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GPCR signaling via β-arrestin-dependent mechanisms

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Abstract

β-arrestin1 (or arrestin2) and β-arrestin2 (or arrestin3) are ubiquitously expressed cytosolic adaptor proteins that were originally discovered for their inhibitory role in G protein-coupled receptor (GPCR) signaling via heterotrimeric G proteins. However, further biochemical characterization revealed that β-arrestins do not just 'block' the activated GPCRs, but trigger endocytosis and kinase activation leading to specific signaling pathways that can be localized on endosomes. The signaling pathways initiated by β-arrestins were also found to be independent of G protein activation by GPCRs. The discovery of ligands that blocked G protein activation but promoted β-arrestin binding, or vice-versa, suggested the exciting possibility of selectively activating intracellular signaling pathways. Additionally, it is becoming increasingly evident that β-arrestin-dependent signaling is extremely diverse and provokes distinct cellular responses through different GPCRs even when the same effector kinase is involved. In this review, we summarize various signaling pathways mediated by β-arrestins and highlight the physiologic effects of β-arrestin-dependent signaling.

Keywords

Arrestin; endocytosis; 7TMR; adaptor; GPCR; biased agonist; kinase

INTRODUCTION

G protein-coupled receptors (GPCRs) or 7-transmembrane receptors (7TMRs) are the most diverse and well-represented group of membrane receptors, with approximately 800 members in the human genome. They recognize a variety of stimuli that include light, hormones, and chemokines and convert these signals to relevant intracellular messages. GPCR activation can significantly influence many cellular biochemical reactions, cell structure, cell motility, and gene expression. GPCR signaling regulates almost every aspect of human physiology and not surprisingly, about 40% of currently prescribed medications act on GPCRs either directly or indirectly.[1–4]

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GPCRs have a conserved heptahelical architecture and interact with at least three families of proteins in an agonist-dependent manner. These three families include (1) the heterotrimeric G proteins, (2) GPCR kinases or GRKs and (3) arrestins.[1,2] The heterotrimeric G proteins are generally divided into four main classes based on sequence similarity of the Gα subunit: $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$ and $G\alpha_{12/13}$.[5] In an activated conformation, GPCRs function as efficient guanine nucleotide exchange factors (GEFs) for bound G proteins and promote the activation of Gα and their dissociation from Gβγ subunits. The activated G protein subunits in turn trigger a signal transduction cascade via various effector molecules and kinases. Seven GRKs (GRK 1–7) are expressed in human cells.[6,7] While GRKs 1 and 7 are confined to the retina, GRK4 is mainly expressed in the brain, kidney and testes, and GRKs 2, 3, 5 and 6 are expressed ubiquitously. The arrestin family includes two visual (arrestin 1 and 4) and two non-visual, ubiquitously expressed arrestins (arrestins 2 and 3, also known β-arrestin 1 and β-arrestin 2 respectively). β-arrestin 1 and 2 share ~80% amino acid sequence identity and present unique as well as redundant roles in GPCR regulation.[8–10] Ablation of both β-arrestin isoforms causes embryonic lethality in mice whereas genetic deletion of a single isoform leads to viable offsprings in which GPCR activation engenders distinct responses, perhaps via individual β-arrestin isoforms.[7,10] The GRKs and arrestins were initially identified as inhibitors of GPCR signaling, but their roles in GPCR signal transduction are multifactorial and continuously evolving.

GRKS AND β-ARRESTINS PROMOTE DESENSITIZATION OF GPCRS

Most agonist-activated GPCRs are rapidly uncoupled from G proteins resulting in signal desensitization by an efficient two-step process: (1) phosphorylation by GRK(s) and (2) β-arrestin binding (Figure 1A).[4] While β-arrestin binding physically obstructs G protein coupling to the GPCR, additional mechanisms are in play by which β-arrestins ensure efficient blockade of G protein signaling. β-arrestins scaffold cyclic nucleotide phosphodiesterases (PDEs) and diacylglycerol kinases (DGKs) that degrade second messengers downstream of activated receptors (Figure 1A).[11,12] PDEs of the PDE4 family terminate cyclic adenosine monophosphate (cAMP) signaling by converting cAMP to AMP. By recruiting PDE4D3 and PDE4D5 to the activated β_2-adrenergic receptor (β_2AR), β-arrestin2 decreases cAMP levels and cAMP-dependent protein kinase (PKA) activity.[13] This in turn attenuates PKA-mediated β_2AR phosphorylation, preventing the switching of β_2AR coupling from G_s to G_i.[14] β-arrestin scaffolding of PDE4D is receptor specific, as constitutive binding of the PDE4D8 isoform to the β_1AR does not require β-arrestins and PDE4D8 dissociates from the β_1AR upon agonist stimulation of the receptor.[13,15] Additionally, β-arrestin can diminish M_1 muscarinic receptor-mediated protein kinase C (PKC) activity by recruiting DGKs.[16] M_1 muscarinic receptors couple to $G_{q/11}$ to stimulate phospholipase C (PLC) which catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) into inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). β-arrestin translocation to the activated receptor leads to the recruitment of DGKs, which decrease DAG-dependent PKC activity by phosphorylating DAG, which is then converted into phosphatidic acid.[16] Phosphatidic acid not only serves as an intermediate for phospholipid synthesis, but also plays important roles in signal transduction and membrane dynamics as a second messenger.[17] Thus while β-arrestin's partnership with PDEs can

terminate second messenger signaling, β-arrestin's association with DGKs can initiate an additional wave of signaling via phosphatidic acid production.

BIMODAL SIGNALING OF GPCRS

Although the β-arrestins were considered as inhibitors of GPCR signaling for nearly a decade after their discovery, a large body of work that followed has changed our appreciation about the functional roles of β-arrestins.[8] β-arrestins have been shown to function as critical adaptors for agonist-induced endocytosis of GPCRs[18,19], and also as important adaptors for agonist-induced ubiquitination of GPCRs[20–24], thus governing the trafficking itinerary of internalized GPCRs (Figure 1B). β-arrestins were shown to form signaling scaffolds for mitogen activated protein kinases (MAPKs) such as the extracellular signaling kinases 1 and 2 (ERK), and c-Jun N-terminal kinase 3 (JNK3) on endosomes with internalized GPCRs (Figure 1C).[25] Subsequently, β-arrestins were shown to promote some of these pathways even when the G protein activity is disabled.[3,26] Thus, GPCR signaling is bimodal, in which the first mode is dependent on G proteins and the second mode dependent on GRK phosphorylation of the GPCR and on β-arrestin-dependent signaling.[4,27–29] GPCRs can be activated to favor one of these modes over the other, thus engendering biased signaling (Figure 2). In some cases, complete bias allows signaling via only one mode: either G protein(s) or β-arrestin(s).

PHOSPHORYLATION BARCODING BY GRKS REGULATES β-ARRESTIN ACTIVITY

Phosphorylation of activated GPCRs by GRKs, originally described as the first step in GPCR desensitization is now known to be a critical regulatory mechanism, which initiates a variety of downstream effects by engaging different conformations of β-arrestins and promoting their various functions.[12,30–32] The correlation between the extent of GPCR phosphorylation and the affinity of GPCR-arrestin complex is inferred by the distinct patterns of β-arrestin recruitment dictated by the abundance and distribution of serine/threonine residues in the GPCR intracellular domains.[33,34] A stable β-arrestin/GPCR endosomal complex is formed by GPCRs that have clustered serine/threonine motifs in their carboxyl tails (for example, the $AT_{1a}R$). In contrast, a transient plasma membrane complex is obtained with GPCRs that possess discontinuous or scattered serines/threonines (for example, the β_2AR). Eliminating all the phosphorylation sites by mutagenesis either weakens a former strong β-arrestin interaction (e.g. $AT_{1a}R$)[35] or eradicates an original weak β-arrestin binding (e.g. β_2AR)[8].

Studies carried out with a number of GPCRs (β_2AR [36], follicle-stimulating hormone receptor[37], somatostatin 2A receptor[38], thyrotropin-releasing hormone receptor[39], dopamine D_1 receptor[40], free fatty acid receptor 4[41], neuropeptide FF2 receptor[42] and other GPCRs [11,43]) showed that elimination of specific phosphorylation sites in a GPCR has distinct downstream effects on β-arrestin binding and/or signaling. Using mass spectrometry and phospho-site antibodies, differential phosphorylation of M3 muscarinic receptor was demonstrated in different cell and tissue types suggesting that particular patterns of phosphorylation engenders tissue-specific signaling.[44] By utilizing quantitative proteomics

chemically distinct ligands were shown to produce qualitatively similar mixture of phosphorylated μ-opioid receptors (MORs).[45] However, multisite phosphorylation of a specific serine-threonine motif is required for MOR internalization as well as β-arrestin recruitment and quantitatively, phosphorylation of this region is greater when MORs are stimulated with DAMGO than when stimulated with morphine.[45] These studies support the idea that distinct phosphorylation motifs may be sensed by GRKs yielding a distinct outcome through subsequent protein interactions, especially via β-arrestin recruitment.

