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Therapeutic potential of β -arrestin- and G protein-biased agonists

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Abstract

Members of the seven-transmembrane receptor (7TMR), or G protein-coupled receptor (GPCR), superfamily represent some of the most successful targets of modern drug therapy, with proven efficacy in the treatment of a broad range of human conditions and disease processes. It is now appreciated that β -arrestins, once viewed simply as negative regulators of traditional 7TMR-stimulated G protein signaling, act as multifunctional adapter proteins that regulate 7TMR desensitization and trafficking and promote distinct intracellular signals in their own right. Moreover, several 7TMR biased agonists, which selectively activate these divergent signaling pathways, have been identified. Here we highlight the diversity of G protein- and β -arrestin-mediated functions and the therapeutic potential of selective targeting of these in disease states.

Signaling by seven transmembrane receptors (7TMRs)

7TMRs represent the largest single family of cell surface receptors [1]. Members of this receptor superfamily are uniquely expressed throughout the body and are activated by a diverse host of ligands, including biogenic amines, hormones, peptides, proteins, growth factors, lipids, nucleic acids, odorants, tastants, protons (H^+), ions (Ca^{2+}) and light (photons) [1]. There are also several 7TMRs for which no clear ligand has yet been identified, the so-called orphan receptors [2]. The combination of ligand diversity and unique tissue expression makes 7TMRs attractive drug targets, with approximately 40% of modern drug therapy targeting 7TMRs, either directly or indirectly [3]. These include blockbuster drugs such as opiates, antihistamines, α - and β -blockers, β -agonists, dopamine receptor blockers, angiotensin receptor blockers, angiotensin-converting enzyme inhibitors and selective serotonin reuptake inhibitors.

Drugs that directly target a 7TMR have been classically described as either agonists or antagonists for G protein signaling. The binding of an agonist to a 7TMR promotes a conformational change that results in the activation of receptor-associated heterotrimeric G proteins and consequent downstream signaling (Figure 1a). In addition to G protein activation, agonist binding promotes a process known as desensitization, which involves rapid phosphorylation of the receptor at the C terminus and intracellular loops, in large part by the G protein-coupled receptor kinases (GRKs). This combination of conformational change and phosphorylation dramatically increases the affinity of the receptor for a small family of multifunctional 7TMR regulatory or adaptor proteins known as the β -arrestins (β -

arrestin1 and β -arrestin2). This association blocks subsequent G protein activation and plays an almost universal role in facilitating traditional 7TMR desensitization (Figure 1b). This process is complemented by the association of β -arrestins with second-messenger-metabolizing or -converting enzymes and components of the internalization machinery, which target the degradation of G protein-mediated signals and facilitate the removal of receptors from the cell surface (Figure 1c).

Classically, agonists have been described as having linear efficacy [4], in which activation of the multiple signaling pathways downstream of a receptor (e.g. G protein signaling, receptor phosphorylation, β -arrestin recruitment and internalization) correlates with the degree of agonist-mediated receptor activation from partial to full (Figure 2a). In this scheme, antagonists have been described almost exclusively by their ability to block agonist-stimulated G protein activation. This view, in which agonists and antagonists can be defined purely in the context of a single form of signaling (most commonly G protein-mediated signaling), has guided the vast majority of modern 7TMR-based drug discovery (Figure 2a,b).

However, it has recently been appreciated that in addition to regulating receptor-stimulated G protein signaling, the β -arrestins are also capable of initiating distinct signals in their own right (Figure 1b). These signals are often both spatially and temporally distinct, and result in unique cellular and physiological or pathophysiological consequences (see [5–7] for a detailed review of β -arrestin signaling). With the discovery of β -arrestin-mediated signaling has come a new appreciation of biased agonism [4,8–10]. Biased agonism is best understood in a model in which different 7TMR active conformations are either competent for the full range of receptor activities or only for a restricted subset of them. Whereas balanced ligands stabilize the conformations that are competent for signaling to all downstream pathways, biased ligands stabilize only those conformations that are capable of promoting a subset of signaling effects [4,8–10]. For example, ligands can show bias for either G protein- (G protein-biased) or β -arrestin-mediated (β -arrestin-biased) signaling (Figure 2c,d). Such bias clearly adds a layer of texture [11] to the definition of ligand action. Using this framework, we could hypothesize that such ligands could more selectively target beneficial signaling and even block or negate detrimental or unwanted actions of receptor activation (e.g. side effects, toxicity or tolerance). Indeed, over the last decade a diversity of biased ligands for 7TMRs have been identified that selectively activate G proteins or β -arrestins (Table 1), and several of these seem to have distinct functional consequences when compared to traditional ligands with more linear efficacy [9]. Realization of the complexity of 7TMR signaling impacts both the way we describe 7TMR functions and how we apply these definitions to future 7TMR-based discovery and drug therapy.

As described above, the β -arrestins are involved in multiple aspects of 7TMR-mediated signaling and regulation, and ligand bias has been described for numerous receptor subtypes (Table 1). Moreover, the β -arrestins have been implicated in numerous aspects of physiology and the pathophysiology of disease [6,7]. In the following sections, we highlight several examples for which targeting of selected β -arrestin-mediated activities might have or actually has therapeutic benefit (Table 2). Although these different activities might be linked functionally, for the purposes of discussion we broadly split the different roles of β -arrestin into desensitization of G protein signaling, receptor internalization or trafficking, and β -arrestin-mediated signaling.

G protein signaling and desensitization

Treatment with 7TMR agonists can be limited by the development of tachyphylaxis, a decrease in responsiveness to a drug with repeated dosing, and tolerance, whereby a higher

drug dose is required with repeated doses to obtain the same effect. Both processes, which limit the utility of therapeutics, are largely thought to be regulated by β -arrestin-dependent receptor desensitization and downregulation. The design of G protein-biased ligands, which avoid β -arrestin activation and the associated negative consequences, represents a novel strategy for the development of improved agonist therapies with more sustained efficacy.

The β_1 adrenergic receptor in heart failure

The β -arrestins are involved in a variety of cardiovascular processes, with a significant body of work focused on the prototypic β -adrenergic receptors (β -ARs). Some of the earliest work in this area involved β -arrestin1 knockout (KO) mice, which were more sensitive to the cardiac stimulatory effects of β -agonist when compared to wild-type (WT) mice, which strongly suggests the involvement of β -arrestin1 in cardiac β -AR desensitization [12]. Clinically, the β -agonist dobutamine is often used to provide inotropic support in patients with severe heart failure, but is associated with the development of tachyphylaxis. A G protein-biased ligand that limits β -arrestin-mediated desensitization and downregulation could provide a more consistent and efficacious therapeutic option.

The β_2 adrenergic receptor in obstructive lung disease

In the pulmonary system, the β_2 -AR plays a major role in regulating airway smooth-muscle-cell contraction and bronchial tone. One major problem in the treatment of asthma is tachyphylaxis to β -agonist-stimulated bronchodilation, whereby repeated doses of either short- or long-acting agonists result in decreased bronchodilation [13]. This decreased responsiveness can lead patients to increase their use of β -agonists, thus increasing the health risks associated with elevated sympathetic activation and decreasing the effectiveness of rescue inhaler use during an asthma attack, and increasing the risk of death from such attacks [14]. β_2 -AR tachyphylaxis is also thought to result from increased receptor desensitization and down-regulation after agonist stimulation, an effect thought to be mediated by β -arrestins. β -Arrestin2 regulates receptor desensitization by sterically preventing the interaction between the receptor and G protein [15], by internalizing receptor [16], and by actively recruiting phosphodiesterases to degrade the cAMP generated by G protein signaling [17]. Studies have shown that the airway relaxation stimulated by the β -agonist albuterol is augmented in β -arrestin2 KO mice compared to WT controls [18]. Moreover, comparable results were obtained in transgenic mice expressing a mutant β_2 -AR deficient in GRK phosphorylation sites; thus, this mutant β_2 -AR is probably resistant to β -arrestin-mediated regulation [19]. These data therefore predict that a G protein-biased ligand that does not promote β -arrestin recruitment would result in less tachyphylaxis and would be a more effective therapeutic agent.

The μ opioid receptor (OR) and analgesia

The influence of β -arrestins on opioid analgesia and tolerance has been the subject of several studies [20–22]. β -Arrestin2 KO mice experienced enhanced and prolonged morphine-induced analgesia [22] (effects not observed in β -arrestin1 KO mice [20]), as well as increased hypothermic responses compared to WT mice [22]. The β -arrestin2 KO mice also showed greater and more efficient central OR G protein coupling [22]. Interestingly, in addition to morphine, heroin also produced augmented analgesic efficacy in the β -arrestin2 KO mice; however, other μ -OR agonists, including etorphine, fentanyl and methadone, did not [20]. Together, these studies show that the analgesic efficacy of μ -OR agonists can be differentially regulated by β -arrestin2 *in vivo*. Consistent with these findings, β -arrestin2 KO mice were also resistant to the development of chronic morphine-induced tolerance, which correlated with preserved central OR G protein coupling [21]. Subsequent studies showed comparable results when β -arrestin2 was knocked down *in vivo* with either antisense [23] or small interfering (si)-RNA [24]. Conversely, adenoviral overexpression of

β -arrestin2 in the brain attenuated morphine analgesia as evaluated by measuring response latencies in the hot-plate test [25]. These findings clearly show that β -arrestin2 is involved in regulating both morphine-stimulated OR desensitization and tolerance *in vivo*, and strongly suggest that a G protein-biased ligand produces enhanced and prolonged morphine-induced analgesia with reduced tolerance.

β -Arrestins and 7TMR internalization and trafficking

It has long been known that β -arrestins regulate the internalization and trafficking of 7TMRs through a variety of mechanisms. β -Arrestins interact with AP-2, an adapter protein for clathrin, thereby regulating receptor endocytosis into clathrin-coated pits [26,27]. Ubiquitination of β -arrestins regulates 7TMR trafficking: transient β -arrestin ubiquitination results in rapid recycling of the receptor, whereas stably ubiquitinated β -arrestin targets the receptor to endosomes [28,29]. More broadly, β -arrestins act as adapters for several E3 ligases that catalyze ubiquitination, such as MDM2 [30], which ubiquitinates β -arrestin2; and other ligases such as NEDD4 [31] and AIP4 [32], which ubiquitinate 7TMRs and regulate their downregulation. The β -arrestins also interact with deubiquitinating enzymes such as the ubiquitin specific protease USP33 [29,33], thus providing a mechanism for the regulation of the β -arrestin–7TMR interaction [33]. Not surprisingly, there are several examples of disease states associated with alterations in receptor trafficking that might benefit from therapies that modulate β -arrestin-mediated functions.

The V2 vasopressin receptor in kidney function

Over 150 mutations of the V2 vasopressin receptor (V2R) have been identified that result in congenital nephrogenic diabetes insipidus (cNDI), a condition in which the kidney is unable to properly reabsorb water because the V2R does not respond appropriately to vasopressin [34]. Most of these mutations result in intracellular retention of a misfolded V2R, which limits its expression on the cell membrane and therefore the cell response to vasopressin. Mutation of one of the critical residues in the highly conserved DRY motif, R137H, results in a receptor that constitutively associates with β -arrestins in intracellular vesicles even in the absence of agonist [34], and is associated with constitutive receptor desensitization [35]. This mutant receptor can be rescued by a cell-permeable V2R antagonist that acts as a pharmacological chaperone and restores the ability of the receptor to fold [36]. However, the constitutive β -arrestin association of R137H is not affected by this chaperone, even though patients with this mutation showed a clinical response to this therapy. Surprisingly, chaperones that are capable of rescuing other V2R mutants restore cAMP signaling in the absence of β -arrestin recruitment or receptor internalization, which suggests a role for G protein-biased agonists in the treatment of cNDI [37].

Conversely, the nephrogenic syndrome of inappropriate antidiuresis, a disorder associated with inappropriate retention of water, results from constitutive activity of the V2R [38]. It is caused by specific mutations of the V2R at R137 (R137C and R137L), the same residue that is mutated in cNDI [38]. Both R137C and R137L V2R mutants interact with β -arrestins in an agonist-independent manner but traffic considerably more efficiently to the plasma membrane than R137H [38], which suggests that receptor expression at the membrane is required for receptor function. As with cNDI, these findings suggest that ligands that restore V2R trafficking might have therapeutic utility in the treatment of the nephrogenic syndrome of inappropriate antidiuresis.

