This review is the first in a thematic series on Novel Aspects of Cardiovascular G-Protein-Coupled Receptor Signaling: Implications for New Therapeutics, which includes the following articles:

Introduction to the Series

Biased Ligands for Better Cardiovascular Drugs: Dissecting GPCR Pharmacology

G-Protein-Dependent and –Independent Signaling Pathways and their Impact on Cardiac Function
Compartmentalization of β-Adrenergic Signals in Cardiomyocytes
G-Protein-Coupled Receptor Kinases (GRK) as Therapeutic Targets in Cardiovascular Disease
Regulators of G-Protein Signaling in the Heart and Their Potential as Therapeutic Targets

Howard Rockman, Guest Editor

Biased Ligands for Better Cardiovascular Drugs
Dissecting G-Protein-Coupled Receptor Pharmacology

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Abstract: Drug discovery efforts targeting G-protein-coupled receptors (GPCR) have been immensely successful in creating new cardiovascular medicines. Currently marketed GPCR drugs are broadly classified as either agonists that activate receptors or antagonists that prevent receptor activation by endogenous stimuli. However, GPCR couple to a multitude of intracellular signaling pathways beyond classical G-protein signals, and these signals can be independently activated by biased ligands to vastly expand the potential for new drugs at these classic targets. By selectively engaging only a subset of a receptor’s potential intracellular partners, biased ligands may deliver more precise therapeutic benefit with fewer side effects than current GPCR-targeted drugs. In this review, we discuss the history of biased ligand research, the current understanding of how biased ligands exert their unique pharmacology, and how research into GPCR signaling has uncovered previously unappreciated capabilities of receptor pharmacology. We focus on several receptors to illustrate the approaches taken and discoveries made, and how these are steadily illuminating the intricacies of GPCR pharmacology. Discoveries of biased ligands targeting the angiotensin II type 1 receptor and of separable pharmacology suggesting the potential value of biased ligands targeting the β-adrenergic receptors and nicotinic acid receptor GPR109a highlight the powerful clinical promise of this new category of potential therapeutics. (Circ Res. 2011;109:205-216.)

Key Words: β-arrestin ■ biased ligand ■ G-protein-coupled receptor ■ functional selectivity

G-protein-coupled receptors (GPCR, also known as 7 transmembrane receptors) remain the largest class of therapeutic targets in medicine, accounting for one-third of marketed pharmaceuticals. The impact of GPCR-targeted drugs is particularly evident in cardiovascular medicine, in which GPCR modulation is used to control hypertension, stimulate inotropy, and prevent thrombosis, among numerous other applications. Widespread appreciation of these examples led to a now classical approach to GPCR-targeted drug discovery, directing research efforts toward either agonist ligands to increase normal receptor activation or antagonist ligands to block inappropriate or deleterious receptor func-
tion. However, the growing understanding of the true complexity of GPCR function has illuminated possibilities for more refined approaches to GPCR pharmacology than simple activation or inhibition. Examples of pharmacological and genetic dissection of GPCR-mediated signal transduction pathways revealed the utility of selectively targeting only the desired subset of signals for a given GPCR. The discovery of “biased ligands,” which selectively activate or block subsets of the signaling repertoire of GPCR demonstrated the feasibility of this concept.1 These compounds offer the potential to stimulate a desired receptor response without activating “on-target” adverse effects or to uncover previously unappreciated pharmacology.

**Brief Review of G-Protein and β-Arrestin–Mediated Signaling From GPCR**

GPCR compose one of the largest superfamilies in the human genome, are expressed in virtually every tissue in the body, and influence nearly all physiological responses.2 They bind extracellular signals such as hormones, neurotransmitters, metabolic products, and odorants, adopting distinct conformations to elicit intracellular responses specified by the complement of intracellular signaling proteins that are available to couple to “activated” receptor. Originally, these intracellular signals were thought to be mediated uniquely by the class of heterotrimeric G proteins, which then modulate second messenger concentrations to elicit downstream responses.3 Now, it is widely appreciated that GPCR can couple to many additional intracellular proteins. Of these, β-arrestins have emerged as the most ubiquitous and general receptor-coupling protein and may rival G proteins in importance to regulating receptor pharmacology.4,5