The differential roles of GRK isoforms in regulating a particular GPCR-activated function of β-arrestin became evident from targeted knockdown of each GRK by RNAi.[46,47] GRK2 and/or GRK3-mediated bulk phosphorylation of the $AT_{1a}R$ and of the vasopressin receptor (V_2R) enables β-arrestin binding and internalization, but not ERK activation. In contrast, GRK5 and/or GRK6 mediated phosphorylation of the same two GPCRs, leads to β-arrestin-dependent ERK activation but does not provoke robust β-arrestin binding nor internalization. The chemokine receptor CCR7, is activated by two endogenous agonists, namely, CCL19 and CCL12, which engage different GRK isoforms and promote different downstream effects via β-arrestin. CCL19 stimulation promotes GRK3 and GRK6-mediated CCR7 phosphorylation whereas CCL21 engages only GRK6 leading to differential consequences: β-arrestin-dependent ERK activation by both agonists and robust β-arrestin binding and desensitization only by CCL19.[48]

Specific phosphorylation barcodes were delineated by mapping the GRK phosphorylation sites on activated β_2AR and the chemokine receptor CXCR4 by employing combinatorial approaches involving stable isotope labeling, mass spectrometry, site-directed mutagenesis, site-specific phospho-antibodies, and RNAi of individual GRKs.[49,50] These studies confirmed the presence of GRK isoform-dependent, phosphorylation bar codes that correspond to the activated conformation of the receptor. Upon CXCL12 treatment, CXCR4 is rapidly phosphorylated on serines 324 and 325 by PKC and GRK6. Additional serines, which are not targeted by GRKs 2, 3 and 5 are also phosphorylated by GRK6 in agonist-activated CXCR4. GRKs 2/3 phosphorylate a small serine cluster at the carboxyl terminus of CXCR4. GRK2, GRK6 phosphorylation and β-arrestin2 binding primarily regulate calcium mobilization triggered by CXCR4 whereas CXCR4-induced ERK activity is dependent on GRK3, GRK6 and β-arrestin1 binding.[50]

Isoproterenol stimulation of the human β_2AR triggers phosphorylation of 13 residues, among which two serines at positions 355 and 356 are phosphorylated by GRK6.[49] Interestingly, carvedilol provokes only phosphorylation of these two residues by GRK6. Both isoproterenol and carvedilol-induced β-arrestin-dependent ERK activation requires that GRK6 phosphorylates the β_2AR. On the other hand, GRK2 phosphorylation of six sites in the carboxyl tail is required for β-arrestin recruitment and receptor internalization. β_2AR phosphorylation at GRK2 as well as GRK6 sites is necessary for efficient desensitization of cAMP signaling.[49]

In summary, barcoding of GPCRs by GRKs that define specific activities through β-arrestins involve hierarchical molecular mechanisms (Figure 3). As a first tier of regulation, each GPCR has distinct phosphorylation sites or motifs distributed in its intracellular domains. As

a second tier of regulation, the recruitment of a GRK is dictated by specific ligand-activated conformation(s) of the GPCR. As a third tier of fine tuning specific β-arrestin-dependent signaling, the expression level of GRK isoforms can be modulated in a cell type or tissue, such that only certain phosphorylation codes are enabled.[51] As a fourth tier of regulation, each GRK phosphorylates a set of target sites, which can be either overlapping or non-overlapping with other GRKs or with other serine/threonine kinases. In some cases, GRKs can compete or antagonize each other's activity suppressing or increasing specific phospho-signatures.[46,47] The resulting unique phosphorylation code on a GPCR engages a particular active conformation of β-arrestin facilitating one or more functions of β-arrestin (i.e. desensitization, internalization or signal transduction).

β-arrestins undergo receptor and ligand-specific conformational changes, and distinct activated conformations of β-arrestins correlate with their trafficking and signaling activities.[30–32] Recently, the existence of such specific β-arrestin conformations that tune with specific receptor phosphorylation barcodes have been demonstrated by employing fluorine-19 nuclear magnetic resonance.[31] These studies were achieved by substituting unnatural amino acid at the 7 sites of β-arrestin1's amino terminus, which were identified as the phosphate binding sites.[31,52] When the phospho-acceptor sites 1, 4, 6 and 7 in β-arrestin1 were involved in engaging GPCR domains, such pattern induced a β-arrestin-1 conformation specific for clathrin recruitment. When the phosphates on the GPCR engaged only the acceptor sites 1 and 5, then the resulting conformation of β-arrestin1 was conducive for c-Src activation.[31] In addition to conformations provoked in β-arrestin by specific phosphorylation patterns on the GPCR interface, additional modifications on a pre-activated β-arrestin by differential ubiquitination or by other post-translational modifications can expand the conformational repertoire of each active form of β-arrestin.[11,22,53,54] This perhaps allows β-arrestin to possess unlimited conformational plasticity enabling its multi-faceted functions in GPCR signaling.

β-ARRESTIN-DEPENDENT SIGNALING VIA SCAFFOLDING

Soon after the identification of β-arrestins as adaptors for endocytosis, they were linked with the activation of signaling kinases.[55] Interestingly, β-arrestins were shown to simultaneously bind and link components of a kinase cascade (i.e. binding to MAPKKK, and MAPK) and act as kinase scaffolds (Figure 1C).[56–58] Upon GPCR activation, β-arrestins also scaffold or sequester other non-kinase proteins such as small GTPases, phosphatases or adaptor proteins, promoting the formation of various signalosomes that regulate cellular activities (Figure 4). Accordingly, β-arrestin's roles as a scaffold in signal transduction can be broadly classified as (A) β-arrestin scaffolding that primarily involves kinases and (B) β-arrestin scaffolding that primarily involves non-kinase proteins with or without a secondary link to a kinase activity.

A) β-arrestin scaffolding that primarily involves kinases

Non-receptor Tyrosine Kinases—Soon after the identification of β-arrestins' roles as endocytic adaptors for activated GPCRs, they were shown to trigger MAPK signaling by binding and recruiting several members of the Src family non-receptor tyrosine kinases.[25] β-

arrestin-dependent Src activity in turn regulates GRK2 levels, providing a negative feedback on GPCR desensitization.[59] Additionally, β-arrestin-Src complexes phosphorylate and modulate the activity of the endocytic proteins, dynamin I and β2-adaptin and regulate clathrin-dependent internalization of GPCRs including $AT_{1a}R$, β_2AR, V_2R and bradykinin B2 receptor.[25] β-arrestin-Src kinase association can also facilitate exocytosis and granule release as shown for the interleukin 8 receptor CXCR1.[60] Additionally, β-arrestin-Src interaction acts as a critical step upstream of MAPK signaling via select GPCRs.[25]

Mitogen-activated protein kinases—Among the MAPKs, the role of β-arrestins has been extensively characterized for ERK signaling. Most GPCRs activate ERK via both β-arrestins and G proteins; however, some GPCRs primarily employ β-arrestins for ERK activation (Figures 1, 2). G protein-mediated ERK activation is transient, localized in the nucleus and promotes gene transcription, whereas β-arrestin-dependent ERK signaling is sustained, localized in clathrin-coated structures (CCSs) and stabilized in endosomes.[8,61–63] The $AT_{1a}R$ and the V_2R which form a stable complex with either β-arrestin isoform, promote sustained ERK signaling in endosomes, whereas the β_2AR forms a transient complex with β-arrestins and induces transient ERK activity.[64] Interestingly, replacement of the C tail of the β_2AR with that of V_2R promotes ERK signaling in endosomes, while the substitution of V_2R C tail with that of β_2AR eliminates ERK signaling in endosomes.[64] These reciprocal effects caused by switching of C tails also correlate with the kinetics of de-ubiquitination of β-arrestins induced by the β_2AR and V_2R.[65,66] Accordingly, β_2AR C tail promotes transient ubiquitination (rapid deubiquitination) of β-arrestin2, while V_2R C tail provokes sustained ubiquitination.[65] Ubiquitinated β-arrestins also display greater binding affinity than non-ubiquitinated β-arrestins with (a) activated GPCRs, (b) clathrin subunits and (c) components of ERK signaling (c-Raf and ERK), which suggests a tight relationship between β-arrestin ubiquitination status and the transmission of β-arrestin-dependent signaling.[11,62,63]

β-arrestins scaffold components of the JNK cascade: apoptosis signal-regulating kinase (ASK1), MKK4, MKK7 as well as JNKs 1, 2 and 3.[58,67] JNK signaling regulates growth arrest, apoptosis and activation of immune cells in response to environmental and hormonal stresses. While all visual and non-visual arrestins bind to JNK3, only β-arrestin2 facilitates JNK3 phosphorylation and activation. Upon $AT_{1a}R$ agonist-stimulation, β-arrestin2 specifically localizes activated JNK3 on endosomes, analogous to its ability to scaffold activated ERK on endosomes.[58] On the other hand, for the metabotropic glutamate 7 (mGlu7) receptor[68], β-arrestin-mediated JNK signaling is regulated by specific GRKs and β-arrestin isoforms: GRK4 activates JNK, whereas GRK2 promotes ERK signaling; β-arrestin2 enables JNK activation, but inhibits ERK, whereas β-arrestin1 activates ERK while inhibiting JNK.[68]

In addition to promoting ERK and JNK signaling, β-arrestins also regulate p38 MAPK activation. CXCR4-induced p38 activity promotes cellular chemotaxis and is primarily mediated by β-arrestins.[69] β_2AR provokes biphasic p38 activation via G protein and β-arrestin-dependent mechanisms. While the late p38 activation is G_s/cAMP-mediated, early p38 activation is dependent on β-arrestin1/Rac1-GTPase/NADPH oxidase signaling and is solely responsible for agonist induced F-actin rearrangement.[70] For the κ-opioid receptors

(KORs), β-arrestin-dependent signaling and p38 activation requires phosphorylation of the receptors by GRK3.[71] While the analgesic effects of kappa opioids are G protein-mediated, dysphoria and addiction is linked with β-arrestin-dependent p38 activation.[72]

PI3K, Akt and GSK3β signaling—The activity of phosphatidylinositol-3 kinase (PI3K) is modulated by β-arrestin as well. However, while β-arrestins inhibit $G\alpha_q/Ca^{2+}$-dependent PI3K activation downstream of the protease activated receptor 2 (PAR2), they facilitate PI3K activity for the chemokine receptor CCR5.[73,74] CCR5 stimulation by the chemokine macrophage inflammatory protein-1β (MIP-1β) promotes macrophage chemotaxis by G_i-dependent as well as by G_i-independent/β-arrestin-dependent signaling mechanisms. The latter signaling involves concurrent activation of ERK, Protein tyrosine kinase 2B (Pyk2), PI3K p85 regulatory subunit and the tyrosine-protein kinase Lyn in a multimolecular complex that is scaffolded by β-arrestins and recruited to the activated CCR5.[74]

