $S1P_1$ and $S1P_3$ receptors [1087]. It interacts with $S1P_1$ during angiogenesis and vasculature maturation [1089]. Gi-Coupled $S1P_1$ receptor regulates cell survival, proliferation, migration, and morphogenesis in response to S1P messenger. Activated $S1P_1$ also stimulates the assembly of cadherin complexes in endothelial cells. In endothelial cells, $S1P_1$ activates $\alpha_V\beta_3$- and β_1-integrins via GTPase Rho [1090]. Liganded $S1P_1$ primes the Gi–PI3K–PKB, PI3K–Rac, PLC–DAG–PKC and $–IP_3–Ca^{++}$, and Ras–ERK pathways. Protein kinase-B then binds to and phosphorylates $S1P_1$, hence regulating GTPase Rac and cortical actin assembly, lamellopodium formation, and chemotaxis [1091]. Activated $S1P_1$ favors cell survival and proliferation of vascular endothelial cells and stimulates nitric oxide synthase in endothelial cells via Gi and PI3K [1092]. Whereas $S1P_2$ inhibits Rac and growth factor-induced chemotaxis [1093], $S1P_3$ activates small GTPase Rho.

Smooth Muscle Cells

In vascular smooth muscle cells, S1P activates Rho GTPase and promotes myosin light-chain phosphorylation that provokes vasoconstriction mainly via $S1P_3$ receptor. On the other hand, S1P can promote the formation of nitric oxide in endothelial cells that, once released, relaxes smooth muscle cells.

7.13.58.4 S1P Receptors in the Immune System

Sphingosine 1-phosphate and lysophosphatidic acid are produced by mastocytes, platelets, and macrophages. Ligands S1P and LPA affect immunocyte survival, differentiation, proliferation, migration, receptor expression, and protein synthesis and secretion (Table 7.104).

The emigration of thymocytes from the thymus, transfer of lymphocytes between blood and secondary lymphoid organs (thus avoiding sequestration in lymph nodes as well as persistence in blood), and immigration of B lymphocytes into splenic follicles (white pulp of spleen) is assisted by S1P and its receptors [1094]. Sphingosine 1-phosphate in dendritic and T cells enhances the IgE production.

Table 7.104. $S1P_1/2$ receptors and their effects on immunocytes (Source: [1094]).

Receptor	Cells	Effects
$S1P_1$	Mast, B, and T cells, macrophage	Cell survival, chemotaxis (at low level)
	Dendritic and NK cells, eosinophil	Chemotaxis
$S1P_2$	Mastocyte	Migration inhibition
	Macrophage	Migration stimulation
	Dendritic cell, eosinophil	
$S1P_3$	Eosinophil, some dendritic and B cells	
$S1P_4$	B and T cells	Cell survival
	Dendritic and NK cells, macrophage	
$S1P_5$	Monocyte, NK cell	

In an autocrine manner, via interactions with different GPCRs, S1P enhances mastocyte migration and release of pro-inflammatory mediators in allergy. Whereas LPA promotes mastocyte development, S1P-bound $S1P_1$ increases mastocyte chemotaxis. In contrast, chemotaxis of mastocytes is prevented by IgE-upregulated S1P-bound $S1P_2$ receptor. In addition, $S1P_2$ augments IgE-mediated release of allergic mediators.

7.13.59 Tachykinin Receptors

Tachykinin receptors are activated by members of the tachykinin family of neuropeptide neurotransmitters: substance-P, neurokinin-A [205] and -B, [206] and neuropeptide-K, [207] and -γ. [208]

Three distinct tachykinin receptors have been identified (NK_1–NK_3, or NK1R–NK3R). Substance-P preferentially activates NK_1, neurokinin-A NK_2, and neurokinin-B NK_3 (Table 7.105). In the respiratory tract, both NK_1 and NK_2 receptors are

205. A.k.a. substance-K, neurokinin-α, and neuromedin-L.
206. A.k.a. neurokinin-β and neuromedin-K.
207. A.k.a. neurokinin-K.
208. The human tachykinin genes TAC1, TAC3 (mouse TAC2 gene), and TAC4 produce tachykinins, as they encode tachykinin precursors, the *preprotachykinins* that undergo proteolytic cleavage to form smaller peptides, and multiple splice variants that leads to different sets of peptides. Each preprotachykinin type is the product of one gene. The TAC1 gene encodes preprotachykinin-A (PPTKa; a.k.a. protachykinin-1 and tachykinin precursor-1), leads to formation by alternative splicing to neurokinins-A and -K and neuropeptide-γ. All TAC1 splice variants (α, β, and γ splice variants) manufacture substance-P, but only β and γ splice variants produce neurokinin-A, neuropeptide-K, and neuropeptide-γ. Neuropeptide-K and -γ are versions of neurokinin-A with a longer N-terminus. The genes TAC3 and TAC4 encode preprotachykinins-B (PPTKb) and -C (PPTKc) that produce neurokinin-B and hemokinin-1, respectively.