β-Arrestins occur in two isoforms and were first characterized as negative regulators of receptor G-protein coupling, mediating receptor desensitization and internalization, but are now known to promote positive signaling as well. By acting as multiprotein scaffolds, β-arrestins bring constituents of intracellular signaling cascades into close proximity and facilitate their activation.6–8 Because β-arrestins translocate from cytosol to bind activated receptors in the cell membrane9 and adopt different conformational states on binding these receptors,10–13 β-arrestin–dependent signaling is, like G-protein signaling, also largely receptor-dependent and ligand-dependent. This set of mechanisms enables a rich complexity of pharmacological responses. The number of signaling pathways modulated by the β-arrestins has grown to include a broad and diverse set, including mitogen-activated protein kinases, tyrosine kinases, and phosphatases.14 As these examples have proliferated and we have grown to appreciate that GPCR do not signal through simple linear pathways but rather through broad networks, researchers have begun to evaluate GPCR pharmacology using a broader array of signaling measurements. Unsurprisingly, this enrichment of molecular pharmacology has led to many striking and unexpected findings that have posed a challenge to standard pharmacological concepts.

**Ligand Bias Theory**

Classical models of receptor pharmacology assume that agonists at a particular GPCR elicit effects through a single mechanism of activation, implying a single activated conformation of the agonist-occupied receptor.15,16 These models account for only a single form of efficacy, in which agonists (and antagonists) of a receptor can differ in the magnitude of their effects but not in the set of downstream responses thereby stimulated. This has yielded a standard nomenclature of GPCR drugs that is specified by affinity and efficacy. Under this paradigm, a single functional assay of receptor response, provided it is appropriately calibrated, is sufficient to determine the nature of a ligand: full agonist, partial agonist, neutral antagonist, or inverse agonist. However, as we have learned to measure broader networks of signals stimulated by agonists, it has become clear that in addition to quantitative differences (eg, partial vs full efficacy), agonists can be qualitatively different by displaying functional selectivity (eg, one ligand selectively simulates one signal whereas a different ligand selectively stimulates a second signal via the same receptor). Thus, not only does a receptor engage a broad range of biochemical responses, but in addition, different agonists can elicit different patterns of these responses.

One of the earliest descriptions of ligand bias reported differing relative efficacies for Gs and Gi coupling of several α2 adrenergic receptor agonists, leading the authors to posit that different ligands could engage distinct receptor conformations.17 Another early example demonstrated that analogs of pituitary adenyl cyclase-activating polypeptide elicited cAMP and inositol phosphate accumulation via the pituitary adenyl cyclase-activating polypeptide receptor with opposite relative potencies.18 These reports were followed by other striking examples, such as receptor internalization caused by ligands that are otherwise antagonists.19 However, the concept was slow to gain broad acceptance because further examples of such ligand bias were clouded by concerns over characteristics such as binding kinetics or compound stability, which can lead to apparent functional selectivity based on experimental conditions. Only in the past decade has functional selectivity been appreciated to be a real and important feature of molecular pharmacology, with an increasing number of groups actively investigating the topic.20–25

The evidence for functional selectivity and ligand bias has been comprehensively reviewed elsewhere;1 this review attempts to concisely summarize the concept of ligand bias and its importance to cardiovascular pharmacology and to review in depth the state of ligand bias research at several therapeutically important GPCR. Although ligand bias is possible
between any two signals at a given receptor, this review largely highlights bias between G-proteins and β-arrestins, because these two protein classes represent general mechanisms of receptor pharmacology and are the direct target of recent drug discovery efforts to bring biased ligand therapies to the clinic.

Because ligand bias is not accounted for in traditional conceptual and quantitative models of GPCR pharmacology, there is little precedence for nomenclature. This has led to a proliferation of names and descriptions for a series of phenomena related to the pharmacological dissection of downstream signaling of GPRC. As discussed later, ligand bias is a subset of the very broad category of functional selectivity. There are several forms of functional selectivity unrelated to ligand bias: partial agonists, which can lead to functional selectivity by differential amplification of downstream signals in different tissues;26 protein agonists, or partial inverse agonists, which activate receptors in the context of very low constitutive activity but inactivate in other contexts of high constitutive activity;27,28 and, most simply, ligands with different pharmacokinetics that reach different target tissues in vivo.29–31 Each of these can result in selective activation of only some of a given receptor’s signaling cascades and can be a valuable strategy to pursue safer, more efficacious drugs. However, each of these forms of functional selectivity is also entirely system-dependent, meaning that it can be difficult to translate in vitro findings to in vivo systems and even more difficult to translate from animal models of disease to a clinical situation. In contrast, ligand bias (as we refer to it here) is an intrinsic property of a ligand–receptor complex, directly stabilizing distinct conformations of a receptor to enable discrete signals without permitting receptor-coupled signaling, and most receptors couple to at least one G-protein 

