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Biased Agonism of G Protein-Coupled Receptors: A Potential Therapeutic Strategy of Cardiovascular Diseases

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Abstract

G Protein-Coupled Receptors (GPCRs) are a family of seven transmembrane receptors, and are major targets of current clinical drugs. Many GPCRs have been successfully targeted by specific drugs which are classified as agonists or antagonists. Classically, the GPCR signal pathways are considered to be regulated by heterotrimeric G proteins. However, many researches have demonstrated that other molecules, β-arrestins, participate in the regulation of GPCRs associated intracellular signaling pathways. The major role of β-arrestins is to desensitize responses to G protein signals and to activate cytoplasmic molecules. GPCR ligands can dominantly activate either the G protein or β-arrestin pathway, which is the basis of the biased agonism of GPCR signaling. Both signals represent independent actions, for example, one is beneficial and the other is related to side effect. In this review, we summarize the signaling pathways of GPCRs and current biased agonist research on ligands with a focus on cardiovascular disease.

Introduction: Brief Review of GPCR Signaling

G Protein-Coupled Receptors (GPCRs) are a family of seven transmembrane proteins, which together are viewed as one of the largest therapeutic targets in clinical medicine [1,2]. In fact, GPCR ligands account for almost 40% of approved drugs such as α- and β-blockers, opiates, β-agonist and angiotensin-converting enzyme inhibitors [3,4]. GPCRs recognize various ligands, including hormones, proteins, peptides, lipids and ions [3-5], and thus, their signals and functions make them attractive drug targets [1,2,6,7].

Classically, agonist binding to GPCRs promotes structural changes that stimulate the activation of heterotrimeric G proteins and lead to the activations of various cytosolic signaling molecules [4,8-10]. It has been demonstrated that binding of agonists to GPCRs promote a desensitization process via GPCR phosphorylation by the G Protein-Coupled Receptor Kinases (GRKs) with the involvement of β-arrestin [11-14]. These structural changes increase the binding affinities of GPCRs for β-arrestins, which are known to block G protein signaling [11-16]. β-arrestins is not only a desensitizer of G protein signals, but also a signaling molecule [9,14,17].

There are many diseases related to GPCRs including Cardiovascular Diseases (CVDs), diabetes and neurologic disorder [1,4,18,19]. Among the diseases, CVDs are one of the most common diseases [20,21]. CVDs, such as, atherosclerosis, coronary artery diseases, stroke, and thrombosis, are leading causes of death [21]. CVDs highly associate with many GPCRs which are expressed in heart [20,22,23], the underlying mechanisms responsible for CVD are poorly understood. In view of the fact that alterations in GPCR pathways are linked to the developments of various CVDs [20,22-26], it would appear drugs that target GPCR pathways might be helpful for the prevention and/or treatment of CVDs. Although drugs used in CVDs bind same receptor, the pharmacologic effects of them are dependent on ligands [4,27]. Here, we discuss a novel GPCR pathway, called biased agonism, and the importance of biased drug development in cardiovascular diseases.

β-Arrestins as Novel Signaling Molecules in GPCR Pathways

Classical paradigms portend G-protein signals act in a linear manner [28-30], to activate various downstream pathways that increase levels of second messengers, such as, cAMP, DAG, and IP3 (Figure 1). In response to agonist binding, G Protein-Coupled Receptors (GPCRs) undergo conformational changes, and combinations of structural changes and receptor phosphorylation causes the recruitment of β-arrestin, which leads to signal desensitization via endocytosis of receptor-protein complexes (Figure 1). Furthermore, sequestration of these receptors from the cell surface is an important component of receptor desensitization and down-regulation [9,11,14].

Recently, many researchers have reported GPCRs activate cytosolic signaling substrates, such as, MAPKs, Tyrosine Kinase (TK), AKT, PI3 Kinase (PI3K), and NF-kB, via β-arrestins, that is, in non-classical G protein independent manner (Figure 2). β-Arrestins act as scaffolds that bind various signaling molecules, including MAPKs, AKT, PI3K in various cells [9,17,31-33]. The proliferation of such findings shows GPCRs have multiple signal networks, and thus, there is a need to evaluate current drugs targeting GPCRs using different experimental tools to elucidate the nature of these networks.