β-arrestin induces the activation of the serine/threonine kinase Akt (also known as protein kinase B) downstream of ghrelin receptor GHS-R1a and α-thrombin receptor.[75,76] Generally, Akt affects components of the apoptotic machinery either directly or indirectly, promotes cell survival, regulates cell proliferation and myocardial angiogenesis.[77] β-arrestin scaffolding of GHS-R1a, c-Src and Akt into a multiprotein complex is necessary for prolonged Akt signaling.[76] In contrast, G protein-mediated Akt activation by ghrelin involves Gβγ triggered-PI3K activation, Akt phosphorylation by c-Src and subsequently by 3-phosphoinositide-dependent protein kinase 1 (PDK1) and mammalian rapamycin-insensitive complex 2 (mTORC2).[76] In the case of the thrombin receptor PAR4, formation of a heterodimer with the purinergic receptor $P2Y_{12}$ promotes sustained β-arrestin-mediated Akt activation.[78] AT_1R stimulation also leads to β-arrestin-dependent PI3K/Akt activation which contributes to phosphorylation and inactivation of Bcl-2 associated death promoter (BAD), a pro-apoptotic protein of the Bcl-2 family.[79] Akt signaling via AT_1R and β-arrestin induces phosphorylation of mTOR and its downstream effector p70/p85 ribosomal S6 kinase (p70/85 S6K).[80] β-arrestin-mediated PI3K/Akt and ERK/p90RSK signaling promotes AT_1R-triggered cell growth and hypertrophy. Chronic catecholamine stimulation of the β_2AR results in the accumulation of chromosomal DNA damage in both somatic and germ cells via β-arrestin1-dependent mechanisms that include: PI3K/AKT activation, AKT mediated phosphorylation and activation of the E3 ubiquitin ligase Mdm2, Mdm2-mediated ubiquitination and proteasomal degradation of p53.[81]

β-arrestin forms a complex with Akt, glycogen synthase kinase 3β (GSK3β) and the protein phosphatase PP2A, upon D_2R activation.[82] Phosphorylation of GSK3β on serine 9 by Akt inhibits GSK3β activity. Akt itself is activated upon phosphorylation of threonine 308 and serine 473 by the phosphatidyl-dependent kinases PDK1 and PDK2/rictor-mTOR, in a PI3K-dependent manner. β-arrestin2, when recruited to the D_2R enhances Akt and PP2A association, leading to Akt dephosphorylation and inactivation.[82] Lithium compounds used as a mood stabilizing medication, inhibit GSK3β activity directly or as shown *in vivo*, by disrupting β-arrestin-PP2A-Akt complexes, which then promotes Akt activation and GSK3 inactivation.[83,84] β-Arrestin–mediated Akt/PP2A/GSK3β signaling cascade presents as a target for antipsychotic drugs aimed at treating schizophrenia.[83,84] On the other hand, β-arrestin2 does not associate with Akt downstream of PAR2, but forms a complex with PP2A/

GSK3β in a Rho kinase dependent manner.[85] β-arrestin-PP2A-GSK3β signaling facilitates stem/progenitor cell survival in normal colon crypts and colon cancer. [85]

Transactivation of receptor tyrosine kinases—GPCR-triggered transactivation of receptor tyrosine kinases (RTKs), namely, epidermal growth factor receptor (EGFR), insulin-like growth factor type 1 receptor (IGF-1R) and vascular endothelial growth factor receptor (VEGFR) are modulated by both β-arrestin and G protein-dependent mechanisms (Figure 4A).[25,29,86] β-arrestin2 inhibits EGFR transactivation by CXCR7 and counteracts mitogenic signaling and cell proliferation.[87] On the other hand, β-arrestins promote sphingosine-1-phosphate-induced transactivation of Fetal Liver Kinase 1 (Flk-1) through S1P1/S1P3 receptors, resulting in mouse embryonic stem cell proliferation.[88] β-arrestins also mediate EGFR transactivation by the β_1AR,[89] a genetic variant of α_1AR,[90] ET_{1A}R,[91] urotensin II receptor (UTR),[92] GPR39 [93] and GPR54.[94] β-arrestin mediated EGFR transactivation may induce diverse cellular responses such as cardioprotection when provoked by β_1AR and UTR, cardiomyoblast hyperproliferation and hypertrophy in the case of α_1AR, myogenesis for GPR39 and cancer cell invasiveness for ET_{1A}R and GPR54.[89–94]

β-arrestin2 and AT_1R-dependent EGFR transactivation and ERK signaling is also evoked by mechanical stimuli such as membrane stretch and leads to load-induced cardiac hypertrophy.[95–97] Hearts from mice lacking either β-arrestin2 or AT_1Rs fail to promote EGFR transactivation and ERK phosphorylation in response to mechanical stretch, while they do promote enhanced myocyte apoptosis.[97] Besides β-arrestin2, Src kinase is also required for the pathologic effects of stretch since Src inhibition prevents stretch-induced activation of ERK, and significantly reduces pressure overload-induced hypertrophy and improves cardiac function.[98]

AngII-induced β-arrestin2 mediated EGFR transactivation contributes to the development of aortic aneurysms, a vascular disease characterized by the dilation, degeneration and eventual rupture of the wall of the aorta. The proaneurysmal effects of β-arrestin2 are independent of TGF-β and of AT_{1a}R-induced G_q signaling.[99] However, AngII-induced abdominal aortic aneurysms (AAAs) and macrophage infiltration into the abdominal aorta are attenuated in the absence of β-arrestin2.[100] This outcome coincides with reduced expression of the inflammatory markers cyclooxygenase-2 (COX-2), monocyte chemoattractant protein-1, and macrophage inflammatory protein 1α.[100] The matrix metalloproteases, MMP2 and MMP9, which disrupt extracellular matrix components and provoke vascular remodeling in aortic aneurysms, are also downregulated in AngII-treated mice lacking β-arrestin2. However, β-arrestin2 deficiency doesn't reverse the increase in blood pressure upon chronic AngII treatment, indicating that β-arrestin2's effects on AAAs are independent of blood pressure. The proaneurysmal effects of β-arrestin2 are also demonstrated in a murine model of Marfan syndrome (MFS).[99,101] MFS is a systemic, inherited disease of connective tissue that is associated with mutations in the fibrillin-1 gene. MFS is responsible for various ocular, skeletal and cardiovascular pathologies and often results in the development of thoracic aortic aneurysms (TAAs). β-arrestin2 deficiency attenuates TAAs as well as the expression of proaneurysmal proteins, MMP-2 and -9 and collagen type 1a1 in aortic tissue of MFS mice carrying the fibrillin-1 C1039G mutation.[99]

(B) β-arrestin scaffolding that primarily involves non-kinases

Rho GTPase and cofilin signaling pathways—GPCRs activate the small GTPase RhoA mainly via $G\alpha_{12/13}$ coupling to Rho guanine nucleotide exchange factors; however, both β-arrestin isoforms also modulate RhoA activity, affecting actin cytoskeletal organization, cell proliferation and cell cycle progression. The growth hormone secretagogue receptor GHSR-1a activates RhoA through $G_{12/13}$, $G_{q/11}$ and β-arrestin2-dependent mechanisms.[102] β-arrestin2 also mediates G_s-independent RhoA activation by the β_2AR: β-arrestin2 forms a complex with a guanine nucleotide exchange factor, p115RhoGEF,[103] enabling p115RhoGEF translocation to the plasma membrane, and concomitant RhoA activation, which promotes focal adhesion remodeling and stress fiber formation.[103] Formyl-Met-Leu-Phe (fMLP) receptor-triggered membrane ruffling is regulated by agonist-induced disassembly of cytosolic β-arrestin- Ral guanine nucleotide dissociation stimulator (RalGDS) complexes, which allows RalGDS to translocate to membrane domains and promote Ras-independent Ral signaling and Rho activation.[104]

RhoA activation and stress fiber formation by the AT_1R are dependent on both $G\alpha_{q/11}$ and β-arrestin1.[102,105] Angiotensin-stimulation promotes β-arrestin1's interaction with Rho GTPase activating protein, ARHGAP21, inhibiting the conversion of RhoA to its inactive GDP-bound state.[106] In addition, β-arrestin2 is required for membrane bleb formation following AT_1R stimulation in a signaling pathway that involves RhoA and its downstream effectors Rho-associated kinase (ROCK) and myosin light chain kinase (MLCK).[107] Alternatively, β-arrestins 1 and 2 can promote angiotensin-induced cell contraction and migration as they interact with myosin light chain phosphatase targeting subunit (MYPT-1) and reciprocally regulate myosin light chain phosphatase (MLCP) activity and myosin light chain (MLC) phosphorylation status.[108]

PAR2-induced β-arrestin-dependent scaffolding of LIM kinase (LIMK), the actin severing protein cofilin, and the phosphatase chronophin decreases phosphorylated-cofilin and increases unphosphorylated cofilin, regulating actin assembly and cell migration (Figure 4B).[109] β-arrestin1 also regulates the phosphorylation-dependent actin association of cofilin by scaffolding ROCK, LIMK and cofilin phosphatases such as slingshot (SSL) following stimulation of the delta opioid receptors (DORs) with the agonist SNC80.[110] β-arrestin1 and PDZ-RhoGEF (postsynaptic density protein 95/disc-large/zonula occludens-RhoGEF) mediate ROCK-LIMK-cofilin signaling upon endothelin-1 (ET-1)/ETA receptor (ET_AR) activation.[111] β-arrestin1 PDZ-RhoGEF interaction facilitates the activation of RhoA and RhoC and subsequent ROCK kinase activity, resulting in enhanced invadopodia and cell invasion of ovarian carcinoma cells.[111]

Wnt and β-catenin signaling pathways—Wnts are secreted lipoglycoproteins that trigger signaling via the 7-transmembrane Frizzled (Fzd) receptors, which function in concert with their single transmembrane co-receptors called low density lipoprotein receptor-related protein 5/6 (Lrp5/6). Wnt signaling is essential for embryonic development; it also regulates cell adhesion, polarity and migration and contributes to cardiac fibrosis.[112,113] Canonical Wnt signaling involves inhibition of GSK3 downstream of Fzd-LRP5/6 and stabilization of β-catenin followed by induction or repression of target genes.

