Published in final edited form as:
Pharmacol Ther. 2009 March ; 121(3): 285–293. doi:10.1016/j.pharmthera.2008.11.005.

Physiological and Pharmacological Implications of Beta-Arrestin Regulation

Cullen L. Schmid and **Laura M. Bohn**
The Ohio State University College of Medicine, Department of Pharmacology and Neuroscience Graduate Studies Program, Columbus, OH, 43210

Abstract

G protein-coupled receptor-targeted drug discovery as well as "compound reassessment" requires the utilization of diverse screens to determine agonist efficacies and potencies beyond the scope of ligand binding and G protein coupling. Such efforts have arisen from extensive studies, both in cellular and animal models, demonstrating that these seven transmembrane domain-spanning, G protein-coupled receptors may engage in more diverse functions than their name suggests and particular focus is drawn to their interactions with beta-arrestins (βarrestins). As regulators, βarrestins are involved in dampening G protein-coupling pathways. βArrestins can also play pro-signaling roles in receptor mediated events and the coupling of receptors to βarrestins may be as important as their potential to couple to G proteins in the physiological setting. In the last decade, the development of βarrestin deficient mouse models has allowed for the assessment of the contribution of individual βarrestins to receptor function in vivo. This review will discuss the current literature that implicates βarrestins in receptor function in respect to physiological and behavioral responses observed in the live animal model.

Keywords

G protein coupled receptors; Seven transmembrane spanning receptors; GRK; beta-arrestin; functional selectivity; knockout mouse models; drug discovery; receptor regulation; physiology

1. Introduction

βArrestins (non-visual arrestins) are ubiquitously expressed proteins that were first described for their role in desensitizing G protein-coupled receptors (GPCRs). There are two βarrestins, namely, βarrestin1 and βarrestin2, which are also referred to as arrestin-2 and arrestin-3, respectively. As their names imply, βarrestins were first identified for their ability to "arrest" agonist-stimulated β2 adrenergic receptor (β2AR) signaling (Lohse, et al., 1990) in a manner similar to arrestin regulation of rhodopsin. The canonical model of GPCR regulation by βarrestins also involves GPCR kinases (GRKs) which phosphorylate receptors and thereby serve to facilitate receptor-βarrestin interactions (Benovic et al., 1987; Sibley et al., 1987; Lohse, et al., 1992; Pitcher et al., 1992). Upon complexing with receptors, βarrestins can serve as inhibitors of signal transduction by preventing further receptor coupling to G protein

Correspondence: Laura M. Bohn, PhD, Associate Professor of Pharmacology, The Ohio State University College of Medicine, 460 W 12th Ave, 798 BRT, Columbus, OH 43210, Laura.Bohn@osumc.edu, 614-292-1303.

signaling cascades (for reviews see: Premont et al., 1995; Freedman and Lefkowitz 1996; Lefkowitz, 1998).

Specific examples of βarrestins serving as negative regulators of GPCR signaling are plentiful in cellular as well as animal model systems (Table 1) (for reviews see: Gainetdinov et al., 2004;Bohn et al., 2004a;Premont and Gainetdinov, 2007;Gurevich and Gurevich 2006). In addition to mediating receptor desensitization, βarrestins can facilitate recruitment and interactions between GPCRs and signaling partners. In this capacity, βarrestins can serve as positive mediators of receptor signaling to downstream targets. Evidence for GPCRs coupling to βarrestins to transduce receptor signaling has also been widely demonstrated in cellular models (for reviews see: Luttrell et al., 1999;Luttrell, 2002;Lefkowitz and Shenoy, 2005;DeWire et al., 2007). Studies in mouse models also support a pro-signaling role for βarrestins (particularly βarrestin2) and these reports are summarized in Table 2.

Arguably, the most studied GPCR is the β2AR. In vitro studies with this receptor have been instrumental in demonstrating the diverse and pleiotropic roles that βarrestins can play in determining agonist-induced receptor responses. The β2AR has been shown to interact with both βarrestin1 and βarrestin2 upon agonist stimulation (Attramadal et al., 1992) and such interactions result in decreased responsiveness to agonist over time. The removal of βarrestins by early anti-sense studies (Mundell et al., 1999), later siRNA studies (Ahn et al., 2003), as well as studies utilizing mouse embryonic fibroblasts devoid of both βarrestin1 and βarrestin2 (Kohout et al., 2001), confirm that βarrestins play a critical role in promoting this waning effect on G protein-coupling and adenylyl cyclase stimulation following agonist activation of the β2AR. Similar studies have been performed for multiple GPCRs of diverse classes and together, these findings support the canonical model wherein the agonist-activated GPCR becomes phosphorylated by GRKs which subsequently increases the binding affinity of the receptor for βarrestins.

βArrestin interactions with activated GPCRs can be detected by co-immunoprecipitation (Groer et al., 2007), confocal microscopy (Barak et al., 1997), bioluminescence resonance energy transfer (BRET) (Hamdan et al., 2005), and fluorescence resonance energy transfer (FRET) (Drake et al., 2008) assays. Such developments, including enzyme complementation assays (von Degenfeld et al., 2007), have facilitated high throughput screens for assessing drug-induced βarrestin-receptor interactions. Looking forward, the interactions between βarrestins and GPCRs may be realized for ultimately determining relative drug efficacies in vivo (Claing and Laporte, 2005; Violin and Lefkowitz, 2007; DeWire et al., 2007).