CXCR4 in autoimmune disease

A cytoplasmic tail truncation mutation in the chemokine receptor CXCR4 is found in the warts, hypogammaglobulinemia, infections, myelokathexis (WHIM) syndrome, and

leukocytes from patients with this disease display defective CXCR4 desensitization and enhanced chemotaxis [39]. The agonist-induced receptor internalization of CXCR4 is regulated specifically by GRK3 and β -arrestin2 and is defective in WHIM syndrome, whereas the chemotaxis of WHIM mutant leukocytes requires β -arrestin2 signaling, which suggests that the truncated receptor is capable of regulating β -arrestin-mediated signaling but not β -arrestin-mediated internalization or endocytosis [39].

CCR5 in HIV

HIV requires cell-surface coreceptors, either the CCR5 or CXCR4 chemokine receptor, to attach and gain entry to target cells. CCR5-tropic viruses are the predominant species in the early stages of infection, and patients with a homozygous truncation mutation of CCR5 (CCR5- Δ 32) are resistant to HIV [40]. Accordingly there has been significant interest in targeting this receptor in the treatment of HIV infection [41]. A modified CCR5 ligand, aminooxypentane-RANTES (AOP-RANTES), promotes receptor internalization similar to endogenous RANTES, but unlike RANTES, AOP-RANTES does not allow the internalized CCR5 to recycle [42,43]. More highly potent derivatives of AOP-RANTES seem to be effective in preventing HIV infection of peripheral blood mononuclear cells [44]. The β -arrestins regulate this CCR5 trafficking, recycling and degradation [45], and these ligands probably regulate CCR5 trafficking via changes in β -arrestin activity. Thus, use of a ligand that modifies β -arrestin-regulated CCR5 trafficking might represent an attractive therapy in the treatment of HIV.

β -Arrestin-mediated signaling

It has now been over a decade since the first reports of β -arrestin-mediated signaling were published [46–48]. β -Arrestin-mediated signaling encompasses a diverse range of pathways [5,49–51], including kinase activation, transcriptional regulation and receptor transactivation. The best-characterized of these responses is the regulation of protein kinases, such as members of the mitogen-activated protein (MAP) and Src kinase families [5]. The list of physiologic responses that are regulated by β -arrestins continues to grow (Table 2), and drugs that selectively target β -arrestin-mediated signaling are currently being developed as therapeutic agents.

The β 1-AR in heart failure

Recent studies have shown that cardiac β 1-ARs can stimulate β -arrestin1- and -2-dependent signaling in the heart that results in transactivation of the epidermal growth factor receptor (EGFR), which is cardioprotective [52]. In addition, it is thought that chronic β -AR activation is cardiotoxic [53–55] and that this in large part involves G_s signaling [55,56]. These combined observations suggest that a β -arrestin-biased ligand acting as a classical antagonist of cardiotoxic G protein signaling, while engaging cardioprotective β -arrestin signaling, could be therapeutically beneficial. Moreover, β -arrestin-biased ligands for both the β 1-AR [57] and β 2-AR [58,59] have recently been identified, with the β -blocker carvedilol a β -arrestin-biased ligand of both receptor subtypes [57,58]. Whether β -arrestin-mediated signaling plays a role in the cardioprotection associated with such compounds remains to be determined.

The angiotensin II type 1A receptor (AT1R) in cardiovascular disease

In addition to the β -ARs, AT1R-stimulated β -arrestin signaling has been the subject of several cardiovascular studies. In the heart and in isolated cardiomyocytes, AT1R-stimulated β -arrestin-dependent and Gprotein-independent signaling promotes growth and hypertrophy [60], myocyte proliferation [61,62] and increased myocyte contractility [63,64]. Moreover, the newly identified β -arrestin-biased ligand Sar¹,D-Ala⁸ angiotensin II (TRV120027)

reduces mean arterial pressure, increases cardiac performance and preserves stroke volume in the anesthetized rat, whereas unbiased antagonists reduce cardiac performance [64]. In vascular smooth muscle cells, β -arrestin signaling increases protein synthesis [65] and is antiapoptotic [66]. Furthermore, cardiovascular function is in large part influenced by changes in body fluid homeostasis and salt balance, and AT1R-stimulated β -arrestin-associated signaling is involved in several related processes. For example, AT1R-stimulated β -arrestin1 signaling promotes adrenocortical aldosterone secretion both *in vitro* (cultured human adrenocortical carcinoma cells, H295) and *in vivo* (adrenal-targeted adenoviral β arr1 overexpression in rats) [67]. Furthermore, central AT1R activation is associated with increased thirst and salt appetite [68]. Interestingly, the β -arrestin-biased AT1R ligand Sar¹,Ile⁴,Ile⁸ angiotensin II (SII) [69] when injected directly into the brain stimulates central MAP kinase activation and salt intake, while blocking G protein-associated increases in central inositol triphosphate production and water intake [70]. Thus, AT1R-stimulated salt appetite would seem to be a β -arrestin-dependent process, at least in part. Overall, it seems that AT1R can stimulate a variety of β -arrestin-mediated cellular signals and associated processes involved in the maintenance of cardiovascular homeostasis.

GPR109A and the regulation of lipid homeostasis

A unique role for β -arrestin1-mediated signaling has recently been identified that involves the niacin receptor, GPR109A. Niacin is one of the most effective therapies for increasing HDL-cholesterol and decreasing triglycerides in the treatment of dyslipidemia [71,72]. However, the therapeutic utility of niacin is limited by the rather unpleasant side effect of cutaneous flushing, which significantly reduces patient compliance [71,72]. Recent studies have shown that the niacin-induced flushing response is dramatically reduced in β -arrestin1 KO mice, whereas the beneficial lipolytic effects remain intact [73]. This supports the hypothesis that a G protein-biased ligand would maintain beneficial effects on plasma lipids in the absence of β -arrestin-mediated flushing. Interestingly, GPR109A agonists have been identified that exhibit antilipolytic actions with significantly reduced cutaneous flushing [74–78]. Some of these compounds also show reduced receptor internalization and extracellular signal-regulated kinase (ERK) activation [74], which could result from non-engagement of β -arrestin.

The parathyroid hormone receptor and bone mineral homeostasis

Bone mineral homeostasis requires a complex process of renewal involving a continuous cycle of bone formation and resorption. The parathyroid hormone (PTH) receptor plays an important role in regulating these processes, with recombinant human PTH 1–34 (Forteo®) currently approved for the treatment of osteoporosis. Previous studies have shown that β -arrestin2 plays a role in regulating PTH-receptor-mediated signaling [79–84], with effects on bone formation and resorption [85,86], as well as related gene expression [87,88]. Furthermore, studies have shown that the PTH receptor can stimulate both G protein- and β -arrestin-mediated signals, and that these signals can be selectively engaged by both G protein- and β -arrestin-biased ligands [89,90]. Most recently, it has been shown that the β -arrestin-biased ligand (D-Trp¹²,Tyr³⁴)-PTH(7–34), which simultaneously stimulates receptor-mediated β -arrestin signaling and blocks G protein signaling, promotes anabolic bone formation in the absence of bone resorption, and that this activity is abolished in β -arrestin2 KO mice [91]. These studies have thus identified a novel β -arrestin-mediated pathway and a unique β -arrestin-biased ligand that positively affects anabolic bone formation. Targeting of this newly identified mechanism of action could represent a novel therapeutic strategy for the treatment of osteoporosis.

Opioid side effects

Opioid therapy is associated with several adverse side effects, including respiratory suppression, constipation, and the development of tolerance and physical dependence. β -Arrestin2 is responsible for desensitization of the receptor after chronic morphine treatment, and β -arrestin2 KO mice are protected from the development of tolerance, the requirement for increasing doses of opioids to maintain the same antinociceptive effect [21]. Studies have shown that β -arrestin2 KO mice are also resistant to morphine-induced respiratory suppression when compared to WT mice [92]. In addition, β -arrestin2 KO mice were less sensitive to some of the adverse gastrointestinal effects of μ -OR agonists. Specifically, β -arrestin2 KO mice were resistant to the effects of morphine on fecal boli accumulation and to a lesser extent colonic propulsion when compared to WT mice [92,93]. The β -arrestin2 KO mice were not, however, resistant to the inhibitory effects of morphine on small intestinal transit. Interestingly, the peripherally restricted μ -OR agonist and antidiarrheal agent loperamide significantly reduced colonic propulsion in WT mice, and these effects were completely abolished in β -arrestin2 KO mice [92]. This suggests that peripherally restricted β -arrestin-biased agonists might be useful in the treatment of diarrhea and other hypermotility disorders.

Cancer cell metastasis and cell motility

The β -arrestins are involved in several cancer-related signals and processes via a range of receptor subtypes. Moreover, elevated β -arrestin mRNA levels have been observed in cancerous tumors and tumorigenic cancer cell lines [94]. Of note, β -arrestin1 plays a role in nicotine-induced proliferation of non-small-cell lung cancer cells via a process involving a β -arrestin1-scaffolded complex of the nicotinic acetylcholine receptor and Src, as well as downstream signaling via the MAP kinase and Rb-Raf-1 pathways [95]. Prostaglandin-E2-stimulated EP2- and EP4-receptor-mediated transactivation of EGFR also involves a complex of receptor, β -arrestin1 and Src, which is involved in mouse skin papilloma development [96] and colorectal carcinoma cell migration and metastasis [97], respectively. Similarly, in ovarian cancer cells the β -arrestins mediate endothelin type A (ET_A)-receptor-stimulated EGFR transactivation via Src, as well as effects on β -catenin, which are thought to be involved in both cell invasion and metastasis [98,99]. Lysophosphatidic acid (LPA) receptor activation is associated with enhanced breast cancer cell metastasis [94], and siRNA-mediated knockdown of either β -arrestin 1 or 2 in breast cancer cells reduces LPA-stimulated transwell migration and 3D Matrigel invasion [94]. Conversely, transgenic overexpression of β -arrestin1 in mice promotes tumor angiogenesis and progression [100].

In addition to activating proliferative signaling pathways, β -arrestins are also involved in several pathways that suppress tumor growth and metastasis. For example, transforming growth factor (TGF)- β inhibits epithelial and cancer cell migration via β -arrestin2-mediated activation of cell division cycle (CDC)-42 [101]. β -Arrestin2 KO mice also have increased tumor growth and metastasis mediated by host changes in inflammation and angiogenesis in a heterotopic model of lung cancer in which both WT and β -arrestin2 KO mice were injected with cells derived from a spontaneously occurring lung cancer tumor in C57BL/6 mice [102]. In androgen-dependent prostate cancer cells, β -arrestin2 acts as an androgen receptor corepressor and promotes the association of MDM2 with the receptor and its consequent ubiquitylation and degradation [103]. The above studies clearly demonstrate that the β -arrestins play roles in a variety of pro- and anticancer-related signals and processes in both neoplastic cells and the surrounding host environment via several different receptor subtypes. The diversity of this influence might provide insight into potential targets for future chemotherapeutic strategies.

Emerging areas of interest

In addition to the specific examples highlighting β -arrestin-mediated functions (Table 2), several reports describe processes that are probably β -arrestin-mediated. One recent example, which might have broad therapeutic implications in the treatment of cognitive disorders, involves the M3 muscarinic receptor. Specifically, M3-receptor-dependent learning and memory require processes that depend on receptor phosphorylation, consistent with β -arrestin recruitment and apparently independent of G protein signaling [104]. These data suggest that a β -arrestin-biased ligand at the M3 receptor might promote both learning and memory, and might thus be beneficial in the treatment of cognitive disorders such as Alzheimer's disease. The exact role of β -arrestin in such processes and the therapeutic potential of targeting β -arrestin-mediated signaling will require further study.

Concluding remarks

The β -arrestins are intimately involved in numerous aspects of 7TMR signaling and regulation, and accordingly influence manifold physiological and pathophysiological processes. β -Arrestin-mediated signaling is a relatively new area of 7TMR research, especially when compared to the study of more traditional G protein signals, and continued efforts in this area will undoubtedly lead to the discovery of additional roles for β -arrestins in 7TMR biology. Of particular interest, the ability of biased ligands to differentiate between β -arrestin and G protein functions at the receptor level should facilitate selective engagement of a subset of signals from a particular 7TMR. In most instances, identification of biased ligands for a specific 7TMR target is rather straightforward. The major bottleneck in determining the therapeutic potential of G protein- and β -arrestin-biased agonists is the lack of knowledge regarding the roles of these distinct signaling pathways in both health and disease. Therefore, studies should focus on the development and use of biased agonists as tool compounds in cellular and animal models of disease to more clearly delineate the physiologic consequences of these two signaling mechanisms. We expect that clearer definitions of the role of β -arrestins and G proteins in 7TMR signaling will facilitate the development of improved therapeutic agents that target 7TMRs (and potentially β -arrestin- and G protein-mediated signals directly) with improved efficacy and fewer side effects.