Biased Agonism of GPCRs

The classical GPCRs signaling pathway presumes that bindings of ligands elicit their effects through one mechanism [28-30]. According to this paradigm, although agonists differ in terms of efficacy, their
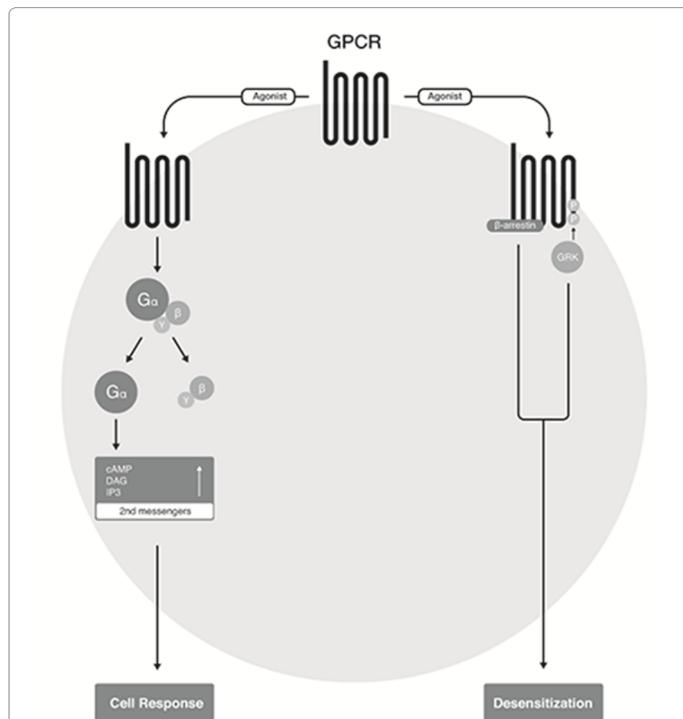
Figure 1: Classical paradigm of G Protein-Coupled Receptors (GPCRs) signaling pathway.

After agonist binding to GPCRs, activated receptor stimulates G proteins and is phosphorylated by G Protein-Coupled Receptor Kinases (GRKs). The β-arrestin binds phosphorylated receptor and leads to desensitization.
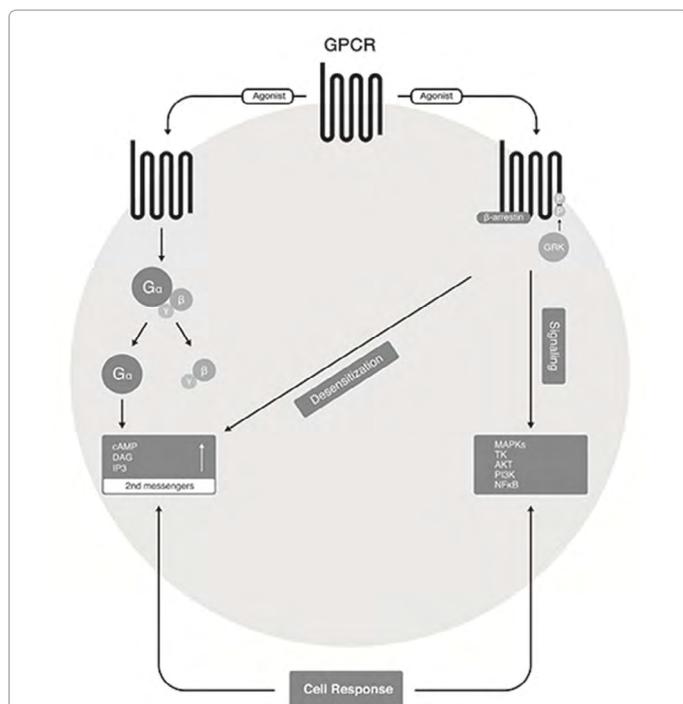

Figure 2: New paradigm of G Protein-Coupled Receptors (GPCRs) signaling pathway.