2. βArrestin Regulation of GPCRs *in vivo*

While cellular model systems have been particularly useful for determining which receptors can possibly interact with βarrestins, in many cases, the question remains as to whether such interactions are pharmacologically and physiologically relevant. Assessing βarrestin function in vivo is challenging as there are no selective inhibitors of βarrestins. Some attempts have been made to develop selective inhibitors to GRKs as a means to prevent subsequent βarrestin recruitment, yet the degree of selectivity for these kinase inhibitors may not exclude inhibition of other serine/threonine kinases involved in alternate signaling cascades.

To overcome these limitations, Drs. Robert J. Lefkowitz and Marc G. Caron of Duke University, undertook the challenge of generating gene knockout mice deficient in βarrestin2. At that same time, the βarrestin1 knockout (βarr1-KO) mouse was generated in the Harvard laboratories of Drs. Jonathan Seidman and Christine Seidman. Five tables included within this review summarize the baseline (Table 4) and drug-induced behavioral and physiological alterations induced by ablating either βarrestin1 or βarrestin2 in the mouse (Table 1–Table 5). Of note, attempts to generate a mouse lacking both βarrestins by crossing heterozygotes of

each genotype were not successful as the double deletion proved to be embryonically lethal (Kohout et al., 2001).

3. βArrestin1 Knockout Mice

3.1 β2 Adrenergic Receptor Regulation

Given the considerable evidence demonstrating βarrestin-mediated regulation of the β2AR, it is not surprising that the initial studies were focused on unveiling a cardiac phenotype in these animals. While the βarr1-KO mice present no overt gross phenotypes, they do display an altered response to β2AR agonist challenge (Conner et al., 1997). In this study, heart rates and ejection fractions between anesthetized wildtype (WT) and βarr1-KO mice were not different, as assessed by echocardiography; however treatment with isoproterenol produced significantly greater increases in cardiac ejection fraction in those mice lacking βarrestin1, while heart rates increased to a similar extent in both genotypes. These results are consistent with a role of βarrestin1 in β2AR desensitization, since receptor signaling was enhanced in its absence.

3.1.1. Neointimal Hyperplasia—Mice lacking βarrestin1 (which were originally generated on a Black Swiss X 129 SvJ background (Conner et al., 1997) and are currently maintained on a congenic C57Bl/6 mouse background), have also been shown to display enhanced neointimal hyperplasia (Kim et al., 2008). Neointimal hyperplasia refers to smooth muscle cell proliferation and migration in the innermost layer of the carotid artery. It is a component of atherosclerosis and in this paradigm, it is initiated by endothelial denudation (scraping the endothelium of the artery with a guidewire). In ex vivo preparations of the carotid artery, there is enhanced neointimal hyperplasia and smooth muscle cell proliferation in the absence of βarrestin1 compared to WT mice. These studies suggest that βarrestin1 expression attenuates reendothelialization of the carotid artery. While a single receptor is not directly implicated as the target of βarrestin1 regulation in this response, the authors propose a model involving βarrestin1-mediated attenuation of mitogenic signaling evoked through activation of lysophosphatidic acid, protease-activated and sphingosine-1-phosphate receptors.

3.1.2. Normal Responses in βArr1-KO Mice—In these published reports involving βarr1-KO mice, βarrestin1 is implicated as a negative regulator of receptor signaling. As such, removal of the negative regulator enhances the physiological response mediated by the receptor. It is somewhat surprising that there are not more studies demonstrating βarrestin1 regulation of behavioral responses. However, βarrestin1 is not alone in regulating receptors as βarrestin2 may serve to compensate somewhat for its absence. Unfortunately, a lack of phenotypic variation in a knockout mouse model rarely earns publication; a list of publications wherein no changes in physiological responses were observed has been included in Table 3. While it may be tempting to conclude that the limited phenotypes produced by the deletion of βarrestin1 might reflect a lesser role for βarrestin1 in GPCR regulation, it may simply indicate that these animals require further evaluation and more widespread physiological, behavioral and therapeutic challenges to more fully reveal the function of this regulator. Of note, since this is an early knockout mouse line, the full genomic sequence was not known at the time of the construct development and therefore, genotyping of this particular line is difficult as PCR primers are not available. The difficulty in genotyping may also contribute to the limited profile of phenotypes.

4. βArrestin2 Knockout Mice

The original colony of βarrestin2 knockout (βarr2-KO) mice was generated in a C57Bl/6 X 129 SvJ mixed mouse strain (Bohn et al., 1999) and has subsequently been backcrossed onto a congenic C57Bl/6 strain. Both the original mixed strain and the backcrossed strain are used in current studies. There are no overt gross phenotypes that distinguish βarr2-KO mice from

their WT counterparts. However, there are subtle differences between the two genotypes that have been found upon closer evaluation of physical properties in the absence of pharmacological challenge and these include altered bone absorption, body mass, nociceptive response latencies in a warm-water tail immersion assay, and locomotor activity (Ferrari et al., 2005; Bohn et al., 2002; 2003; Table 4).