Glossary

Antinociceptive effect	effect that leads to a decreased sensation of pain
Congenital nephrogenic diabetes insipidus (cNDI)	condition in which the kidney is unable to properly reabsorb water because it does not respond appropriately to vasopressin
Inotropic support	therapy aimed at improving the contractile function of the heart
Nephrogenic syndrome of inappropriate antidiuresis	disorder in which the body inappropriately retains water due to a defect in signaling in the kidney
Tachyphylaxis	decrease in responsiveness to a drug with repeated dosing
Warts, hypogammaglobulinemia, infections, myelokathexis (WHIM) syndrome	congenital immunodeficiency due to mutation of CXCR4 that results in neutropenia

References

1. Lagerstrom MC, Schioth HB. Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nat. Rev. Drug Discov.* 2008; 7:339–357. [PubMed: 18382464]
2. Chung S, et al. Orphan GPCR research. *Br. J. Pharmacol.* 2008; 153(Suppl 1):S339–S346. [PubMed: 18071299]
3. Ma P, Zimmel R. Value of novelty? *Nat. Rev. Drug Discov.* 2002; 1:571–572. [PubMed: 12402497]
4. Kenakin T. New concepts in drug discovery: collateral efficacy and permissive antagonism. *Nat. Rev. Drug Discov.* 2005; 4:919–927. [PubMed: 16264435]
5. DeWire SM, et al. beta-Arrestins and cell signaling. *Annu. Rev. Physiol.* 2007; 69:483–510. [PubMed: 17305471]
6. Luttrell LM, Gesty-Palmer D. Beyond desensitization: physiological relevance of arrestin-dependent signaling. *Pharmacol. Rev.* 2010; 62:305–330. [PubMed: 20427692]
7. Schmid CL, Bohn LM. Physiological and pharmacological implications of beta-arrestin regulation. *Pharmacol. Ther.* 2009; 121:285–293. [PubMed: 19100766]
8. Rajagopal S, et al. Teaching old receptors new tricks: biasing seven-transmembrane receptors. *Nat. Rev. Drug Discov.* 2010; 9:373–386. [PubMed: 20431569]
9. Violin JD, Lefkowitz RJ. beta-Arrestin-biased ligands at seven-transmembrane receptors. *Trends Pharmacol. Sci.* 2007; 28:416–422. [PubMed: 17644195]
10. Kenakin T, Miller LJ. Seven transmembrane receptors as shapeshifting proteins: the impact of allosteric modulation and functional selectivity on new drug discovery. *Pharmacol. Rev.* 2010; 62:265–304. [PubMed: 20392808]
11. Kenakin T. Inverse, protean, and ligand-selective agonism: matters of receptor conformation. *FASEB J.* 2001; 15:598–611. [PubMed: 11259378]
12. Conner DA, et al. beta-Arrestin1 knockout mice appear normal but demonstrate altered cardiac responses to beta-adrenergic stimulation. *Circ. Res.* 1997; 81:1021–1026. [PubMed: 9400383]
13. Haney S, Hancox RJ. Recovery from bronchoconstriction and bronchodilator tolerance. *Clin. Rev. Allergy Immunol.* 2006; 31:181–196. [PubMed: 17085792]
14. Abramson MJ, et al. Adverse effects of beta-agonists: are they clinically relevant? *Am. J. Respir. Med.* 2003; 2:287–297. [PubMed: 14719995]
15. Lohse MJ, et al. beta-Arrestin: a protein that regulates beta-adrenergic receptor function. *Science.* 1990; 248:1547–1550. [PubMed: 2163110]
16. Ahn S, et al. Desensitization, internalization, and signaling functions of beta-arrestins demonstrated by RNA interference. *Proc. Natl. Acad. Sci. U.S.A.* 2003; 100:1740–1744. [PubMed: 12582207]
17. Perry SJ, et al. Targeting of cyclic AMP degradation to beta 2- adrenergic receptors by beta-arrestins. *Science.* 2002; 298:834–836. [PubMed: 12399592]
18. Deshpande DA, et al. beta-Arrestins specifically constrain beta2-adrenergic receptor signaling and function in airway smooth muscle. *FASEB J.* 2008; 22:2134–2141. [PubMed: 18337459]
19. Wang WC, et al. Targeted transgenesis reveals discrete attenuator functions of GRK and PKA in airway beta2-adrenergic receptor physiologic signaling. *Proc. Natl. Acad. Sci. U.S.A.* 2009; 106:15007–15012. [PubMed: 19706446]
20. Bohn LM, et al. Relative opioid efficacy is determined by the complements of the G protein-coupled receptor desensitization machinery. *Mol. Pharmacol.* 2004; 66:106–112. [PubMed: 15213301]
21. Bohn LM, et al. mu-Opioid receptor desensitization by beta-arrestin-2 determines morphine tolerance but not dependence. *Nature.* 2000; 408:720–723. [PubMed: 11130073]
22. Bohn LM, et al. Enhanced morphine analgesia in mice lacking beta-arrestin 2. *Science.* 1999; 286:2495–2498. [PubMed: 10617462]
23. Przewlocka B, et al. Knockdown of spinal opioid receptors by antisense targeting beta-arrestin reduces morphine tolerance and allodynia in rat. *Neurosci. Lett.* 2002; 325:107–110. [PubMed: 12044633]

24. Li Y, et al. Improvement of morphine-mediated analgesia by inhibition of beta-arrestin 2 expression in mice periaqueductal gray matter. *Int. J. Mol. Sci.* 2009; 10:954–963. [PubMed: 19399231]
25. Jiang B, et al. Decreased morphine analgesia in rat overexpressing beta-arrestin 2 at periaqueductal gray. *Neurosci. Lett.* 2006; 400:150–153. [PubMed: 16563622]
26. Goodman OB Jr, et al. beta-Arrestin acts as a clathrin adaptor in endocytosis of the beta2-adrenergic receptor. *Nature.* 1996; 383:447–450. [PubMed: 8837779]
27. Laporte SA, et al. The beta2-adrenergic receptor/beta-arrestin complex recruits the clathrin adaptor AP-2 during endocytosis. *Proc. Natl. Acad. Sci. U.S.A.* 1999; 96:3712–3717. [PubMed: 10097102]
28. Shenoy SK, et al. Ubiquitination of beta-arrestin links seven-transmembrane receptor endocytosis and ERK activation. *J. Biol. Chem.* 2007; 282:29549–29562. [PubMed: 17666399]
29. Shenoy SK, et al. Beta-arrestin-dependent signaling and trafficking of 7-transmembrane receptors is reciprocally regulated by the deubiquitinase USP33 and the E3 ligase Mdm2. *Proc. Natl. Acad. Sci. U.S.A.* 2009; 106:6650–6655. [PubMed: 19363159]
30. Shenoy SK, et al. Regulation of receptor fate by ubiquitination of activated beta 2-adrenergic receptor and beta-arrestin. *Science.* 2001; 294:1307–1313. [PubMed: 11588219]
31. Shenoy SK, et al. Nedd4 mediates agonist-dependent ubiquitination, lysosomal targeting, and degradation of the beta2-adrenergic receptor. *J. Biol. Chem.* 2008; 283:22166–22176. [PubMed: 18544533]
32. Bhandari D, et al. Arrestin-2 interacts with the ubiquitin-protein isopeptide ligase atrophin-interacting protein 4 and mediates endosomal sorting of the chemokine receptor CXCR4. *J. Biol. Chem.* 2007; 282:36971–36979. [PubMed: 17947233]
33. Berthouze M, et al. The deubiquitinases USP33 and USP20 coordinate beta2 adrenergic receptor recycling and resensitization. *EMBO J.* 2009; 28:1684–1696. [PubMed: 19424180]
34. Barak LS, et al. Constitutive arrestin-mediated desensitization of a human vasopressin receptor mutant associated with nephrogenic diabetes insipidus. *Proc. Natl. Acad. Sci. U.S.A.* 2001; 98:93–98. [PubMed: 11134505]
35. Wilbanks AM, et al. Apparent loss-of-function mutant GPCRs revealed as constitutively desensitized receptors. *Biochemistry.* 2002; 41:11981–11989. [PubMed: 12356298]
36. Bernier V, et al. Functional rescue of the constitutively internalized V2 vasopressin receptor mutant R137H by the pharmacological chaperone action of SR49059. *Mol. Endocrinol.* 2004; 18:2074–2084. [PubMed: 15166253]
37. Jean-Alphonse F, et al. Biased agonist pharmacochaperones of the AVP V2 receptor may treat congenital nephrogenic diabetes insipidus. *J. Am. Soc. Nephrol.* 2009; 20:2190–2203. [PubMed: 19729439]
38. Kocan M, et al. Agonist-independent interactions between beta-arrestins and mutant vasopressin type II receptors associated with nephrogenic syndrome of inappropriate antidiuresis. *Mol. Endocrinol.* 2009; 23:559–571. [PubMed: 19179480]
39. Balabanian K, et al. WHIM syndromes with different genetic anomalies are accounted for by impaired CXCR4 desensitization to CXCL12. *Blood.* 2005; 105:2449–2457. [PubMed: 15536153]
40. Samson M, et al. Resistance to HIV-1 infection in Caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature.* 1996; 382:722–725. [PubMed: 8751444]
41. Simmons G, et al. Co-receptor use by HIV and inhibition of HIV infection by chemokine receptor ligands. *Immunol. Rev.* 2000; 177:112–126. [PubMed: 11138769]
42. Mack M, et al. Aminooxypentane-RANTES induces CCR5 internalization but inhibits recycling: a novel inhibitory mechanism of HIV infectivity. *J. Exp. Med.* 1998; 187:1215–1224. [PubMed: 9547333]
43. Signoret N, et al. Endocytosis and recycling of the HIV coreceptor CCR5. *J. Cell Biol.* 2000; 151:1281–1294. [PubMed: 11121442]
44. Hartley O, et al. Medicinal chemistry applied to a synthetic protein: development of highly potent HIV entry inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* 2004; 101:16460–16465. [PubMed: 15545608]
45. Oppermann M. Chemokine receptor CCR5: insights into structure, function, and regulation. *Cell Signal.* 2004; 16:1201–1210. [PubMed: 15337520]