After agonist binding to GPCRs, the β-arrestin which was bound to receptor not only terminate G-protein signaling, but acts as scaffolds to activate several signaling molecules.

elicited downstream effects are identical [34,35]. This concept is sufficient to determine the character of a ligand, i.e., full, partial, inverse agonist or neutral antagonist (Figure 3) [28,36,37]. However, agonists differ qualitatively (e.g., one agonist selectively activates one signal whereas another predominantly stimulates a different signal via the same receptor) and quantitatively (e.g. partial verses full) [28,36,37]. Thus, according to this expansion of the classical paradigm, agonists recognized by same GPCR elicits different types of responses.

Because ligands exhibit functional specificity via the same GPCR, the molecular pharmacologic concept of 'biased ligand' was proposed [27,38,39]. Interactions between GPCRs and G proteins and β-arrestins represent general mechanisms of GPCR pharmacology and can initiate distinct signals, which are associated with specific physiological or pathophysiological consequences [20,22-26,40-42]. As shown in Figure 4, ligands cause bias toward the G protein- or β-arrestin-mediated pathways, which suggests that biased ligands can be used to selectively to achieve greater beneficial or even negate unwanted results of GPCR activation, like side effects [43-45]. In past decades, several biased GPCR ligands have been identified that selectively target G proteins or β-arrestins [4,27]. Thus, the characterization of GPCRs signaling pathways related to specific drugs is an essential prerequisite to the development of optimal therapeutic approaches.

The biased ligand concept proffers GPCRs adopt specific ligand-dependent conformations [10,46-49]. In particular, different ligand biases could stabilize distinct receptor conformations, and result in the transmissions of different signals to intracellular components [46,47,50,51]. Moreover, the bindings of different β-arrestin biased ligands to same GPCRs might activate other effectors in different ways [18,52,53]. About 400 GPCRs have pharmacologically relevant in human, only 30 GPCRs of structures have been reported [18]. It

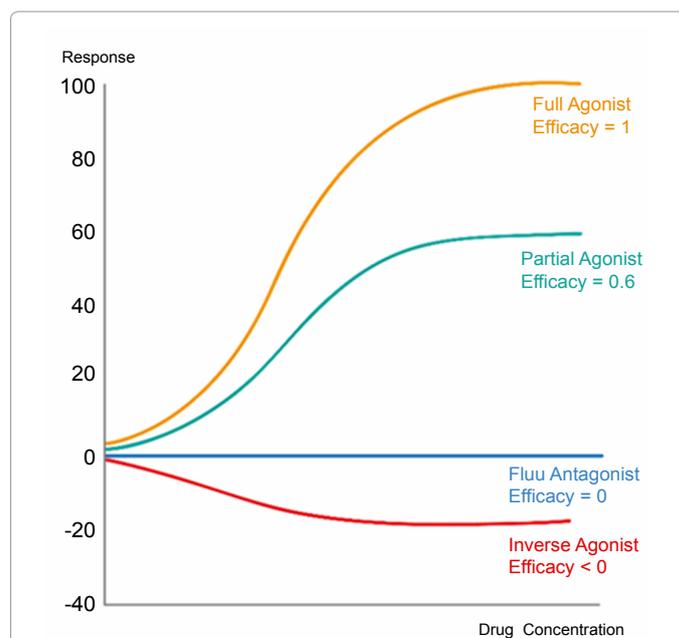**Figure 3:** Interaction between ligand and receptor.

Full agonist: It binds to the certain receptor, and leads to produce full efficacy.
Partial agonist: Although it binds to the same receptor, it produces only partial efficacy.
Full antagonist: It inhibits agonist-mediated pharmacological functions.
Inverse agonist: It binds to the same receptor, however, it has opposite effect compared to agonist.

is helpful to reveal 3-dimensioinal structures of GPCRs for designing biased ligands. Thus, understanding of structure-activity relationships is required for the developments effective therapeutics.