Mechanistically, ligand bias can occur either competitively (ie, orthostERICALLY) by interacting with the same receptor binding site as the endogenous agonist or allosterically by exerting effects through a distinct binding site. Biased ligands described to date largely couple to a subset of the signals elicited by reference ligands—the endogenous ligand or, in some cases, commonly used drugs. Thus, although functionally selective receptor conformations have not been directly demonstrated yet, orthosteric biased ligands are likely to engage a subset of the receptor conformational changes elicited by full agonists and thereby do not stimulate the full range of receptor-activating conformations. This could occur either through failure to engage specific ligand–receptor contacts or by engaging new contacts that restrict “normal” receptor conformational changes. However, it remains possible that biased ligands at the orthosteric site can engage new “active” conformations, endowing a receptor with novel signal transduction responses. In contrast to orthosteric ligands, allosteric biased ligands likely engage functional selectivity either by directly stabilizing select “active” receptor conformations via a nonstandard binding site (known as allosteric agonism) or by restricting a receptor from adopting some of the conformations normally engaged by an orthosteric agonist (known as allosteric modulation). This type of ligand–receptor interaction is exemplified by a CRTH2 ligand that blocks agonist-induced β-arrestin engagement without affecting agonist-induced G-protein signaling.41 Other examples include the M1 allosteric modulator BQCA, which unlike other allosteric modulators promotes β-arrestin recruitment, and the NK2 receptor ligand LP805, which modulates receptor coupling away from Gas and toward Gq.37,41 We refer to this type of functional selectivity as “biasing allosteric modulation,” in keeping with the mechanistic concept of allosteric modulation, which has gained widespread use in drug discovery.42–43 The difference between orthosteric and allosteric ligand bias is illustrated in Figure 1.

Together, these different forms of ligand–receptor interaction provide a broad opportunity for novel GPCR pharmacology that is only now gaining pervasive attention. This discussion does not encompass the burgeoning field of GPCR dimerization, which introduces a further dimension of complexity by adding possible allosteric actions of individual protomers within a dimer on each other. The evidence for such interactions, the challenges facing the field, and the potential utility of GPCR dimerization in pharmacology and drug discovery are reviewed elsewhere.44–46

**Approaches to GPCR-Biased Ligand Pathway Validation**

Traditional drug discovery target validation attempts to demonstrate that a potential molecular target is relevant to, and can potentially modulate, a disease state. Biased ligand drug discovery is more complex because it requires not only target validation but also pathway validation, an understanding of which receptor signals should be activated and which should be inactivated to create optimal pharmacological intervention in a disease. As discussed, biased ligands parse GPCR responses to offer improvements over classic agonists or antagonists by two means: (1) reducing an unwanted “on-target” side effect of an agonist or (2) uncovering pharma-
but now also appear to be potential therapeutic targets for biased ligands. These well-studied receptors are rapidly being joined by more novel GPCRs, such as the nicotinic acid receptor GPR109a, which despite considerable uncertainty over its role in human physiology and disease has already yielded substantial data suggesting that biased ligands will have distinct and perhaps more beneficial effects than an unbiased or “balanced” agonist.

**AT1R**

The peptide hormone AngII is a classical vasopressor that regulates salt and fluid homeostasis by modulating vasoconstriction and aldosterone secretion, as well as a range of other functions such as thirst and inflammation. AngII is a key product of the renin-angiotensin system, and its levels are elevated in pathological states such as hypertension and heart failure. Many of the physiological roles of AngII are mediated by the AT1R. The importance of this GPCR system is illustrated by the benefit of angiotensin-converting enzyme inhibitors to lower AngII levels and angiotensin receptor blockers to block AngII effects on the AT1R. These therapies are widely used in treating hypertension and other cardiovascular diseases.

