Differential Assembly of GPCR Signaling Complexes Determines Signaling Specificity

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Abstract	Recent proteomic and biochemical evidence indicates that cellular signaling is organized in protein modules. G protein-coupled receptors (GPCRs) are privileged entry points for extracellular signals that are transmitted through the plasma membrane into the cell. The adequate cellular response and signaling specificity is regulated by GPCR-associated protein modules. The composition of these modules is dynamic and might depend on receptor stimulation, the proteome of a given cellular context, the subcellular localization of receptor-associated modules, the formation of GPCR oligomers and the variation of expression levels of components of these modules under physiological, for example circadian rhythm, or pathological conditions. The current article will highlight the importance of GPCR-associated protein modules as a biochemical basis for signaling specificity.
Keywords (separated by ',')	Circadian rhythm - Compartmentalization - Complex assembly - Dimerization - Therapeutic

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Pascal Maurice, Abba Benleulmi-Chaachoua, and Ralf Jockers4

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27 **Keywords** Circadian rhythm • Compartmentalization • Complex assembly •
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29 12.1 Introduction

30 Membrane receptors are at the interface between the extra- and intra-cellular
31 environment and are thus crucial for cellular communication. The superfamily of G
32 protein-coupled receptors (GPCRs) constitutes the largest family of membrane
33 receptors with approximately 800 members in humans (Vassilatis et al. 2003;
34 Fredriksson and Schiöth 2005). They are composed of seven transmembrane
35 domains connected by short intra- and extra-cellular loops and respond to a large
36 panel of signals: photons, ions, metabolites, amino acids, lipids, peptides and
37 proteins. Despite this ligand diversity, the overall architecture and activation mechanism
38 is believed to be highly conserved for these receptors. Many GPCRs are able
39 to activate heterotrimeric G proteins composed of α , β and γ subunits. G proteins are
40 classified according to the activation of various effectors: $G_{i/o}$ and G_s proteins are
41 typically involved in the inhibition and stimulation of adenylyl cyclase, respectively;
42 $G_{q/11}$ proteins in the stimulation of phospholipase C and $G_{12/13}$ proteins in the activation
43 of small G proteins and cytoskeleton rearrangements. According to the type of
44 G protein activated, GPCRs have been initially classified as $G_{i/o}$ -, G_s -, $G_{q/11}$ - or $G_{12/13}$ -
45 coupled receptors. However, replication of these studies in different cell types and
46 experimental conditions clearly show that most GPCRs do not couple to one single
47 G protein but often to several of them. Activation of additional G protein-independent
48 signaling events, i.e. β -arrestin-dependent pathways, are also increasingly
49 described. The capacity of GPCRs to signal through multiple pathways together
50 with the fact that signaling pathways are highly interconnected within networks
51 raises the fundamental and still largely unsolved question of how signaling specificity
52 is obtained upon stimulation of cells with a given ligand.

53 A simple way to generate signaling specificity is the expression of different
54 receptor isoforms responding to the same ligand but triggering different signaling
55 events (Fig. 12.1a). This strategy is widely used and applies to multiple neurotransmitters
56 (serotonin, dopamine, glutamate, etc.). In other more limited circumstances,
57 signaling specificity is generated by the expression of different natural ligands targeting
58 the same receptor with different functional outcomes (Fig. 12.1c). Prominent
59 examples are the β_2 -adrenergic receptors (β_2 AR) that is activated by epinephrine
60 and norepinephrine (Reiner et al. 2010) and the parathyroid hormone receptor
61 (PTHrP) that is activated by PTH and the PTH-related peptide (PTHrP) (Ferrandon
62 et al. 2009). Epinephrine and norepinephrine induce different conformational
63 changes within the receptor with different kinetics as monitored with a fluorescently
64 tagged β_2 AR FRET sensor. These differences translate into different capacities to
65 activate G_s - and β -arrestin-dependent signaling events. In the case of the PTHrP,
66 both natural ligands trigger different durations of the cAMP responses in which
67 PTHrP-promoted actions are short-lived and restricted to the cell surface and

