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RGS proteins destroy spare receptors: Effects of GPCR-interacting proteins and signal deamplification on measurements of GPCR agonist potency

Peter Chidiac *

Department of Physiology and Pharmacology, University of Western Ontario, Canada

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ABSTRACT

Many GPCRs are able to activate multiple distinct signaling pathways, and these may include biochemical cascades activated via either heterotrimeric G proteins or by β-arrestins. The relative potencies and/or efficacies among a series of agonists that act on a common receptor can vary depending upon which signaling pathway is being activated. This phenomenon is known as biased signaling or functional selectivity, and is presumed to reflect underlying differences in ligand binding affinities for alternate conformational states of the receptor. The first part of this review discusses how various cellular GPCR interacting proteins (GIPs) can influence receptor conformation and thereby affect ligand–receptor interactions and contribute to signaling bias. Upon activation, receptors trigger biochemical cascades that lead to altered cellular function, and measuring points along the cascade (*e.g.*, second messenger production) conveys information about receptor activity. As a signal continues along its way, the observed concentration dependence of a GPCR ligand may change due to amplification and saturation of biochemical steps. The second part of this review considers additional cellular factors that affect signal processing, focusing mainly on structural elements and deamplification mechanisms, and discusses the relevance of these to measurements of potency and functional selectivity.

1. GPCRs isomerize between multiple conformational states

The concentration dependence, or *potency*, of a pharmacological signal initiated by an agonist acting on its receptor will reflect a combination of four factors: (1) the availability of receptors to activate downstream signaling, or *receptor density* (2), the concentration of agonist relative to its *affinity* for the receptor, (3) the proclivity of the agonist to promote/sustain a relevant activated receptor state, or in other words the *intrinsic efficacy* of the agonist and (4) whatever biochemical steps lie between the activated receptor and the endpoint being measured to gauge its activity. In terms of simple mass action (*i.e.*, a single ligand binding to a uniform population of monomeric receptors), the affinity between a ligand and its receptor is signified by the *equilibrium dissociation constant* (K_D), which is typically expressed in molar units. K_D is equal to the ratio of the dissociation rate constant to the association rate constant [1]. Since the receptor rapidly isomerizes between multiple conformational states (*i.e.,* at least one active and one inactive), each with its own distinct agonist binding properties, K_D as measured in equilibrium binding experiments actually represents an amalgam of the affinities of the ligand for each individual conformation. Agonists bind with higher affinity to activated receptor conformations and also promote isomerization toward those states, whereas inverse agonists analogously favor inactive states and neutral antagonists show no preference. A highly efficacious agonist is one that shows a strong preference for binding to activated receptor, and once bound, it will also tend to disfavor isomerization back to an inactive state; thus, it will be more likely to initiate a signaling cascade once bound to the receptor than would a weakly efficacious agonist.

Early evidence for the ability of GPCRs to spontaneously isomerize between active and inactive states followed upon the successful sequencing of GPCR-encoding genes, as the heterologous expression of cloned receptors revealed G protein and effector activities to be elevated in transfected as compared to nontransfected cells [2]. The observed effects of agonists and inverse agonists in such systems were initially formalized in terms of a two-state model [3], wherein a receptor is presumed to isomerize between a single active state and a single inactive one. While many observed GPCR-mediated effects appeared consistent with such a model,

* Address: Department of Physiology and Pharmacology, Medical Sciences Building, University of Western Ontario, London, Ontario N6A 5C1, Canada.
E-mail address: peter.chidiac@schulich.uwo.ca

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some findings implied that it was overly simplistic [4,5], and it is now generally accepted that GPCRs can assume multiple active conformations and signal pleiotropically [6–8]. Over time, multiple studies have shown that a group of agonists that act on a common receptor can differ among themselves in terms of their relative abilities to stimulate one or another signaling pathway [7], a phenomenon that has been termed *biased agonism* (alternatively *functional selectivity* or *agonist trafficking*). Occasionally drugs are identified that have stimulatory effects on some receptor-mediated signals but act as inverse agonists on other pathways mediated via the same receptor, and such ligands are referred to as *protean agonists* [9]. In addition to the effects of orthosteric ligands, which bind to the same site on the receptor targeted by its endogenous agonist(s), GPCR signaling can also be increased or decreased by allosteric drugs which bind elsewhere on the receptor, and it is now clear that such drugs can similarly exhibit signaling bias among pathways activated via the targeted receptor [10].