46. DeFea KA, et al. The proliferative and antiapoptotic effects of substance P are facilitated by formation of a beta-arrestin-dependent scaffolding complex. *Proc. Natl. Acad. Sci. U.S.A.* 2000; 97:11086–11091. [PubMed: 10995467]
47. DeFea KA, et al. beta-Arrestin-dependent endocytosis of proteinase-activated receptor 2 is required for intracellular targeting of activated ERK1/2. *J. Cell Biol.* 2000; 148:1267–1281. [PubMed: 10725339]
48. Luttrell LM, et al. beta-Arrestin-dependent formation of beta2 adrenergic receptor-Src protein kinase complexes. *Science.* 1999; 283:655–661. [PubMed: 9924018]
49. Christensen GL, et al. Quantitative phosphoproteomics dissection of seven-transmembrane receptor signaling using full and biased agonists. *Mol. Cell Proteomics.* 2010; 9:1540–1553. [PubMed: 20363803]
50. Xiao K, et al. Functional specialization of beta-arrestin interactions revealed by proteomic analysis. *Proc. Natl. Acad. Sci. U.S.A.* 2007; 104:12011–12016. [PubMed: 17620599]
51. Xiao K, et al. Global phosphorylation analysis of beta-arrestin-mediated signaling downstream of a seven transmembrane receptor (7TMR). *Proc. Natl. Acad. Sci. U.S.A.* 2010; 107:15299–15304. [PubMed: 20686112]
52. Noma T, et al. beta-Arrestin-mediated beta1-adrenergic receptor transactivation of the EGFR confers cardioprotection. *J. Clin. Invest.* 2007; 117:2445–2458. [PubMed: 17786238]
53. Bristow MR. beta-Adrenergic receptor blockade in chronic heart failure. *Circulation.* 2000; 101:558–569. [PubMed: 10662755]
54. Cohn JN, et al. Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. *N. Engl. J. Med.* 1984; 311:819–823. [PubMed: 6382011]
55. Lohse MJ, et al. What is the role of beta-adrenergic signaling in heart failure? *Circ. Res.* 2003; 93:896–906. [PubMed: 14615493]
56. Xiao RP. beta-Adrenergic signaling in the heart: dual coupling of the beta2-adrenergic receptor to Gs and Gi proteins. *Sci. STKE.* 2001:re15. [PubMed: 11604549]
57. Kim IM, et al. beta-Blockers alprenolol and carvedilol stimulate beta-arrestin-mediated EGFR transactivation. *Proc. Natl. Acad. Sci. U.S.A.* 2008; 105:14555–14560. [PubMed: 18787115]
58. Wisler JW, et al. A unique mechanism of beta-blocker action: carvedilol stimulates beta-arrestin signaling. *Proc. Natl. Acad. Sci. U.S.A.* 2007; 104:16657–16662. [PubMed: 17925438]
59. Drake MT, et al. beta-Arrestin-biased agonism at the beta2-adrenergic receptor. *J. Biol. Chem.* 2008; 283:5669–5676. [PubMed: 18086673]
60. Zhai P, et al. Cardiac-specific overexpression of AT1 receptor mutant lacking G alpha q/G alpha i coupling causes hypertrophy and bradycardia in transgenic mice. *J. Clin. Invest.* 2005; 115:3045–3056. [PubMed: 16276415]
61. Aplin M, et al. Differential extracellular signal-regulated kinases 1 and 2 activation by the angiotensin type 1 receptor supports distinct phenotypes of cardiac myocytes. *Basic Clin. Pharmacol. Toxicol.* 2007; 100:296–301. [PubMed: 17448114]
62. Hansen JL, et al. The human angiotensin AT1 receptor supports G protein-independent extracellular signal-regulated kinase 1/2 activation and cellular proliferation. *Eur. J. Pharmacol.* 2008; 590:255–263. [PubMed: 18565507]
63. Rajagopal K, et al. beta-Arrestin2-mediated inotropic effects of the angiotensin II type 1A receptor in isolated cardiac myocytes. *Proc. Natl. Acad. Sci. U.S.A.* 2006; 103:16284–16289. [PubMed: 17060617]
64. Violin JD, et al. Selectively engaging beta-arrestins at the AT1R reduces blood pressure and increases cardiac performance. *J. Pharmacol. Exp. Ther.* 2010; 335:572–579. [PubMed: 20801892]
65. DeWire SM, et al. beta-Arrestin-mediated signaling regulates protein synthesis. *J. Biol. Chem.* 2008; 283:10611–10620. [PubMed: 18276584]
66. Ahn S, et al. beta-Arrestin-2 mediates anti-apoptotic signaling through regulation of BAD phosphorylation. *J. Biol. Chem.* 2009; 284:8855–8865. [PubMed: 19171933]
67. Lymperopoulos A, et al. An adrenal beta-arrestin 1-mediated signaling pathway underlies angiotensin II-induced aldosterone production *in vitro* and *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.* 2009; 106:5825–5830. [PubMed: 19289825]

68. Johnson AK, Thunhorst RL. The neuroendocrinology of thirst and salt appetite: visceral sensory signals and mechanisms of central integration. *Front Neuroendocrinol.* 1997; 18:292–353. [PubMed: 9237080]
69. We H, et al. Independent beta-arrestin 2 and G protein-mediated pathways for angiotensin II activation of extracellular signal-regulated kinases 1 and 2. *Proc. Natl. Acad. Sci. U.S.A.* 2003; 100:10782–10787. [PubMed: 12949261]
70. Daniels D, et al. Divergent behavioral roles of angiotensin receptor intracellular signaling cascades. *Endocrinology.* 2005; 146:5552–5560. [PubMed: 16123155]
71. Guyton JR. Extended-release niacin for modifying the lipoprotein profile. *Expert Opin. Pharmacother.* 2004; 5:1385–1398. [PubMed: 15163282]
72. Guyton JR. Niacin in cardiovascular prevention: mechanisms, efficacy, and safety. *Curr. Opin. Lipidol.* 2007; 18:415–420. [PubMed: 17620858]
73. Walters RW, et al. beta-Arrestin1 mediates nicotinic acid-induced flushing, but not its antilipolytic effect, in mice. *J. Clin. Invest.* 2009; 119:1312–1321. [PubMed: 19349687]
74. Richman JG, et al. Nicotinic acid receptor agonists differentially activate downstream effectors. *J. Biol. Chem.* 2007; 282:18028–18036. [PubMed: 17452318]
75. Semple G, et al. 3-(1*H*-Tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole (MK-0354): a partial agonist of the nicotinic acid receptor, G-protein coupled receptor 109a, with antilipolytic but no vasodilatory activity in mice. *J. Med. Chem.* 2008; 51:5101–5108. [PubMed: 18665582]
76. Shen HC, et al. Discovery of novel tricyclic full agonists for the G-protein-coupled niacin receptor 109A with minimized flushing in rats. *J. Med. Chem.* 2009; 52:2587–2602. [PubMed: 19309152]
77. Shen HC, et al. Discovery of biaryl anthranilides as full agonists for the high affinity niacin receptor. *J. Med. Chem.* 2007; 50:6303–6306. [PubMed: 17994679]
78. Shen HC, et al. Discovery of a biaryl cyclohexene carboxylic acid (MK-6892): a potent and selective high affinity niacin receptor full agonist with reduced flushing profiles in animals as a preclinical candidate. *J. Med. Chem.* 2010; 53:2666–2670. [PubMed: 20184326]
79. Castro M, et al. Dual regulation of the parathyroid hormone (PTH)/PTH-related peptide receptor signaling by protein kinase C and beta-arrestins. *Endocrinology.* 2002; 143:3854–3865. [PubMed: 12239097]
80. Ferrari SL, et al. Endocytosis of ligand–human parathyroid hormone receptor 1 complexes is protein kinase C-dependent and involves beta-arrestin2. Real-time monitoring by fluorescence microscopy. *J. Biol. Chem.* 1999; 274:29968–29975. [PubMed: 10514480]
81. Ferrari SL, Bisello A. Cellular distribution of constitutively active mutant parathyroid hormone (PTH)/PTH-related protein receptors and regulation of cyclic adenosine 3',5'-monophosphate signaling by beta-arrestin2. *Mol. Endocrinol.* 2001; 15:149–163. [PubMed: 11145746]
82. Sneddon WB, Friedman PA. beta-Arrestin-dependent parathyroid hormone-stimulated extracellular signal-regulated kinase activation and parathyroid hormone type 1 receptor internalization. *Endocrinology.* 2007; 148:4073–4079. [PubMed: 17525124]
83. Vilardaga JP, et al. Differential conformational requirements for activation of G proteins and the regulatory proteins arrestin and G protein-coupled receptor kinase in the G protein-coupled receptor for parathyroid hormone (PTH)/PTH-related protein. *J. Biol. Chem.* 2001; 276:33435–33443. [PubMed: 11387315]
84. Vilardaga JP, et al. Internalization determinants of the parathyroid hormone receptor differentially regulate beta-arrestin/receptor association. *J. Biol. Chem.* 2002; 277:8121–8129. [PubMed: 11726668]
85. Bouxsein ML, et al. beta-Arrestin2 regulates the differential response of cortical and trabecular bone to intermittent PTH in female mice. *J. Bone Miner Res.* 2005; 20:635–643. [PubMed: 15765183]
86. Ferrari SL, et al. Bone response to intermittent parathyroid hormone is altered in mice null for beta-arrestin2. *Endocrinology.* 2005; 146:1854–1862. [PubMed: 15705780]
87. Bianchi EN, Ferrari SL. Beta-arrestin2 regulates parathyroid hormone effects on a p38 MAPK and NFkappaB gene expression network in osteoblasts. *Bone.* 2009; 45:716–725. [PubMed: 19560570]

88. Pierroz DD, et al. beta-Arrestin2 regulates RANKL and ephrins gene expression in response to bone remodeling in mice. *J. Bone Miner Res.* 2009; 24:775–784. [PubMed: 19113915]
89. Bisello A, et al. Selective ligand-induced stabilization of active and desensitized parathyroid hormone type 1 receptor conformations. *J. Biol. Chem.* 2002; 277:38524–38530. [PubMed: 12107160]
90. Gesty-Palmer D, et al. Distinct beta-arrestin- and G protein-dependent pathways for parathyroid hormone receptor-stimulated ERK1/2 activation. *J. Biol. Chem.* 2006; 281:10856–10864. [PubMed: 16492667]
91. Gesty-Palmer D, et al. A beta-arrestin-biased agonist of the parathyroid hormone receptor (PTH1R) promotes bone formation independent of G protein activation. *Sci. Transl. Med.* 2009; 1:1ra1.
92. Raehal KM, et al. Morphine side effects in beta-arrestin 2 knockout mice. *J. Pharmacol. Exp. Ther.* 2005; 314:1195–1201. [PubMed: 15917400]
93. Bohn LM, Raehal KM. Opioid receptor signaling: relevance for gastrointestinal therapy. *Curr. Opin. Pharmacol.* 2006; 6:559–563. [PubMed: 16935560]
94. Li TT, et al. beta-Arrestin/Ral signaling regulates lysophosphatidic acid-mediated migration and invasion of human breast tumor cells. *Mol. Cancer Res.* 2009; 7:1064–1077. [PubMed: 19609003]
95. Dasgupta P, et al. Nicotine induces cell proliferation by beta-arrestin- mediated activation of Src and Rb-Raf-1 pathways. *J. Clin. Invest.* 2006; 116:2208–2217. [PubMed: 16862215]
96. Chun KS, et al. The prostaglandin receptor EP2 activates multiple signaling pathways and beta-arrestin1 complex formation during mouse skin papilloma development. *Carcinogenesis.* 2009; 30:1620–1627. [PubMed: 19587094]
97. Buchanan FG, et al. Role of beta-arrestin 1 in the metastatic progression of colorectal cancer. *Proc. Natl. Acad. Sci. U.S.A.* 2006; 103:1492–1497. [PubMed: 16432186]
98. Rosano L, Bagnato A. Convergent pathways link the endothelin A receptor to the beta-catenin: the beta-arrestin connection. *Cell Cycle.* 2009; 8:1462–1463. [PubMed: 19395853]
99. Rosano L, et al. Beta-arrestin links endothelin A receptor to beta-catenin signaling to induce ovarian cancer cell invasion and metastasis. *Proc. Natl. Acad. Sci. U.S.A.* 2009; 106:2806–2811. [PubMed: 19202075]
100. Zou L, et al. Rapid xenograft tumor progression in beta-arrestin1 transgenic mice due to enhanced tumor angiogenesis. *FASEB J.* 2008; 22:355–364. [PubMed: 17890288]
101. Myhre K, Blobe GC. The type III TGF-beta receptor regulates epithelial and cancer cell migration through beta-arrestin2-mediated activation of Cdc42. *Proc. Natl. Acad. Sci. U.S.A.* 2009; 106:8221–8226. [PubMed: 19416857]
102. Raghuwanshi SK, et al. Depletion of beta-arrestin-2 promotes tumor growth and angiogenesis in a murine model of lung cancer. *J. Immunol.* 2008; 180:5699–5706. [PubMed: 18390755]
103. Lakshmikanthan V, et al. Identification of β arrestin2 as a corepressor of androgen receptor signaling in prostate cancer. *Proc. Natl. Acad. Sci. U.S.A.* 2009; 106:9379–9384. [PubMed: 19458261]
104. Poulin B, et al. The M3-muscarinic receptor regulates learning and memory in a receptor phosphorylation/arrestin-dependent manner. *Proc. Natl. Acad. Sci. U.S.A.* 2010; 107:9440–9445. [PubMed: 20439723]
105. Gao ZG, Jacobson KA. Translocation of arrestin induced by human A3 adenosine receptor ligands in an engineered cell line: comparison with G protein-dependent pathways. *Pharmacol. Res.* 2008; 57:303–311. [PubMed: 18424164]
106. Lee DK, et al. The fate of the internalized apelin receptor is determined by different isoforms of apelin mediating differential interaction with beta-arrestin. *Biochem. Biophys. Res. Commun.* 2010; 395:185–189. [PubMed: 20353754]
107. Ahn S, et al. Differential kinetic and spatial patterns of beta-arrestin and G protein-mediated ERK activation by the angiotensin II receptor. *J. Biol. Chem.* 2004; 279:35518–35525. [PubMed: 15205453]
108. Ahn S, et al. Reciprocal regulation of angiotensin receptor-activated extracellular signal-regulated kinases by beta-arrestins 1 and 2. *J. Biol. Chem.* 2004; 279:7807–7811. [PubMed: 14711824]

