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Biased agonism

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Abstract

Seven-transmembrane receptors are commonly coupled to multiple signaling pathways in cells. The simple model describing agonists for these receptors as producing a common active state to induce uniform activation of the pathways linked to the receptor has been shown to be untenable in light of a large body of data that suggest that some agonists produce activation of some but not all available pathways. These agonists are referred to as 'biased' in that they select which signaling pathways become activated upon binding to the receptor. The data to support this mechanism as well as ideas on the possible therapeutic application of this effect will be discussed.

Introduction and context

Seven-transmembrane receptors are a family of signaling proteins that bind hormones, autacoids, and neurotransmitters to mediate a myriad of cellular functions. Like all proteins, these exist in collections of tertiary conformations called protein ensembles; receptors sample these conformations according to changes in the thermal energy in the system. Some of these conformations can be designated as 'active states', denoting their ability to activate cytosolic signaling mechanisms. The current model of pharmacological seven-transmembrane receptor agonism describes the selective binding of agonists to these active states to enrich their presence within the ensemble through a process referred to as 'conformational selection' [1]. Thus, through Le Chatelier's principle, upon agonist binding, the make-up of the ensemble is directed toward the conformations possessing the highest affinity for the agonist and these are stabilized at the expense of other conformations. The product of this thermodynamic process is a collection of membrane proteins that activate cellular signaling processes and agonism ensues. Early discussions of this mechanism and agonist efficacy in general used the most simple assumptions that the receptor is the minimal unit of control for this process and that a receptor activated by any agonist triggers all cellular signaling processes mediated by that particular receptor type.

Two experimental findings required the modification of this idea. First, it was observed that seven-transmembrane receptors pleiotropically interact with a wide range of cytosolic coupling proteins. Second, new technology revealed multiple biochemical behaviors of receptors such as the propensity to be phosphorylated and to interact with multiple G-proteins and β-arrestin, internalization, and desensitization. Subsequent studies have indicated that not all agonists uniformly produce activation of these multiple receptor behaviors. For example, the peptides $PACAP_{1-27}$ and $PACAP_{1-38}$ activate PACAP (pituitary adenylate cyclase-activating polypeptide type 1) receptors to elevate cyclic AMP and increase production of IP_3. However, the relative potency of these agonists for these responses is reversed; the relative efficacy of $PACAP_{1-27}$ for cyclic AMP elevation is higher than that of $PACAP_{1-38}$ but lower for elevation of IP_3 [2]. This indicates that the receptor is not the minimal unit of control of agonism, it is the agonist-receptor complex that controls the ultimate signaling event; the data leading to this conclusion also clearly indicate that agonist activation of multiple signaling mechanisms is not uniform but rather is often 'biased' toward some but not all signaling pathways. This concept has been put into a formal model showing that agonist-selective states can produce biased agonism [3].

In terms of the molecular mechanism of biased agonism, two additional factors are relevant to the discussion, namely the nature of the receptor-active state and the interaction of the activated receptor with multiple cytosolic signaling proteins. The selective activation of cellular pathways with biased agonists is consistent with there being 'agonist-specific' ensembles of receptor conformations mediating these events; subsequent studies have given independent corroboration of this mechanism through separate lines of experimentation that directly show that ligands can stabilize unique receptor conformations [4-9]. Similarly, molecular dynamics predicts that when proteins such as receptors change conformation, different regions of the receptor change independently (i.e., the protein does not form uniform global conformations) [10-13]. Therefore, the fact that signaling proteins interact with different regions of the receptor (e.g. [14,15]) suggests that different conformations would not produce uniform coupling to all signaling proteins; that is, the unique receptor conformations stabilized by agonists most likely will result in differential (biased) activation of cell signaling pathways [16]. In fact, the activation of a receptor that interacts with multiple signaling components in a cell most likely will never produce equal activation of all pathways; therefore, from this standpoint, every agonist will have a bias in signaling. However, a useful point of reference is the natural agonist for the receptor; this will have a natural signaling bias and can be used as a standard with which other agonists can be compared. Within this scale, functionally selective agonists are defined as having a signaling bias different from that of the natural agonist.

Major recent advances

Studies using a wide variety of technologies now indicate that biased agonism is a common phenomenon. Agonists have been shown to differentially activate different G-proteins and β-arrestin and have differing susceptibility to phosphorylation, desensitization, and internalization in a wide variety of receptor systems (for reviews, see [17-23]). In particular, many studies now specifically show how differential activation of G-proteins versus β-arrestin results from biased stabilization of receptor conformation [24,25]. Observed most often with synthetic agonist ligands, bias can also be detected in natural systems such as the chemokine CCR7 receptor. Thus, CCL19 and CCL21, two natural agonists for the CCR7 chemokine receptor, differ in the type of pathway stimulation they elicit; although both agonists produce G-protein activation, only CCL19 (not CCL21) causes receptor agonist-dependent phosphorylation and recruitment of β-arrestin to terminate the G-protein stimulus [26]. In addition to G-proteins and β-arrestin, seven-transmembrane receptors have been shown to couple to many other signaling proteins such as JAK/STATs (Janus kinase/signal transducers and activators of transcription), Src-family tyrosine kinases, G-protein receptor kinases (GRKs), and PDZ domain-containing proteins [27]. Finally, other receptor types also are involved in biased signaling. For example, different proteases have been shown to differentially activate protease-activated receptors through stabilization of distinct conformation (much like biased ligands [28]).