4.1 βarrestin2 as a Negative Regulator of GPCR Signaling

4.1.1. Mu Opioid Receptors

4.1.1.1. Morphine-Induced Antinociception: CNS: The first reported behavioral responses in the βarr2-KO mice were revealed when mice were challenged with the potent opioid analgesic, morphine. Upon challenge with morphine, βarr2-KO mice display enhanced and prolonged response latencies in the hot plate test (Bohn et al., 1999), a classical measure of a nociceptive response to thermal stimuli (Heinricher and Morgan, 1999). Morphine mediates most of its physiological effects by activation of the mu opioid receptor (MOR) as demonstrated by a number of studies using MOR-KO mice (for reviews see:Kieffer, 1999; Kieffer and Gavériaux-Ruff, 2002).

4.1.1.1.1. Biochemical Correlations: As negative regulators, βarrestins contribute to the desensitization of GPCRs in respect to G protein-coupling (Premont et al., 1995). Biochemical analysis reveals that MOR coupling to G proteins in brain regions associated with morphine effects on MOR-mediated pain processing, such as periaqueductal grey area and brainstem, are significantly elevated in the βarrestin2-KO mice as compared to the WT littermates (Bohn et al. 1999; 2000). Collectively these findings indicate a negative regulatory role for βarrestin2 at the MOR as removal of βarrestin2 enhances MOR function.

4.1.1.2. Morphine-Induced Antinociceptive Tolerance: CNS: Following repeated administration, morphine loses therapeutic efficacy in a condition referred to as opiate tolerance. Morphine tolerance can be seen in WT mice following a single high dose of morphine, chronic daily doses of morphine or continuous infusions of the drug via time release pellets. However, none of these routes of morphine administration produce morphine tolerance in mice lacking βarrestin2 (Bohn et al., 2000). Therefore, in addition to revealing enhanced MOR function in response to morphine, βarr2-KO mice do not “desensitize” to the antinociceptive benefits of morphine over time.

4.1.1.2.1. Biochemical Correlations: Removal of βarrestins in cellular cultures delays the onset of receptor desensitization and amplifies maximal coupling efficacies (Kohout et al., 2001). This phenomenon is also observed in vivo for the MOR. Upon chronic activation via repeated or continuous administration of morphine, the MOR expressed in brain regions associated with pain perception, becomes desensitized in WT animals and this has been correlated with the onset of antinociceptive tolerance in response to subsequent morphine challenges. Mice lacking βarrestin2 do not display morphine-induced antinociceptive tolerance in the hot plate nociceptive test and the MOR retains its ability to couple to G proteins in periaqueductal grey area and brainstem (Bohn et al., 2000). Collectively these studies support a negative regulatory role for βarrestin2 in desensitizing the morphine-bound MOR as an important step in morphine-induced antinociceptive tolerance.

4.1.1.3. Morphine-Induced Antinociception: Spinal Cord: While the hot plate analgesia test is used to assess CNS processing of pain perception, the tail flick test is used to model spinal reflexes to painful stimuli (LeBars et al., 1976; Yaksh, 1997). In βarr2-KO mice, morphine-induced antinociception assessed by the tail flick test is enhanced and prolonged (Bohn et al., 2002), results which are similar to the observations made in the hot plate studies. Similarly, selective knockdown of βarrestin2 in rats via treatment with intrathecal injections of βarrestin2

antisense mRNA produces enhanced responses to morphine in tail-flick nociceptive assays (Przewlocka et al., 2002). These results further demonstrate the role of βarrestin2 in "negatively" regulating the MOR.

4.1.1.3. Morphine-Induced Antinociceptive Tolerance: Spinal Cord: By the seventh day of chronic morphine treatment, the βarr2-KO mice develop some tolerance to the analgesic properties of morphine as assessed by the tail flick test, though their sensitivity to morphine remains 2-fold greater than that observed in the WT mice receiving the same treatment regimen (Bohn et al., 2002). Therefore in the tail-flick nociceptive assay, in contrast to the hot-plate studies, the βarr2-KO mice experience an attenuated tolerance to morphine which is delayed in its onset compared to WT mice. These studies suggest that βarrestin2 contributes significantly to the adaptations underlying morphine tolerance in the tail flick antinociceptive response, yet it is not the sole regulator determining this response potential. Interestingly, the remaining antinociceptive tolerance in the βarr2-KO mice displayed in the tail flick test could be reversed by a systemic injection of chelerythrine, a commonly used protein kinase C (PKC) inhibitor. Therefore, in the absence of βarrestin2-mediated desensitization mechanisms, the contribution of PKC is emphasized. Taken together, these findings demonstrate a significant role for βarrestin2 in mediating morphine tolerance in the tail flick test and highlight the importance of PKC regulation of MOR in the spinal cord.

4.1.1.5. Other Morphine-Induced Physiological Effects: The differential regulation of the MOR in the CNS versus the spinal cord following morphine treatment, as assessed by the hot-plate and tail flick analgesic tests, suggests that βarrestin2 may differentially impact MOR signaling depending on the site of action of the drug in vivo. The cellular environment in which the receptor is expressed may therefore ultimately determine the role that βarrestins play in regulating a particular receptor (i.e. the deletion of βarrestin2 has a different impact on MOR regulation in the brain vs. the spinal cord). The importance of the receptor's cellular environment was further echoed when other morphine-induced behaviors and physiologies were monitored in the βarrestin2-KO mice. While some behaviors are enhanced in the βarr2-KO mice following morphine treatment, such as striatal dopamine release, drug reinforcement and hypothermia, other behaviors, such as physical dependence, are seemingly not affected by the loss of βarrestin2 (Bohn et al., 1999, 2000, 2003). Intriguingly, some responses induced by morphine are actually diminished, such as constipation, respiratory suppression and hyperlocomotor activity (Bohn et al., 2003; Raehal et al., 2005). A decrease in morphine-induced behaviors might suggest that βarrestin2 could be playing a pro-signaling role for the MOR; however a direct demonstration of this relationship has yet to be shown in vivo. More direct evidence of βarrestin2 function as a signaling facilitator has been reported and will be discussed.