Small molecule effects on GPCR isomerization are not limited to orthosteric and allosteric drugs. Cellular or assay constituents such as ions and phospholipids can influence GPCR conformational states and thus affect ligand binding, as can experimental factors such as temperature, pH and osmolality [11,10]. Thus when comparing the effects of a ligand on different signaling pathways, it is best to keep buffer components, etc., as consistent as possible. On a similar note, the experimenter should be aware when doing transfection-based receptor assays that exogenous proteins included to assess signaling might themselves also influence receptor state, particularly if they bind directly to the receptor, and this in turn can influence agonist binding as well as resultant pharmacological outputs. As well, different types of cells or tissues may express different amounts of or different varieties of receptor-interacting proteins, and this in turn may affect agonist concentration dependence. The following section will review effects of cellular proteins on receptor conformation.

1.1. GPCR–GIP interactions affect receptor conformation

The binding of any other protein to a receptor would be expected alter its conformational properties [12], and evidence of this can be seen in the changes in measured binding affinities that occur when GPCRs bind to G proteins, other receptors, or other GPCR-interacting proteins (GIPs). A GIP-induced alteration in the conformational properties of a GPCR could potentially change the relative affinities of a receptor for one agonist versus another and thereby contribute to functional selectivity.

1.1.1. Receptor activity modifying proteins

Some of the clearest examples of GIP-induced conformation-altering effects on GPCRs are found with the receptor activity modifying protein (RAMP) family. These are single transmembrane-spanning proteins that associate with certain receptors, predominantly members of Family B1 (secretin-like) GPCRs. While not receptors themselves, RAMPs can form stable complexes with GPCRs and modify their binding properties, in some cases altering selectivity from one endogenous activator to another [13]. For example, the calcitonin receptor on its own exhibits relatively high affinity for calcitonin and relatively low affinity for amylin, but when bound to RAMP1 or RAMP3 this agonist preference is reversed, and moreover the same RAMP-induced switch in rank order is also exhibited in the potencies of these agonists to stimulate cAMP production [14]. Comparable effects are observed upon the binding of RAMPs to the calcitonin receptor-like receptor (CRLR), as these can combine to yield receptors for calcitonin gene related peptide (CRLR + RAMP1) or adrenomedullin (CRLR + RAMP2 or RAMP3) [15]. These findings indicate that the binding of a GIP to a GPCR can change the agonist rank order with respect to both binding and effector activation.

The ability of RAMPs to alter GPCR conformation suggests the possibility that they could potentially alter interactions with G proteins or other intracellular proteins, and there is some evidence for this. Morfis and co-workers [16] showed that the association of either RAMP1 or RAMP3 with the calcitonin receptor led to a 20–30-fold increase in amylin potency with respect to Gs-mediated signaling while amylin potency in activating ERK1/2 or calcium transients was increased only 2–5-fold. Taken together with their observed effects on ligand binding [14], these results imply that RAMP1 and RAMP3 impart functional selectivity by favoring the ability of amylin to selectively activate Gs-mediated signaling through the calcitonin receptor [16].

1.1.2. GPCR oligomerization

Can proteins other than RAMPs bind to GPCRs and govern agonist binding preferences? One would expect so, and indeed there is much evidence which shows that GPCR binding properties, and thus presumably conformational state, are influenced by the binding of other proteins. When receptors form into homo-oligomers or hetero-oligomers (*e.g.*, dimers or tetramers), there can be cooperative interactions wherein the affinity of one binding site is increased or decreased by the binding of a ligand to another orthosteric site within the oligomer [17,18]. This also holds for allosteric sites within GPCR oligomers, and furthermore there can be cooperativity between an orthosteric site on one protomer and an allosteric site on another [19]. Apart from ligand-dependent effects, protein–protein interactions within a GPCR oligomer also appear to influence conformation. With hetero-oligomeric GPCRs, agonist efficacies, potencies or binding affinities in many cases are found to differ from their homomeric counterparts (reviewed in [20]), suggesting effects analogous to those observed with RAMP-GPCR complexes. For example, the various opioid receptor subtypes can assemble into μ-δ, μ-k, and δ-κ heteromers, and these show agonist responses that are distinct from those of the parent homomeric receptors [21], with morphine demonstrating greater potency at μ-δ heteromers than at either μ or δ homomers [22]. Conversely, with β1-β2 adrenergic [23] and D2-D3 dopaminergic heteromers [24], agonist potency has been found instead to be decreased relative to the corresponding homomeric receptors. A reasonable interpretation of such heteromer/homomer differences is that binding between associated GPCRs impacts their conformational states. This also suggests the possibility that heterooligomerization could affect G protein (or β-arrestin) affinities for GPCRs, however evidence for that so far appears to be limited.