Classically, the AT1R couples primarily to $G_{q,i}$ signaling, leading to phosphatidylinositol bisphosphate hydrolysis, generating diacylglycerol, mobilizing calcium, and activating signaling enzymes such as protein kinase C. These pathways were first thought to mediate the majority of AT1R pharmacology and to be the only intracellular signals relevant to the clinical effects of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers. Reports of coupling to $G_{q,i}$ signaling and inhibition of adenyl cyclase were understood to reflect the same agonist-induced receptor state and to be engaged to the extent that a particular cell expressed an appropriate portfolio of G-protein subtypes. Later work demonstrated that the AT1R is also subject to a wide range of regulatory and coupling mechanisms. These include receptor phosphorylation by protein kinase C and G-protein-coupled receptor kinase (GRK). However, the most striking finding of AT1R biasing has been the engagement of $G_{i}$-dependent pathways.

A series of studies have shown that many of these pathways are independent of G-protein coupling and can, in some cases, be selectively engaged by biased ligands. One body of evidence for distinct AT1R signal-coupling mechanisms came from receptor mutagenesis. These focused largely on mutating amino acids either in the cytosolic face of the receptor and precluding engagement of intracellular proteins or on the extracellular face or binding pocket of the receptor, altering how AngII binds and influences receptor conformation. For example, in the rat AT1aR, mutation of the conserved DRY motif (amino acids 125–127) eliminates receptor coupling to $G_{q,i}$ but does not block AngII-stimulated receptor phosphorylation, internalization, or recruitment of and signaling via $G_{q}$.

**Biased Ligands for Receptors of Cardiovascular Importance**

Because GPCR regulating cardiovascular physiology are among the best studied and most successfully targeted with therapeutic ligands, it is perhaps not surprising that they are also among the receptors best characterized for biased ligand pharmacology. In particular, two of the most well-known cardiovascular GPCR provide broad precedent for the potential therapeutic power of biased ligands: the angiotensin II (AngII) type 1 receptor (AT1R) and the $\beta$-adrenergic receptors, all of which have been targeted by widely used drugs
cardiac overexpression of this mutant receptor to the wild-type AT1R, the mutant resulted in an exacerbation of cardiac hypertrophy but also displayed reduced fibrosis, apoptosis, and AngII-stimulated cardiac chronotropy.69 These findings were aligned with a distinct profile of biochemical signals in cardiac tissue from the two transgenic lines such as altered subcellular localization of active ERK1/2. It is difficult to discern developmental versus pharmacological effects in these transgenic studies, but this work clearly indicated that AT1R effects can be divided into distinct G-protein–dependent and G-protein–independent signals in vivo.

In parallel to these efforts, a distinct line of investigation began to illuminate the contributions of β-arr2 to AT1R signaling. Reduction or elimination of β-arr1 or β-arr2 expression with siRNA in vitro or genetic deletion in vivo showed that β-arrs are required for activation of ERK1/2, Rho, Mnk1, MDM2, and many other signaling pathways.6,59,61,70–72 These targeted studies have been complemented by broader screening approaches to describe genomic and proteomic effects of β-arrs.73–76 In addition, studies in knockout animals have begun to elucidate the physiological role of β-arrs in AT1R signaling; mechanical stretch activates AT1R–dependent and β-arr2–dependent prosurvival signaling without stimulating G-protein-stimulated diacylglycerol translocation.77 Knockout of either AT1R or β-arr2 worsened stretch-induced apoptosis, as did an angiotensin receptor blockers in wild-type mice, indicating that β-arr2 engaged to the AT1R is cardioprotective.77

These discoveries of β-arr contribution to AT1R function have been reinforced, often in the same studies, by the use of biased ligands that selectively engage β-arr signals without promoting detectable G-protein signaling. The first, and most widely used, of these biased ligands was 1Sar, 4Ile, 8Ile-AngII (SII).78 This peptide was first described to promote AT1R phosphorylation, internalization, and ERK activation, but not phosphatidylinositol turnover;78 these behaviors were later shown to reflect positive efficacy for β-arr in the absence of any detectable G-protein signaling.67 It is now clear that β-arrs mediate AT1R signal pathways that reduce apoptosis,60,77 stimulate chemotaxis,79,80 promote cellular protein synthesis, growth, and proliferation,59,60,81 and cardiac contractility.82 These signaling effects are detectable from endogenous receptor, as demonstrated by SII stimulation of ERK1/2 activation in Langendorff-perfused hearts.83 In addition, SII stimulated proliferation via cytosolic ERK1/2 activation in rat neonatal myocytes without engaging hypertrophic nuclear ERK1/2 activation elicited by AngII.84 Interestingly, SII-stimulated contractility was not detectable in the langendorff-perfused rat heart, contrasting with the enhanced contractility seen in isolated mouse myocytes85 and in vivo in rats (discussed later). It is unclear if this discrepancy reflects differences in the experimental preparations, the weak affinity of SII for the AT1R, or the generally modest amount of contractility stimulated by the AT1R compared to classical inotropes such as dobutamine.