Table 7.105. Tachykinin receptors, main G-protein subunit transducers, and ligands (Source: [5]; NKa(b): neurokinin-A(B); SP: substance P).

Type	Main transducer	Potency order
NK_1	Gq/11	SP > NKa > NKb
NK_2	Gq/11	NKa > NKb ≫ SP
NK_3	Gq/11	NKb > NKa > SP

Table 7.106. Trace amine-associated receptors, main G-protein subunit transducers, and ligands (Source: [5]; PEA: phenylethylamine).

Type	Other names	Main Transducer	Potency order
TA_1	TAA_1, TAR1, TAAR1	Gs	Tyramine ≤ PEA > octopamine ∼ dopamine
TA_2	TAA_2, TAR2, TAAR2 TAAR3, GPR57, GPR58	Gs	PEA > tryptamine

detected in bronchial glands and vessels, as well as bronchial smooth muscle, but not in the respiratory epithelium [1095]. [209]

7.13.60 Trace Amine Receptors

Trace amine receptors, or trace amine-associated receptors, (TA_1–TA_2) are bound by trace amines (Table 7.106). Trace amines are endogenous compounds that are structurally related to classical biogenic amines. They are produced in the nervous system. They colocalize in neurons in which these biogenic amines serves as neurohormones, neuromodulators, and/or neurotransmitters, such as monoamines that encompass catecholamines, which derive from phenylalanine and tyrosine [210] (adrenaline, dopamine, and noradrenaline), serotonin, histamine, and melatonin.

Trace amines include: (1) tyramine, [211] a monoamine derived from tyrosine; [212] (2) phenylethylamine (PEA), a natural monoamine alkaloid that is synthesized from

209. Tachykinin receptors can also be found in nerves (NK_1) and inflammatory cells (NK_2), such as T lymphocytes, macrophages, and mastocytes [1095].

210. Tyrosine is either generated from phenylalanine by phenylalanine hydroxylase or processed from ingested proteins.

211. A.k.a. 4-hydroxyphenethylamine and para-tyramine.

212. Tyramine releases catecholamines (adrenaline, dopamine, and noradrenaline). It is metabolized by monoamine oxidase. A large dietary intake of tyramine can cause the tyramine pressor response, with an increase in systolic blood pressure associated with vasoconstriction, but repeated exposure to tyramine reduces tyramine pressor response.

Table 7.107. Thyrotropin-releasing hormone receptors and their main G-protein subunit transducers (Source: [5]).

Type	Main transducer
TRH_1	Gq
TRH_2	Gq

phenylalanine; (3) tryptamine, another monoamine alkaloid;[213] (4) octopamine;[214] (5) 3-iodothyronamine that has negative inotropic and chronotropic effects [1097]; and (6) psychostimulant amphetamines[215] (α-methylphenethylamines).

Endogenous amphetamines, like all trace amines, bind to trace amine receptors, or trace amine-associated receptors. The TA_1 receptor localizes to the central nervous system as well as peripheral organs such as the kidney. It may be targeted by thyronamines, endogenous derivatives of thyroid hormones (decarboxylated and deiodinated metabolites).

7.13.61 Thyrotropin-Releasing Hormone Receptors

Thyrotropin-releasing hormone receptors (Table 7.107) are activated by the endogenous tripeptide thyrotropin-releasing hormone (TRH) that is produced by medial neurons of the hypothalamic paraventricular nucleus to stimulate the release of thyroid-stimulating hormone and prolactin by the adenohypophysis (endocrine anterior lobe of the pituitary gland).

7.13.62 Urotensin-2 Receptor

Urotensin-2 receptor[216] is a Gq/11-coupled receptor. In humans, its highest levels are detected in skeletal muscle and cerebral cortex and lower levels in kidney cortex and left ventricle [1098]. Urotensin-2 is synthesized from preprourotensin-2 isoforms that contain 124 and 139 amino acids. Urotensin-2 causes vasoconstriction in human arteries (e.g., coronary, mammary, and radial arteries) and veins (e.g.,

213. Tryptamine serves as a backbone for members of the tryptamine group. Tryptamine derivatives comprise neurotransmitter serotonin (5-hydroxytryptamine) and sleep–wake cycle regulator neurohormone melatonin (^{N}acetyl 5-methoxytryptamine). Serotonin is synthesized from tryptophan (α-carboxyltryptamine) successively by tryptophan hydroxylase and amino acid decarboxylase. Serotonin, in turn, can be converted to melatonin by ^{N}acetyltransferase and 5-hydroxyindole ^{O}methyltransferase.