Wnts can also trigger β-catenin-independent signaling via the activation of Rho, Rac-1 or an atypical receptor tyrosine kinase called ROR2.[114] β-arrestins have been identified as important scaffolds in both aforementioned Wnt signaling pathways. β-arrestin1 as well as its association with the Fzd adaptor disheveled (Dvl) are required for Wnt3a-induced, β-catenin-dependent TCF/LEF-mediated transcription. β-arrestins are necessary for maximal Wnt3a-induced phosphorylation of the Dvl by casein kinase 1δ/ε (CK1δ/ε), which is critical for Fzd internalization and for endosomal signaling of internalized Fzd complexes.[115] β-arrestin2 interacts with two Dvl-associated kinases PI4KIIα and PIP5KIβ which stimulate PIP2 production and provoke β-arrestin2 interaction with PIP2-binding protein Amer1 (APC membrane recruitment protein 1). β-arrestin2's scaffolding of Amer1 facilitates Wnt3a-induced Lrp6 phosphorylation, which stabilizes β-catenin.[116] β-arrestin also forms a trimeric complex with phosphorylated Dvl and axis inhibition protein 1 (Axin1) and associated GSK3, thus blocking the phosphorylation and degradation of β-catenin[114]

Alternatively, β-arrestin regulates Wnt-activated, β-catenin-independent signaling cascades such as Wnt/Planar Cell Polarity (PCP) pathway and Wnt/Ca^{2+} pathway. β-arrestin dependent Wnt/PCP signaling induces intracellular cytoskeletal rearrangements by scaffolding phosphorylated Dvl and dishevelled-associated activator of morphogenesis 1 (DAAM1).[117] β-arrestin connects the C-terminal diaphanous autoregulatory domain (DAD) in DAAM1 with the PDZ domain of Dvl engendering RhoA activation.[117] β-Arrestin forms a multiprotein signaling complex with Dvl and Gβγ to promote Wnt/Ca^{2+} signaling and provoke convergent extension movements during Xenopus gastrulation.[114,118,119]

β-arrestin2 also associates with Dvl-2 downstream of PAR1 upon binding of activated protein C to a coreceptor called endothelial protein C receptor (EPCR).[120] The binding of activated protein C with EPCR facilitates the cleavage of PAR1 at a site different from the thrombin cleavage site and stimulates PAR1 signaling.[121] While the EPCR/PAR1 signaling has anticoagulant and anti-inflammatory effects in endothelial cells, thrombin-induced activation of PAR1 promotes coagulation and inflammation.[122–124] β-arrestin2 enables activated protein C-triggered PAR1 signaling via a mechanism that involves 1) EPCR-dependent recruitment of GRK5 to the cytoplasmic domain of PAR1, 2) β-arrestin2 scaffolding of Dvl-2, and 3) induction of Rac1 signaling, which confers protection against thrombin-induced endothelial barrier permeability.[120,125]

β-arrestin1 forms two trimeric signaling complexes following ET_AR activation to induce β-catenin signaling pathways and promote cell invasion. First, β-arrestin activates Src, leading to EGFR transactivation, MAPK signaling and β-catenin stabilization via Tyr phosphorylation. Second, β-arrestin inhibits the "destruction complex" formed by GSK3β, Axin1, adenomatous polyposis coli (APC) and the E3 ligase β-transducin repeat containing protein (β-TrCP), preventing Ser/Thr phosphorylation and subsequent degradation of β-catenin.[91] By acting as a transcriptional regulator of endothelin-1-induced β-catenin signaling, β-arrestin1 promotes epithelial to mesenchymal transition (EMT), cancer invasion and metastasis.[126,127]

Hedgehog signaling—Hedgehog (Hh) signaling regulates stem cell maintenance, embryonic development and adult tissue integrity; dysregulation of this signaling is

associated with congenital birth defects and various types of cancers.[128] β-arrestin recruitment to Smoothened (Smo), a GPCR of the frizzled family is essential for Hh signaling and for the subsequent activation the Gli family of transcription factors in vertebrates.[129,130] In the absence of Hh, Smo activity is inhibited by the 12-pass transmembrane protein, Patched (Ptch). Binding of Hh to Ptch relieves Smo inhibition by provoking not only Ptch dissociation from Smo, but also Ptch internalization and degradation.[128] β-arrestin2 preferentially interacts with activated Smo in a GRK2-dependent manner, and promotes Smo endocytosis into clathrin-coated pits.[131] Activated Smo also translocates to the primary cilium, which promotes Gli-mediated transcription and upregulation of Hh-responsive genes.[132] Chemical agonists that directly bind to Smo such as SAG (Smoothened agonist) or 20(S)-hydroxycholesterol (20-OHC) provoke Smo accumulation in the cilium independently of Hh and Ptch.[133,134] β-arrestins translocate to the primary cilia and associate with KIF3A and with integrin-linked kinase (ILK) which are both linked to Smo ciliary translocation.[132,135] Both β-arrestin isoforms interact with a recently identified negative regulator of Smo, the orphan GPCR, GPR161, which represses Hh signaling by constitutively activating cAMP.[136,137] In a Smo, GRK2 and clathrin-dependent manner, β-arrestin binds to GPR161 and promotes GPR161 clearance from the cilium.[136]

NFκB and inflammatory signaling—The transcription factor nuclear factor kappa B (NF-κB) regulates the expression of target genes involved in immune and inflammatory responses, cell proliferation, and tumorigenesis. The lysophosphatidic acid receptor (LPAR) activates NF-κB via a G protein-mediated, PKCα-dependent mechanism that induces IκB kinase (IKK) phosphorylation, or via an alternative pathway in which β-arrestin2 promotes IKK activation by recruiting the adaptor protein CARD and MAGUK domain-containing protein 3 (CARMA3) (Figure 4C).[138] Upon LPAR activation, CARMA3 forms a complex with B-cell chronic lymphocytic leukemia-lymphoma 10 (Bcl10), mucosa-associated lymphoid tissue lymphoma translocation gene 1 (MALT1), and the E3 ligase tumor necrosis factor receptor-associated factor 6 (TRAF6) to form the CBM signalosome, which associates with IKK to ubiquitinate the regulatory subunit of the IKK complex called NF-κB essential modulator (NEMO).[138] NF-κB activation elicited by GPCRs such as $AT_{1a}R$, ET_1R, CXCR4 and PAR1 or by RTKs like EGFR and VEGFR2 requires CARMA3; however, NF-κB activated by cytokine receptors such as the tumor necrosis factor receptor (TNFR) is not dependent on CARMA3.[138] β-arrestin2, and not β-arrestin1, is required for LPAR and PAR1-mediated NF-κB signaling and CARMA3-containing CBM signalosome assembly, as it recruits CARMA3 to the activated GPCR complexes (Figure 4C).[138] On the other hand, both β-arrestin isoforms are known to interact with TRAF6, the NFκB inhibitor IκBα, and regulatory kinases such as IKKs α and β and NFκB-inducing kinase (NIK).[139–141]

Interestingly, β-arrestin isoforms reciprocally regulate NFκB signaling, inflammation and consequently atherosclerosis. For instance, β-arrestin2 promotes aortic smooth muscle cell (SMC) proliferation and migration whereas β-arrestin1 inhibits these processes.[142] Moreover, although β-arrestin1 inhibits NFκB signaling by preventing IκB degradation and TRAF6 ubiquitination downstream of TNFR and the Toll-like receptor/interleukin-1 receptor respectively, it promotes NFκB signaling in endothelin-1 stimulated epithelial

ovarian cancer (EOC) cells by forming a complex with the p65 subunit of NF-κB.[139,141,143] On the other hand, β_2AR stimulation inhibits NFκB signaling by promoting β-arrestin2 interaction with IκB.[140] β-arrestin2 effects on NF-κB signaling are closely associated with its ubiquitination status.[54] Ubiquitinated β-arrestin2 promotes NF-κB signaling in SMCs, whereas deubiquitinated β-arrestin2 attenuates NF-κB signaling by scaffolding the deubiquitinase USP20, that in turn deubiquitinates TRAF6, which blocks the NF-κB signaling cascade.[54] Although these effects of reversible β-arrestin ubiquitination are demonstrated for the Toll-like receptor pathway, these mechanisms may potentially regulate NFκB signaling downstream of GPCRs.