4.1.1.5.1. Morphine-Induced Constipation: Morphine delays gastrointestinal transit in mice; however, βarr2-KO mice display greatly attenuated constipation following an acute dose of the drug. Further, morphine injected directly into the intracerebroventricular space delays gastrointestinal transit to a similar extent in both WT and βarr2-KO mice (Bohn and Raehal, 2006), while a systemic injection of loperamide, the peripherally restricted MOR agonist, delays transit times only in the WT mice (Raehal et al., 2005). Though the cellular signaling has not yet been elucidated to directly demonstrate βarrestin2 function in the enteric nervous system neurons of the gastrointestinal tract, the current behavioral evidence suggests that βarrestin2 may be playing a different role in regulating MOR in this population than in the neurons of the CNS. Most interestingly, it appears that βarrestin2 may play a pro-signaling role in peripheral MOR regulation while it does not seem to effect the CNS contribution of the MOR to regulation of gastrointestinal function.

4.1.1.5.2. Morphine Impacts on Dopaminergic Responses: The reinforcing properties of morphine are enhanced in the absence of βarrestin2 (Bohn et al., 2003) as demonstrated by conditioned place preference assays. The reinforcing properties of morphine have been shown are correlated with the drug's ability to increase striatal dopamine release which then activates of mesolimbic dopamine receptors. Accordingly, morphine induces more striatal dopamine release in βarr2-KO mice compared to WT mice which may account for the enhanced preference for the drug (Bohn et al., 2003). In contrast, although striatal dopamine levels are increased by morphine, hyperlocomotor activity is not enhanced in the βarr2-KO mice (Bohn et al., 2003). This effect may be due to dysfunctional striatal D2 dopamine receptor as discussed later in this review (Bealieau et al., 2005). How the function of the D2 dopamine receptor in locomotor activity differs from the dopaminergic responsiveness involved in developing conditioned place preference remains to be determined.

4.1.1.6. Functional Selectivity at the Mu Opioid Receptor: The concept of functional selectivity (also referred to as "ligand-directed signaling", "biased agonism", or "collateral efficacy") has been a topic of considerable interest among pharmacologists in the last several years (Urban et al., 2007; Violin and Lefkowitz, 2007; Mailman, 2007; Kenakin, 2007). The concept is based on the idea that the chemical characteristics of the ligand may alter the conformation of the receptor such that it will interact preferentially with certain cellular proteins to mediate distinct biological responses. Therefore, the nature of the receptor-protein interactions will dictate the signal transduction pathway based on the properties of the ligand bound (Table 5).

Certain agonists at the MOR produce behaviors that are sensitive to βarrestin2 expression while other agonists do not. For example, morphine, methadone, etorphine, and fentanyl can, at different potencies, produce equi-efficacious MOR-G protein coupling and a similar extent of analgesia in mice. However, these ligands differ in their propensity to regulate the receptor. While etorphine, methadone and fentanyl robustly promote receptor phosphorylation, βarrestin recruitment and receptor internalization, morphine weakly promotes these events (Zhang et al., 1998; Whistler and von Zastrow, 1998; Bohn et al., 2004b). Moreover, while βarr2-KO mice display enhanced antinociceptive responses to morphine, their responses to methadone, fentanyl and etorphine do not differ from WT mice. Since etorphine, methadone and fentanyl robustly induce receptor phosphorylation, it is likely that βarrestin1 compensates for regulating the receptor while the morphine bound, weakly phosphorylated receptor may be a weaker substrate for βarrestin1 binding (Bohn et al., 2004b). Taken together, these studies suggest that distinct agonists at the MOR differentially depend upon βarr2 to mediate the same physiological response. Moreover, MOR expression in different tissue and neuronal populations could be differentially sensitive to the regulation of βarrestin2.

4.1.2. β2 Adrenergic Receptors—As work has progressed with the βarr2-KO mice, multiple phenotypes, beyond those associated with opiates, have been realized. For example, treatment with the bronchodilator albuterol results in airway smooth muscle relaxation and this behavioral response is mediated in part by by βarrestin2 regulation of the β2AR (Deshpande et al., 2008). In the βarr2-KO mice, albuterol-mediated relaxation of the methacholine-constricted airway is enhanced in βarr2-KO mice as compared to WT littermates. Methacholine, which nonselectively activates muscarinic GPCRs, equally induces smooth muscle constriction in both genotypes suggesting that while βarrestin2 appears to be negatively regulating β2AR in airway smooth muscle it does not appear to be critical for maintaining function of muscarinic receptors in this system. Furthermore, assessment of tracheal ring contraction in an ex vivo model system does not reveal differences in tension generation following methacholine treatment between genotypes (Deshpande et al., 2008). However, isoproterenol-mediated relaxation of methacholine-induced contraction is greater in the βarr2-KO tracheal rings compared to the WTs. These findings may have implications regarding

β2AR receptor function in response to adrenergic agonist therapies for asthma and suggest that βarrestin2 may desensitize the responsiveness of these receptors in albuterol therapies.