The discovery that some ligands can produce activation of some but not all receptor-linked stimulus mechanisms can introduce ambiguity in the classification of drugs [29]. For instance, the active internalization of receptors by some antagonists indicates that a label of 'agonist' should be placed on this one aspect of receptor behavior (internalization) [17,30,31]. Similarly, while propranolol is a well-known antagonist and inverse agonist of agonist activation of G_s-coupled effects of β-adrenoceptors [32], it has also been shown to be an active activator (agonist) of extracellular signal-regulated kinase (ERK) [33]. Biased agonism can be complex and need not necessarily involve excitatory signaling events; that is, some ligands can be positive agonists for one pathway and antagonists or inverse agonists for another [34]. Such divergences in efficacy have been used to propose that efficacy be considered 'pluridimensional'; that is, ligands may have a range of different efficacies to cause a range of receptor behaviors [35].

Future directions

The therapeutic relevance of biased agonism is still unknown. The emphasis has clearly shifted from 'does biased agonism occur?' to 'when it does occur, how can it be harnessed therapeutically?' This trend is consistent with the improving technology to detect whole-cell effects through label-free technology; these data provide cell-specific and detailed information regarding signaling patterns of ligands [36-40]. Thus, while assay technology is making it increasingly possible to detect and characterize agonist bias, focus is shifting to the mechanisms responsible for this effect.

Emphasis has also shifted from the cell surface to the cytosol in an effort to understand biased signaling. For example, detailed studies of biased ligand-induced β-arrestin-mediated signaling indicate significant differences in functionally distinct pools of β-arrestin accessed through conformational control of receptor sensitivity to phosphorylation by GRK isoforms [41]. These unique phosphorylation patterns result in effective 'bar coding' of ligand-bound receptors that lead to further downstream instruction of β-arrestin partners within the

cell [41]. Moreover, just as receptors adopt different conformations to achieve selective signaling, it has now been shown that β-arrestin itself forms different conformations and that these are associated with selective functions within the cell [42].

The significance of biased signaling is increasingly appreciated as signaling pathways become linked to normal physiology and pathophysiology. For example, the reported ERK activation through β-arrestin by some antagonists (i.e. propranolol [33]) is interesting in light of recent data that suggest that angiotensin receptor-mediated β-arrestin stimulation may be beneficial in the treatment of heart failure [43] or, alternatively, may be linked to the progression of heart failure through aldosterone pathways [44]. Biased signaling has been implicated in a unique profile of antagonism and agonism in breast cancer. Specifically, substance-P analogues SP-D and SP-G have been shown to produce biased signaling at vasopressin V_{1A} receptors and receptors for gastrin-releasing peptide to yield a profile of blocked G_q-protein-mediated calcium release and concomitant activation of ERK. This is postulated to produce a unique antiproliferative profile of activity [45].

In terms of specific examples in which bias may be a practical aspect of drug activity, there are interesting divergences in activity for opioid agonists to produce analgesia with reduced propensity to produce desensitization and δ-opioid receptor internalization [46-50] and intriguing functionally selective dopamine [51] and serotonin [52,53] agonists for the treatment of psychiatric disorders. Similarly, there are data that suggest that functionally selective thyrotropin agonists may be able to differentiate thyroid growth and thyroid hormone synthesis [54] and orexin receptor functionally selective agonists may differentiate effects on catecholamine release and adrenal steroid production [55,56]. The consequences of biased agonism currently are an intense subject of study. While initial work in the field considered acute effects such as desensitization, receptor internalization, and differential activation of G-proteins, more recent studies center on long-term effects of biased signaling such as prolonged signaling through β-arrestin activation and effects such as G-protein regulation [57].

In general, it is not yet clear to what extent these biased stimulations of cellular signaling pathways will yield favorable therapeutic phenotypic agonism. What is clear is that seven-transmembrane receptors cannot be considered on/off switches and synthetic agonists cannot be considered surrogates of natural agonists. Stabilization of receptor-active states by different molecules has the potential to traffic receptor stimulus in unique ways to cause complex patterns of cellular activation. The challenge now is to harness this powerful effect.

Abbreviations

CCR7, chemokine (C-C motif) receptor 7; CCL, chemokine (C-C motif) ligand; ERK, extracellular signal-regulated kinase; GRK, G-protein receptor kinase; PACAP, pituitary adenylate cyclase-activating polypeptide type 1.

Competing interests

The author declares that he has no competing interests.

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