Fig. 12.1 Schematic illustration of different means to generate GPCR signaling specificity. (a) Stimulation of different receptor isoforms by the same ligand may result in the activation of different signaling pathways; (b) receptor polymorphisms may modify the functional properties of receptors; (c) different natural ligands may activate the same receptor differently; (d) receptor function may vary depending on the cellular context; (e) GPCR dimerization may modify receptor function and the recruitment of different GPCs; (f) association of GPCRs with ion channels and neurotransmitter transporters may modify receptor function; (g) the subcellular localization of receptors may impact on receptor function; (h) the functional response of receptors may vary depending on physiological or pathological changes of receptor or GPCR expression. Changes in GPCR composition as a function of the circadian rhythm are illustrated

PTH-promoted actions are long-lived and dependent on receptor internalization. 68
This idea can be also extended towards inverse agonists as illustrated by melanocortin 69
MC3/MC4 receptors and their naturally occurring inverse agonists agouti and 70
agouti-related protein (Agrp) that diminish the action of the melanocortin receptor 71
agonist α -melanocyte-stimulating hormone (Adan and Kas 2003). 72

The same strategy is increasingly applied by pharmaceutical industry to design synthetic ligands with biased signaling properties. The idea behind biased ligands is to develop drugs that activate only a subset of signaling pathways (ideally only those important for the therapeutic effect) thus significantly decreasing unwanted side effects of drugs due to the activation of therapeutically irrelevant pathways. The proof-of-concept for such a strategy has been shown for the nicotinic acid receptor. Nicotinic acid is one of the most effective agents for both lowering triglycerides and raising HDL levels. However, the side effect of cutaneous flushing severely limits patient compliance. Recent evidence suggests that effects on lipid metabolism are mediated by $G_{i/o}$ proteins and flushing by β -arrestin (Walters et al. 2009). Drugs with reduced flushing profiles are currently developed (Shen et al. 2010).

Furthermore, receptor polymorphisms might modify the signaling profile of the receptor and thus redirect the functional outcome (Fig. 12.1b). This aspect has to be considered in the context of personalized drug design.

Although the abovementioned cases participate in the generation of signaling specificity, they only apply to a subset of situations and leave the majority of cases unexplained. Indeed, the signaling outcome of the stimulation of a particular receptor might largely depend on the cellular context, the precise subcellular localization of receptors, their clustering and their interaction with signaling modules, whose composition might vary depending on different physiological or pathological conditions. The present article describes how signaling specificity can be generated under these different circumstances. We will focus our attention on the importance of GPCR-associated protein complexes (GAPCs) that might be either involved in the trafficking and subcellular localization of the receptor or directly in receptor signaling. Importantly, integration of receptors into different preformed signaling complexes might be a biochemical basis for the generation of signaling specificity.

12.2 Impact of the Cellular Context

Activation of specific signaling pathways and the functional outcome of receptor activation depend on the cellular context. This may include differences in the functional response depending on the differentiation state of cells, for example preadipocytes vs. adipocytes, or on the cell types (immune cells, epithelial cells, neurons, etc.). Differences may occur at the level of receptor expression, receptor localization, the expression pattern of effector systems and downstream signaling pathways as well as expression of components of GAPCs (Fig. 12.1d).

This is well illustrated by the regulator of G protein signaling (RGS) proteins. RGS proteins are GTPase-activating proteins that accelerate the GTPase activity of $G\alpha$ subunits, thereby modulating G protein signaling (Sjogren 2011). In addition to their selectivity for certain $G\alpha$ subtypes, one obvious source of specificity is the tissue- or cell-specific expression pattern of RGS. A good example is the distinct localization of the two splice variants of RGS9. RGS9, a member of the R7 family of RGS proteins, is expressed as two splice variants, RGS9-1 in the retina and the

longer form RGS9-2 primarily postsynaptic in dopamine receptor-rich brain regions, such as the striatum (Gold et al. 1997; Mancuso et al. 2010). In the retina, RGS9-1 is exclusively found in the outer segment of photoreceptor cells in which it regulates signaling through transducin α subunits in rods and cones (Cowan et al. 1998; He et al. 1998). This protein seems to be essential for normal visual perception as demonstrated by the RGS9 knockout mice and humans with mutations in RGS9 (Nishiguchi et al. 2004; Michaelides et al. 2010). In contrast, RGS9-2 is highly expressed in the striatum where it regulates opiodergic and dopaminergic responses (Gold et al. 1997). RGS proteins have been also implicated in regulating cancer progression. In many cases, the link is related to changes in expression. For instance, RGS2 and RGS4 mRNA are both down-regulated in ovarian cancer cell lines, in contrast to RGS6 mRNA which is upregulated, indicating different and specific functional roles for these proteins (for review, see Hurst and Hooks 2009).

