Methods 92 (2016) 87–93

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

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

ARTICLE INFO

Article history:
Received 24 June 2015
Received in revised form 13 August 2015
Accepted 14 August 2015
Available online 18 August 2015

Keywords:
Agonist potency
Receptor conformation
G protein-mediated signaling
GPCR-interacting proteins (GIPs)
Regulator of G protein signaling proteins (RGS proteins)
Functional selectivity

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

http://dx.doi.org/10.1016/j.ymeth.2015.08.011
1046-2023/

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.