109. Aplin M, et al. The angiotensin type 1 receptor activates extracellular signal-regulated kinases 1 and 2 by G protein-dependent and -independent pathways in cardiac myocytes and Langendorff-perfused hearts. *Basic Clin. Pharmacol. Toxicol.* 2007; 100:289–295. [PubMed: 17448113]
110. Barnes WG, et al. beta-Arrestin 1 and Galphaq/11 coordinately activate RhoA and stress fiber formation following receptor stimulation. *J. Biol. Chem.* 2005; 280:8041–8050. [PubMed: 15611106]
111. Hunton DL, et al. beta-Arrestin 2-dependent angiotensin II type 1A receptor-mediated pathway of chemotaxis. *Mol. Pharmacol.* 2005; 67:1229–1236. [PubMed: 15635042]
112. Kim J, et al. Independent beta-arrestin2 and Gq/protein kinase Czeta pathways for ERK stimulated by angiotensin type 1A receptors in vascular smooth muscle cells converge on transactivation of the epidermal growth factor receptor. *J. Biol. Chem.* 2009; 284:11953–11962. [PubMed: 19254952]
113. Morinelli TA, et al. Angiotensin II-induced cyclooxygenase 2 expression in rat aorta vascular smooth muscle cells does not require heterotrimeric G protein activation. *J. Pharmacol. Exp. Ther.* 2009; 330:118–124. [PubMed: 19351865]
114. Tilley DG, et al. Troglitazone stimulates beta-arrestin-dependent cardiomyocyte contractility via the angiotensin II type 1A receptor. *Biochem. Biophys. Res. Commun.* 2010; 396:921–926. [PubMed: 20460106]
115. Lu R, et al. Epitope-tagged receptor knock-in mice reveal that differential desensitization of alpha2-adrenergic responses is because of ligand-selective internalization. *J. Biol. Chem.* 2009; 284:13233–13243. [PubMed: 19276088]
116. Kukkonen JP, et al. Agonist trafficking of G_{i/o}-mediated alpha_{2A}-adrenoceptor responses in HEL 92.1.7 cells. *Br. J. Pharmacol.* 2001; 132:1477–1484. [PubMed: 11264241]
117. Azzi M, et al. beta-Arrestin-mediated activation of MAPK by inverse agonists reveals distinct active conformations for G protein-coupled receptors. *Proc. Natl. Acad. Sci. U.S.A.* 2003; 100:11406–11411. [PubMed: 13679574]
118. Reiner S, et al. Differential signaling of the endogenous agonists at the beta2-adrenergic receptor. *J. Biol. Chem.* 2010; 285:31688–31698.
119. MacKinnon AC, et al. Bombesin and substance P analogues differentially regulate G-protein coupling to the bombesin receptor. Direct evidence for biased agonism. *J. Biol. Chem.* 2001; 276:28083–28091. [PubMed: 11323408]
120. Breivogel CS, et al. Sensitivity to delta9-tetrahydrocannabinol is selectively enhanced in beta-arrestin2 $-/-$ mice. *Behav. Pharmacol.* 2008; 19:298–307. [PubMed: 18622177]
121. Wu DF, et al. Role of receptor internalization in the agonist-induced desensitization of cannabinoid type 1 receptors. *J. Neurochem.* 2008; 104:1132–1143. [PubMed: 17986216]
122. Roettger BF, et al. Antagonist-stimulated internalization of the G protein-coupled cholecystokinin receptor. *Mol. Pharmacol.* 1997; 51:357–362. [PubMed: 9058588]
123. Rafei M, et al. An engineered GM-CSF-CCL2 fusokine is a potent inhibitor of CCR2-driven inflammation as demonstrated in a murine model of inflammatory arthritis. *J. Immunol.* 2009; 183:1759–1766. [PubMed: 19592643]
124. Blanpain C, et al. CCR5 and HIV infection. *Receptors Channels.* 2002; 8:19–31. [PubMed: 12402506]
125. Vila-Coro AJ, et al. Characterization of RANTES- and aminooxypentane-RANTES-triggered desensitization signals reveals differences in recruitment of the G protein-coupled receptor complex. *J. Immunol.* 1999; 163:3037–3044. [PubMed: 10477567]
126. Blanpain C, et al. Multiple active states and oligomerization of CCR5 revealed by functional properties of monoclonal antibodies. *Mol. Biol. Cell.* 2002; 13:723–737. [PubMed: 11854425]
127. Byers MA, et al. Arrestin 3 mediates endocytosis of CCR7 following ligation of CCL19 but not CCL21. *J. Immunol.* 2008; 181:4723–4732. [PubMed: 18802075]
128. Kohout TA, et al. Differential desensitization, receptor phosphorylation, beta-arrestin recruitment, and ERK1/2 activation by the two endogenous ligands for the CC chemokine receptor 7. *J. Biol. Chem.* 2004; 279:23214–23222. [PubMed: 15054093]

129. Zidar DA, et al. Selective engagement of G protein coupled receptor kinases (GRKs) encodes distinct functions of biased ligands. *Proc. Natl. Acad. Sci. U.S.A.* 2009; 106:9649–9654. [PubMed: 19497875]
130. Watson C, et al. The use of stimulus-biased assay systems to detect agonist-specific receptor active states: implications for the trafficking of receptor stimulus by agonists. *Mol. Pharmacol.* 2000; 58:1230–1238. [PubMed: 11093758]
131. Hall DA, et al. Signalling by CXC-chemokine receptors 1 and 2 expressed in CHO cells: a comparison of calcium mobilization, inhibition of adenylyl cyclase and stimulation of GTPgammaS binding induced by IL-8 and GROalpha. *Br. J. Pharmacol.* 1999; 126:810–818. [PubMed: 10188995]
132. Sachpatzidis A, et al. Identification of allosteric peptide agonists of CXCR4. *J. Biol. Chem.* 2003; 278:896–907. [PubMed: 12417595]
133. Mathiesen JM, et al. Identification of indole derivatives exclusively interfering with a G protein-independent signaling pathway of the prostaglandin D2 receptor CRTH2. *Mol. Pharmacol.* 2005; 68:393–402. [PubMed: 15870392]
134. Lewis MM, et al. Homologous desensitization of the D1A dopamine receptor: efficacy in causing desensitization dissociates from both receptor occupancy and functional potency. *J. Pharmacol. Exp. Ther.* 1998; 286:345–353. [PubMed: 9655879]
135. Ryman-Rasmussen JP, et al. Functional selectivity of dopamine D1 receptor agonists in regulating the fate of internalized receptors. *Neuropharmacology.* 2007; 52:562–575. [PubMed: 17067639]
136. Ryman-Rasmussen JP, et al. Differential activation of adenylate cyclase and receptor internalization by novel dopamine D1 receptor agonists. *Mol. Pharmacol.* 2005; 68:1039–1048. [PubMed: 15985612]
137. Klewe IV, et al. Recruitment of beta-arrestin2 to the dopamine D2 receptor: insights into anti-psychotic and anti-parkinsonian drug receptor signaling. *Neuropharmacology.* 2008; 54:1215–1222. [PubMed: 18455202]
138. Masri B, et al. Antagonism of dopamine D2 receptor/beta-arrestin-2 interaction is a common property of clinically effective antipsychotics. *Proc. Natl. Acad. Sci. U.S.A.* 2008; 105:13656–13661. [PubMed: 18768802]
139. Mottola DM, et al. Functional selectivity of dopamine receptor agonists. I. Selective activation of postsynaptic dopamine D2 receptors linked to adenylate cyclase. *J. Pharmacol. Exp. Ther.* 2002; 301:1166–1178. [PubMed: 12023552]
140. Kilts JD, et al. Functional selectivity of dopamine receptor agonists. II. Actions of dihydrexidine in D2L receptor-transfected MN9D cells and pituitary lactotrophs. *J. Pharmacol. Exp. Ther.* 2002; 301:1179–1189. [PubMed: 12023553]
141. Graler MH, Goetzl EJ. The immunosuppressant FTY720 down-regulates sphingosine 1-phosphate G-protein-coupled receptors. *FASEB J.* 2004; 18:551–553. [PubMed: 14715694]
142. Wetter JA, et al. Utilization of the Tango beta-arrestin recruitment technology for cell-based EDG receptor assay development and interrogation. *J. Biomol. Screen.* 2009; 14:1134–1141. [PubMed: 19726785]
143. Sensken SC, et al. Selective activation of G alpha i mediated signalling of S1P3 by FTY720-phosphate. *Cell Signal.* 2008; 20:1125–1133. [PubMed: 18313900]
144. Leduc M, et al. Functional selectivity of natural and synthetic prostaglandin EP4 receptor ligands. *J. Pharmacol. Exp. Ther.* 2009; 331:297–307. [PubMed: 19584306]
145. Bhowmick N, et al. The endothelin subtype A receptor undergoes agonist- and antagonist-mediated internalization in the absence of signaling. *Endocrinology.* 1998; 139:3185–3192. [PubMed: 9645692]
146. Wehbi V, et al. Selective modulation of follicle-stimulating hormone signaling pathways with enhancing equine chorionic gonadotropin/antibody immune complexes. *Endocrinology.* 2010; 151:2788–2799. [PubMed: 20332198]
147. Wehbi V, et al. Partially deglycosylated equine LH preferentially activates {beta}-arrestin-dependent signaling at the follicle-stimulating hormone receptor. *Mol. Endocrinol.* 2010; 24:561–573. [PubMed: 20107152]

