Review

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Biased ligands at G-protein-coupled receptors: promise and progress

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Drug discovery targeting G protein-coupled receptors (GPCRs) is no longer limited to seeking agonists or antagonists to stimulate or block cellular responses associated with a particular receptor. GPCRs are now known to support a diversity of pharmacological profiles, a concept broadly referred to as functional selectivity. In particular, the concept of ligand bias, whereby a ligand stabilizes subsets of receptor conformations to engender novel pharmacological profiles, has recently gained increasing prominence. This review discusses how biased ligands may deliver safer, better tolerated, and more efficacious drugs, and highlights several biased ligands that are in clinical development. Biased ligands targeting the angiotensin II type 1 receptor and the μ opioid receptor illustrate the translation of the biased ligand concept from basic biology to clinical drug development.

New frontiers for G protein-coupled receptors

G protein-coupled receptors (GPCRs) have long occupied a central role in pharmacology. They are the targets of many of the most widely prescribed drugs in central nervous system (CNS), cardiovascular, pulmonary, gastrointestinal, and many other disease areas. Originally conceived as theoretical entities to explain the drug-specific responses of tissues, GPCRs are now known to be a superfamily of membrane proteins that bind hormones, neurotransmitters, and other extracellular cues to transmit information about the external world to the interior machinery of cells [1]. In the simplest conceptual model, GPCRs operate as switches, normally residing in an inactive state, and are only activated to engender intracellular response when bound by agonists. Antagonists in this paradigm bind the receptor and prevent activation by preventing agonist binding. This light-switch model dominated basic research and drug discovery efforts for many years. However, receptors are in fact capable of much more than simple binary signals. Illumination of these more subtle mechanisms has unveiled new opportunities for research and drug discovery targeting GPCRs. This review summarizes these possibilities, with an emphasis on the class of biased ligands, which provide a previously unattainable level of pharmacological selectivity by targeting not just specific receptors, but specific signaling pathways downstream of those receptors.

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Pleuripotency of signaling: pathway validation

Classically, agonist-occupied GPCRs mediate cellular responses by binding heterotrimeric G proteins, which initiate cascades of second-messenger responses to alter metabolism, cytoskeletal structure, transcription and translation, and tissue-specific responses such as myocyte contractility and neuron polarization among many others. Almost every GPCR couples to one or more G protein classes, which dictates different cellular responses. Almost every receptor also engages a parallel set of regulatory mechanisms initiated by recruitment of GPCR kinases (GRKs), which phosphorylate agonist-occupied receptor and other targets, and mediate a variety of regulatory protein–protein interactions [2]. Following phosphorylation, GPCRs engage β-arrestins, scaffolding proteins expressed as two isoforms that directly bind GPCRs by recognizing both the agonist-occupied receptor conformation and phosphorylated receptor regions [3]. Typically, β-arrestin recruitment inhibits further G protein coupling, promotes receptor internalization by coupling receptors to endocytic machinery, and triggers signaling by recruiting scaffolded signaling proteins to the receptor. Thus, β-arrestins both regulate G protein signaling and initiate G protein-independent signals. The dual pathways of G protein and β-arrestin coupling are ubiquitous and generic, and have been described in a wide variety of *in vitro* and *in vivo* systems [3,4], but GPCRs can also engage many other mechanisms of signal transduction that translate to a diversity of molecular and cellular responses [5].

Several approaches have been used to delineate the contributions of G proteins and β-arrestins to GPCR function, including targeted genetic deletion of GRKs or β-arrestins, RNA silencing of G protein and β-arrestin pathway components, and application of small-molecule inhibitors of specific signal transduction pathways. These tools have been invaluable in dissecting the pharmacology of specific receptors, and in some cases, described below, have uncovered previously unappreciated signal transduction paradigms. The different experimental strategies taken for such pathway validation are reviewed elsewhere [6,7] and can be thought of as complementary to traditional target validation in assessing potential drug discovery efforts.

Concept of ligand bias

Classically, agonists were thought to entrain the entire signal repertoire of a receptor, and thus pharmacological

selectivity was specified as selectivity of a drug, hormone, or neurotransmitter for a particular receptor type. Receptors were either 'on' or 'off' as envisioned in the classic two-state model of receptor function [8]. The discovery of partial agonists and inverse agonists revealed new levels of pharmacological properties, often differentiating these agonists from full agonists and neutral antagonists, but still consistent with the two-state model. Thus, it was envisioned that receptors adopt two states, and ligand binding preferentially stabilizes the inactive state (for antagonists and inverse agonists) or the active state (weakly for partial agonists, more strongly for full agonists).