Importance of Biased Ligands in the Background of CVD

Numerous studies have been conducted on relations between GPCRs and disease states [19,20,22-26,42,54-59]. Angiotensin II (AngII) Type 1 Receptor (AT1R) and β-adrenergic receptors are probably the most important and well-studied GPCRs in the context of CVDs [60-65]. The Platelet-Activating Factor (PAFR) has an important role in the development of atherosclerosis (Figure 5 and Table 1) [32,66,67].

Angiotensin II (Ang II) Type 1 Receptor (AT$_1$R)

As shown in Figure 6, AT1R, which is stimulated by Ang II, plays an important regulatory role in the cardiovascular system [60,62,64,68-72]. Excessive stimulation by Ang II is detrimental and causes arterial hypertension, myocardial hypertrophy, and cardiac dysfunction [60,62,64,68-72]. Like other well-studied GPCRs, signaling of AT1R also occurs via G proteins and β-arrestins pathways [28,73], the characteristics of which have been investigated using mutant ligands that activate one pathway, signal inhibitors, siRNAs, and knockout mice [60,62,64,65,68].

TRV120027, a β-arrestin biased ligand, reduces arterial pressure and increases cardiac contractility whereas G protein biased ligand, angiotensin II, causes vasoconstriction and fluid retention [72,74,75]. Insufficient cardiac output causes vasoconstriction by activating the Renin-Angiotensin System (RAS), which further impairs cardiac function because of the additional work required to maintain end organ perfusion [76,77]. It has been shown TRV120027 can block vasoconstriction induced by G protein activation and increase cardiac performance via β-arrestin biased signaling [72,74,75]. TRV120027 also suppresses angiotensin II-induced cardiac hypertrophy but enhances cardiac contractility [70]. Other β-arrestin biased ligand, TRV120026, has similar effects [4,64]. In this regard, β-arrestin biased ligands of AT1R offer a potentially efficient means of improving patient outcomes (Figure 6A).

β-Adrenergic Receptors (β-AR)

The adrenergic receptors are GPCR family members that target catecholamines, and the β-adrenergic receptors have been well studied [78-80]. Antagonists and agonists of adrenergic receptors are among the most clinically important drugs for the treatment of cardiovascular diseases [81,82]. In the cardiovascular system, β1-adrenergic receptor (β1-AR) specifically increases cardiac output by increasing heart rate, conduction velocity, and stroke volume [83-85], whereas β2-AR controls vascular tone [86].

Recent research has revealed that carvedilol, a widely used β-blocker, activates ERK1/2 via β-arrestin in the absence of G protein activation, and classical antagonists of G protein signaling transactivate EGFR via a β-arrestin-dependent pathway [87-91]. These transactivations show that β-blocker, which acts as a β-arrestin biased ligand, has cardioprotective effects *in-vitro* and *in-vivo* systems [87-90]. However, it is thought that sustained β1-AR activation is cardiotoxic such as increasing apoptosis, heart rate, blood pressure via Gs signaling (Figure 6B) [92]. Moreover, carvedilol (a well-known β-blocker) acts as a β-arrestin-biased ligand for β1-AR and β2-AR [88,91]. Thus, the β-arrestin-dependent signaling of β-AR is expected to have a cardioprotective effect (Figure 6B).
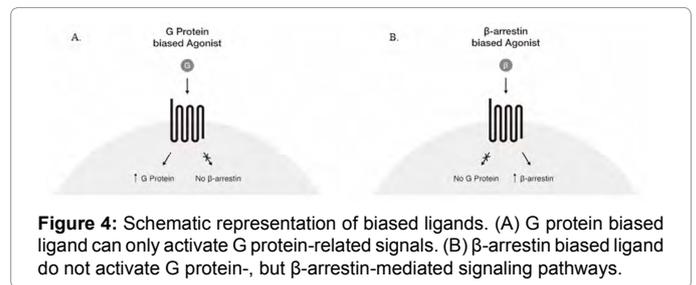