148. Jorgensen R, et al. Oxyntomodulin differentially affects glucagon-like peptide-1 receptor beta-arrestin recruitment and signaling through Galpha. *J. Pharmacol. Exp. Ther.* 2007; 322:148–154. [PubMed: 17395766]
149. Koole C, et al. Allosteric ligands of the glucagon-like peptide 1 receptor (GLP-1R) differentially modulate endogenous and exogenous peptide responses in a pathway-selective manner: implications for drug screening. *Mol. Pharmacol.* 2010; 78:456–465. [PubMed: 20547734]
150. Jennes L, et al. Receptor-mediated binding and uptake of GnRH agonist and antagonist by pituitary cells. *Peptides.* 1984; 5(Suppl 1):215–220. [PubMed: 6091067]
151. Jennes L, et al. Receptor-mediated uptake of GnRH agonist and antagonists by cultured gonadotropes: evidence for differential intracellular routing. *Peptides.* 1986; 7:459–463. [PubMed: 3022254]
152. Millar RP, et al. Diversity of actions of GnRHs mediated by ligand-induced selective signaling. *Front Neuroendocrinol.* 2008; 29:17–35. [PubMed: 17976709]
153. Davis AA, et al. Differential effects of allosteric M₁ muscarinic acetylcholine receptor agonists on receptor activation, arrestin 3 recruitment, and receptor down-regulation. *ACS Chem. Neurosci.* 2010; 1:542–551. [PubMed: 20835371]
154. Davis CN, et al. Differential regulation of muscarinic M1 receptors by orthosteric and allosteric ligands. *BMC Pharmacol.* 2009; 9:14. [PubMed: 19951444]
155. Ma L, et al. Selective activation of the M1 muscarinic acetylcholine receptor achieved by allosteric potentiation. *Proc. Natl. Acad. Sci. U.S.A.* 2009; 106:15950–15955. [PubMed: 19717450]
156. Fisher A, et al. Selective signaling via unique M1 muscarinic agonists. *Ann. N. Y. Acad. Sci.* 1993; 695:300–303. [PubMed: 8239299]
157. Gurwitz D, et al. Discrete activation of transduction pathways associated with acetylcholine M1 receptor by several muscarinic ligands. *Eur. J. Pharmacol.* 1994; 267:21–31. [PubMed: 8206127]
158. Gregory KJ, et al. Identification of orthosteric and allosteric site mutations in M2 muscarinic acetylcholine receptors that contribute to ligand-selective signaling bias. *J. Biol. Chem.* 2010; 285:7459–7474. [PubMed: 20051519]
159. Mitselos A, et al. Delineation of the motilin domain involved in desensitization and internalization of the motilin receptor by using full and partial antagonists. *Biochem. Pharmacol.* 2007; 73:115–124. [PubMed: 17074305]
160. Bradbury FA, et al. G protein independent phosphorylation and internalization of the delta-opioid receptor. *J. Neurochem.* 2009; 109:1526–1535. [PubMed: 19344370]
161. Zhang J, et al. Agonist-specific regulation of delta-opioid receptor trafficking by G protein-coupled receptor kinase and beta-arrestin. *J. Recept. Signal. Transduct. Res.* 1999; 19:301–313. [PubMed: 10071766]
162. Molinari P, et al. Morphine-like opiates selectively antagonize receptor–arrestin interactions. *J. Biol. Chem.* 2010; 285:12522–12535. [PubMed: 20189994]
163. Li JG, et al. Differential regulation of the human kappa opioid receptor by agonists: etorphine and levorphanol reduced dynorphin A-and U50,488H-induced internalization and phosphorylation. *J. Pharmacol. Exp. Ther.* 2003; 305:531–540. [PubMed: 12606694]
164. Bruchas MR, et al. Long-acting kappa opioid antagonists disrupt receptor signaling and produce noncompetitive effects by activating c-Jun N-terminal kinase. *J. Biol. Chem.* 2007; 282:29803–29811. [PubMed: 17702750]
165. Yu Y, et al. mu Opioid receptor phosphorylation, desensitization, and ligand efficacy. *J. Biol. Chem.* 1997; 272:28869–28874. [PubMed: 9360954]
166. Zhang J, et al. Role for G protein-coupled receptor kinase in agonist-specific regulation of mu-opioid receptor responsiveness. *Proc. Natl. Acad. Sci. U.S.A.* 1998; 95:7157–7162. [PubMed: 9618555]
167. Whistler JL, von Zastrow M. Morphine-activated opioid receptors elude desensitization by beta-arrestin. *Proc. Natl. Acad. Sci. U.S.A.* 1998; 95:9914–9919. [PubMed: 9707575]
168. Reversi A, et al. The oxytocin receptor antagonist atosiban inhibits cell growth via a “biased agonist” mechanism. *J. Biol. Chem.* 2005; 280:16311–16318. [PubMed: 15705593]

169. Hoffmann C, et al. Agonist-selective, receptor-specific interaction of human P2Y receptors with beta-arrestin-1 and -2. *J. Biol. Chem.* 2008; 283:30933–30941. [PubMed: 18703513]
170. Velazquez B, et al. Differential agonist-induced desensitization of P2Y2 nucleotide receptors by ATP and UTP. *Mol. Cell Biochem.* 2000; 206:75–89. [PubMed: 10839197]
171. Dupre DJ, et al. Inverse agonist-induced signaling and down-regulation of the platelet-activating factor receptor. *Cell Signal.* 2007; 19:2068–2079. [PubMed: 17609120]
172. Ramachandran R, et al. Agonist-biased signaling via proteinase activated receptor-2: differential activation of calcium and mitogen-activated protein kinase pathways. *Mol. Pharmacol.* 2009; 76:791–801. [PubMed: 19605524]
173. Pauwels PJ, Colpaert FC. Ca²⁺ responses in Chinese hamster ovary-K1 cells demonstrate an atypical pattern of ligand-induced 5-HT1A receptor activation. *J. Pharmacol. Exp. Ther.* 2003; 307:608–614. [PubMed: 12970382]
174. Gettys TW, et al. Selective activation of inhibitory G-protein alpha-subunits by partial agonists of the human 5-HT1A receptor. *Biochemistry.* 1994; 33:4283–4290. [PubMed: 8155646]
175. Akin D, et al. Agonist-directed trafficking explaining the difference between response pattern of naratriptan and sumatriptan in rabbit common carotid artery. *Br. J. Pharmacol.* 2002; 136:171–176. [PubMed: 12010764]
176. Schmid CL, et al. Agonist-directed signaling of the serotonin 2A receptor depends on beta-arrestin-2 interactions *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.* 2008; 105:1079–1084. [PubMed: 18195357]
177. Kurrasch-Orbaugh DM, et al. Serotonin 5-hydroxytryptamine 2A receptor-coupled phospholipase C and phospholipase A2 signaling pathways have different receptor reserves. *J. Pharmacol. Exp. Ther.* 2003; 304:229–237. [PubMed: 12490596]
178. Berry SA, et al. Rapid agonist-induced internalization of the 5-hydroxytryptamine2A receptor occurs via the endosome pathway *in vitro*. *Mol. Pharmacol.* 1996; 50:306–313. [PubMed: 8700138]
179. Bhatnagar A, et al. The dynamin-dependent, arrestin-independent internalization of 5-hydroxytryptamine 2A (5-HT2A) serotonin receptors reveals differential sorting of arrestins and 5-HT2A receptors during endocytosis. *J. Biol. Chem.* 2001; 276:8269–8277. [PubMed: 11069907]
180. Cussac D, et al. Agonist-directed trafficking of signalling at serotonin 5-HT2A, 5-HT2B and 5-HT2C-VSV receptors mediated Gq/11 activation and calcium mobilisation in CHO cells. *Eur. J. Pharmacol.* 2008; 594:32–38. [PubMed: 18703043]
181. Hanley NR, Hensler JG. Mechanisms of ligand-induced desensitization of the 5-hydroxytryptamine_{2A} receptor. *J. Pharmacol. Exp. Ther.* 2002; 300:468–477. [PubMed: 11805206]
182. Newton RA, Elliott JM. Mianserin-induced down-regulation of human 5-hydroxytryptamine_{2A} and 5-hydroxytryptamine_{2C} receptors stably expressed in the human neuroblastoma cell line SH-SY5Y. *J. Neurochem.* 1997; 69:1031–1038. [PubMed: 9282925]
183. Willins DL, et al. Clozapine and other 5-hydroxytryptamine-2A receptor antagonists alter the subcellular distribution of 5-hydroxytryptamine-2A receptors *in vitro* and *in vivo*. *Neuroscience.* 1999; 91:599–606. [PubMed: 10366017]
184. Berg KA, et al. Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: evidence for agonist-directed trafficking of receptor stimulus. *Mol. Pharmacol.* 1998; 54:94–104. [PubMed: 9658194]
185. Stout BD, et al. Rapid desensitization of the serotonin_{2C} receptor system: effector pathway and agonist dependence. *J. Pharmacol. Exp. Ther.* 2002; 302:957–962. [PubMed: 12183652]
186. Werry TD, et al. Characterization of serotonin 5-HT_{2C} receptor signaling to extracellular signal-regulated kinases 1 and 2. *J. Neurochem.* 2005; 93:1603–1615. [PubMed: 15935077]
187. Urban JD, et al. Functional selectivity and classical concepts of quantitative pharmacology. *J. Pharmacol. Exp. Ther.* 2007; 320:1–13. [PubMed: 16803859]
188. Lesche S, et al. Differential effects of octreotide and pasireotide on somatostatin receptor internalization and trafficking *in vitro*. *J. Clin. Endocrinol. Metab.* 2009; 94:654–661. [PubMed: 19001514]

189. Cescato R, et al. Internalization of sst2, sst3, and sst5 receptors: effects of somatostatin agonists and antagonists. *J. Nucl. Med.* 2006; 47:502–511. [PubMed: 16513620]
190. Rihakova L, et al. VRQ397 (CRAVKY): a novel noncompetitive V2 receptor antagonist. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2009; 297:R1009–R1018. [PubMed: 19641130]
191. Rakesh K, et al. beta-Arrestin-biased agonism of the angiotensin receptor induced by mechanical stress. *Sci. Signal.* 2010; 3:ra46. [PubMed: 20530803]
192. Mederos y Schnitzler M, et al. Gq-coupled receptors as mechanosensors mediating myogenic vasoconstriction. *EMBO J.* 2008; 27:3092–3103. [PubMed: 18987636]
193. Kim J, et al. Beta-arrestins regulate atherosclerosis and neointimal hyperplasia by controlling smooth muscle cell proliferation and migration. *Circ. Res.* 2008; 103:70–79. [PubMed: 18519945]
194. Morris GE, et al. G protein-coupled receptor kinase 2 and arrestin2 regulate arterial smooth muscle P2Y-purinoreceptor signalling. *Cardiovasc Res.* 2010
195. Hollingsworth JW, et al. Both hematopoietic- and non-hematopoietic- derived {beta}-arrestin-2 regulates murine allergic airway disease. *AmJRespir. Cell Mol. Biol.* 2009; 43:269–275.
196. Walker JK, et al. beta-Arrestin-2 regulates the development of allergic asthma. *J. Clin. Invest.* 2003; 112:566–574. [PubMed: 12925697]
197. Kuhr FK, et al. beta-Arrestin 2 is required for B1 receptor-dependent post-translational activation of inducible nitric oxide synthase. *FASEB J.* 2010; 24:2475–2483. [PubMed: 20228252]
198. Manson ME, et al. cAMP-mediated regulation of cholesterol accumulation in cystic fibrosis and Niemann-Pick type C cells. *Am. J. Physiol. Lung Cell Mol. Physiol.* 2008; 295:L809–L819. [PubMed: 18790990]
199. Fong AM, et al. Defective lymphocyte chemotaxis in beta-arrestin2- and GRK6-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 2002; 99:7478–7483. [PubMed: 12032308]
200. Kizaki T, et al. beta2-Adrenergic receptor regulates Toll-like receptor-4-induced nuclear factor-kappaB activation through beta-arrestin-2. *Immunology.* 2008; 124:348–356. [PubMed: 18194271]
201. Li Y, et al. Morphine promotes apoptosis via TLR2, and this is negatively regulated by beta-arrestin 2. *Biochem. Biophys. Res. Commun.* 2009; 378:857–861. [PubMed: 19071087]
202. Loniewski K, et al. Toll-like receptors differentially regulate GPCR kinases and arrestins in primary macrophages. *Mol. Immunol.* 2008; 45:2312–2322. [PubMed: 18180038]
203. Mo W, et al. Nuclear beta-arrestin1 functions as a scaffold for the dephosphorylation of STAT1 and moderates the antiviral activity of IFN-gamma. *Mol. Cell.* 2008; 31:695–707. [PubMed: 18775329]
204. Yu MC, et al. An essential function for beta-arrestin 2 in the inhibitory signaling of natural killer cells. *Nat. Immunol.* 2008; 9:898–907. [PubMed: 18604210]
205. Basher F, et al. beta-Arrestin 2: a negative regulator of inflammatory responses in polymorphonuclear leukocytes. *IntJClin. Exp. Med.* 2008; 1:32–41.
206. Shi Y, et al. Critical regulation of CD4+ T cell survival and autoimmunity by beta-arrestin 1. *Nat. Immunol.* 2007; 8:817–824. [PubMed: 17618287]
207. Wang Y, et al. Association of beta-arrestin and TRAF6 negatively regulates Toll-like receptor-interleukin 1 receptor signaling. *Nat. Immunol.* 2006; 7:139–147. [PubMed: 16378096]
208. Fan H, et al. Beta-arrestin 2 negatively regulates sepsis-induced inflammation. *Immunology.* 2010; 130:344–351. [PubMed: 20465566]
209. Porter KJ, et al. Regulation of lipopolysaccharide-induced inflammatory response and endotoxemia by beta-arrestins. *J. Cell Physiol.* 2010; 225:406–416. [PubMed: 20589830]
210. Tsutsui S, et al. Glucocorticoids regulate innate immunity in a model of multiple sclerosis: reciprocal interactions between the A1 adenosine receptor and beta-arrestin-1 in monocytoic cells. *FASEB J.* 2008; 22:786–796. [PubMed: 17965263]
211. DeFea KA. Stop that cell! beta-Arrestin-dependent chemotaxis: a tale of localized actin assembly and receptor desensitization. *Annu. Rev. Physiol.* 2007; 69:535–560. [PubMed: 17002593]
212. Su Y, et al. Altered CXCR2 signaling in beta-arrestin-2-deficient mouse models. *J. Immunol.* 2005; 175:5396–5402. [PubMed: 16210646]