cAMP/Epac/CaMKII pathway and hypertrophic signaling—Both β-arrestins 1 and 2 are required for β_1AR-induced CaMKII activation (Figure 4D).[144] β-arrestin-mediated CaMKII activation by the β_1AR increases cardiac excitation-contraction coupling via phosphorylation of ryanodine receptors, phospholamban, cardiac troponin I and myosin-binding protein C and induces myocardial hypertrophy following pressure overload and chronic isoproterenol stimulation.[145–147] β-arrestins form a complex with CaMKII and the cAMP sensor protein exchange protein directly activated by cAMP 1 (Epac1), enabling their translocation to the plasma membrane upon β_1AR activation. CaMKII activation via Epac involves Rap–PLC-ε–PKC-ε signaling and is independent of cAMP-dependent protein kinase A (PKA).[144] Epac1 binding to cAMP activates the small GTPases of Ras superfamily Rap1 and Rap2. Epac1 also couples to H-Ras in a β-arrestin2 dependent manner, promoting H-Ras activation via Rap2B and subsequent hypertrophic signaling.[146] Interestingly, CaMKII is activated only by the β_1AR and not by the β_2AR, even though both βARs stimulate cAMP production. This specificity rests on the C terminal domain of the β_1AR and constitutive interaction of β-arrestin2 with PDE4D5.[144,146] PDE4D5 and Epac1 share common β-arrestin2 binding sites and compete for interaction with β-arrestin2. Disruption of PDE4D5–β-arrestin2 interaction allows the recruitment of Epac1 to β_2AR, causing a switch in β_2AR signaling from non-hypertrophic to pro-hypertrophic mode. β-arrestin2–induced CaMKII signaling also provokes the nuclear export of histone deacetylase 4 (HDAC4) which is critical for the development of cardiac hypertrophy.[146]

NON-CANONICAL SIGNALING VIA β-ARRESTINS

β-arrestin and G protein signaling at early endosomes

Paradoxically, β-arrestins can prolong G protein-mediated signaling instead of blocking it for a subset of GPCRs.[148,149] This newly identified function of β-arrestins appears to challenge their conventional role in inhibiting G protein coupling and in promoting receptor desensitization. However, because β-arrestin's binding with activated GPCRs causes dynamic changes in its structure, it is likely that certain conformations of β-arrestins may inhibit G protein coupling, while other conformations of β-arrestins may fail to uncouple G proteins from GPCRs.

Prolonged G protein activity and cAMP response have been observed in early endosomes for the following GPCRs: thyroid-stimulating hormone receptor (TSHR), parathyroid hormone receptor type 1 (PTH1R), and V_2R which couple with G_s and for the sphingosine-1-

phosphate receptor (S1PR) which couples to G_i.[149–153] The aforementioned GPCRs belong to the group termed 'class B', since they have equal affinity for either β-arrestin isoform and promote stable β-arrestin interaction and sustained ERK signaling in endosomes.[34,64] In contrast, the class A receptor β_2AR,[34] which prefers to bind β-arrestin2, forms transient complexes with β-arrestin2 and fails to induce cAMP signaling in endosomes unless the β_2AR is forced to have the carboxyl tail of V_2R (β_2V_2R chimera).[148] For the S1P receptor, sustained endosomal signaling after washout is ligand-specific and is triggered by FTY720-phosphate but not by the native ligand SIP.[153] Similar ligand dependence is observed for the V_2R since vasopressin, but not oxytocin induces β-arrestin recruitment to endosomes, as well as prolonged cAMP signaling in endosomes.[150] β-arrestins, by sequestering the activated receptors away from phosphodiesterases at the cell surface or by inhibiting PDE4 activity through ERK activation in endosomes, may promote sustained cAMP production.[154,155] Intriguingly, desensitization of vasopressin-mediated cAMP generation involves the retromer complex (VPS26/29/35) that regulates trafficking of internalized receptor from endosomes to the Golgi; of these retromer subunits, VPS26 crystal structures have shown a strong structural homology with β-arrestins.[156]

Super complexes, termed "megaplexes" consisting of the activated receptor, β-arrestin, Gα and Gβγ subunits have been characterized by cryo-electron microscopy (EM) and bioluminescence resonance energy transfer, suggesting that G protein and β-arrestin interactions with activated GPCRs are not mutually exclusive.[148] A ternary complex containing PTH1R, β-arrestin and the Gβγ dimer was observed in endosomes in response to PTH stimulation.[152] β-arrestin is known to embrace two different conformations when bound to GPCRs as observed in negative-stain EM study of the β_2V_2R chimera.[52,157] Of the two detected β-arrestin conformations, the "core" conformation involves tight interaction with the activated receptor via full integration of β-arrestin into the transmembrane core of the receptor while concurrently binding with the phosphorylated C-terminal tail of the receptor. The other conformation involves loose receptor interaction as β-arrestin "hangs" from the phosphorylated C-terminal tail of the activated receptor ("tail" conformation).[157] β-arrestin1 configuration in the megaplex of signaling endosomes assumes the 'tail' conformation of the receptor complex which perhaps enables concurrent interaction of the heterotrimeric G_s with the transmembrane core of the receptor for signal transduction.[148] The core interaction of β-arrestin in the β_2V_2R-β-arrestin complex hinders G protein coupling to the receptor but is also found to be expendable for agonist-induced receptor internalization as well as for β–arrestin binding and activation of ERK.[148,158] Thus, the tail conformation by simultaneously enabling G protein coupling to the internalized receptor-β–arrestin complex and β–arrestin association with ERK may allow concurrent cAMP production and ERK signaling in endosomes.

β-arrestin signaling distant from GPCRs

Intriguingly, sustained interaction with the activated receptor is not mandatory for β-arrestin-mediated signal transduction. When β-arrestin2 was forced to translocate to the plasma membrane, it triggered ERK activation even in the absence of GPCR activity.[159] β-arrestin2 was recently found to undergo receptor-specific conformational changes that outlast β-arrestin/receptor interaction and enable β-arrestin signaling activity after dissociation from

the activated receptor.[32] The receptor-specific activation/deactivation cycles of β-arrestins can optimize signaling by freeing the receptor to allow activation of additional β-arrestins and by permitting β-arrestin trafficking for activation of effectors located at distal sites from the receptor.[32] β-arrestin activation at distance was also demonstrated for β_1AR-induced signaling.[61] While both β_1AR and β_2AR form transient complexes with β-arrestin2 and trigger equal β-arrestin2 recruitment to clathrin-coated structures (CCSs), only β_2AR internalizes readily and accumulates in CCSs in a complex with β-arrestin. Although β_1AR does not traffic with β-arrestin, it promotes β-arrestin accumulation in the CCSs to elicit clathrin-dependent ERK activation, which persists in endosomes.[61]

β-arrestin-mediated transfer of functional GPCRs to exosomes

Exosomes are extracellular vesicles that play an important role as intercellular messengers, influencing the cellular activities of neighboring cells via the transfer of bioactive molecules such as lipids, proteins, RNAs and microRNAs.[160] Intriguingly, β-arrestin regulates the intercellular trafficking of functional GPCRs via exosomes. Mice subjected to cardiac pressure overload, release functional AT_1Rs into circulation and these receptor are packaged into exosomes in a β-arrestin2-dependent manner.[161] Although β-arrestin2 is not required for the intracellular maturation and exocytosis of exosomes, it is necessary for the sorting and packaging of the AT_1R and possibly other GPCRs into these vesicular bodies. The exosomes released from cardiomyocytes during cardiac pressure overload are transferred to recipient cells that include cardiomyocytes, skeletal myocytes, and mesenteric resistance vessels and could serve as reservoirs of functional AT_1Rs to preserve cardiovascular homeostasis.[161]

β-ARRESTIN-BIASED AGONISTS

The therapeutic potential of β-arrestin-dependent signaling has attracted tremendous research interests in academia as well as pharmaceutical companies and a number of ligands with bias for β-arrestin have been discovered and their biological properties described (TABLE 1). [27,29] The following sections include some of the well-studied GPCR examples for β-arrestin-biased agonism highlighting the biological effects and therapeutic potential of β-arrestin-biased signaling.

AT_1R

AT_1R and the renin-angiotensin-aldosterone system (RAAS) are targeted for renal and hypertensive vascular diseases because of their predominant role in stimulating vasoconstriction. Angiotensin receptor blockers (ARBs) are commonly used to alleviate hypertension, diabetic nephropathy, cardiac hypertrophy and congestive heart failure.

An AngII analog, SII ([Sar^1,Ile^4,Ile^8]Ang II) is a modified AngII peptide, which does not activate G_q-dependent calcium influx and IP3 production.[162] However, SII functions as a β-arrestin-biased agonist (Figure 2) and triggers AT_1R phosphorylation, β-arrestin recruitment as well as β-arrestin-mediated receptor internalization and signaling. Elegant phosphoproteomics studies have revealed that while AngII and SII provoke matching phosphorylation of common proteins, SII also triggers unique phosphorylation patterns

(based on phospho-peptide identification).[163,164] SII elicits GRK6 and β-arrestin2-dependent ERK activation in endosomes and promotes β-arrestin-regulated Akt activity and mTOR phosphorylation to stimulate protein synthesis.[80,165]

β-arrestin1-dependent signaling triggered by the $AT_{1a}R$ increases aldosterone production and also upregulates steroidogenic acute regulatory protein (StAR), which catalyzes mitochondrial cholesterol uptake in adrenocortical zona glomerulosa (AZG) cells.[166] β-arrestin1 overexpression or $AT_{1a}R$ stimulation by the β-arrestin-biased agonist SII increases circulating aldosterone levels. β-arrestin1-mediated aldosterone elevation worsens cardiac function after myocardial infarction and accelerates cardiac remodeling. Inhibition of adrenal β-arrestin1 is cardioprotective because it decreases circulating aldosterone levels and regresses remodeling of the infarcted heart.[167] Three novel AngII peptide analogs: CORML: [Met^5, Leu^8]-AngII, CORSML: [Sar^1, Met^5, Leu^8]-AngII, and CORET: [Sar^1, $Cys(Et)^5$, Leu^8]-AngII are more potent than SII at inducing β-arrestin coupling and provoking AT_1R-triggered aldosterone secretion.[168] CORET, the most potent and efficient out of the three, significantly increases aldosterone levels after post-myocardial infarction in mice. While the above β-arrestin1-dependent signaling triggered by elevated aldosterone leads to adverse cardiac remodeling downstream of the AT_1R, whether β-arrestin2 has a reciprocal effect through these biased ligands remains to be defined.