4.1.3. Parathyroid Hormone Receptor—βArrestin2-dependent phenotypes have also been observed in bone homeostasis in response to parathyroid hormone challenges (Ferrari et al., 2005). Administration of intermittent parathyroid hormone increases bone mass by directly stimulating osteoblast-mediated bone formation, however it can also stimulate bone resorption through the coupling of osteoblasts to osteoclasts. Daily injections of parathyroid hormone increases total body bone mineral content and vertebral trabecular bone volume and thickness in WT mice. In contrast, parathyroid hormone treatment does not increase bone formation in βarr2-KO mice (Ferrari et al., 2005). Further, histomorphic analysis of the femurs of treated animals revealed that while both WT and βarr2-KO mice have marked increases in osteoblast number and activity, parathyroid hormone also increases osteoclast numbers on trabecular surfaces in βarr2-KO mice. These studies suggest that βarrestin2 may be involved in limiting skeletal osteoclastogenesis following chronic treatment with parathyroid hormone.

4.1.4. Immunocyte Receptors

4.1.4.1. Toll-like Receptor 4: In the immune system, several βarrestin2-dependent phenotypes have been described. Activation of toll-like receptor 4 induces activation of transcription factors which control the expression of immunoregulatory genes. Activation of this receptor by lipopolysaccharide (LPS) and D-galactosamine can induce endotoxin shock in WT mice. Mice deficient in βarrestin2, however, are more susceptible to endotoxin shock than their WT littermates. Furthermore, assessment of mRNA transcript populations from the livers of WT and βarr2-KO mice demonstrate that *Ccl2*, *Il1b*, *Tnf*, *Il6* and *Nfkbiz* cytokine expression is enhanced in the absence of βarrestin2 and βarr2-KO mice have higher TNF and IL-6 protein levels in their plasma than their WT littermates (Wang et al., 2006). Therefore, these studies suggest that βarrestin2 may be an essential negative regulator of toll-like receptor 4-mediated pro-inflammatory cytokine production and immune response.

4.1.4.2. CXCR-2 Receptors: βarrestins may also play a role in immune-cell migration and proliferation stimulated by chemokine activation of GPCRs. To determine the role that βarrestin2 plays in CXCR2-mediated neutrophil migration, dorsal air pouches were raised in mice by subcutaneous injection of air and inflammation was induced by local injection of CXCL1 (Su et al., 2005). CXCL1 injection induced neutrophil accumulation into the air pouches to a greater extent in the βarr2-KO mice, compared to their WT littermates. The role βarrestin2 plays in neutrophil chemotaxis and wound healing was further assessed with the excisional wound healing model. The recruitment of neutrophils into the wound beds of βarr2-KO mice was also increased compared to WT controls. Further, the re-epithelialization of the wound was significantly faster in the absence of βarrestin2. These studies indicate that βarrestin2 is a negative regulator for CXCR2 signaling in vivo. Moreover, Fong et al. (2002) demonstrated that βarrestin2 is involved in negatively regulating chemokine receptors in lymphocytes isolated from the spleens of WT and βarr2-KO mice, as CXCL12-stimulated GTPase activity was higher in the absence of βarrestin2 and CXCL12-stimulated chemotaxis of βarrestin2-deficient T and B lymphocytes was decreased compared to WTs.

4.1.5. Cannabinoid CB_1 Receptors—Finally, βarrestin2 has also been shown to negatively regulate cannabinoid-mediated mouse behaviors (Breivogel et al., 2008). Agonists to the cannabinoid CB_1 receptors, such as Δ^9-tetrahydrocannabinol (Δ^9-THC) and CP 55,940, induce antinociception, as assessed by the tail flick test and decreases in body temperature in mice. In the absence of βarrestin2, Δ^9-THC produces enhanced antinociception and a greater decrease in body temperature. However, CP 55,940 induces both antinociception and hypothermia to the same extent in both genotypes. These studies suggest that βarrestin2 may

regulate cannabinoid-stimulated behaviors in an agonist-dependent manner; however, these studies were performed in mice generated from homozygous breeding pairs so that differences may be somewhat attributed to inbred strains differences. Further studies of the βarrestin2-KO mice for differences in CB1 receptor functions will be of interest.

4.2. βArrestin2 as a Positive Mediator of GPCR Signaling

βArrestins can also mediate GPCR signaling and this has been demonstrated in cell culture models for vasopressin, angiotensin, and βadrenergic receptors; this list continues to grow (Ren et al., 2005; Charest et al., 2007; Ahn et al., 2003; Tohgo et al., 2002; Shenoy et al., 2006; Luttrell et al., 2001). Selective coupling to βarrestin-mediated signaling may ultimately prove to be as essential as (or even more important than) G protein mediated signal transduction for some receptors. To date, there have been no reports indicating βarrestin1 as a positive mediator of receptor signaling in vivo; however, this phenomenon has been repeatedly demonstrated in the βarr2-KO mice.