12.3 GPCR Dimerization 127

Organization of GPCRs into dimeric clusters has been considered for more than 10 years and is now supported by numerous studies (Milligan 2009) and the crystal structure of two GPCRs (Palczewski et al. 2000; Wu et al. 2010). GPCR heterodimerization is a topic of great interest due to the potential importance of the functional consequences of such interactions on signal transduction pathways. Depending on the two protomers constituting the dimer (the dimer being the minimal functional oligomeric unit), the composition of the GAPCs can vary and generate signaling specificity (Fig. 12.1e). In addition, GPCR heterodimerization offers a plausible solution for the simultaneous binding of many interaction partners to the receptor and would increase the diversity of the activation response of such complexes.

Accumulating evidence in the literature indicates that heterodimerization may modify the G protein coupling profile. For instance, signaling of μ - and δ -opioid receptor heterodimers is insensitive to pertussis toxin (PTX) treatment in contrast to the individually expressed receptors, suggesting a switch from PTX-sensitive to PTX-insensitive G protein coupling (George et al. 2000). Other studies on dopamine D_1 and D_2 receptor heterodimers demonstrated that the $G\alpha_s$ -coupled D_1 and the $G\alpha_s$ -coupled D_2 receptors become $G\alpha_{q/11}$ -coupled in the corresponding heterodimer (Rashid et al. 2007). These examples illustrate how heterodimerization between two different GPCRs can determine signaling specificity.

[AU3] Asymmetry between the two protomers of a GPCR dimer may also contribute to signaling specificity (for review, see Maurice et al. 2011; Kamal et al. 2011). The recent development of a functional complementation assay allowed to explore the individual contribution of each protomer of class A GPCR dimers (Han et al. 2009). A *trans*-activation model was proposed in which the agonist-occupied protomer can *trans*-activate the second protomer that associates with the $G\alpha$ subunit to modulate the signal transduction pathway. Another example in which asymmetry downstream of the G protein determines signaling specificity was recently reported for the

melatonin MT₁/MT₂ heterodimer and RGS20 (Maurice et al. 2010). In contrast to the MT₂ receptor, which only couples to G_i proteins, the MT₁ receptor forms a constitutive ternary complex with both G_i and RGS20. By using a ligand binding-deficient MT₁* mutant and bioluminescence resonance energy transfer (BRET) studies with energy transfer probes at different sites of the MT₁/Gi/RGS20 complex, Maurice et al. showed that activation of the MT₂ protomer, within the MT₁*/MT₂ heteromer, can *cis*-activate G_i, which then interacts functionally with RGS20 bound to the MT₁* protomer. This shows that receptors, such as MT₂, that are insensitive to a specific GPCR-interacting protein (GIP) can become sensitive to the action of this GIP in the appropriate heterodimer complex.

Agonist binding to GPCRs and G protein activation are followed by the recruitment of G protein-coupled receptor kinases (GRKs) that phosphorylate the receptor, and of arrestins triggering receptor endocytosis and arrestin-dependent signal transduction (Reiter and Lefkowitz 2006). The receptor/GRK stoichiometry remains unknown but several studies have reported that binding of only one GRK molecule to the receptor dimer might be sufficient for full receptor phosphorylation of both protomers by cross-phosphorylation. This phenomenon has been shown for rhodopsin for which recruitment of the rhodopsin kinase to an activated rhodopsin can cross-phosphorylate multiple nearby non-activated rhodopsin molecules (Shi et al. 2005) and for several other class A GPCR heteromers (Pfeiffer et al. 2002, 2003). In these studies, GRK cross-phosphorylation of the unligated GPCR within the heterodimer was accompanied by co-internalization of this unligated protomer together with the ligand-bound protomer of the dimer (Pfeiffer et al. 2002, 2003). Cross-internalization of receptors within GPCR heterodimers may have important functional consequences, as shown in the case of the μ/δ -opioid receptor heterodimer. Activation of the δ -opioid receptor cross-internalizes μ -opioid receptors, thereby severely limiting μ -opioid receptor-associated morphine analgesia (He et al. 2011).