TRV023 (Sar-Arg-Val-Tyr-Lys-His-Pro-Ala-OH) and TRV027 (Sar-Arg-Val-Tyr-Ile-His-Pro-D-Ala-OH) are additional β-arrestin-biased agonists of AT_1R that have cardioprotective effects.[169] They bind AT_1R with higher affinity than SII and antagonize AngII-stimulated G-protein signaling. Binding of these peptide ligands to the $AT_{1a}R$ also promotes β-arrestin recruitment but with less efficiency than AngII. Nevertheless, TRV023 and TRV027 provoke receptor internalization more efficiently than AngII and promote ERK, Src, and endothelial nitric-oxide synthase phosphorylation in a β-arrestin-dependent manner.[170] These compounds have cardiovascular benefits of reducing blood pressure and promoting cardiac contraction. Thus, they provide an alternative for angiotensin converting enzyme (ACE) inhibitors and ARBs which, despite their vasodilative effects, are contraindicated in acute heart failure as they may induce hypotension and reduce cardiac output.[170]

Functional selectivity has recently been investigated in natural renin-angiotensin-aldosterone system peptides which include AngII, AngIII, AngIV, and Ang-(1–7).[171] Interestingly, Ang-(1–7) presents β-arrestin biased agonism and acts as a competitive antagonist for AngII as it fails to stimulate G protein signaling. Ang-(1–7) also negatively regulates vasoconstriction in vivo as its binding to the AT_1R attenuates phenylephrine-induced aorta contraction.

βAR

β_1AR is the dominant βAR in the heart, which promotes catecholamine-induced cardiac inotropy, lusitropy and chronotropy, and represents 75–80% of the total cardiac βARs. On the other hand, β_2AR expression contributes 20–25% of the total cardiac βARs in normal hearts.[172] In failing human hearts, β_1ARs are substantially downregulated, whereas β_2AR levels remain unaltered.[173,174] Chronic induction of G protein signaling via the β_1AR promotes harmful apoptotic signaling whereas β_2AR activation in failing hearts promotes cell survival.[175] On the other hand, β-arrestin isoforms may regulate cardiac function and

βAR-induced cardiac contractility differentially. While βarrestin1 seems to be mainly responsible for βAR desensitization, β-arrestin 2 is cardioprotective with beneficial effects on β_1AR-dependent contractility.[29,176,177]

βAR-signaling via β-arrestin-dependent mechanism is induced by carvedilol, a nonselective βAR antagonist that also blocks α_1-adrenergic receptors. Carvedilol is more effective than most traditional β-blockers in reducing mortality and morbidity of patients with congestive heart failure as it presents additional cardiovascular benefits associated with its anti-oxidative, anti-inflammatory and anti-proliferative properties.[178] While both carvedilol and the β-blocker propranolol act as inverse agonists for G_s-dependent adenylyl cyclase activation by the β_2AR, only carvedilol activates ERK signaling in a β-arrestin-dependent manner.[49,179] Carvedilol-induced effects are insensitive to pertussis toxin pretreatment and hence are independent of β_2AR coupling to G_i.[179,180] Carvedilol induces EGFR transactivation by the β_1AR in a GRK5/6 and β-arrestin-dependent manner.[89,181] β-arrestins recruit the tyrosine-protein kinase Src to carvedilol-activated β_1AR, promoting membrane-bound MMP activation, heparin-binding EGF (HB-EGF) cleavage and release into the extracellular space for EGFR binding, resulting in EGFR tyrosine phosphorylation, EGFR internalization and subsequent ERK signaling. β-arrestin-mediated β_1AR transactivation of EGFR is cardioprotective in mice, highlighting the therapeutic benefits of carvedilol.[89]

β_1AR activation by carvedilol also promotes microRNA (miR) maturation in a β-arrestin-dependent fashion.[182] miRs are small noncoding RNAs that regulate post-transcriptional gene expression bearing a variety of effects on cardiac physiology and function. Irregularities in their expression are associated with myocardial infarction, hypertrophy, and cardiac remodeling. As a result, miRs are emerging as biomarkers and therapeutic targets for cardiovascular diseases and heart failure.[183,184] Carvedilol upregulates a subset of mature and pre-miRs that includes miRs 125a-5p, 125b-5p, 150, 190, 199a-3p and 214.[182,185] Among them, miR-150 has cardio-protective effects and is down-regulated during acute myocardial infarction, atrial fibrillation, dilated cardiomyopathy, ischemic injury and heart failure.[185–190] miR-199a-3p and miR-214 are activated by carvedilol in cardiomyocytes subjected to ischemia/reperfusion and promote cardiomyocyte survival by activating p-Akt survival signaling, inducing the expression SRY (sex determining region Y)-box 2 (Sox2), a transcription factor that promotes pluripotency and also by repressing the apoptosis-associated genes ddit4 (DNA damage-inducible transcript 4) and ing4 (inhibitor of growth family member 4).[191] miR-190 upregulation by carvedilol occurs via the β_1AR and is independent of β_2AR and α_1AR activation. It also requires specific activated conformations of the receptor, β_1AR's phosphorylation by GRKs 5 or 6 and the recruitment of β-arrestin1 to the carvedilol bound-receptor complex. Ablation of β_1AR or knockdown of GRK 5, 6 or β-arrestin1 blocks the effects of carvedilol on miR-190 expression.[182] β-arrestin's effects on miR maturation are attributed to its ability to interact with enzymes that regulate miR processing such as Drosha and hnRNPA1.[182]

Recent studies have shown that pepducins, which are cell-permeable lipidated peptides representing the intracellular loop regions of GPCRs can trigger signaling by acting as agonists or allosteric modulators although in general they act as inhibitors of GPCR activity.[192,193] Of the various pepducins generated from the β_2AR intracellular loop

sequences, the pepducin called ICL1-9 surprisingly elicits β-arrestin-biased signaling via the β_2AR.[194] Like carvedilol, ICL1–9 promotes β_2AR phosphorylation by GRKs, β-arrestin recruitment, receptor internalization, ERK activation, and EGFR transactivation. Nevertheless, unlike carvedilol, ICL1–9 does not block the orthosteric-binding site of β_2AR and does not induce β_1AR signaling and trafficking. Moreover, ICL1–9 also provokes cardiomyocyte contraction mediated by β-arrestin1. The β-arrestin-bias and inotropic abilities of ICL1–9 offer a new therapeutic option for cardiac dysfunction.[194]

D_2R

Three β-arrestin-biased D_2R ligands designated as UNC9975, UNC0006 and UNC9994 were identified from a small library of compounds generated based on the chemical structure of aripiprazole, which is an FDA-approved antipsychotic drug.[195] Aripiprazole was originally defined as a partial D_2 selective agonist, but later studies showed that based on the assay platform utilized, it had a wide range of efficacy (partial agonist, full agonist and antagonist) as a D_2R ligand.[196] UNC9975, UNC0006 and UNC9994 antagonize D_2R/G_i coupling, but serve as partial agonists for D_2R/β-arrestin2 association.[195] Interestingly, when administered to mice, UNC9975 and UNC9994 displayed potent antipsychotic-like activity, which required β-arrestin2 expression. When mice lacked β-arrestin2, UNC9975 had little antipsychotic actions, but instead provoked catalepsy or motor dysfunction, that is generally observed as a side effect of antipsychotic drugs.[195] These studies convey that β-arrestin2-D_2R interactions provoke signaling that not only reduces psychotic responses in the brain, but also suppress motoric side effects of D_2R activation. Accordingly, β-arrestin-biased D_2R agonists could be valuable in the treatment of schizophrenia and related disorders.

PTH1R

Biased ligands have been characterized for the PTH1R, a GPCR mainly expressed in kidneys and bones. PTH1R stimulation activates G protein and β-arrestin-mediated signaling pathways to regulate calcium homeostasis and/or bone formation.[197–199] Binding of conventional, nonselective agonist PTH(1–34) to PTH1R triggers G protein activation, and also induces β-arrestin recruitment, resulting in receptor internalization and ERK signaling. On the other hand, β-arrestin-biased agonist, D-Trp(12), Tyr(34)-bPTH(7–34), antagonizes G protein coupling and triggers β-arrestin-mediated ERK activity and PTH1R internalization. These ligands also present distinct effects on bone formation. While both PTH(1–34) and PTH(7–34) stimulate anabolic bone formation in mice, only PTH(1–34) increases markers of bone resorption and promote hypercalcemia.[197] PTH(7–34)-triggered β-arrestin signaling, by its limiting effects on hypercalcemia, is a potential therapeutic target for the treatment of metabolic bone diseases like osteoporosis.

CONCLUSIONS

GPCRs are major therapeutic targets because their signal activation or inactivation affects almost all physiological pathways. β-arrestins are at the nexus of a variety of GPCR signal transduction modules and largely influence the signaling activities, vesicular trafficking and fate of GPCRs by scaffolding a wide range of proteins to the activated receptor complex. It has become evident that β-arrestin molecules embrace different activated conformations

upon association with the activated GPCR.[30,32] These conformations of β-arrestins are dictated by ligand-triggered "barcoding" of the receptor tail by GRKs or by agonist-induced posttranslational modifications of β-arrestins. Biased agonists present an unmatched opportunity to selectively target one mode of signaling (via a G protein or β-arrestin); however, such compounds can have unique pharmacological and biological profiles than those observed when the particular signaling mode is stimulated by a 'balanced' agonist. Therefore, in order to fully harness the potential benefits of biased agonism, it becomes critical to chart the signal transduction network(s) induced by the biased agonist in a cell-specific manner and further uncover the molecular mechanisms that regulate (or desensitize) biased signaling.