214. Tyramine is hydroxylized to octopamine by dopamine β-hydroxylase to be subsequently packaged in synaptic vesicles with noradrenaline. In mammals, octopamine may release lipids from adipocytes. Whereas serotonin increases peristaltic movements in the digestive tract, octopamine suppresses them [1096].

215. Endogenous amphetamines are manufactured in the central and peripheral nervous system. They modulate the level of excitement and alertness.

216. A.k.a. hypocretin receptor; UTR, UTR2, UTs2R, or GPR14.

Table 7.108. Arginine vasopressin (AVP) and oxytocin (OxT) receptors, main G-protein subunit transducers, and order of potency of ligands (Source: [5]; CNS: central nervous system, CVS: cardiovascular system).

Type	Other names	Main transducer	Potency order	Location
V_{1A}	AVPR1a	Gq/11	AVP > OxT	CNS, CVS, kidney, liver
V_{1B}	AVPR1b	Gq/11	AVP > OxT	CNS
V_2	AVPR2	Gs	AVP > OxT	Kidney
OT	OXTR	Gq/11, Gi/o	AVP ≤ OxT	CNS, uterus (at birth), mammary gland

Table 7.109. Vasopressin and oxytocin receptors, mediators, and effects (Source: Wikipedia; ACTH: adrenocorticotropic hormone; vWF: von Willebrand factor). Cullin-5 has been originally named vasopressin-activated, calcium-mobilizing receptor VACM1. It reaches its highest levels in cardiac and skeletal tissues. It is specifically expressed in vascular endothelium and renal collecting tubules.

Type	Mediators	Effects
V_{1A}	Calcium	Vasoconstriction, gluconeogenesis, platelet aggregation, release of factor VIII and vWF,
V_{1B}	Calcium	ACTH secretion in response to stress
V_2	ACase–cAMP	Water reabsorption, release of vWF from endothelial cells
Cullin-5	Calcium	Calcium signaling

saphenous and umbilical veins) [1098]. On the other hand, urotensin-2 can trigger a potent vasodilation in human small muscular pulmonary and abdominal resistance arteries [1099].

7.13.63 Vasopressin and Oxytocin Receptors

Magnocellular neurosecretory cells of the paraventricular and supraoptic nuclei of the hypothalamus synthesize one of the neurohypophysial hormones – nonapeptides arginine vasopressin (AVP),[217] or antidiuretic hormone (ADH), and oxytocin (OT) – along with different neuropeptides or neuromodulators. Vasopressin and oxytocin receptors are activated by vasopressin and oxytocin. Vasopressin signals via 3 cognate receptors (Tables 7.108 and 7.109). Oxytocin receptor requires Mg^{++} ion and cholesterol.

Hormones oxytocin and vasopressin not only act on peripheral organs via the blood circulation, but are also released in the brain, where they influence social be-

217. Lysine vasopressin (LVP), or lypressin, plays the same role in pigs.

havior.[218] Vasopressin contributes to water reabsorption in the renal collecting ducts via aquaporin-2. Vasopressin also causes a moderate vasoconstriction. Neuropeptide oxytocin acts as a hormone with several peripheral actions and a neurotransmitter in the central nervous system released from centrally projecting oxytocin neurons. Oxytocin intervenes in parturition initiation and milk ejection during lactation, in addition to psychosocial behavior.

Oxytocin and vasopressin are stored in vesicles in and released by the neurohypophysis into the blood circulation.[219] Oxytocin is synthesized as an inactive precursor that includes its carrier neurophysin-1.[220] It is stored with neurophysin-1 in Herring bodies at axon terminals in the neurohypophysis. Vasopressin and its carrier neurophysin-2 also derives from the same precursor.[221]

Regulation of water transport across the epithelium of the collecting duct of the nephron enables a precise control of renal water excretion, thereby regulating the osmolality of body fluids. Water transport control is mainly achieved by vasopressin that binds to V_2 receptor in the basolateral plasma membrane of principal cells of collecting duct to cause an increase in intracellular cAMP and Ca^{++} concentrations. Vasopressin, indeed, favors via V_2 the transfer of aquaporin-2 to the apical plasma membrane, as it triggers phosphorylation by myosin light-chain kinase of the myosin regulatory light chain, as well as aquaporin-2 phosphorylation (Ser256, and Ser264, and S269) and dephosphorylation (Ser261) [1101]. Vasopressin primes activation of several kinases of the AGC family, such as cAMP-dependent protein kinase-A and calmodulin-dependent kinase-2 (via autophosphorylation at Thr286), but lowers phosphorylation of cyclin-dependent kinases and members of the mitogen-activated protein kinase family, such as JNK1 and JNK2 (Thr183 and Tyr185) and ERK1 and ERK2 (Thr183 and Tyr185; Thr203 and Tyr205). In normal circumstances, vasopressin attenuates activities of ERK1 and -2 and JNK1 and -2, but not P38MAPK. Aquaporin-2 is strongly phosphorylated (Ser261) by JNK, P38MAPK, and CDK5 and -9, as well as weakly by ERK kinases. It is phosphorylated (Ser256) by protein kinase-A.