Taken together, these examples indicate how GPCR heterodimerization, by acting as scaffolds and by assembling distinct GAPCs, can generate signaling properties that differ from those observed in the corresponding homodimers thus illustrating the diversification of the functional properties of receptors by heterodimerization.

12.4 Complex Formation with Membrane Proteins Other Than GPCRs

GPCRs have not only been reported to cluster into homo- and heterodimeric complexes but also with other major transmembrane proteins as diverse as neurotransmitter transporters and ion channels, which are privileged pharmacological targets by themselves (Fig. 12.1f). A physical and functional interaction was reported between the γ -aminobutyric-acid A (GABA_A) -ligand gated channel and the dopamine D₅ receptor (Liu et al. 2000). Interestingly, this association was shown to enable mutually inhibitory effects resulting in D₅ receptor-attenuated GABA_A-mediated inhibitory postsynaptic currents and GABA_A-stimulated reductions of D₅

receptor cAMP accumulation. These findings are of primary importance since GABA_A receptors mediate fast inhibitory synaptic transmission at the vast majority of inhibitory synapses in the brain, thereby playing a fundamental role in brain physiology and pathology.

The ionotropic *N*-methyl-D-aspartate (NMDA) receptor was also reported to physically interact with the D₁ receptor (Lee et al. 2002) and more recently with the mGlu5a receptor (Perroy et al. 2008). NMDA receptors play an important role in the regulation of activity-dependent neuroplasticity and excitotoxicity and the interaction with D₁ was reported to modulate NMDA receptor-mediated functions in hippocampal culture neurons (Lee et al. 2002). Interaction with mGlu5a allows reciprocal modulation of both receptors; the mGlu5a receptor decreases NMDA receptor currents and the NMDA receptor strongly reduces the ability of the mGlu5a receptor to release intracellular calcium (Perroy et al. 2008). These studies illustrate the richness in the functional interactions between a GPCR and other unrelated transmembrane receptors, leading to a much higher degree of effector specificity and subcellular signaling pathways.

The nociceptin (ORL1) receptor has been shown to physically and specifically interact with another type of ion channel, the voltage-gated N-type Ca_v2.2 calcium channel (Beedle et al. 2004). Calcium influx through N-type calcium channels in dorsal root ganglion (DRG) neurons is an essential step in the transmission of nociceptive signals at the spinal level. Specific interaction between ORL1 and Ca_v2.2 has been shown to result in an agonist-independent, receptor concentration-dependent inhibition of the channel *in vitro* and in DRG neurons (Beedle et al. 2004). These findings raised the possibility that the activity of N-type calcium channels, and consequently transmission of pain signals, may be regulated by difference in GPCR (ORL1) receptor density. Further characterization of the ORL1/Ca_v2.2 interaction showed that prolonged exposure of ORL1 to its cognate agonist nociceptin triggers co-internalization of ORL1 and the ion channel thus down-regulating calcium entry in DRG neurons (Altier et al. 2006).

Preferential interactions have also been reported between GPCRs and neurotransmitter transporters. The dopamine D₂ and orphan GPR37 receptor have been shown to interact with the dopamine transporter (DAT), one of the key presynaptic components involved in regulating dopaminergic tone. The physical coupling between DAT and D₂ facilitates the recruitment of intracellular DAT to the plasma membrane, thus enhancing dopamine reuptake in primary cultures of rat midbrain neurons. Disruption of the D₂/DAT interaction in mice decreases synaptosomal dopamine uptake and increases locomotor activity (Lee et al. 2007). Interestingly, opposite effects have been reported following interaction of DAT with GPR37 (Marazziti et al. 2007). *Gpr37*-null mutant mice show enhanced DAT-mediated dopamine uptake in striatal membrane samples, with a significant increase in the number of membrane DAT molecules. Taken together, these two studies demonstrate how, according to the nature of the GPCR engaged in a complex with a neurotransmitter transporter, opposite functional effects can be observed.