4.2.1. α2 Adrenergic Receptors—Alpha2 adrenergic receptor (α2AR) agonists induce sedation in mice and this behavior can be assessed by determining performance in a roto-rod test in which the mouse must coordinate reflex and balance. Upon challenge with the α2AR agonist, UK-14,304 (5-Bromo-6-(2-imidazolin-2-ylamino)quinoxaline), βarr2-KO mice were more resistant to sedation than WT mice. To test whether βarr2-KO mice were simply resistant to sedation, an agonist to the adenosine 1 receptor (R-PIA; R(-)N6-(2-phenylisopropyl) adenosine) was also tested and it produced sedation in both genotypes further strengthening the claim that α2AR responsiveness is selectively altered in these animals (Wang et al., 2004). These studies indicate that βarrestin2 may be acting as a signal-transducer at the α2AR for this particular behavioral response.

4.2.2. Dopamine Receptors—βArr2-KO mice have been shown to have altered responses to dopamine-induced locomotor activity when dopamine levels are elevated by morphine or amphetamine (Bohn et al., 2003; Beaulieu et al., 2005). Beaulieu et al. (2005) have shown both behavioral and biochemical evidence that support a positive signaling role for βarrestin2 in mediating D2 dopamine receptor (D2 DAR) signaling in mice. More recent biochemical studies from this group demonstrate that a common property of atypical antipsychotic action is to disrupt the D2 DAR-βarrestin2 interaction; therefore implicating the receptor coupling to βarrestin2 as an important signaling complex for therapeutic targeting (Masri et al., 2008).

4.2.2.1. Locomotor activity: Dopamine activation of the D2 DAR promotes motor activity in rodents (Crespi et al., 1997; Chausmer and Katz, 2001). Behavioral evidence for βarrestin2 and D2 DAR signaling is best demonstrated by the lack of both amphetamine-induced hyperlocomotor activity and apomorphine-induced stereotypy in βarr2-KO mice (Beaulieu et al., 2005; Gainetdinov et al., 2004). Interestingly, cocaine, which blocks the dopamine transporter, dose-dependently induces equivalent hyperlocomotor activity in both genotypes suggesting that the mechanisms of action of cocaine and amphetamine may differ in their respect to βarrestin2-dependent regulation (Bohn et al., 2003; Gainetdinov et al., 2004).

4.2.2.1.1. Biochemical Correlations: In an elegant series of studies, Beaulieu et al. (2005) have correlated the decreased locomotor activity following amphetamine treatment in the βarr2-KO mice to a disruption in an AKT-mediated signaling cascade in vivo using brain region preparations from WT and βarr2-KO mice. Stimulation of the D2 DAR by dopamine or dopaminergic drugs leads to the formation of an AKT, βarrestin2 and protein phosphatase 2A (PP2A) signaling complex in the mouse striatum. Further, in the absence of βarrestin2, the formation of this signaling complex is disrupted. These studies suggest that decreased

behavioral responses in βarr2-KO mice may be attributed to a disruption in βarrestin2 mediated signaling.

4.2.2.1.2. Lithium Disruption of βArrestin2 Signaling Complex: Beaulieu et al. (2008) have described a signaling complex involving βarrestin2 and AKT as integral in mediating the function of D2 DAR in regulation of ambulatory behaviors. In a recent series of studies, they demonstrate that lithium, a commonly used treatment for bipolar disorder, may exert its mechanism of action by disrupting the βarrestin2-AKT complex thereby disrupting signal transduction downstream of the D2 DAR. Behavioral studies designed to assess D2 DAR function in the βarr2-KO mice revealed a lack of lithium effects on novelty-induced locomotor activity, tail suspension immobility and light-dark compartment emergence latencies. While their work has focused on the role of the βarrestin2 complex in the D2 DAR signaling cascade it will be interesting to determine what other receptors may utilize this particular cascade. Such studies may not only improve our understanding of lithium's therapeutic benefits, but may also lend insight as to the cause of unwanted side-effects associated with this treatment.

4.2.3. Serotonin 2A Receptors—The activation of the serotonin 2A receptors (5-HT2AR) in humans is associated with hallucinations as all serotonergic hallucinogens have affinity for the 5-HT2AR. In rodents, 5-HT2AR activation induces a head twitch response and mice lacking this receptor fail to produce this response when challenged with a panel of hallucinogenic 5-HT2AR agonists (Gonzalez-Maseo et al., 2007). Serotonin-induced behaviors in mice can be observed after increasing brain serotonin levels by administering serotonin precursors such as tryptophan (or the more metabolically stable 5-HTP (5-hydroxytryptophan)); selective serotonin reuptake inhibitors (SSRI) such as fluoxetine; amphetamines such as MDMA; or inhibitors of serotonin degradation such as monoamine oxidase A inhibitors (Corne and Pickering, 1967). Elevations of serotonin by systemic 5-HTP injections or direct intracerebroventricular injections of serotonin will also induce a head twitch response in mice.

4.2.3.1. Head Twitch Responses: 5-HT2AR function is substantially altered in βarr2-KO mice. While treatment with the serotonin precursor, 5-HTP, produces the expected display of the head twitch response in WT mice (Corne et al., 1963; Corne and Pickering, 1967), the response is greatly attenuated in βarr2-KO mice (Schmid et al., 2008). A gene dosage effect is also seen in the βarrestin2 heterozygous mice as they display significantly fewer head twitches compared to WT mice. A surge in endogenous serotonin produced by the systemic 5-HTP injection would be expected to produce multiple physiological responses in mice and may reveal phenotypes associated with multiple serotonin receptor types. However, hypothermia (~3.5°C decrease in body temperature in 30 min) and the onset and severity of diarrhea, did not differ between the two genotypes following 5-HTP treatment (Schmid et al., 2008). Hypothermia and gastrointestinal secretion are generally attributed to actions of other serotonin receptor subtypes and not the 5-HT2AR (Fiorica-Howells et al., 2002; Hedlund et al., 2003), indicating some degree of selectivity in determining the regulation of one serotonin receptor subtype over others.