This paradigm dominated receptor theory and drug discovery for several decades, and afforded a level of simplicity for investigators: one assay of receptor function was sufficient to predict pharmacological responses in other systems. However, several examples were found that did not fit this paradigm: compound sets yielded different relative potencies in different assays, which could not be explained by off-target pharmacology [9,10]. These findings, controversial at first, were slowly replicated for other receptors, challenging the notion that receptors are only capable of a single spectrum of responses. As these examples have proliferated and been replicated, contemporary pharmacological theory has been revised to incorporate the possibility that receptors engage distinct subsets of their full signaling repertoire. Receptors are now envisioned as occupying myriad discrete conformations, and nonoverlapping sets of these conformations are associated with signaling mechanisms such as G protein coupling, β-arrestin recruitment, receptor trafficking, and other signals. When conformations linked to different signals are distinct, ligands that selectively stabilize these conformations will promote pharmacological responses that differ not just quantitatively but also qualitatively from responses to traditional agonists. These compounds are thus biased towards subsets of receptor function relative to a reference ligand. By convention, the reference ligand is defined as unbiased or balanced; this reference is usually an endogenous agonist or clinically validated drug, but examples of endogenous biased ligands have been described [11,12].

Different receptor conformations permitting ligand bias were first inferred as a parsimonious explanation for measurement of signals downstream of receptors [9,10,13,14], but biophysical techniques have since demonstrated different receptor states for different ligands [15,16]. In addition, several studies of GPCR crystal structures have shown potential structural bases for ligand bias [17,18]. However, there is not yet direct structural evidence for distinct receptor conformations linked to specific signals such as β-arrestin recruitment or distinct G protein classes. Future studies comparing crystal structures of a receptor bound to biased and unbiased ligands may establish these relationships.

Definition of ligand bias versus functional selectivity: not just semantics

The notion of selective signaling in pharmacology has a long history predating the isolation of receptors, beginning with classification of drugs according to differential pharmacology in different tissues. As molecular biology and genetics have uncovered the many mechanisms by which compounds can exert distinct pharmacology, pharmacological nomenclature has evolved, so that drugs are now described by their efficacy (agonist, antagonist, partial agonist, or inverse agonist) and target (receptor type and subtype). For ligand bias the nomenclature is not yet settled, and the phenomenon has been described as ligand bias, functional selectivity, and protean agonism, among other terms [19]. It is helpful to discriminate between the many forms of differential pharmacology on a mechanistic basis. Thus, functional selectivity refers to differential pharmacological effects across any number of assays in any number of systems, and is therefore a very broad category that can include mechanistic differentiation at the level of pharmacokinetics, molecular target(s), intrinsic efficacy, and target receptor conformation. There are numerous examples of each type of functional selectivity driving important differences in *in vivo* pharmacology (Table 1). Ligand bias, however, is solely related to target receptor conformation and is intrinsic to the ligand–receptor complex when a compound stabilizes different receptor conformations compared to a reference ligand.

One of the important features of intrinsic ligand bias is that it is more likely to be system-independent. Unlike functional selectivity, which is driven by pharmacokinetics, engagement of multiple targets, or differential amplification of signals downstream of a partial agonists, each of which largely depends on tissue-specific factors, ligand bias reflects different thermodynamic contributions of ligand binding energy to stabilize distinct conformations of the ligand-bound receptor, and thus the unmasking of different receptor-coupling mechanisms. Although membrane composition and expression/regulation of receptor-coupling proteins such as G proteins, β-arrestins, and receptor trafficking machinery can influence the pharmacology downstream of a biased ligand, the core differentiation may be preserved across experimental systems, as noted in several examples of biased ligands described below. Thus, *in vitro* assays, measured appropriately (see below), can identify intrinsic bias associated with specific ligand-receptor states that are somewhat insulated from cellular or tissue factors; this may explain the successes to date in translating data from *in vitro* overexpressed receptor systems to *in vivo* differentiation of biased and unbiased ligands.

The different GPCR conformations stabilized by biased and unbiased ligands are most often inferred from differences in receptor-selective signals measured *in vitro*. This has been done using comprehensive approaches such as proteomics and with more focused assays [20–22]. These approaches represent both integrative downstream signals, which capture the influences of multiple receptor-coupling mechanisms such as proteome pathway clustering, cellular impedance, and MAPK activation, and discriminating proximal readouts, which more directly measure receptor-coupling mechanisms such as second messenger signals, receptor–effector coupling (e.g., β-arrestin recruitment), and receptor trafficking [23,24]. Either approach can effectively identify biased ligands, but it is important to recognize the shortfalls of each.