Formation of stable GPCR-effector complexes within the plasma membrane is also exemplified by GPCRs and G protein-gated inwardly-rectifying K⁺ (GIRK)

channels, particularly of family 3 (Kir3). Kir3 channels represent a family of ion channels that mediate the postsynaptic inhibitory effect of many neurotransmitters and drugs of abuse. In 2002, a first study reported the formation of stable complexes of Kir3 with D_2 -like, D_4 and β_2 adrenergic receptors using co-immunoprecipitation and BRET experiments (Lavigne et al. 2002). Further studies reported formation of such complexes with other GPCRs such as $GABA_B$ receptors (David et al. 2006; Ciruela et al. 2010; Fowler et al. 2007), and muscarinic M_2 , serotonin 1_A , adenosine A_1 , and LPA_1 receptors (Jaen and Doupnik 2006).

Overall, these different studies suggest that assemblies containing these components may represent a common organizational premise needed to determine specificity and efficacy of signal transduction and may be critical to assure the speed of signaling.

12.5 Physiological Changes, Illustrated by Circadian Rhythmicity

The cellular state is constantly changing depending on the cell cycle and the developmental stage of the organism. An interesting regular modification of biological systems is imposed by the biological clock that generates circadian rhythmicity at the cellular level. It is estimated that at least 10 % of all genes are cycling in a circadian manner, which has obviously important functional consequences on cellular homeostasis (Lowrey and Takahashi 2004). Two recent examples illustrate the direct impact of circadian rhythmicity on GPCR function (Fig. 12.1h).

In mammals, the hypothalamic suprachiasmatic nucleus (SCN) is the central pacemaker, generating an endogenous and self-sustained circadian activity and coordinating rhythms of numerous peripheral tissues (Sujino et al. 2003). Circadian rhythmicity is generated at the cellular level but how the phase and period of circadian oscillation of different SCN neurons is maintained remains largely unknown. Oscillation in intracellular cyclic adenosine monophosphate (cAMP) levels is known to be an important component of circadian rhythmicity. The cAMP system can be manipulated by several means. cAMP production is controlled by stimulating G_s or G_i protein-coupled receptors that activate or inhibit adenylyl cyclases, respectively. Degradation of cAMP occurs through phosphodiesterases. In addition, RGS proteins such as RGS16 may inhibit the function of G_i proteins by accelerating their GTP-GDP exchange rate. Interestingly, RGS16 is expressed in a circadian manner in the dorsomedial part of the SCN with peak levels during dawn modulating cAMP signaling in a highly regulated spatiotemporal manner (Doi et al. 2011). Ablation of the *Rgs16* gene eliminates the circadian cAMP production and, as a result, lengthened the circadian period of the locomotor activity rhythm thus demonstrating the importance of defined circadian activation patterns for behavioral outputs. This is further underlined by the fact that cAMP levels in *Rgs16* knockout mice remained constant at an elevated level demonstrating that not elevated cAMP levels *per se* but rather their rhythmicity are important. Taken together, rhythmic

changes in the expression of an important component of GPCR signaling, RGS16, may define signaling specificity in a temporal manner.

Another example illustrating the impact of the circadian rhythm on GPCR signaling has been shown in a peripheral organ, the liver (Zhang et al. 2010). In hepatic cells, intracellular cAMP levels appear to be regulated by a core component of the circadian rhythm generating cellular machinery, cryptochrome proteins (Cry). Circadian control of gene expression is achieved by two transcriptional activators, Clock and Bmal1, which stimulate Cry1, Cry2 and Period repressors that feedback on Clock-Bmal1 activity (Reppert and Weaver 2002). Recent evidence indicates that Cry may have additional, extra-nuclear, functions in hepatocytes (Zhang et al. 2010). Cry protein expression is rhythmic with peak levels during the night-day transition. By interacting with G_s proteins, Cry diminishes intracellular cAMP levels in a circadian manner due to its circadian expression pattern. This may be of physiological relevance as the liver clock regulates the gluconeogenic genetic program through Cry-mediated inhibition of CREB activity during fasting. Furthermore, hepatic overexpression of Cry1 lowered blood glucose concentrations and improved insulin sensitivity in insulin-resistant db/db mice suggesting that enhancing Cry activity might be beneficial in type 2 diabetes treatment. Taken together, circadian expression of proteins involved in the regulation of GPCR signaling provides a powerful means to control GPCR signaling specificity.