1.1.3. G proteins and β-arrestins

Agonist affinity has been shown to increase when GPCRs are coupled to G proteins [17], which presumably reflects alterations in receptor conformation due to the allosteric effects of G protein binding [12]. Such GPCR conformational changes are expected to vary from one G protein to the next, as they do from one agonist or inverse agonist to the next [25]. Apart from their G protein-mediated effects, many GPCRs can also signal in a G protein-independent manner via β-arrestins, a family of proteins originally identified through their role in receptor desensitization [26]. Interestingly, β-arrestins have been shown to increase agonist affinity when bound to GPCRs in a manner analogous to that of G proteins [27], again implying an effect on receptor isomerization. It is well established that agonist rank orders at a common GPCR target can vary from one G protein- or β-arrestin-mediated signal to the next, and this is taken to indicate that the mutual allostery between a ligand and a G protein or β-arrestin is unique for each combination.

A common conception (or perhaps misconception) is that an agonist can selectively promote a particular receptor conformation and subsequently "traffic" the receptor to an appropriate G protein or other signaling partner. Although there is no disputing that agonist rank orders can vary depending on which signaling pathway is being activated via a common GPCR target, at present there seem to be few if any direct observations of an agonist causing a receptor to "choose" through which G protein or β-arrestin it will signal. An alternative view is that a state-selective agonist may differentially recognize pre-formed complexes that include the same type of receptor coupled to different signaling partners [28]. There is considerable evidence that receptors and G proteins pre-couple in the plasma membrane [17,29–31], although there may be uncoupled receptors as well [32]. Pre-coupling would presumably limit the range of conformations available to the receptor and the reciprocal allosteric interactions between a GPCR and its physically associated G protein might be expected to engender selectivity toward some agonists over others.

1.1.4. Signaling complexes

The above examples demonstrate that cellular proteins that bind to GPCRs are capable of producing allosteric effects that alter ligand binding properties. Many other GIPs have been identified all of which could potentially influence GPCR conformational states as well [12]. Known GIPs include effectors, various kinases, phosphatases, scaffolding/adapter proteins (see Sections 2.2 and 2.2.1, below), chaperones, and sorting proteins, as well as signaling modulators such as regulator of G protein signaling (RGS) proteins (see Section 2.1.1, below), calmodulin, and periplakin [29,33–36]. Each of these may in turn bind to other proteins and bring them into close proximity with the GPCR, and this could further affect receptor conformation, either directly or indirectly. The choreography regarding how and when (and if) all of these additional proteins interact with GPCRs within the context of a single signal transduction event for the most part remains to be worked out. It seems unlikely either that a GPCR molecule would be able to interact with all of its binding partners simultaneously, or conversely that signal transduction could be accomplished exclusively through a series of transient steps involving bimolecular protein interactions.

While some GIP–GPCR interactions may be transient, there is now a large body of data that points to the existence of GPCR signaling complexes (also variously called receptosomes, signalosomes, etc.), as summarized in several previous reviews [28,29,34], and as well the implications of this with respect to signaling bias have been considered in depth by Piñeyro [28]. Whether GPCR signaling through multiprotein complexes is the norm is not yet clear, but broadly speaking, the more components that are contained within such a complex, the less likely it seems that its formation could be driven by the mere binding of an agonist to a receptor. Formally, any protein within a complex could affect the conformation of the resident GPCR(s), however given that many of these proteins also influence signaling outcomes (see examples below) it may be difficult to discern to what degree a particular GIP governs the ligand binding properties of the receptor as opposed to modulating agonist concentration dependence via post-receptor effects.