The realization that β-arr–dependent effects could be elicited by AT1R ligands such as SII that were derived from AngII, and thus likely bound competitively with AngII to the AT1R, led to the hypothesis that biased ligands could simultaneously antagonize some AT1R functions while stimulating others.60,82 In particular, the simultaneous blockade of AngII-dependent hypertension while increasing cardiomyocyte contractility and promoting cytoprotective/antiapoptotic signals might be of great benefit in acute heart failure, a syndrome of reduced cardiac function, and, in most patients, neurohormonal activation and elevated systemic vascular resistance.85 This hypothesis drove the recent discovery and development of TRV120027, a selective and β-arr2–biased AT1R ligand with increased potency and β-arr2 efficacy compared to SII.90 TRV120027 is competitive with AngII at the AT1R both in cellular signaling assays and in rats, where it reduces AngII-mediated hypertension; in addition, TRV120027 increases contractility of isolated cardiomyocytes and promotes cardiac performance in vivo (Figure 2). This is in striking contrast to classical angiotensin receptor blockers, which have similar effects on blood pressure but have either no effect on cardiac performance or, in some cases, decrease it.86–88 TRV120027 elicits some, but not all, of the kinase signaling pathways that are stimulated in vitro by AngII, consistent with the notion that biased ligands in general engage a subset of a receptor’s normal repertoire. Consistent with the effects observed in rats, TRV120027 showed benefits in a canine heart failure model, reducing blood pressure and pulmonary capillary wedge pressure while promoting cardiac output and preserving kidney function, a unique profile that may be of benefit in acute heart failure.89 TRV120027 is now in clinical trials for the treatment of acute heart failure and may provide precedent for the targeted discovery and development of differentiated biased ligand therapeutics with unique pharmacological profiles.

The AT1R also provides some important lessons for how the structure of both the ligand and receptor can guide functionally selective responses. Although there is not yet a crystal structure of the AT1R available, many studies have used mutagenesis to map key contact points for both affinity and efficacy of peptide ligands.65,66 Striking differences in the residues contributing to agonist and antagonist peptide binding and efficacy support the expectation that ligand binding stabilizes or destabilizes particular receptor structural features, and that integration of these interactions permit or restrict AT1R signal-coupling mechanisms.90,91 Inconsistent with a simpler “lock and key” model of agonist and antagonist efficacy. Intriguingly, these findings have been extended by homology modeling of the AT1R to crystal structures of rhodopsin, β2 adrenergic, and adenosine A2 receptors to hypothesize several key residues that may be engaged in β-arr2 coupling and the difference between balanced and β-arr2–biased AT1R ligands.34 In particular, a proline near the extracellular terminus of the second transmembrane helix (position 82 in the rat AT1aR) forms a “proline kink” that could influence global receptor conformation. Substitution of alanine for this proline prevented AngII signaling via Gαq without altering receptor–ligand affinity, suggesting that this motif is important for transmitting conformational rearrangements to intracellular receptor regions responsible for G-protein coupling.92 This site may also modulate the balance between β-arr2 and G-protein coupling states of the receptor; in striking contrast to the effects on AngII signaling, the same...
alanine substitution reportedly increases G-protein coupling of SII, effectively "unbiasing" the normally β-arrestin–biased ligand. This site thus may be a key focal point for determining balanced vs biased pharmacology. Testing a range of receptor mutations and a more complete set of biased and balanced ligands will refine models of how the AT1R conformational changes differentially couple to G proteins and β-arrestins, and may generate hypotheses for how to build a broader range of biased AT1R ligands, including small-molecule β-arrestin–biased ligands. However, predictive models of biased ligand structure–activity relationships may require crystal structures of the AT1R in active, inactive, and biased conformations. In the meantime, further characterization of AT1R-biased ligands, including clinical evaluation of TRV120027, will elaborate the utility of parsing AT1R signaling for both basic research and pharmacotherapy.