12.6 Localization of GPCRs at Different Subcellular Compartments

GPCR signaling specificity can be modulated depending on receptor localization at different subcellular compartments (Fig. 12.1g). Targeting of receptors to these compartments determines receptor function due to the specific physicochemical properties of each compartment (membrane rigidity and lipid composition) and the enrichment of GAPCs and signaling pathway components in these compartments. Microdomains (lipid rafts and caveolae) are specialized compartments of the plasma membrane that are highly structured zones of low fluidity due to their enrichment in cholesterol and sphingolipids. The structural integrity of caveolae is further maintained by a coat composed of caveolin protein at the cytoplasmic side. Microdomains have been suggested to constitute a scaffolding zone of GPCR signaling cascades that also modify GPCR trafficking and favor crosstalk with other signaling pathways. The precise mechanism of GPCR targeting into these microdomains remains unclear, in particular whether some GPCRs show higher intrinsic affinity for microdomains than others or whether microdomain-enriched GAPCs target GPCR to these domains (Insel et al. 2005). Furthermore, the impact of microdomains on GPCR localization and signaling specificity may vary depending on the tissue, as some cells, such as adipocytes, are richer in microdomains than others.

An obvious role of caveolae in determining the functional outcome of GPCR signaling is provided by caveolae-localized PAR1 receptors that phosphorylate

caveolin-1 in a Src-dependent manner. Phosphocaveolin-1 then binds to the SH2 domain of the C-terminal Src kinase (Csk) resulting in a rapid decrease in Src kinase activity. Thus, PAR1-induced Src activation is down-regulated by recruiting Csk specifically in caveolin-1 containing microdomains (Lu et al. 2006).

GnRH receptors have been shown to constitutively localize within microdomains as part of a preformed signaling complex composed of caveolin-1, c-Src, protein kinase C, Ras, MAPK kinase 1/2, ERK1/2, tubulin, FAK, paxillin, vinculin, and kinase suppressor of Ras-1 (Dobkin-Bekman et al. 2009). Stimulation by GnRH induces molecular rearrangement of the complex, the rapid dissociation of some components and the phosphorylation of FAK and paxillin by ERK1/2. Whereas activation of ERK1/2 typically induces their nuclear translocation and phosphorylation of transcription factors, scaffolding into microdomain-associated multiprotein signaling complexes, as described here, helps to maintain the activated ERK1/2 pool within the cytoplasm and to phosphorylate FAK and paxillin at focal adhesions.

Primary cilia are appendages present at the cell surface on most mammalian cells involved in signal sensing and transmission (Goetz and Anderson 2010). Some GPCRs, including somatostatin receptor 3, serotonin receptor 6, smoothened, dopamine D₁ receptor and melanin-concentrating hormone receptor 1 localize to cilia. The importance of the localization and signaling of GPCRs in primary cilia is suggested by altered signaling caused by the absence of cilia formation (Berbari et al. 2009). Some molecular determinants directing localization of GPCRs to cilia have been identified in the third intracellular loop, which most likely binds to cytoskeleton proteins involved in the transport into and out of cilia (Berbari et al. 2008). The functions of cilia are defined by the signaling proteins localized to the ciliary membrane such as adenylyl cyclases, heterotrimeric G proteins and β -arrestins. What makes primary cilia so special compared to other cell surface-exposed membrane regions, remains still an open question and warrants further investigation.

Many GPCRs contain a PSD-95/Disc large/Zonula occludens 1 (PDZ) ligand sequence composed of three amino acids at their carboxyl-terminal extremity (Heydorn et al. 2004). These PDZ ligands are recognized by PDZ domains, which are present in almost 200 proteins. PDZ domains are scaffolding domains per excellence that are involved in GPCR trafficking and subcellular localization. The post-synaptic density protein 95 (PSD95) contains three PDZ domains and interacts with the serotonin 5-HT_{2C} receptor and other GPCRs in post-synaptic membranes (Becamel et al. 2002). PSD95 is also part of large molecular networks such as the PSD95/GKAP/Shank/Homer3 complex that physically and functionally connects NMDA receptors (PSD95 binding partner) to metabotropic glutamate 5 receptors (mGluR5) (Homayoun and Moghaddam 2010). Homer three proteins assemble into tetramers bridging GKAP and mGluR5. Interestingly, this complex can be disrupted by the monomeric immediate early gene Homer1a that blocks the interaction between GKAP and mGluR5 by competing with Homer3. This indicates a dynamic regulation of the presence of mGluR5 in the complex and possibly its localization to post-synaptic membranes (Bertaso et al. 2010).