1.2. Post-translational modification of GPCRs – a game changer?

Covalent modifications such as phosphorylation and palmitoylation can strongly influence GPCR signaling properties [37–39], although, strictly speaking, covalently modified receptors should be viewed as distinct subpopulations, since such changes are not as readily reversible as ligand binding. The phosphorylation of GPCRs by second messenger-activated kinases (protein kinase A, protein kinase C) and by GPCR kinases (GRKs) was originally identified as a general mechanism to decrease G protein coupling and thus cause receptor desensitization, with phosphorylation by GRKs also promoting GPCR binding to β-arrestins [26] (although a few receptors appear to be able to bind to β-arrestins in a phosphorylation-independent manner [39]). Apart from their roles in desensitization, β-arrestins are now recognized as essential elements in G protein-independent signaling pathways [40]. β-Arrestin-mediated receptor responses may display different agonist rank orders than G protein-dependent signals [40], and in some cases ligands that are inverse agonists with respect to G protein-mediated functions have been found to promote activation of β-arrestin-dependent signals [41–43]. Although a common supposition, it may be a fallacy to expect that a GPCR ligand could drive receptor isomerization toward either a "G protein-binding conformation" or a "β-arrestin-binding conformation" as the two would tend to be chemically distinct (*i.e.*, non-phosphorylated *vs* phosphorylated) and thus could not readily interconvert. If their targets are chemically distinct and not just conformationally distinct, it follows that G proteins and β-arrestins may not compete in any meaningful sense for activated GPCRs.

1.2.1. Will the real non-canonical GPCR partner please stand up?

Based on the above, one might argue that the relevant GPCR species for β-arrestin signaling is actually the non-phosphorylated receptor existing in a conformational state that binds selectively to one or more GRKs. Upon phosphorylation, the receptor would become a suitable binding partner for β-arrestins and have reduced affinity for G proteins, reflecting an essential change in its signaling capabilities. Consistent with this idea, recent findings obtained using BRET-based approaches suggest that GRK and β-arrestin recruitment follow similar time courses [44], and that the presence of kinase-dead GRK mutants can impede GPCR-β-arrestin association [45].

A number of outcomes are possible subsequent to the recruitment of GRKs to GPCRs, and this depends on which GPCR and which GRK isoform are involved. For example, the binding of GRK2 under some circumstances appears to attenuate GPCR signaling via mechanisms that are independent of its kinase activity and do not necessarily involve β-arrestin recruitment [46,47]. Moreover, the various GRK isoforms differ in their modes of regulation and plasma membrane association and to some extent they appear to distinguish between different GPCRs [48]. More importantly, the interactions of a GPCR with different GRK isoforms can lead to different desensitization or signaling outcomes, and as well multiple ligands that act on the same receptor may engage different GRKs [44,45,48]. Furthermore, additional kinases may become involved in a ligand- and/or tissue-specific manner to yield a specific phosphorylation profile that promotes the coupling of a GPCR to a particular pathway [49]. Once phosphorylation has occurred, it is possible that the continued presence of an activating ligand at the receptor may be needed for signaling to continue, but it seems that the key step in this process is likely to be the ability of individual ligands to stabilize GPCR conformations amenable to serving as substrates for relevant kinases.

2. Post-receptor signal processing

When a ligand and its target GPCR interact, the resulting quantity of activated receptors will depend upon the first three of the four factors described above in Section 1 (in short, receptor density, drug affinity, and intrinsic efficacy). Once a GPCR becomes activated, it may set off a cascade of events that leads to a measurable response. It has long been recognized that the pharmacological response to an agonist (relative to its maximal effect) is not always proportional to the fraction of receptors bound [50,51]. When the

K_D value is greater than the corresponding EC_{50} there is said to be *receptor reserve* or *spare receptors*. What this means is that there are more receptors present in a system than are needed for a particular agonist to maximally stimulate a given pharmacological response, *i.e.*, that there is "redundant" signaling upstream of the response being measured. For example, this could happen if a sub-saturating concentration of agonist for a Gs-coupled GPCR population were to stimulate adenylyl cyclase sufficiently to produce all of the cAMP needed to activate essentially all of the cAMP binding sites of its target protein kinase A population, in which case any activation of the remaining receptors would not further increase the activity of PKA. Thus, if we were to measure protein kinase A stimulation as a function of agonist concentration, the resultant EC_{50} value would be less than the K_D, reflecting the disproportionality between response and agonist binding. This same essential phenomenon can be repeated as the receptor signal moves down its biochemical cascade. Continuing with the previous example, if we consider an experimental endpoint that is dependent upon a particular target of PKA, it is possible that the complete phosphorylation of this target would not require all of the available PKA to be activated by cAMP, in which case even less receptor would initially need to be activated, and therefore the EC_{50} value corresponding to the PKA-dependent endpoint would deviate even further from K_D.