Figure 4: Schematic representation of biased ligands. (A) G protein biased ligand can only activate G protein-related signals. (B) β-arrestin biased ligand do not activate G protein-, but β-arrestin-mediated signaling pathways.
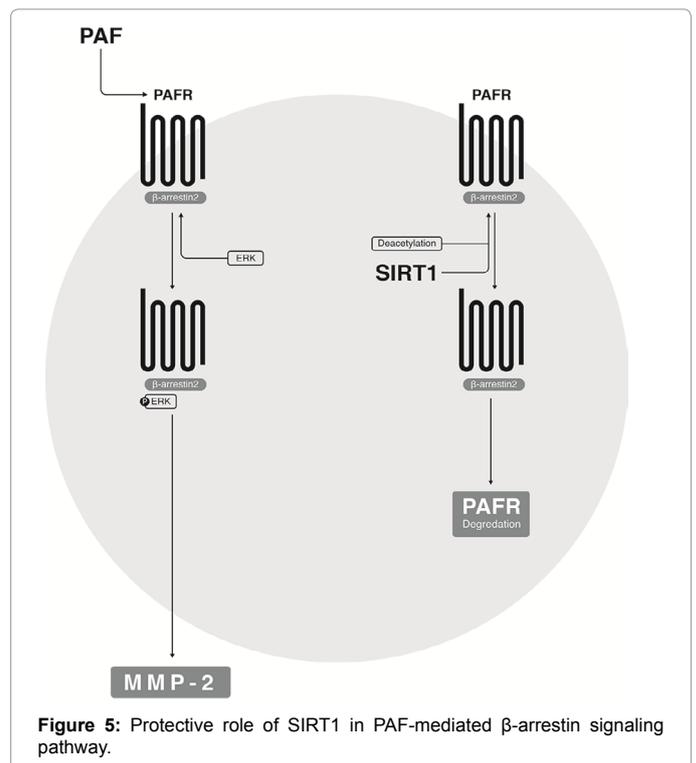

Figure 5: Protective role of SIRT1 in PAF-mediated β-arrestin signaling pathway.
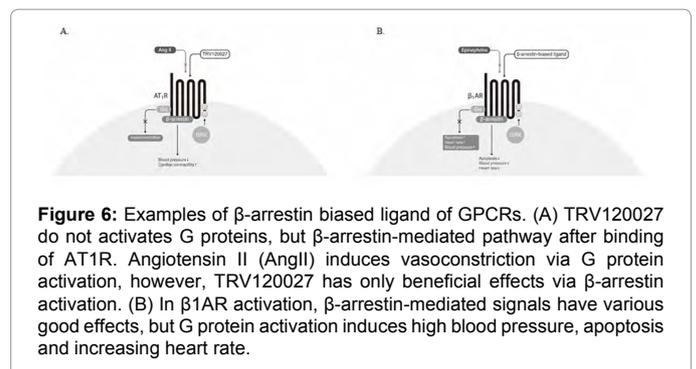
Figure 6: Examples of β-arrestin biased ligand of GPCRs. (A) TRV120027 do not activates G proteins, but β-arrestin-mediated pathway after binding of AT1R. Angiotensin II (AngII) induces vasoconstriction via G protein activation, however, TRV120027 has only beneficial effects via β-arrestin activation. (B) In β1AR activation, β-arrestin-mediated signals have various good effects, but G protein activation induces high blood pressure, apoptosis and increasing heart rate.

Platelet-Activating Factor Receptor (PAFR)

Platelet Activating Factor (PAF), which binds to PAF receptor (a member of the GPCR family), was found to enhance matrixmetalloproteinase-2 (MMP-2) expression levels and MMP-2 is considered to make an important contribution to atherosclerotic plaque instability [32,66,93-95]. PAF-enhanced MMP-2 production has been shown to occur via activation of a β-arrestin-dependent ERK pathway (Figure 5) [32,66]. Interestingly, SIRT1 (silent mating type