From the perspective of drug discovery pathway validation, the AT1R exemplifies the potential for biased ligands to elicit novel pharmacological responses from a receptor already successfully targeted by an important class of drugs. TRV120027 captures the benefits of angiotensin receptor blockers in countering renin-angiotensin system activation to lower blood pressure, but also engages the novel biology of beneficial β-arrestin functions. In this regard, AT1R β-arrestin–biased ligands are a new class of potential therapeutic that aim to provide meaningful clinical benefit by targeting intracellular signals that were invisible until the recent elaboration of β-arrestin–dependent signaling and the appreciation of ligand bias as a means to unmask previously hidden benefits of AT1R signaling.

**β-Adrenergic Receptors**

Since their discovery more than 50 years ago, β-adrenergic receptor antagonists, or "β-blockers," have been one of the most clinically important classes of therapeutics in cardiovascular medicine. β-blockers inhibit endogenous catecholamines, which control inotropy and chronotropy largely through the β1 adrenergic receptor (β1AR). Conversely, the β2 isofrom of the adrenergic receptor (β2AR) controls functions such as vascular tone and airway constriction, with the latter property giving rise to the widespread use of β2AR agonists in asthma. β-blockers have become standard treatment for patients after myocardial infarction and are further used for the management of arrhythmia, hypertension, and angina pectoris.

Because β-blockers have proven so beneficial, it may seem there is little room for biased ligands to offer the potential for improvement to this drug class. However, recent signaling pathway dissection has revealed a novel, distinct cardioprotective set of β1AR signals. These signals are carried by β-arrestins through EKR1/2 phosphorylation and transactivation of the epidermal growth factor receptor. This pathway was further resolved by receptors mutated to engage only selective signals. In mice with transgenic overexpression of the wild-type β1AR or mutant receptors lacking either GRK phosphorylation sites (GRK-, unable to recruit β-arrestins) or lacking protein kinase A phosphorylation sites (PKA-, unable to be desensitized by protein kinase A mechanisms), chronic isoproterenol caused more profound deterioration of cardiac function only in mice expressing the GRK-β1AR. These data imply that β1AR G-protein and β-arrestin pathways normally strike a balance between apoptosis associated with prolonged inotropy and counteracting cardioprotection. When this balance is disrupted in the absence of β-arrestin signaling (as in the case of the GRK- receptor mutant that only signals via G proteins), apoptosis increases and cardiac functions decreases.

Another line of investigation implicated β1AR agonist-mediated activation of the Ca(2+)/calmodulin-dependent kinase II in cardiac dysfunction after myocardial infarction. Further work demonstrated that β-arrestin2 is necessary to scaffold calcium(2+)/calmodulin-dependent kinase II and its signaling partners to the β1AR for activation, resulting in...
decreased cardiac function.\textsuperscript{101} This pathway could counteract cardioprotective β-arrestin functions at the β1AR.\textsuperscript{99} However, because activation of β-arrestin–scaffolded calcium(2+)calmodulin-dependent kinase II by the β1AR requires cAMP,\textsuperscript{101} this pathway is not likely to be engaged by a β-arrestin–biased ligand that antagonizes G-protein–mediated cAMP generation. Thus, current evidence suggests that the net effect of a β-arrestin–biased ligand will be cardioprotective.

In light of these possible benefits of GRK-mediated and β-arrestin–mediated signaling from the β1AR, a β-arrestin–biased ligand, devoid of G-protein coupling, may be a useful therapy for targeting the β1AR in cardiovascular indications of declining heart function (Figure 3). Whereas this hypothesis has not been tested experimentally because of a lack of a strongly biased ligand, there is some clinical and biochemical evidence to support it. Out of 20 β-blockers tested, only carvedilol and alpenolol activated the cardioprotective β-arrestin–mediated epidermal growth factor receptor transactivation signaling pathway.\textsuperscript{102} And whereas alpenolol is not currently approved for use in heart failure, carvedilol has shown potentially superior clinical efficacy over other β-blockers in terms of cardiovascular events after myocardial infarction\textsuperscript{103} and perhaps mortality.\textsuperscript{104} Carvedilol differs in other regards from other β-blockers because it is an α1-adrenergic receptor antagonist\textsuperscript{105} and an antioxidant,\textsuperscript{106} so the contributions of GRK/β-arrestin function to its clinical efficacy remain unclear.