To mathematically describe the phenomenon of receptor reserve, Black and Leff's Operational Model [52] posited that the relationship between agonist-receptor complex and pharmacological effect follows the law of mass action, which in turn allows for the determination of the fraction of receptors that must be occupied by agonist to obtain a half-maximal response. Although receptor density was explicitly included in the model, efficacy was still treated as a "black box", albeit one recognized as reflecting "elements in the response transmission machinery beyond the initial receptor linked steps [52]". Further studies have modeled signaling cascades in terms of individual biochemical steps, showing with simulated data how receptor reserve can increase as a signal moves downstream from the original receptor stimulus [53,54].

While the propagation of receptor-initiated stimuli through biochemical cascades is no doubt a key element in determining the concentration dependence of pharmacological responses, it should be noted that the stepwise progression of a signal can be facilitated or tempered in various ways. For example, whereas signaling cascades tend to be viewed as a series of steps in which a stimulus is serially amplified as it progresses, the transmission of a signal may also be limited by countervailing de-amplification mechanisms. As well, the efficiency with which one step in a signaling cascade leads to the next may be either increased or decreased by structural elements which affect the local concentrations and/or proximity of relevant constituents. Both of these types of factors may influence the potencies of an agonist that activates multiple signaling pathways through a single GPCR, and thus are of interest from the perspective of functional selectivity. Once a receptor is activated, the intervening steps that lead to a measured response will differ depending on the signaling cascade, and the overall impact of these on agonist concentration dependence may differ from one cascade to the next. One consequence of this is that differences in the potency of an agonist in one pathway verses another cannot be taken *a priori* as accurately reflecting differences in the affinity of the agonist for one or another conformational state of the receptor.

A noteworthy examination of two different signals initiated via the same receptor was described in a study on serotoninergic signaling by Kurrasch-Orbaugh and co-workers [55]. That study showed receptor reserve to be greater with respect to 5HT-stimulated phospholipase A2 as compared to 5HT-stimulated phospholipase C activation in NIH3T3 fibroblasts stably expressing the 5HT2a receptor. Those authors also compared a series of 5HT2a receptor agonists and found clear evidence of signaling bias toward one or the other pathway, notwithstanding that most agonists showed greater potency with respect to PLA2 signaling, as might be expected given the greater receptor reserve [55]. A practical implication of this is that it may be challenging to identify biased ligands that have greater potency toward pathways with relatively little receptor reserve than toward pathways with greater reserve.

2.1. Effects of de-amplification processes on measurements of agonist potency

When a receptor signal is initiated in a cell, mechanisms to limit it come into play almost immediately. For example, cAMP produced in response to Gs-coupled receptor activation in turn promotes the activity of cAMP phosphodiesterases (via protein kinase A) to rapidly reduce cellular cAMP levels [56]. Similarly, the effects of kinases activated by receptor signaling are reversed by phosphatases [57,58]. De-amplification processes may be ongoing or may be acutely turned on to serve as negative feedback mechanisms, but either way they have the potential to minimize signal propagation and in doing so serve to limit efficacy or, figuratively, "destroy spare receptors." This could theoretically eliminate whatever reserve had been "created" by upstream signaling.

