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Pilot the pulse: controlling the multiplicity of receptor dynamics

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G protein-coupled receptors (GPCRs) are involved in almost every (patho)physiological process, which explains their importance as drug targets. GPCRs have long been regarded as on/off-switches, which is reflected by direct activation or blockade of these receptors through the majority of marketed GPCR drugs. In recent years, however, our view of GPCRs has changed dramatically. GPCRs are now appreciated as integrative and highly dynamic signaling machines which can adopt numerous distinct conformations enabling them to initiate a highly ramified signaling network. We argue here that it may be possible to chemically encode distinct signaling profiles into ligands by rational ligand design. We exemplify our hypothesis by fine-tuning partial and biased agonism, thereby exploiting two new principles of GPCR modulation – dynamic and dualsteric ligand binding. We propose that the emerging understanding of the multiplicity of receptor dynamics will eventually lead to rationally designed new drugs which pilot the pulse; in other words, that stabilize distinct receptor states to fine-tune GPCR signaling.

GPCRs as integrative and dynamic signaling machines

GPCRs are ubiquitously expressed integral seven-transmembrane proteins comprising a superfamily of approximately 800 members in humans [1,2]. GPCRs recognize a plethora of chemically diverse ligands and hence mediate almost every (patho)physiological process in humans, underscoring why they make up one of the most important groups of drug targets [3].

Upon binding their endogenous agonists at the so-called orthosteric binding site, GPCRs undergo conformational rearrangements leading to closure of the ligand-binding pocket towards the extracellular space (from partial to complete depending on the receptor) and, concomitantly, to an opening of the intracellular domains, which permits binding of cytosolic adaptor proteins [4,5]. Moreover, many GPCRs contain topographically distinct allosteric binding sites, and this allows modulation of receptor function not only by classical orthosteric drugs but also by allosteric [6,7] and dualsteric (i.e., bitopic orthosteric/allosteric) ligands [8–10] (Figure 1).

Canonical GPCR signaling is mediated by G proteins [11], but receptors may also engage β-arrestins [12] with their plethora of downstream interacting proteins [13] and other GPCR-interacting proteins (GIPs) [14,15] (Figure 1). The recruitment of effector and adaptor proteins by GPCRs may thus initiate a highly ramified signaling network leading to changes in second messenger levels (cAMP, cGMP, IP_3, DAG, and Ca^{2+}), activation of kinases, modulation of ion channels, and regulation of transcription [16]. Receptor signaling can change dynamically in space and time: a single GPCR can engage different signaling proteins sequentially and may even continue signaling from intracellular compartments after sequestration from the plasma membrane [17]. It is most likely that this multiplicity of receptor dynamics results from the ability of the receptor to adopt numerous distinct conformations [18,19]. An agonist may stabilize ensembles of distinct receptor conformations [20] which are unique to the nature (orthosteric, allosteric, or dualsteric) and molecular structure of the agonist. Consequently, every agonist specifically determines the receptor signaling profile in space and in time.

It is our opinion that it may become feasible to address and stabilize distinct receptor conformations in a predictable fashion. We hope that the emerging understanding of the structural basis of GPCR activation will allow us to chemically encode specific signaling facets into a ligand by rational ligand design. Our opinion is supported by several recent studies, including our own work, which have succeeded in setting the degree of GPCR activation (partial agonism) and fine-tuning signaling at multiple levels (biased agonism). Based on these studies we propose here general molecular mechanisms of partial and biased agonism, and suggest that it might even be achievable to manipulate signaling compartmentalization by extracellular ligands. Some of the proposed mechanisms require ligand engagement with allosteric sites of the receptor whereas others appear to occur via the orthosteric site. We argue that exploiting the multiplicity of receptor