213. Seregin SS, et al. beta-Arrestins modulate adenovirus-vector-induced innate immune responses: differential regulation by beta-arrestin-1 and beta-arrestin-2. *Virus Res.* 2010; 147:123–134. [PubMed: 19896992]
214. Luan B, et al. Deficiency of a beta-arrestin-2 signal complex contributes to insulin resistance. *Nature.* 2009; 457:1146–1149. [PubMed: 19122674]
215. Quoyer J, et al. GLP-1 mediates antiapoptotic effect by phosphorylating Bad through a beta-arrestin 1-mediated ERK1/2 activation in pancreatic beta-cells. *J. Biol. Chem.* 2010; 285:1989–2002. [PubMed: 19915011]
216. Sonoda N, et al. beta-Arrestin-1 mediates glucagon-like peptide-1 signaling to insulin secretion in cultured pancreatic beta cells. *Proc. Natl. Acad. Sci. U.S.A.* 2008; 105:6614–6619. [PubMed: 18445652]
217. Oakley RH, et al. Molecular determinants underlying the formation of stable intracellular G protein-coupled receptor-beta-arrestin complexes after receptor endocytosis*. *J. Biol. Chem.* 2001; 276:19452–19460. [PubMed: 11279203]
218. Phaneuf S, et al. Effect of oxytocin antagonists on the activation of human myometrium *in vitro*: atosiban prevents oxytocin-induced desensitization. *Am. J. Obstet. Gynecol.* 1994; 171:1627–1634. [PubMed: 7802080]
219. Phaneuf S, et al. Oxytocin signalling in human myometrium. *Adv. Exp. Med. Biol.* 1995; 395:453–467. [PubMed: 8713998]
220. Phaneuf S, et al. Loss of myometrial oxytocin receptors during oxytocin-induced and oxytocin-augmented labour. *J. Reprod. Fertil.* 2000; 120:91–97. [PubMed: 11006150]
221. Robinson C, et al. Oxytocin-induced desensitization of the oxytocin receptor. *Am. J. Obstet. Gynecol.* 2003; 188:497–502. [PubMed: 12592262]
222. Yun SP, et al. Interaction of profiling-1 and F-actin via a beta-arrestin-1/JNK signaling pathway involved in prostaglandin E₂-induced human mesenchymal stem cell migration and proliferation. *J. Cell Physiol.* 2010
223. Jacob C, et al. Mast cell tryptase controls paracellular permeability of the intestine. Role of protease-activated receptor 2 and beta-arrestins. *J. Biol. Chem.* 2005; 280:31936–31948. [PubMed: 16027150]
224. Moussa O, et al. Novel role of thromboxane receptors beta isoform in bladder cancer pathogenesis. *Cancer Res.* 2008; 68:4097–4104. [PubMed: 18519668]
225. Fan X, et al. Acute and chronic morphine treatments and morphine withdrawal differentially regulate GRK2 and GRK5 gene expression in rat brain. *Neuropharmacology.* 2002; 43:809–816. [PubMed: 12384166]
226. Fan XL, et al. Differential regulation of beta-arrestin 1 and beta-arrestin 2 gene expression in rat brain by morphine. *Neuroscience.* 2003; 117:383–389. [PubMed: 12614678]
227. Terwilliger RZ, et al. Chronic morphine administration increases beta-adrenergic receptor kinase (beta ARK) levels in the rat locus coeruleus. *J. Neurochem.* 1994; 63:1983–1986. [PubMed: 7931357]
228. Bohn LM, et al. Enhanced rewarding properties of morphine, but not cocaine, in β arrestin-2 knock-out mice. *J. Neurosci.* 2003; 23:10265–10273. [PubMed: 14614085]
229. Beaulieu JM, et al. An Akt/beta-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. *Cell.* 2005; 122:261–273. [PubMed: 16051150]
230. Beaulieu JM, et al. A beta-arrestin 2 signaling complex mediates lithium action on behavior. *Cell.* 2008; 132:125–136. [PubMed: 18191226]
231. Gainetdinov RR, et al. Desensitization of G protein-coupled receptors and neuronal functions. *Annu. Rev. Neurosci.* 2004; 27:107–144. [PubMed: 15217328]
232. Ahmed MR, et al. Haloperidol and clozapine differentially affect the expression of arrestins, receptor kinases, and extracellular signal-regulated kinase activation. *J. Pharmacol. Exp. Ther.* 2008; 325:276–283. [PubMed: 18178904]
233. Amar S, et al. Possible involvement of post-dopamine D2 receptor signalling components in the pathophysiology of schizophrenia. *Int. J. Neuropsychopharmacol.* 2008; 11:197–205. [PubMed: 17681085]

234. Bezard E, et al. L-DOPA reverses the MPTP-induced elevation of the arrestin2 and GRK6 expression and enhanced ERK activation in monkey brain. *Neurobiol. Dis.* 2005; 18:323–335. [PubMed: 15686961]
235. Bychkov ER, et al. Arrestins and two receptor kinases are upregulated in Parkinson's disease with dementia. *Neurobiol. Aging.* 2008; 29:379–396. [PubMed: 17125886]
236. Guigoni C, et al. Pathogenesis of levodopa-induced dyskinesia: focus on D1 and D3 dopamine receptors. *Parkinsonism Relat. Disord.* 2005; 11(Suppl 1):S25–S29. [PubMed: 15885624]
237. Chen J, et al. Dopamine promotes striatal neuronal apoptotic death via ERK signaling cascades. *Eur. J. Neurosci.* 2009; 29:287–306. [PubMed: 19200235]
238. Bruchas MR, et al. Stress-induced p38 mitogen-activated protein kinase activation mediates kappa-opioid-dependent dysphoria. *J. Neurosci.* 2007; 27:11614–11623. [PubMed: 17959804]
239. Bruchas MR, et al. Kappa opioid receptor activation of p38 MAPK is GRK3- and arrestin-dependent in neurons and astrocytes. *J. Biol. Chem.* 2006; 281:18081–18089. [PubMed: 16648139]
240. Bjork K, et al. Modulation of voluntary ethanol consumption by beta-arrestin 2. *FASEB J.* 2008; 22:2552–2560. [PubMed: 18367649]
241. Rimondini R, et al. Long-lasting increase in voluntary ethanol consumption and transcriptional regulation in the rat brain after intermittent exposure to alcohol. *FASEB J.* 2002; 16:27–35. [PubMed: 11772933]
242. Correll JA, et al. Nicotine sensitization and analysis of brain-derived neurotrophic factor in adolescent beta-arrestin-2 knockout mice. *Synapse.* 2009; 63:510–519. [PubMed: 19224602]
243. Sun D, et al. Beta-arrestins 1 and 2 are associated with nicotine dependence in European American smokers. *Mol. Psychiatry.* 2008; 13:398–406. [PubMed: 17579607]
244. Wang Q, et al. Spinophilin blocks arrestin actions *in vitro* and *in vivo* at G protein-coupled receptors. *Science.* 2004; 304:1940–1944. [PubMed: 15218143]

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Figure 1.

7TMR signaling and regulation by the GRKs and β -arrestins. (a) Agonist-stimulated, 7TMR-mediated G protein activation. (b) Subsequent desensitization of 7TMR-mediated G protein signaling and activation of β -arrestin-mediated signals. (c) β -Arrestin-mediated 7TMR receptor internalization involving clathrin and AP-2.

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Figure 2.

Differential 7TMR-stimulated G protein- and β -arrestin-mediated signaling. (a) Traditional agonist with linear efficacy. (b) Traditional antagonist, blocking all aspects of 7TMR signaling. (c) G protein-biased ligand, promoting 7TMR G protein signaling in the absence of β -arrestin-mediated desensitization, internalization and signaling. (d) β -Arrestin-biased ligand, promoting β -arrestin-mediated signaling and internalization in the absence of G protein activation.

Table 1

7TMR ligands showing β -arrestin or G protein bias. For effects that are merely consistent with bias, ligands are in italics

Receptor	Biased ligands	Bias	Reference(s)
A3A adenosine	CCPA, DPMA, MRS542, MRS1760	β -Arrestin	[105]
	DBXRM	G protein	[105]
<i>APJ (Apelin)</i>	<i>Apelin 13, Apelin 36</i>	<i>Differential receptor trafficking</i>	[106]
AT ₁	Sar1,Ile4,Ile8 Ang II (SII), Sar1,Lys5,Ala8 Ang II (TRV120023)	β -arrestin	[61–64,66,67,69,107–114]
	Sar1,Tyr5,Pro7-NH2 Ang II (TRV120026)		
	Sar1,D-Ala8 Ang II (TRV120027), troglitazone		
α_{2A} -Adrenergic	<i>Clonidine, guanfacine</i>	<i>Differential signaling, regulation and trafficking</i>	[115]
	<i>Multiple ligands</i>	<i>Differential G protein signaling</i>	[116]
β_1 -Adrenergic	Alprenolol, carvedilol	β -Arrestin	[57]
β_2 -Adrenergic	Carvedilol, ICI118551, propranolol cyclophenylbutanephine	β -Arrestin	[58,59,117]
	Norepinephrin	G protein	[118]
<i>Bombesin</i>	<i>[D-Arg1,D-Phe5,D-Trp7,9,Leu11] substance P</i>	<i>Differential signaling</i>	[119]
<i>CB₁</i>	<i>Δ^9-Tetrahydrocannabinol</i>	<i>Differential tolerance (WT vs β-arrestin2 KO mice)</i>	[120]
	<i>Δ^9-Tetrahydrocannabinol, anandamide, WIN 55,212-2, CP 55940, 2-arachidonoyl glycerol</i>	<i>Differential receptor trafficking</i>	[121]
CCK _A	D-Tyr-Gly-([Nle ^{28,31} ,D-Trp ³⁰]CCK-26–32)-phenethyl ester	<i>Internalizing antagonist</i>	[122]
CCR2	GMME1	G protein	[123]
CCR5	<i>AOP-RANTES, CAP-RANTES, MET-RANTES, NNY-RANTES, PSC-RANTES</i>	<i>Differential internalization and trafficking</i>	[42–44,124,125]
	<i>Monoclonal antibody MC-1</i>	<i>Internalizing antagonist</i>	[126]
	<i>Monoclonal antibody MC-4</i>	<i>Allosteric blocker of internalization</i>	[126]
CCR7	CCL19, CCL21	<i>G protein, differential GRK</i>	[127–129]
<i>CTR2</i>	<i>Multiple ligands</i>	<i>Reversal of potency</i>	[130]
<i>CXCR2</i>	<i>IL-8, GROα</i>	<i>Reversal of potency</i>	[131]
<i>CXCR4</i>	<i>ASLW</i>	<i>G protein (allosteric)</i>	[132]
CRTH2	1-(4-Ethoxyphenyl)-5-methoxy-2-methylindole-3-carboxylic acid, <i>N_α</i> -tosyltryptophan	G protein (allosteric)	[133]
<i>D₁ dopamine</i>	<i>Multiple ligands</i>	<i>G protein, desensitization and internalization</i>	[134–136]
<i>D₂ dopamine</i>	<i>Multiple ligands</i>	Block D ₂ stimulated β -arrestin recruitment	[137,138]
	<i>Dihydropyridine (DHX), N-n-propyl-DHX</i>	<i>Differential signaling</i>	[139,140]
EDG1	FTY720 (fingolimod)	β -Arrestin	[141,142]
EDG3	FTY720-P (<i>in vivo</i> activated FTY720)	G protein	[142,143]
EP4	Multiple ligands	β -Arrestin and G protein	[144]
<i>ET_AR</i>	<i>BQ123</i>	<i>Internalizing antagonist</i>	[145]
FSH	Multiple ligands	β -Arrestin and G protein	[146,147]
GLP-1	Oxyntomodulin, glucagon	G protein	[148]