For the closely related β2AR, several reports of biased ligands exist. As with the β1AR, carvedilol is weakly β-arrestin–biased at the β2AR.\textsuperscript{107} In addition, β2AR agonists have been identified with efficacy at both G and β-arrestin signaling pathways, with a preferential activation of one pathway.\textsuperscript{108–109} The clinical utility of biased β2AR ligands is unclear but could extend to diseases such as asthma, sarcopenia, and hypertension. More robustly biased ligands are required for the field to understand the in vivo relevance of biased signaling at β-adrenergic receptors.

GPR109a/Niacin Receptor

Blood lipid modulation, particularly through the use of statins, and the correlated reduction in cardiovascular events, has profoundly influenced how patients with cardiovascular risk are managed.\textsuperscript{110} Despite the success of statin therapy, there remains a population displaying low high-density lipoprotein cholesterol (HDL-C), which is an independent risk factor for cardiovascular disease.\textsuperscript{111} Niacin, also known as nicotinic acid or vitamin B3, has been used as a powerful lipid modulation agent for decades, particularly for its ability to increase HDL-C.\textsuperscript{112} Niacin is used as either a stand-alone therapy for dyslipidemia or as adjunctive therapy to statins.\textsuperscript{113} Both over-the-counter and prescription formulations of niacin are available, although all preparations are poorly tolerated because they cause a highly uncomfortable cutaneous flush in many individuals.\textsuperscript{114} Because of this, few patients maintain long-term compliance with niacin therapy. The flushing response is mediated by niacin activation of the GPCR GPR109a (also known as PUMA-G or HM74) on Langerhans cells of the epidermis.\textsuperscript{115} In response to GPR109a stimulation, Langerhans cells release prostaglandin D2, which causes a local vasodilation that manifests in redness and itching. Despite this drawback and intense effort to develop alternative therapies or improve niacin tolerability, niacin remains the most powerful agent clinically available for increasing HDL-C, and the only single agent to modify all four classes of serum lipids (triglycerides, low-density lipoproteins, HDL-C, and very-low-density lipoproteins).\textsuperscript{112} Although the effect of niacin on HDL has been definitively proven in patients, whether these lipid changes are mediated through GPR109a is less clear.\textsuperscript{116} Niacin has other proposed targets, such as diacylglycerol acyltransferase-2, which could mediate its effects on HDL-C.\textsuperscript{117} In contrast, both the acute plasma free fatty acid reduction and the peripheral vasodilation that results from niacin treatment are strongly linked with GPR109a, because GPR109a knockout mice display neither of these responses.\textsuperscript{118,119} Unfortunately, niacin has no effect on HDL-C in mice, so it is difficult to use GPR109a ablation to test the mechanism of niacin effects on HDL-C. However, if cholesteryl ester transfer protein is overexpressed in transgenic mice, the ability of niacin to increase HDL-C is restored, suggesting an action of cholesteryl ester transfer protein modulation by niacin in other species.\textsuperscript{120} Addition-
ally, one group demonstrated the necessity of GPR109a for HDL-C efflux from the liver in vitro, adding further support to the niacin-GPR109a-HDL-C link.121

Early attempts to eliminate the flushing effect of niacin have centered on pharmacokinetics122 and coformulation with prostaglandin inhibitors,123 with marginal success in flush reduction in both cases.114,123 Several groups have reported novel GPR109a agonists capable of causing free fatty acid reductions without induction of flush in mice; however, none of these compounds have been successfully developed to date.124 Most striking is the example of MK0354, a partial agonist of GPR109a, which showed strong antilipolytic activity without flush in mice but had no effect on HDL in humans.125,126 This cast some doubt on the hypothesis that GPR109a mediates the therapeutic effects of niacin. This was congruent with previous work suggesting a separation of niacin flushing and lipid responses was possible but did not elaborate a mechanistic understanding of how GPR109a mediates niacin flush or whether GPR109a activation is sufficient for increasing HDL-C.127

More recently, a mechanism for GPR109a-induced flushing was reported. Walters et al128 described that β-arrestin1 expression was necessary for the full flushing response elicited by niacin. Whereas wild-type mice exhibited both flushing and antilipolytic free fatty acid reduction after niacin treatment, β-arrestin1 knockout mice retained free fatty acid lowering but had markedly reduced flushing. The mechanism elucidated in this study involved β-arrestin1 scaffolding of cPLA2, which signals to arachidonic acid and eventual release of prostaglandin. These data suggest a G-biased ligand at GPR109a, capable of signaling through G-proteins but not β-arrestins (specifically β-arrestin1), would provide lipid-modulating benefits without flushing (Figure 4).