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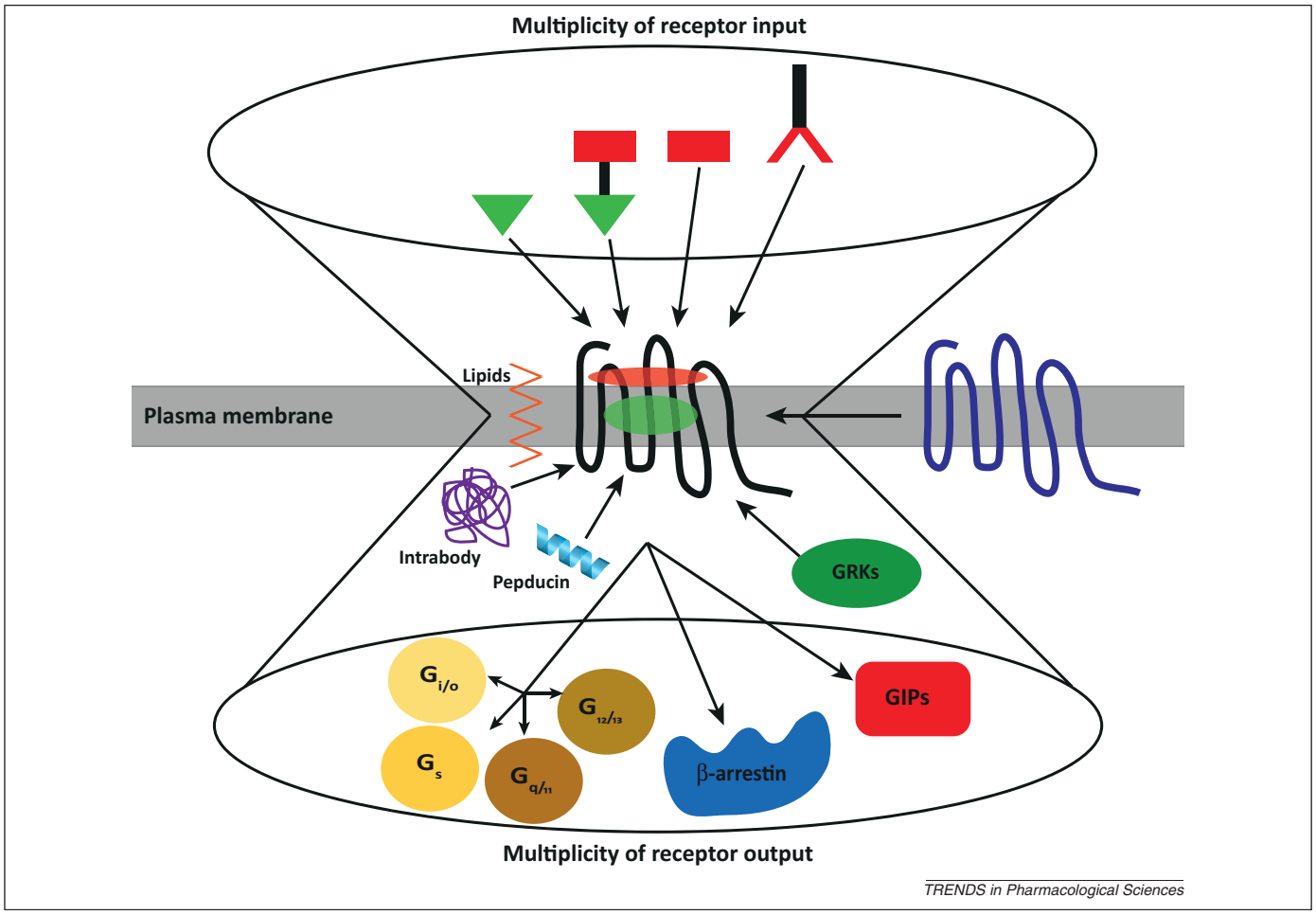

Figure 1. A G protein-coupled receptor (GPCR) is a dynamic signaling hub. Extracellular ligands for GPCRs can be classified as orthosteric (green triangle), allosteric (red rectangle), or dualsteric ligands (i.e., bitopic orthosteric/allosteric), which bind to the orthosteric site (green ellipse), the allosteric site (red ellipse), or simultaneously to both sites on the receptor protein, respectively. Moreover, GPCRs can be activated by antibodies targeting extracellular receptor domains. GPCR function can be diversified either by lateral homo- or hetero-oligomerization with other receptors (indicated by a GPCR depicted in blue) [73] or by interference with lipids present within the plasma membrane [93,94]. GPCR signaling can be fine-tuned by 'phosphorylation barcodes' specifically attached by endogenously expressed GPCR kinases (GRKs) [95,96]. Moreover, GPCR signaling can be initiated or regulated from the cell interior by heterologous expression of pepducins [83,84,97] or conformationally selective intrabodies [85], respectively. Canonically, GPCRs can activate up to four classes of G proteins ($G_{i/o}$, G_s, $G_{q/11}$, and $G_{12/13}$) [11], which further leads to alterations of both second messenger levels and activity of ion channels. Additionally, GPCRs are able to recruit β-arrestins, and this shuts off G-protein signaling but initiates a second wave of β-arrestin-mediated signaling [12]. GPCR signaling is further diversified by an increasing number of GPCR interacting proteins (GIPs) [14] such as the Na^+/H^+ exchanger regulatory factor (NHERF) [15].

dynamics to rationally design drugs may eventually create new and beneficial therapeutic opportunities.

Fine-tuning GPCR efficacy

Depending on their degree of receptor activation, agonists are subclassified as full or partial agonists. Salbutamol, buprenorphine, and aripiprazole are well-established and efficacious drugs in the treatment of bronchial asthma [21], pain [22,23], and schizophrenia [24], respectively. Intriguingly, these drugs all behave as partial agonists. *In vivo*, an orthosteric partial agonist will compete with and thereby limit receptor activation by endogenous agonists. Thus, it is conceivable that partial agonists are less likely to lead to chronic overstimulation of receptors and, at least in some instances, might induce less internalization of receptors and, hence, less tolerance [25]. Vice versa, in the absence of endogenous agonist a partial agonist will maintain a level of receptor activity.

Hence, partial agonists might, at least in some instances, offer some degree of superiority over classical full agonists, and this makes them interesting drug candidates. Until now, however, the rational design of partial GPCR agonists has hardly been achieved. In the following chapter we propose a molecular mechanism of partial agonism that may allow the rational design of such ligands.

Concepts of partial agonism

Partial agonists have been shown to stabilize distinct receptor conformations different from those stabilized by classical full agonists [26,27]. At the level of the receptor itself, crystal structures of the turkey β_1-adrenoceptor co-crystallized with the partial agonists salbutamol and dobutamine revealed that these ligands did not induce rotamer conformational changes of the epitope $Ser215^{5.46}$, in contrast to the full agonists isoprenaline and carmoterol [28]. This resulted in a weaker contraction of the orthosteric binding pocket by the partial agonists as compared with the crystal structures of the full agonists isoprenaline and carmoterol, and hence led to submaximal receptor activation [28]. At the level of signaling it was shown very