2.1.1. RGS proteins

Activated GPCRs turn on heterotrimeric G proteins by promoting the dissociation of GDP, which allows the activating nucleotide GTP to bind. The regulator of G protein signaling (RGS) proteins are GTPase accelerating proteins (GAPs) that hasten the deactivation of GTP-bound G proteins [33]. Thus they have the ability to nip G protein-mediated signaling in the bud. With the exception of RGS2, which selectively acts on Gq [59,60], all RGS proteins are GAPs for Gi/o subfamily members and about half of them are also GAPs for Gq [61]. As well, a number of RGS proteins can inhibit GPCR signaling by acting as "effector antagonists" for Gs-activated adenylyl cyclase or Gq-activated phospholipase Cβ [33]. When the expression of RGS proteins is induced [62] or they are artificially overexpressed [63], agonist-stimulated responses tend to exhibit decreased maximal effects, lower potency, or both, and correspondingly the reduction or elimination of a particular RGS protein will tend to increase agonist activity at some receptors [64].

Although RGS proteins can be upregulated [62,65] or recruited [66] to attenuate GPCR signals, they also play an ongoing modulatory role in GPCR signaling [67]. One technique that dramatically reveals the importance of RGS proteins in the control of GPCR signaling is the use of Gi/o mutants that bear a single amino acid residue substitution which greatly reduces their affinity for RGS proteins and thus renders their signaling insensitive to all endogenous RGS proteins. The expression of RGS-insensitive G proteins is associated with leftward shifts in agonist concentration dependence of an order of magnitude or more [68,69]. Such changes correspond to a substantive *increase* in receptor reserve when the negative contribution of endogenous RGS proteins to G protein-mediated signaling is removed. Furthermore, the loss of G protein inactivation due to the introduction into cells of RGS-insensitive G proteins can also increase the duration of G protein-mediated signaling effects [70].

Clearly not all GPCR signaling pathways are governed by endogenous RGS proteins, as many of them have no discernible effect on G12/13-, Gs-, or Gq-mediated signaling [61,71]. As well, there is some RGS protein selectivity within the Gi/o subfamily, and signals initiated by some GPCRs seem to be more amenable than others to regulation by RGS proteins [67,71,72]. Observed differences from one receptor to the next imply that

receptor-dissociated Gα-GTP is not the only target (or perhaps not even the primary target) of RGS protein inhibitory activity, however the mechanisms that confer GPCR selectivity are not entirely clear. In some cases RGS proteins may be recruited to their target G proteins by binding directly to the activated receptor or to an associated effector or scaffolding protein [33].

If a receptor is capable of activating two (or more) different signaling pathways, it is possible that they could be differentially governed by RGS proteins. For example, if a GPCR were to activate both an RGS-sensitive G protein and an RGS-insensitive G protein, the former signal but not the latter would be de-amplified at the G protein-effector step. It follows that the RGS-sensitive G protein population might provide a less robust impetus to downstream signaling events than the RGS-insensitive G protein population, which could contribute to differences in the relative potencies of the agonist for one G protein-mediated signaling pathway versus the other. In other words, the presence of RGS proteins would tend to bias signaling in favor of pathways that are insensitive to their effects.

Apart from their inhibitory effects on signaling, it is possible that RGS proteins could affect GPCR function *per se*, and indeed it has been suggested that RGS proteins could play a role in biased agonism [73]. As with other binding partners, the binding of an RGS protein either directly or indirectly to a GPCR [33] could influence the conformational state of the receptor [12], and this in turn could conceivably favor the binding of some ligands over others.

2.2. Organization of G protein-mediated signals by scaffolding/anchoring proteins

Scaffolding or anchoring proteins are structural proteins that generally lack functional domains but contribute to signaling by bringing together components within a signaling cascade. For GPCR signaling the most important structural proteins are members of the PDZ (postsynaptic density protein 95, Drosophila disc large tumor suppressor, zonula occludens-1 protein) and AKAP (A kinase anchoring protein) families. (Leaving aside the question of whether β-arrestins are merely scaffolds or signaling proteins in their own right.) The general mechanisms by which AKAPs and PDZ proteins coordinate signaling are well known and have been reviewed in depth elsewhere [74,75]. Briefly, scaffolding proteins bind simultaneously to multiple components within a signaling cascade. This increases signaling specificity and efficiency, and promotes spatial and temporal focusing. For example the PDZ protein INAD coordinates visual signaling in *Drosophila* by tethering together phospholipase C (NORPA), protein kinase C (INAC), and the cation channel TRP and brings them into proximity with the photon receptor rhodopsin and its associated G protein Gq [76]. This arrangement increases the efficiency of signaling as diacylglycerol produced by phospholipase C rapidly reaches protein kinase C, which then phosphorylates and activates TRP, resulting in the fastest known G protein-coupled signaling cascade in nature [76]. In addition to improving signaling specificity and sensitivity, scaffolding proteins can also facilitate signal turnoff or deamplification, for example there are AKAPs that juxtapose protein kinase A with a phosphodiesterase or phosphatase, which respectively limit activation of the kinase and dephosphorylate its targets [74], while the PDZ protein GIPC binds to both D3 dopaminergic receptors and RGS19 and appears to decrease G protein activation [75]. By increasing agonist signaling efficiency, scaffolding proteins have the potential to increase cellular efficacy and receptor reserve, while increasing the proximity of negative regulators of signaling would be expected to decrease the duration of signaling and could impact measured potency as well.