4.2.3.2. Functional Selectivity of 5-HT2AR Signaling via βArrestins: Although 5-HTP injections fail to promote the robust head twitch response in βarr2-KO mice, the hallucinogenic 5-HT2AR selective agonist, 2,5-dimethoxy-4-iodoamphetamine (DOI; Glennon, 1986), produces head twitch responses of equal magnitude in both genotypes (Schmid et al., 2008). Importantly, the actions of both DOI and 5-HTP are blocked by administration of the highly selective 5-HT2AR antagonist, M100907 (R(+)-alpha-(2,3-dimethoxyphenyl)-1-[2(4-fluorophenylethyl)]-4-piperidinemethanol; Schmidt et al. 1992; Sorenson et al., 1993; Schmid et al., 2008). Taken together, these findings suggest that βarrestin2 mediates serotonin-induced

head twitches while DOI produces the head twitch response in a βarrestin2-independent manner. In this scenario, 5-HT2AR mediates signal transduction via βarrestin2 when serotonin is the agonist, but is not a critical component of DOI-induced head twitch responses.

4.2.3.3. Biochemical Correlations: Further evidence for the divergence of the serotonin and DOI mediated signal transduction via the 5-HT2AR was gained utilizing primary cortical cultures from WT and βarrestin2-KO mice, mouse embryonic fibroblasts (MEFs) derived from βarrestin1 and βarrestin2 double KO mice and tissue procured from frontal cortex of drug treated animals (Schmid et al., 2008). In WT cortical neurons, the 5-HT2AR can predominantly be found intracellularly while the receptor in the βarr2-KO neurons localizes to the soma membrane, suggesting an integral role for βarrestin2 in trafficking of the 5-HT2AR. The same distribution was observed in WT and double KO MEFs. When WT cells are deprived of serum, the 5-HT2AR returns to the cell surface and can be internalized upon addition of serotonin or DOI. In the absence of βarrestins, the receptor resides on the surface of the cells, even when treated with serotonin. DOI, however, is capable of inducing receptor internalization in the βarrestin null cells demonstrating a critical role for βarrestins in determining 5-HT2AR internalization in response to serotonin but not DOI. Moreover, in mice, treatment with either DOI or 5-HTP induces ERK activation in the frontal cortex, yet only DOI induces ERK activation in βarr2-KO mice. These studies further implicate βarrestin2 interaction with the 5-HT2AR as a point of divergence in signaling transduction for different agonists activating the 5-HT2AR: either βarrestin2-dependent (serotonin) or independent (DOI).

4.2.4. Ethanol Preference—βArrestin2 may also serve as a positive regulator for ethanol-mediated behaviors as deletion of βarrestin2 results in decreased behavioral responses to ethanol. Microarray experiments have linked βarrestin2 to ethanol preference in rodents, as genetic variations in the *ARRB2* gene can affect alcohol preference in rats (Arlinde et al., 2004; Sommer et al., 2006). To further confirm these findings, Bjork et al. (2008) utilized βarr2-KO mice to determine how the genetic knockdown of this protein affects the consumption and psychostimulant actions of ethanol in mice. They determined that βarr2-KO and heterozygous mice have decreased voluntary ethanol intake and lowered ethanol preference compared to their WT littermates. Importantly, there were no differences in the taste preferences for either sucrose or quinine across genotypes. Further, βarr2-KO and heterozygous mice exhibit decreased ethanol-induced locomotion compared to WT controls. However, given that βarr2-KO mice do not display the characteristic increase in locomotor activity following treatment with psychostimulants (Beaulieu et al., 2005), it is unsurprising that βarr2-KO mice are also insensitive to the stimulating effects of ethanol. These data suggest that βarrestin2 may modulate these acute responses to ethanol.

4.2.5. Lysophosphatidic Acid, Protease Activated and Sphingosine-1-Phosphate Receptors—While βarr1-KO displayed less more severe neointimal hyperplasia in ex vivo preparations of carotid artery (Kim et al., 2008); βarr2-KO mice displayed less severe neointimal hyperplasia in similar preparations. Studies in the βarr2-KO were also carried out in vivo by feeding a high fat diet and monitoring atherosclerosis formation over time. In these studies, a deletion of βarrestin2 appeared to decrease arterial plaque formation. The authors propose the involvement of lysophosphatidic acid, protease activated and sphingosine-1-phosphate receptors in smooth muscle cells. Interestingly, their data support a bidirectional role for βarrestin1 versus βarrestin2 in injury-provoked neointimal hyperplasia suggesting that inhibition of βarrestin2 while stimulation of βarrestin1 mediated pathways might be therapeutically beneficial (Kim et al., 2008).