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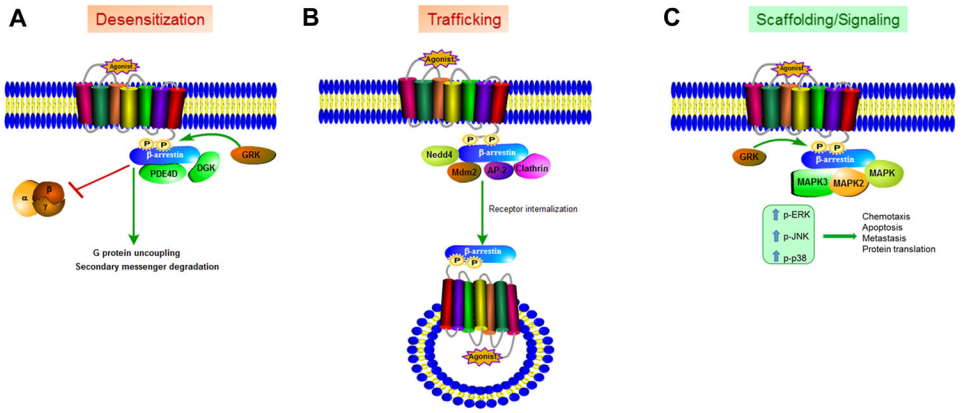

Figure 1. β-arrestins act as multifunctional adaptors for activated GPCRs

(**A**) Agonist-occupied GPCRs are rapidly phosphorylated by specific GPCR kinases (GRKs) on target serine(s) and threonine(s) in the intracellular domain(s) of GPCRs. The pattern(s) of phosphorylation dictates the stability and duration of β-arrestin-GPCR association. β-arrestin-binding interdicts G protein coupling and blocks G protein-mediated signaling. This results in desensitization of GPCR signaling. β-arrestins scaffold enzymes namely, phosphodiesterase (PDE) and diacylglycerol kinase (DGK) that degrade second messengers generated by G protein activity. This process boosts the efficiency and fidelity of β-arrestin-mediated desensitization. (**B**) β-arrestin undergoes conformational rearrangement(s) upon its association with GPCRs; so activated β-arrestin interacts with adaptors of the endocytic machinery as well as with specific E3 ubiquitin ligases that ubiquitinate either β-arrestin itself (Mdm2) or ubiquitinate the GPCR (Nedd4). These and other protein interactions (not shown, see text and cited references) promote specific trafficking itineraries of internalized GPCRs. (**C**) Activated β-arrestin scaffolds multiple components of a kinase cascade such as the MAPKKK, MAPK thus propagating specific linear pathways of signaling. During this process, β-arrestin-GPCR complex can be localized to endosomes along with the MAPK scaffold allowing compartmentalization of signaling.

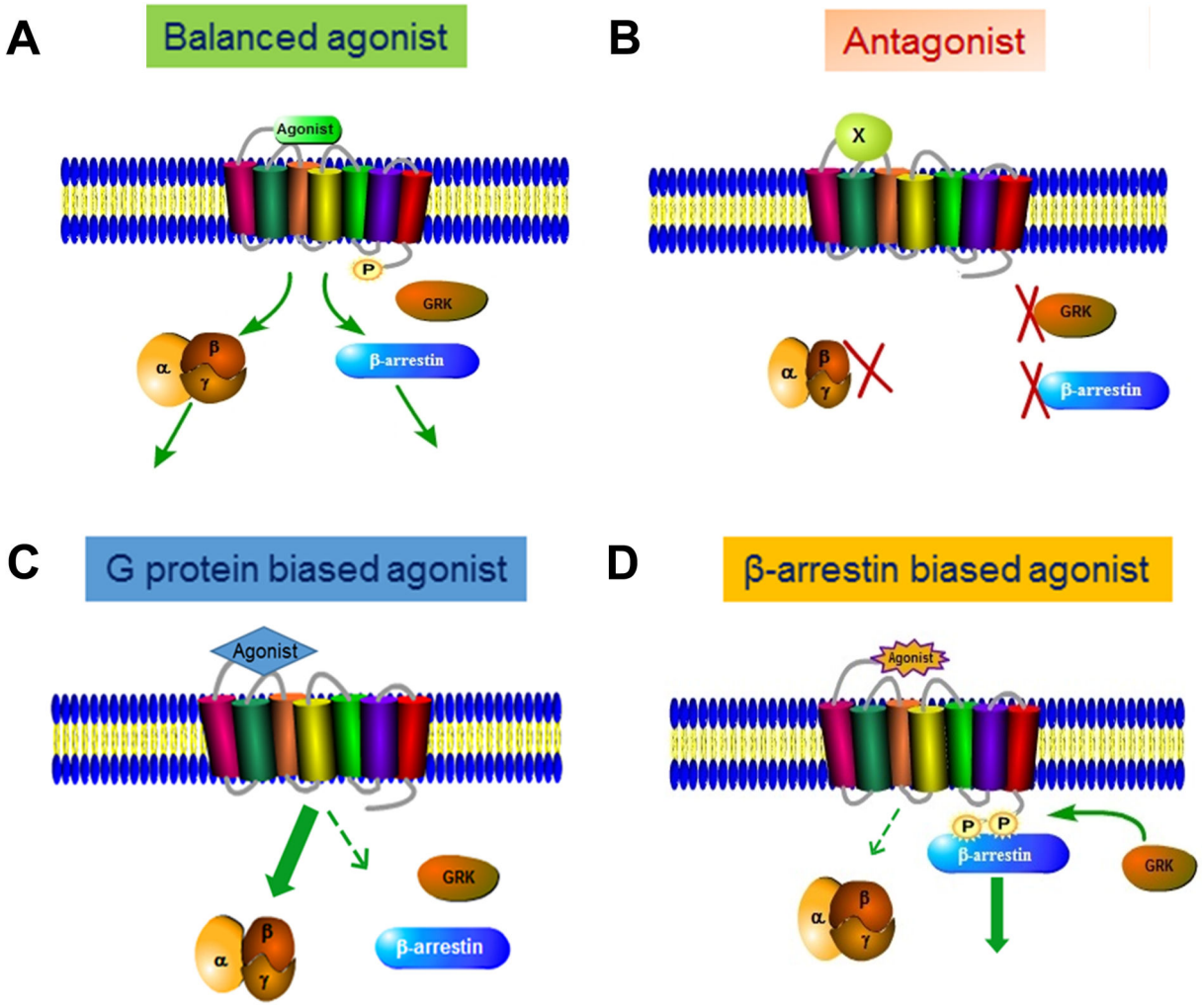

Figure 2. GPCR ligands and their effects

(**A**) Balanced agonist stabilizes receptor conformation(s) that induces both G-protein and β-arrestin-dependent signaling with variable efficacies and kinetic displacement. (**B**) Non-selective antagonist locks the receptor in an inactive state, and neither G protein nor β-arrestins promote signaling. (**C** and **D**) Biased agonists promote an active conformation of the receptor that selects either G-proteins or β-arrestins as the transducer for signaling, potentiating (thick arrow) certain signaling events while attenuating (thin arrow) others.

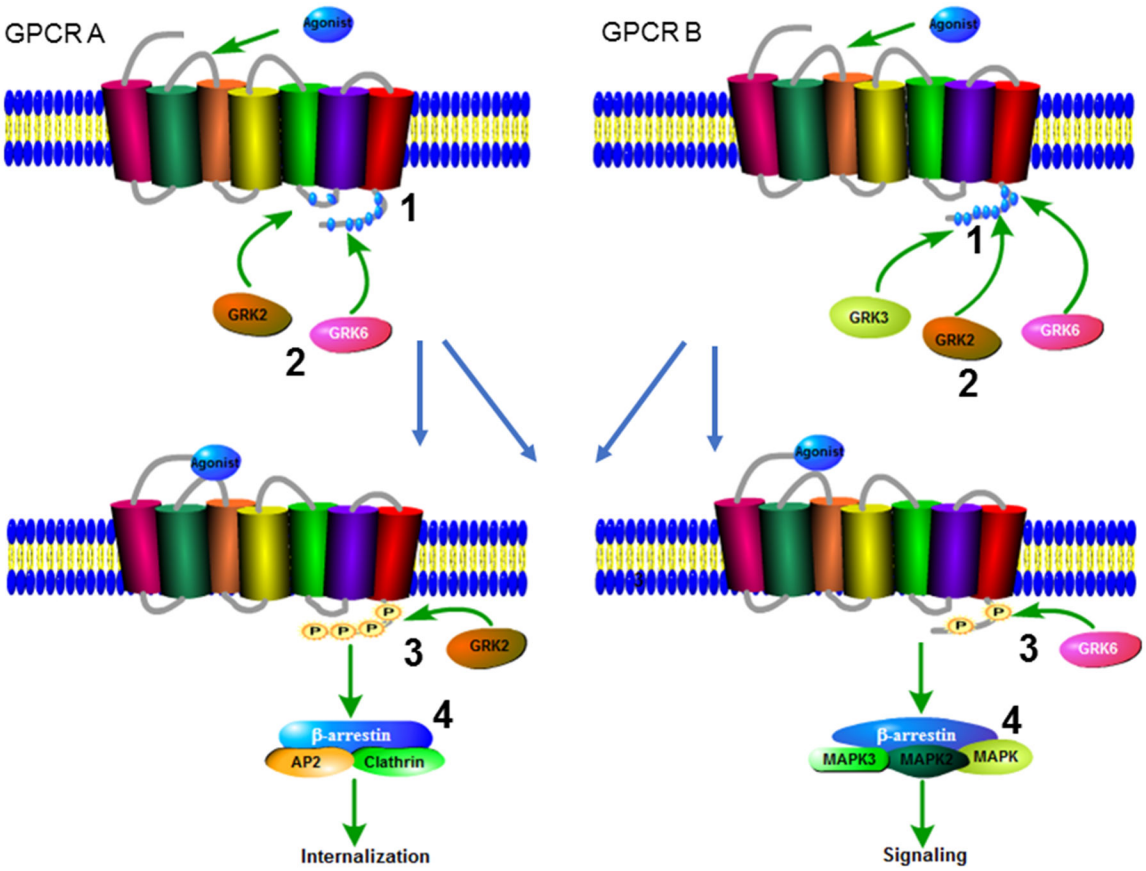

Figure 3. GRK phosphorylation and barcoding of GPCRs

(1) Individual GPCRs possess unique phosphorylation sites that are targets for GRK phosphorylation. (2) Each GRK phosphorylates specific sites in a GPCR. Expression and activity of each GRK isoform is cell and tissue-specific. Recruitment of a GRK isoform to activated GPCR is ligand-specific. (3) When a GPCR is phosphorylated on particular sites a specific phosphorylation code emerges. (4) This barcode is sensed by the recruited β-arrestin, resulting in specific conformational orientations of β-arrestins. Additional conformational signals are appended by other post-translational modifications such as ubiquitination. The activated β-arrestin then propagates specific signals downstream of a GPCR.