2.2.1. Role of scaffolding proteins in functional selectivity

Can scaffolding proteins contribute to biased agonism? While it is unknown whether these structural proteins can influence the binding of a GPCR to one agonist versus another, it is clear that they can limit or increase the chances that a particular signaling pathway will be utilized once a receptor has been activated. For example, the type 1 parathyroid hormone receptor (PTHR1) can activate both adenylyl cyclase signaling via Gs and phospholipase Cβ signaling via either Gq or Gi [77], and as well it can inhibit adenylyl cyclase via Gi and activate ERK1/2 via both β-arrestin and G protein-mediated pathways [78]. PTHR1 also can bind to the PDZ proteins Na^+/H^+ exchanger regulatory factors 1 and 2 (NHERF1 and NHERF2) [79]. This can affect PTHR1 signaling, as NHERF2 also binds to phospholipase Cβ1 and promotes the ability of PTHR1 to stimulate this effector enzyme [79]. Furthermore, a study focusing on the effects of the agonist PTH1-34 on G protein activation showed PTHR1-stimulated guanine nucleotide exchange on Gs to be reduced by the binding of the receptor to NHERF2 whereas exchange on Gq and Gi was increased, while NHERF1 similarly enhanced agonist- and receptor-promoted nucleotide exchange on Gq but had little or no effect on either Gs or Gi [77]. Thus the binding of a scaffolding protein to a GPCR can govern the G protein selectivity of the receptor.

3. Summary and concluding remarks

When a receptor becomes activated by an extracellular chemical or physical signal, it transduces that signal to the inside of the cell, initiating a chain of biochemical events that ultimately leads to a change in cellular function (and at a higher level, a tissue response). The observed concentration dependence of a receptor-activating ligand is the end result of its interaction with the target receptor population and whatever steps occur between there and the signaling endpoint being measured. This overview has focused on how GPCR-binding proteins can influence ligand–receptor interactions (Section 1), and how, in addition to signal propagation and amplification, structural components of the signaling machinery and deamplification processes can have effects on agonist potency (Section 2).

Biased agonism pertains to the ability of a group of ligands that bind to a common receptor to differentially promote functionally distinct states that lead to divergent signaling outcomes. As outlined in Section 1, the conformational states available to a receptor may be limited by other proteins that bind to the receptor, and it is suggested that the binding of a receptor to its signaling partners influences its binding to ligands more than the other way around. When multiple signaling pathways are available to a receptor, each drug that binds to it will have its own intrinsic efficacy with respect to the activation of each signaling pathway, and it is the relative differences between these that underlie the phenomenon of functional selectivity.

While the ability to promote the assumption of a particular conformational state is arguably the most important property in determining the signaling outcome when an agonist binds to its receptor, the concentration dependence of a response may differ from the concentration dependence of the agonist–receptor interaction *per se*. Discrepancies between agonist affinity and potency can be accounted for by various tissue factors, the earliest recognized of which was receptor density [50–52]. While the enzymes and other proteins that convey signals would presumably be similar from one cell type to another, the abundance of each may not be. As well, the presence or absence of specific scaffolding proteins and mitigating factors can also influence signal propagation, and thereby potency. Ultimately the effects of a GPCR agonist on a particular tissue will depend upon multiple factors. Thus while the goal of developing receptor state-selective drugs to increase

therapeutic specificity and decrease untoward effects holds much promise, it should be recognized that some receptor-initiated outcomes may be intrinsically better connected than others.

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