Receptor	Biased ligands	Bias	Reference(s)
<i>GnRH</i>	<i>Novo Nordisk compound 2 (6,7-dichloro2-methylsulfonyl-3-tert-butylaminoquinoxaline), quercetin</i>	<i>Allosteric ligands promote differential signaling</i>	[149]
	<i>Multiple ligands</i>	<i>Internalizing antagonists, altered trafficking and differential signaling</i>	[150–152]
GPR109a	<i>Multiple ligands</i>	<i>Reduced internalization</i>	[74]
<i>M₁ muscarinic</i>	AC260584, TBPB	Allosteric G protein	[153]
	<i>AC-42, AC-260584, N-desmethylozapine, xanomeline, BQCA</i>	<i>Allosteric ligands promote differential signaling and receptor trafficking</i>	[154,155]
<i>M₂ muscarinic</i>	<i>AF102B, AF150, AF151, pilocarpine</i>	<i>Differential signaling</i>	[156,157]
	<i>Multiple ligands</i>	<i>Allosteric ligands promote differential signaling</i>	[158]
<i>Motilin (GPR38)</i>	<i>ABT-229</i>	<i>Differential receptor trafficking</i>	[159]
δ -OR	TAN-67, morphine	G protein	[158,160–162]
κ -OR	Etorphine, levorphanol	<i>G protein</i>	[163]
	<i>norBNI, JDTic</i>	<i>Differential signaling</i>	[164]
μ -OR	Morphine, heroin	G protein	[20,161,162,165–167]
<i>OX₁</i>	<i>Atosiban</i>	<i>Differential signaling</i>	[168]
P2Y ₂	ATP, UTP	<i>Differential β-arrestin trafficking</i>	[169,170]
<i>PAF</i>	<i>WEB2086</i>	<i>Internalizing antagonist</i>	[171]
PAR-2	rPAR2-Ala ^{37–38} , rPAR2-Leu ³⁷ Ser ³⁸ (receptor-tethered mutant ligands)	G protein	[172]
PTH1	(D-Trp ¹² , Tyr ³⁴)-PTH(7–34)	β -Arrestin	[90,91]
	[Trp ¹]PTHrp-(1–36)	G protein	[89,90]
<i>5-HT_{1A}</i>	<i>Ipsaperone, F14,679, flesinoxan</i>	<i>Differential signaling</i>	[173,174]
<i>5-HT_{1B}</i>	<i>Naratriptan</i>	<i>Differential signaling</i>	[175]
<i>5-HT_{2A}</i>	<i>Multiple ligands</i>	<i>Internalizing antagonist and differential signaling</i>	[176–184]
<i>5-HT_{2C}</i>	<i>Multiple ligands</i>	<i>Differential signaling</i>	[184–187]
<i>SST2</i>	Pasireotide (SOM230)	G protein	[188]
<i>SST5</i>	<i>KE108, BIM-23244, L-817,818</i>	<i>Reduced internalization</i>	[189]
V2 vasopressin	MCF14, MCF18, MCF57	G protein and chaperones that modulate trafficking	[37]
	<i>SR49059</i>	<i>Chaperone that modulates trafficking</i>	[36]
	<i>VRQ397 (CRA VKY)</i>	<i>Allosteric ligand promoting differential signaling</i>	[190]

Table 2Physiology and pathophysiology of β -arrestin-mediated processes

Therapeutic area	β -Arrestin-mediated process (isoform)	Function	References
Cardiovascular	AT ₁ R-stimulated increase in cardiac performance and preserved stroke volume	Signaling	[64]
	AT ₁ R-stimulated cardiomyocyte growth or hypertrophy, proliferation and contractility	Signaling	[60–63,114]
	AT ₁ R-stimulated vascular smooth-muscle-cell protein synthesis and antiapoptosis	Signaling	[65,66]
	AT ₁ R-stimulated salt appetite	Signaling	[70]
	A G protein-uncoupled AT ₁ R mutant stimulated cardiac hypertrophy	Signaling	[60]
	Mechanical stress induces AT ₁ R-stimulated β -arrestin recruitment and signaling	Signaling	[191,192]
	Differential effects on experimental vascular injury (1 and 2)	Signaling	[193]
	Cardiac β AR desensitization (1)	Desensitization	[12]
	β_1 AR-stimulated EGFR transactivation and cardioprotection (1 and 2)	Signaling	[52]
	β -Arrestin1 regulates vascular smooth muscle P2Y-purinoreceptor signaling	Desensitization	[194]
Renal	AT ₁ R-stimulated adrenocortical aldosterone secretion (1)	Signaling	[67]
	cNDI and the nephrogenic syndrome of inappropriate antidiuresis are associated with V2R mutations that alter receptor and β -arrestin dynamics, signaling, desensitization and trafficking	Desensitization and trafficking	[34–38]
Pulmonary	β -Arrestin2 KO mice are resistant to airway inflammation and airway smooth-muscle hyperactivity associated with experimental allergic asthma	Signaling	[195,196]
	Airway smooth-muscle cell and bronchial β_2 AR desensitization (1 and 2)	Desensitization	[18,19]
	β -Arrestin2 involved in B1-bradykinin-stimulated iNOS activation in human lung microvascular endothelial cells	Signaling	[197]
	Significant β -arrestin2 upregulation in a cellular model of cystic fibrosis (2)	Unknown	[198]
Rheumatology	Chemokine-stimulated leukocyte chemotaxis involves β -arrestins	Signaling	[199]
and immunology	Toll-like receptors involved in innate immunity signaling through β -arrestins	Signaling	[200–202]
	Knockdown of β -arrestin1 enhances IFN- γ -induced antiviral response (1)	Signaling	[203]
	β -Arrestin2 KO mice show decreased susceptibility to mouse CMV infection	Signaling	[204]
	β -Arrestins play important roles in the regulation of neutrophils, natural killer cells and lymphocytes	Signaling and desensitization	[204–206]
	β -Arrestin2 KO mice show greater inflammatory responses and are more susceptible to endotoxin-mediated shock (2)	Desensitization	[207]
	β -Arrestin2 negatively regulates the inflammatory response to microbial sepsis	Unknown	[208]
	β -Arrestin KO mice are protected from LPS stimulated TLR4-mediated endotoxic shock and lethality (1 and 2)	Signaling	[209]
	CCR2-stimulated β -arrestin-mediated signaling is a mediator of inflammation in a mouse model of arthritis	Signaling	[123]

Therapeutic area	β -Arrestin-mediated process (isoform)	Function	References
Endocrinology <i>Metabolic</i>	β -Arrestin1 expression of is increased in an animal model of multiple sclerosis (MS) and MS patients	Unknown	[210]
	A C-terminal truncation mutation of CXCR4 affects receptor signaling and β -arrestin-mediated regulation and is associated with WHIM syndrome	Desensitization and trafficking	[39]
	Defective chemotaxis observed in lymphocytes from β -arrestin2 KO mice	Signaling	[199,211]
	Significant increase in neutrophil chemotaxis and wound re-epithelialization in β -arrestin2 KO mice, which is thought to involve CXCR2	Desensitization	[212]
	Differential regulation of adenovirus-vector-induced innate immune responses	Unknown	[213]
	The niacin-induced flushing response is dramatically reduced in β -arrestin1 KO mice whereas the beneficial lipolytic effects remain intact	Signaling	[73]
	β -Arrestin2 is down-regulated in both <i>db/db</i> and high-fat diet mouse models of type 2 diabetes	Unknown	[214]
	β -Arrestin2 KO mice exhibit significant insulin resistance, whereas mice overexpressing	Signaling	[214]
	β -arrestin2 show improved glucose metabolism and insulin sensitivity		
	GLP-1 receptor-stimulated pancreatic β -cell insulin secretion, as well as ERK activation and associated antiapoptotic signals, involve β -arrestin1	Signaling	[215,216]
Endocrinology <i>Bone mineral homeostasis</i>	β -Arrestin2 plays a role in regulating PTH-receptor-mediated signaling, with effects on bone formation and resorption, as well as related gene expression	Signaling	[79–90]
Endocrinology <i>Reproductive</i>	The β -arrestin-biased ligand (D-Trp12,Tyr34)-PTH(7–34) promotes anabolic bone formation in the absence of bone resorption, and this activity is absent in β -arrestin2 KO mice	Signaling	[91]
	The use of oxytocin to induce or augment labor is often limited by tachyphylaxis, and oxytocin receptor regulation is thought to involve β -arrestin	Desensitization	[217–221]
	β -Arrestin1 involved in prostaglandin E2-stimulated human mesenchymal stem cell growth	Signaling	[222]
Gastrointestinal	GnRH receptor β -arrestin-biased ligands have potent antiproliferative activities	Signaling	[152]
	PAR2-receptor-mediated β -arrestin-signaling provides a mechanistic link between inflammation and stress-induced alterations in colonic permeability	Signaling	[223]
Oncology	β -Arrestin2 KO mice are resistant to the effects of morphine and loperamide (Imodium®) on fecal bolus accumulation	Signaling	[92,93]
	Elevated β -arrestin1 mRNA levels have been observed in multiple tumorigenic cancer cell lines, and elevated β -arrestin2 mRNA has been observed in advanced breast cancer tumors	Unknown	[94]
	β -Arrestin1 plays a role in nicotine induced proliferation of non-small-cell lung cancer cells	Signaling	[95]
	Prostaglandin E2-stimulated EP2- and EP4-receptor-mediated transactivation of EGFR uses β -arrestin1, and is involved in mouse skin papilloma development and colorectal carcinoma cell migration and metastasis	Signaling	[96,97]
	β -Arrestins mediate ET _A -receptor-stimulated EGFR transactivation in ovarian cancer cells, which is thought to be involved in both cell invasion and metastasis	Signaling	[98,99]
	Knockdown of either β -arrestin1 or -2 reduces LPA-stimulated cancer cell migration and invasion, and transgenic	Signaling	[100,102]

Therapeutic area	β -Arrestin-mediated process (isoform)	Function	References
Neurology and behavior <i>Opioids</i>	overexpression of β -arrestin1 promotes tumor angiogenesis and progression		
	TGF- β inhibits epithelial and cancer cell migration via β -arrestin2-mediated activation of CDC42	Signaling	[101]
	β -Arrestin2 is involved in thromboxane-receptor- β -stimulated bladder cancer cell migration	Signaling	[224]
	β -Arrestin2 KO mice show increased tumor growth and metastasis in an experimental model of lung cancer	Unknown	[102]
	In androgen-dependent prostate-cancer cells, β -arrestin2 acts as an androgen receptor corepressor	Desensitization	[103]
	β -Arrestin2 KO mice experience enhanced and prolonged morphine-induced analgesia and are resistant to the development of chronic morphine-induced tolerance	Desensitization	[20–22]
	β -Arrestin2 KO mice are resistant to morphine-induced respiratory suppression and constipation	Signaling	[7,92,93]
	β -Arrestin2 knockdown <i>in vivo</i> with either antisense or siRNA results in reduced tolerance, whereas overexpression in the brain attenuates morphine analgesia	Desensitization	[23–25]
	Chronic morphine treatment affects β -arrestin and GRK expression in the brain	Unknown	[225–227]
	β -Arrestin2 is involved in limiting opioid-induced reward	Unknown	[228]
Neurology and behavior <i>Dopamine</i>	D2 dopamine receptor signals via a β -arrestin2-scaffolded complex of protein phosphatase 2A and Akt, acting as a positive mediator of dopamine-associated signals and behavior	Signaling	[229]
	A common feature of clinically effective antipsychotics, acting at D2 dopamine receptors, is their ability to block D2-receptor-stimulated β -arrestin recruitment	Desensitization	[137,138]
	The antidepressant effects of lithium involve disruption of a β -arrestin2 signaling complex	Signaling	[230]
	β -Arrestin2 KO mice are resistant to both direct or indirect dopamine-receptor-stimulated locomotor sensitization	Signaling	[229,231]
	Changes in central β -arrestin and GRK expression are observed in several neural and behavioral disorders	Unknown	[232–236]
	Data suggest that D1 dopamine-receptor-stimulated increases in striatal neuron apoptosis involve β -arrestin	Signaling	[237]
	Studies suggest that κ -OR dysphoria involves GRK3 and β -arrestin, which can be differentiated from κ -OR-stimulated analgesic effects	Signaling	[238,239]
<i>Other effects</i>	β -Arrestin2 KO mice show enhanced Δ^9 -tetrahydrocannabinol-stimulated CB ₁ cannabinoid-receptor-mediated analgesic and hypothermic responses	Desensitization	[120]
	β -Arrestin2 plays a role in alcohol exposure, consumption and reward	Unknown	[240,241]
	β -Arrestin2 plays a positive role in nicotine sensitization and dependence	Unknown	[242,243]
	β -Arrestin2 plays a role in 5-HT _{2A} -receptor-stimulated hallucinations	Signaling	[176]
	β -Arrestin2 KO mice are resistant to α ₂ -AR-stimulated sedation	Signaling	[244]