Signaling of GPCRs has been reported not only at the plasma membrane or specialized plasma membrane sub-compartments such as microdomains, primary

cilia and synapses, but also in intracellular membranes such as endosomes and nuclear membranes (Boivin et al. 2008; Calebiro et al. 2010). GAPCs appear to play an important role under these circumstances as current observations are compatible with the joint translocation of both receptors and their GAPCs from the cell surface to intracellular compartments to prolong their signaling capacity. It remains however unclear why these complexes remain stable during receptor endocytosis and which component is responsible for the targeting of the complex to specific intracellular sites. Although it is tempting to speculate that specific GAPCs participate in this new paradigm of signaling specificity, experimental proof for this hypothesis is still lacking.

12.7 Targeting GPCR-GIP Interaction In Vivo: A Promising Approach for Specific Therapeutic Intervention?

An important issue concerns the pathophysiological relevance of such differential assembly of GAPCs. Indeed, the potential of targeting GPCR-GIP interactions as a novel approach for therapeutic intervention is of particular interest and specific disruption of a GPCR/GIP interaction in a given tissue without affecting the interaction of this same GPCR with other GIPs in another tissue represents an attractive strategy for drug discovery. Emerging evidence that illustrate this concept came from studies based on the use of cell-penetrating HIV TAT-conjugated peptides that clearly showed the potential of peptides in disrupting a specific GPCR/GIP interaction.

A first example was proposed by the study of Ji et al. (2006) reporting that intravenous injection in rats of a TAT-conjugated peptide encompassing the PTEN-binding motif located within the i3 loop of 5-HT_{2C} receptor suppresses the firing rate of ventral tegmental area (VTA) dopaminergic neurons induced by d9-tetrahydrocannabinol (THC), thereby reproducing the effect of 5-HT_{2C} receptor agonists. As 5-HT_{2C} receptor agonists, this TAT-peptide also inhibits the rewarding effects of THC (and nicotine) mediated by VTA dopaminergic neurons. However, it does not reproduce the side effects (anxiogenic effects, penile erection, hypophagia, and motor functional suppression) observed following administration of 5-HT_{2C} agonists (Ji et al. 2006).

Disruption of the mGluR7a/PICK1 interaction by intravenous injection of a TAT-conjugated peptide was able to trigger absence epilepsy-like seizures in mice and rats, providing evidence that a single PDZ protein/GPCR interaction can result in a specific neurological disorder (Bertaso et al. 2008). More recently, disruption of the interaction between 5-HT_{2A} and PDZ proteins in diabetic neuropathic rats by a TAT-conjugated peptide encompassing the nine C-terminal residues of the 5-HT_{2A} receptor was shown to induce an antihyperalgesic effect and to strongly enhance antihyperalgesia induced by the selective serotonin reuptake inhibitor (SSRI) fluoxetine. This study thus suggests that 5-HT_{2A} receptor/PDZ protein interactions might contribute to the resistance to SSRI-induced analgesia in painful diabetic neuropathy and that disruption of these interactions might be a valuable strategy to

design novel treatments for neuropathic pain and to increase the effectiveness of SSRIs (Pichon et al. 2010).

The importance of D_1/D_2 heterodimers in human disease was suggested in a recent study showing that physical interaction between dopamine D_1 and D_2 receptors is markedly increased in postmortem brains of patients suffering from major depression. Administration of an interfering peptide consisting of a sequence located in the third intracellular loop of D_2 that disrupts the D_1 - D_2 receptor complex reduced immobility in the forced swim test without affecting locomotor activity, and decreased escape failures in learned helplessness tests in rats (Pei et al. 2010). This study thus provided evidence for potential implication of GPCR heterodimers and associated signaling specificity in human diseases.

12.8 Conclusions

Evolution has selected several ways to achieve signaling specificity, by generating receptor isoforms, receptor polymorphisms, differential localization and targeting of receptors and pre-assembly of receptors and GAPCs into defined and distinct signaling modules. The latter holds the potential to explain many of the observed signaling specificity. However, we are only beginning to define the precise composition and impact of these modules on receptor signaling. Given the dynamic nature of these modules, a precise analysis has to be performed for each receptor in a specific cellular context and under defined physiological and pathological conditions.

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Author Queries

Chapter No.: 12   0001645432

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