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218. In rats, vasopressin signals in the olfactory system for proper social recognition [1102]. A population of vasopressin neurons resides in the olfactory bulb. In humans, oxytocin participates in the processing of social stimuli [1100]. Oxytocin reduces activation in lateral and dorsal regions of the anterior amygdala in the presence of negative social stimuli, but enhances activity for positive social stimuli. Furthermore, oxytocin increases activity in the posterior amygdala and promotes the coupling of this subregion to the superior colliculi [1100]. Oxytocin also operates in behavioral and endocrine stress responses. It contributes to increase trust and reduce betrayal aversion.

219. The pituitary gland consists of adeno- (anterior pituitary) and neurohypophysis (posterior pituitary). The pituitary secretes hormones that regulate water homeostasis, blood pressure, development, and reproduction.

220. Neurophysin-1 and oxytocin result from the cleavage of a common precursor: preprooxyphysin, or preprooxytocin-neurophysin-1.

221. Neurophysins also act as neurohypophysial hormone. Neurophysin-2 stimulates prolactin secretion. It is generated from the same precursor as vasopressin: preproArg vasopressin–neurophysin-2.

Table 7.110. Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating peptide (PACAP) receptors, main G-protein subunit transducers, and ligands (Source: [5]; GRF: growth hormone-releasing factor; PHI: peptide histidine isoleucineamide). Two – short and long – PACAP isoforms (PACAP27 and PACAP38) derive from proteolytic cleavage of a precursor (proPACAP(131–157) and proPACAP(131–168), respectively).

Type	Other names	Main transducer	Potency order
$VPAC_1$	VIP_1, VIPR1, PACAPR2	Gs	VIP, PACAP27 $\sim$ PACAP38 $>$ GRF $\gg$ $\gg$ PHI $\gg$ secretin
$VPAC_2$	VIP_2, VIPR2, PACAPR3	Gs	VIP, PACAP38 $>$ PACAP27 $>$ PHI $\gg$ GRF, secretin
PAC_1	PACAPR1, AdCyAP1R1	Gs	PACAP27, PACAP38 $\gg$ VIP $>$ PHI

7.13.64 Receptors for VIP and PACAP Peptides

Vasoactive intestinal peptide and pituitary adenylate cyclase-activating peptide receptors are activated by endogenous peptides vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating peptides PACAP27 and PACAP38, peptide histidine isoleucineamide (PHI), peptide histidine methionine amide (PHM), peptide histidine valine (PHV), and growth hormone-releasing factor (GRF) [5]. Vasoactive intestinal peptide causes smooth muscle relaxation, exo- and endocrine secretion, and water and ion flux in respiratory and intestinal epithelia. Pituitary adenylate cyclase-activating polypeptides are neuropeptides that belong to the VIP–PACAP–glucagon–secretin family. They can operate as neurotransmitters and neuromodulators. In addition, they contribute to para- and autocrine regulation of some cell types.

The VIP–PACAP receptors constitute a family ($VPAC_1$, $VPAC_2$, and PAC_1; Table 7.110). The PAC_1 receptor is expressed in brain, adrenal medulla, pancreatic acini, uterus, and myenteric plexus [1106].

The $VPAC_1$ receptor is widely distributed, but predominantly detected in the lung, small intestine, thymus, and, within brain, in the cerebral cortex and hippocampus [1103]. (It is also found in the heart, kidney, liver, spleen, colon, prostate, testis, and placenta, as well as T lymphocytes [1104]). The $VPAC_1$ receptor is also activated by secretin.

The $VPAC_2$ receptor is also broadly distributed (central nervous system, skeletal and smooth muscles, heart, kidney, adipose tissue, pancreas,[222] stomach, and testis). In the central nervous system, $VPAC_2$ receptor is present in regions associated with neuroendocrine function, such as several hypothalamic nuclei. It is also detected in the pituitary gland and pancreatic islets [1103]. In the human respiratory tract, $VPAC_2$ is observed in tracheal and bronchial ciliated epithelial cells as well

222. It has been isolated from a mouse insulin-secreting β-cell line.

as mucous and serous cells of submucosal glands, bronchiolar epithelial cells, and alveolar macrophages, but not airway and vascular smooth muscle and endothelial cells [1105].