GPCRs	Ligand	Bias	Function	Dose for biased action	References
Angiotensin II Type I Receptor (AT$_1$R)	Sar^1,Lys^5,Ala^8 Ang II (TRV120023)	β-arrestin-biased	Increased cardiac contractility	~44 nM (EC$_{50}$)	[4,70]
	Sar^1,Tyr^5,Pro^7-NH2 Ang II (TRV120026)	β-arrestin-biased	Reduced BP Increased cardiac contractility	~230 nM (EC$_{50}$)	[4,64]
	Sar^1,D-Ala^8 Ang II (TRV120027)	β-arrestin-biased	Reduced BP Increased cardiac contractility	~44 nM (EC$_{50}$)	[4,72]
β$_1$-adrenergic receptor	Carvedilol	β-arrestin-biased	Cardioprotection (against catecholamine) Increased cardiac contractility	~2 nM (EC$_{50}$)	[84,88,89]
	Alprenolol			~59 nM (EC$_{50}$)	
Platelet Activating Factor Receptor (PAFR)	Platelet activating factor	β-arrestin-biased	Increased matrix metalloproteinase-2 (MMP-2)	~1 nM	[32]
Apelin receptor	Stretch	β-arrestin-biased	Cardiac hypertropy	-	[26,99,100-105]
Sphingosine 1-phosphate receptor-1 (S1P$_1$)	ApoM$^+$HDL-S1P	β-arrestin-biased	Decreased vascular inflammation	~100 nM	[109]

Table 1: Introduction of β-arrestin biased ligands of GPCRs related to cardiovascular diseases.

information regulation 2 homolog 1), is a key regulator of protection against vascular disorders and down-regulates PAFR via β-arrestin-mediated internalization [66]. These results suggest deacetylation, a function of SIRT1, might be involved in GPCR regulation (Figure 5).

PAFR also plays a critical role in platelet aggregation [96,97]. Arachidonic acid, which is known to potent aggregation factor, induced platelet aggregation and thrombosis formation through PAFR [98]. SIRT1 down-regulates PAFR in platelets via proteasomal and/or lysosomal pathway [66]. SIRT1 activation suppresses platelet activation *ex-vivo* and pulmonary thromboembolism *in-vivo* [98]. Thus, elucidating G protein-related signaling pathway of PAFR is important to develop new cardiovascular drugs targeting PAFR.

Apelin receptor

Apelin receptor, which is also one of the GPCR family, has similar sequence with the AT1 receptor [26,99]. However, the receptor does not respond to angiotensin II [26,100]. In Apelin receptor signaling pathway on vascular endothelial cells, biased toward G protein (MM07, Elabela/Toddler) signals has beneficial effects like vasodilation, increasing cardiac output [26,101-104]. MM07 or Elabela/Toddler showed β-arrestin biased actions at around 45 nM or 100 nM. β-arrestin-related signaling by mechanical stretch in Apelin receptor causes cardiac hypertrophy whereas G protein prevents the cardiac hypertrophy [26,105]. In case of Apelin receptor, G protein signal is beneficial compared to β-arrestin pathway.

Sphingosine-1-Phosphate 1 receptor (S1P1 receptor)

Chronic inflammation induced by inflammatory macrophages plays critical role in the initiation and progression of cardiovascular diseases including atherosclerosis [106,107]. S1P enhances phenotypic changes of macrophages to anti-inflammatory phenotype [108]. In recent research, ApoM+HDL act as a β-arrestin biased ligand and it decreases vascular inflammation [109]. These results can support the cardiovascular protective role of HDL [109].

Conclusion and Perspectives

To make ideal drugs which offer safer, more efficacious for GPCRs, studies should focus on revealing the signals in detail and binding of ligand-receptor. Biased agonism is relatively novel concept, and despite the examples described above, comparatively few studies have been undertaken to elucidate the natures of GPCR pathway signals. Some research suggested functional bias is dependent on structures of ligands. Identification of receptor structures will support the structure-based drug design. These approaches will undoubtedly lead to the discovery of developing ideal biased ligands for therapeutics.

This review places emphasis on the G protein/β-arrestin bias concept because G protein and β-arrestin are being directly targeted for drug discovery. We hope this article provides the motivation to better define the natures of GPCRs signals and aids the developments of more effective drugs on the basis of structures.

Acknowledgment

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