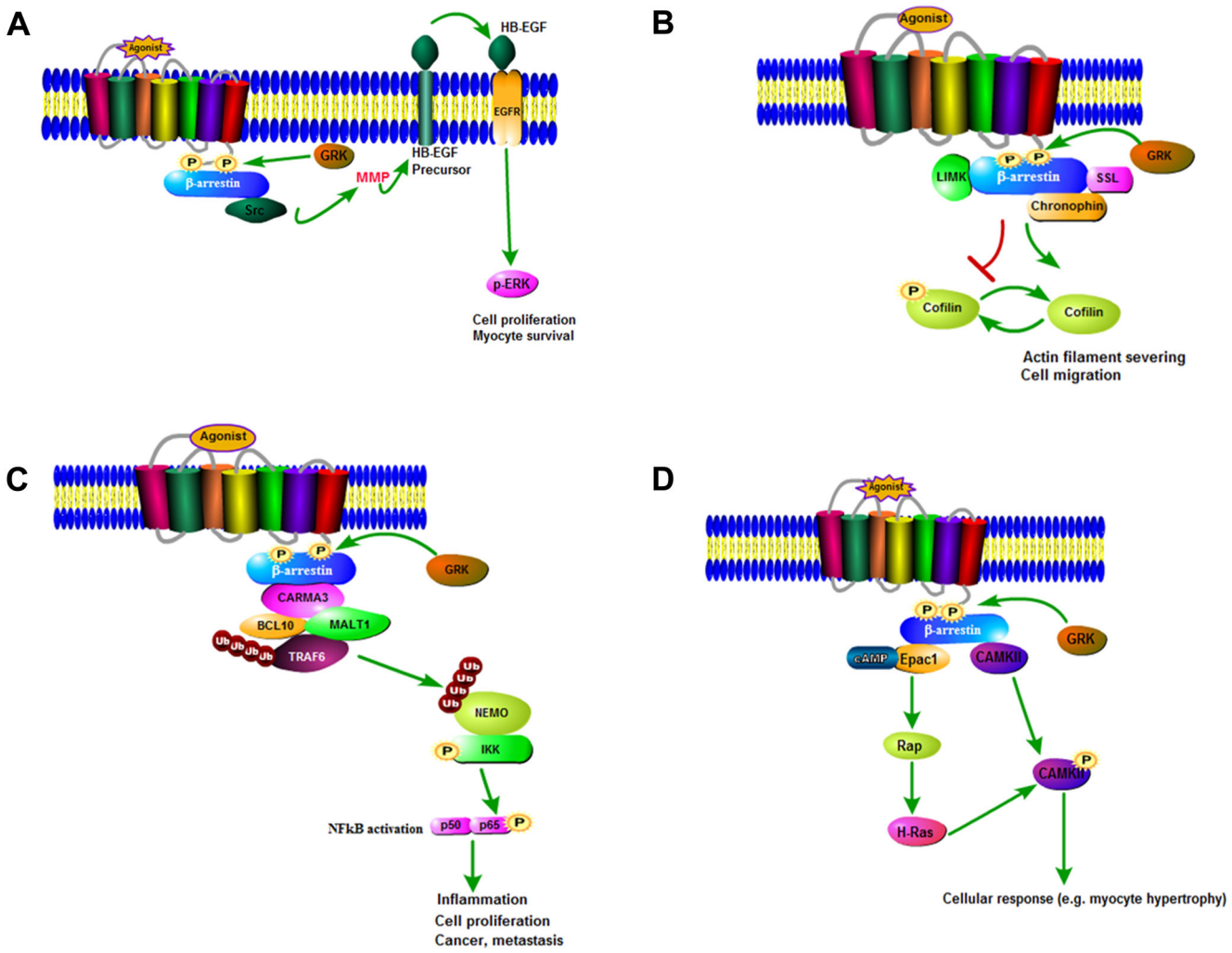

Figure 4. Examples of β-arrestin-dependent signaling mechanisms

(**A**) Agonist-stimulated GPCR when phosphorylated by GRK5/6 recruits β-arrestin and bound active c-Src, which activates matrix metalloproteases (MMP) engendering cleavage and shedding of HB-EGF, which in turn binds and activates EGFR and promotes its signal transduction. (**B**) By binding to LIMK, β-arrestin blocks cofilin phosphorylation by LIMK. By scaffolding phosphatases such as chronophin or slingshot β-arrestin promotes cofilin dephosphorylation. This facilitates cofilin association with actin, and actin filament severing at the leading edge of a migrating cell. (**C**) β-arrestin2 recruits CARMA3 to activated GPCRs, enabling the CBM signalosome to ubiquitinate the NEMO subunit of IKK via TRAF6 which leads to NFκB activation. (**D**) β-arrestin forms a complex with CaMKII and exchange protein directly activated by cAMP 1 (Epac1), enabling their translocation to the plasma membrane upon activation of β_1ARs. Epac1 binding to cAMP activates the small GTPases of Ras superfamily Rap1 and Rap2 and thence CAMKII activation.

TABLE 1

LIST OF β-ARRESTIN-BIASED LIGANDS

GPCR	LIGAND	EFFECT	THERAPEUTIC AREA	References
α_2-Adrenergic Receptor (α_2AR)	Desipramine	Blockade of vasoconstriction via α_2AR down-regulation	Hypertension	200
Angiotensin Receptor (AT_1R)	Sar1,Ile4,Ile8-AngII (SII)	β-arrestin2-dependent signaling and protein synthesis. Increased circulating aldosterone levels via β-arrestin1	Heart Failure	162
	Sar1,Gly4,Gly8- AngII (SGG)	β-arrestin2-dependent signaling		201
	TRV023,TRV027	Reduction of blood pressure and increase in cardiac contraction	Hypertension & Acute heart failure	169
	Ang-(1–7)	Attenuation of phenylephrine-induced aorta contraction	Hypertension	171
	CORML: [Met5, Leu8]-AngII, CORSML: [Sar1, Met5, Leu8]-AngII, and CORET: [Sar1, Cys(Et)5, Leu8]-AngII	Post-MI hyperaldosteronism	Heart Failure	168
β-Adrenergic Receptors (βARs)	Carvedilol	Cardioprotection, anti-oxidative, anti-inflammatory and anti-proliferative, anti- apoptotic.	Congestive Heart Failure Hypertension	179,181,202
	Nebivolol	Vasodilation by inducing nitric oxide (NO) production	Hypertension	203
β_2-Adrenergic Receptors (β_2AR)	ICL1–9 (pepducin)	Cardiomyocyte contraction	Congestive Heart Failure	194
CXCR3 (Chemokine Receptor)	FAUC1036	Weak chemotaxis, β-arrestin2 recruitment and receptor internalization without G protein activation	Rheumatoid Arthritis, Multiple Sclerosis, Psoriasis, Cancer	204,205
Dopamine D_2 Receptor (D_2R)	UNC9975, UNC0006, and UNC9994	Reduction of psychotic responses in the brain	Schizophrenia	195
Free Fatty Acid Receptor 1 FFAR1/GPR40	TAK-875	Increased glucose-stimulated insulin secretion	Type 2 Diabetes	206
Histamine H4 Receptor	1-[(5-chloro-1H-indol-2-yl)carbonyl]-4-methyl-piperazine (JNJ7777120)	Unknown	Chronic inflammatory diseases	207
Metabotropic Glutamate 1a (mGlu1aR)	Glutaric acid; Succinic acid	Cytoprotective signaling	Schizophrenia Melanoma	208
Neurotensin 1 Receptor NTR1	2-cyclopropyl-6,7-dimethoxy-4-(4-(2-methoxyphenyl)- piperazin-1-yl)quinazoline, 32(ML314)	Attenuation of methamphetamine-induced hyper-locomotion, and associated conditioned place preference.	Drug abuse, addiction	209,210
κ-Opioid Receptor (KOR)	GR89696, ICI 199,441	Sedation, dysphoria, hallucination	Psychotic disorders	211
Protease Activated Receptor 1 (PAR1)	Anticoagulant protease-activated protein C (APC)	Endothelial barrier protection	Thrombosis, Wound healing	120,121,125
Parathyroid Hormone Receptor (PTH1R)	[d-Trp12,Tyr34]-bPTH (7–34)	Stimulate anabolic bone formation with limited bone resorption	Osteoporosis	197