If the HDL-C modulation properties of niacin act through GPR109a, then it is intriguing to consider what a high-efficacy intrinsically biased GPR109a agonist might accomplish. It may retain the flushless behavior of MK-0354 while increasing HDL-C by virtue of more robust G-protein coupling. It is possible that the partial efficacy displayed by MK-0354 manifests as functional selectivity in in vitro assays and for free fatty acid reduction but is insufficient to increase HDL-C in humans. This highlights one benefit of intrinsic ligand bias over functional selectivity of partial agonists. It is difficult to translate partial agonist functional selectivity without a thorough understanding of receptor densities and signal amplification in both humans and disease model species. Unfortunately, a lack of good preclinical animal models or clinical validation still leaves unanswered the question of how much influence GPR109a can exert on HDL-C levels.

More generally, GPR109a exemplifies a reverse genetic approach to biased ligand pathway validation, an attempt to dissect the pharmacology of a known agonist. By working backwards from therapeutically relevant end points to signal transduction “nodes,” the selective target of a biased ligand can be hypothesized. For example, β-arrestin1 or β-arrestin2 or various GRK knockout animals are compared to their wild-type littermates in standard models of GPCR physiology or pharmacology. This approach has been used for a wide range of receptors.129 Because β-arrestins simultaneously inhibit G-protein signals and stimulate β-arrestin–dependent signals, changes in agonist pharmacology in β-arrestin knockout animals can begin to discriminate distinct signaling pathways: G-protein-linked responses often increase and β-arrestin–linked responses decrease in GRK or β-arrestin knockout animals compared to wild-type littermates. This, in turn, informs hypotheses for the differentiation of balanced and biased ligands.

**Major Challenges to Biased Ligand Drug Discovery**

These examples highlight some of the conceptual and technical progress made to date in discovering and developing biased ligands for cardiovascular medicine. As with any paradigm shifting discovery, advances have been fitful and required progress on multiple fronts, from developing tools to dissect receptor signaling to identifying biased ligands to appreciating unmet medical need that aligns with pharmacological profiles that a biased ligand might deliver. Beyond the AT1R, β-adrenergic receptors, and GPR109a, several other receptors of cardiovascular importance have revealed clues to suggest separable pharmacology and potential opportunities...
for new biased ligands.\textsuperscript{130–132} More thorough enumeration of reported biased ligands and physiological roles of \(\beta\)-arrestins in receptor pharmacology are found elsewhere.\textsuperscript{1,129,133}

A particular problem confronting the field is the absence of simple criteria to determine when a ligand is intrinsically biased, functionally selective in meaningful settings, or merely selective only in the model system undergoing investigation but completely undifferentiated in more physiologically relevant settings. Several attempts have been made to standardize nomenclature and criteria for determining ligand bias,\textsuperscript{71,134} and recently several groups have introduced quantitative methods to assess ligand bias.\textsuperscript{135–137} Further effort to develop these approaches is warranted, as is diligence in recognizing their assumptions and applying their criteria, to most efficiently move the field of ligand bias and functional selectivity forward to clinical relevance.

In addition, despite the examples described, there are still relatively few well-characterized examples of pathway validation. We hope that as biased ligands proliferate and progress, more research will be dedicated to thorough pathway validation, particularly for “problematic” GPCR that elicit potentially beneficial effects but have been less successful in drug development because of on-target adverse events.\textsuperscript{138,139} These are the “low-hanging fruit” of unbiased ligand drug discovery. From the perspective of functional selectivity and ligand bias, these are highly attractive targets for basic research to separate agonist responses to test if a biased ligand might “rescue” the receptor for drug discovery.

In the final reckoning, however, the field will likely accelerate only after clinical validation of a biased ligand, i.e., that it is pharmacologically distinct from a balanced ligand and offers valuable therapeutic benefit. TRV120027 and other biased ligands offer a chance to achieve just that and may open a window to a new generation of GPCR-targeted drugs aimed at a wide range of unmet medical need.

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Both authors are employees of Trevena, a biotechnology company developing TRV120027 for acute heart failure.

References


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