4.2.6. Chemokine Receptors—Finally, studies using βarr2-KO mice also demonstrate that in addition to the negative regulatory roles βarrestins play in mediating cell migration,

βarrestins may also play positive regulatory roles for those chemokine receptors involved in the development of allergic asthma. When treated with ovalbumin, normal mice develop symptoms of allergic asthma. In the absence of βarrestin2, however, mice do not exhibit symptoms of increased airway responsiveness. OVA treatment leads to the accumulation of T lymphocytes and the release of inflammatory cytokines in the lungs of WT, but not βarr2-KO mice (Walker et al., 2003). βArrestin2 does not appear to play a role in mediating endotoxin-mediated asthma, since LPS induced neutrophilic inflammation and increased airway responsiveness in WT and βarr2-KO mice to a similar extent.

5. Summary

Cell model characterizations have been critical for determining which proteins can interact to influence cellular function, and studies of this nature have opened new avenues for considering drug function in respect to receptor-pathway engagement. Although receptor-βarrestin interactions may be observed in cells, the role that βarrestins play in vivo, wherein receptor levels may be quite low and GRK levels may vary between tissues, may be difficult to predict. The use of genetically modified animals, lacking βarrestins, has allowed a gross assessment of which receptor signaling pathways are most sensitive to individual βarrestins and such sensitivities have been most apparent upon drug challenges. When using animal models to predict receptor function, it is important to study a robust phenotype that is most directly linked to the control of a certain receptor as well as pharmacological and genetic evidence to definitively implicate the receptor. Finally, it is essential to consider that behavioral modifications may be due to indirect drug actions (i.e. the induction of neurotransmitter release) on other receptors that are dysregulated in the absence of βarrestins.

In this review, we have grouped examples of βarrestins serving as negative or positive influencers on certain drug functions. While the literature we have reviewed here support the notion that βarrestins may play such inhibititory or facillitory roles in the regulation of the indicated receptors, the interpretations of in vivo studies must be approached with some caution. We must consider the complexity and system-affected biology that ensues following drug treatment and expect that some of the observed behaviors may not be due to drugs acting differently at the predicted target but rather at another point in the system.

Using the current global knockout models, it is critical to directly assess receptor dysregulation in tissues and in ex vivo preparations to restrict potential systemic effects or unanticipated contributions of the deletion of βarrestins to other aspects of the behavioral response (for example, a lack of a pain response may not be due to a disruption in pain processing, but could simply result from a disruption in reflex response when the drug is on board). As gene delivery and gene silencing techniques improve, the selective deletion of βarrestins in particular tissues or cellular populations may prove useful in order to somewhat limit the impact of protein deletion to a particular system under study.

Bibliography

Ahn S, Nelson CD, Garrison TR, Miller WE, Lefkowitz RJ. Desensitization, internalization, and signaling functions of beta-arrestins demonstrated by RNA interference. Proc Natl Acad Sci U S A 2003;100 (4):1740–1744. [PubMed: 12582207]

Arlinde C, Sommer W, Bjork K, Reimers M, Hyytia P, Kiianmaa K, et al. A cluster of differentially expressed signal transduction genes identified by microarray analysis in a rat genetic model of alcoholism. Pharmacogenomics J 2004;4(3):208–218. [PubMed: 15052257]

Attramadal H, Arriza JL, Aoki C, Dawson TM, Codina J, Kwatra MM, et al. Beta-arrestin2, a novel member of the arrestin/beta-arrestin gene family. J Biol Chem 1992;267(25):17882–17890. [PubMed: 1517224]

Barak LS, Ferguson SS, Zhang J, Caron MG. A beta-arrestin/green fluorescent protein biosensor for detecting G protein-coupled receptor activation. J Biol Chem 1997;272(44):27497–27500. [PubMed: 9346876]

Beaulieu JM, Marion S, Rodriguiz RM, Medvedev IO, Sotnikova TD, Ghisi V, et al. A beta-arrestin 2 signaling complex mediates lithium action on behavior. Cell 2008;132(1):125–136. [PubMed: 18191226]

Beaulieu JM, Sotnikova TD, Marion S, Lefkowitz RJ, Gainetdinov RR, Caron MG. An Akt/beta-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. Cell 2005;122(2): 261–273. [PubMed: 16051150]

Benovic JL, Kuhn H, Weyand I, Codina J, Caron MG, Lefkowitz RJ. Functional desensitization of the isolated beta-adrenergic receptor by the beta-adrenergic receptor kinase: potential role of an analog of the retinal protein arrestin (48-kDa protein). Proc Natl Acad Sci U S A 1987;84(24):8879–8882. [PubMed: 2827157]

Bjork K, Rimondini R, Hansson AC, Terasmaa A, Hyytia P, Heilig M, et al. Modulation of voluntary ethanol consumption by beta-arrestin 2. Faseb J 2008;22(7):2552–2560. [PubMed: 18367649]

Bohn LM, Gainetdinov RR, Caron MG. G protein-coupled receptor kinase/beta-arrestin systems and drugs of abuse: psychostimulant and opiate studies in knockout mice. Neuromolecular Med 2004a;5 (1):41–50. [PubMed: 15001811]

Bohn LM, Dykstra LA, Lefkowitz RJ, Caron MG, Barak LS. Relative opioid efficacy is determined by the complements of the G protein-coupled receptor desensitization machinery. Mol Pharmacol 2004b;66(1):106–112. [PubMed: 15213301]

Bohn LM, Gainetdinov RR, Lin FT, Lefkowitz RJ, Caron MG. Mu-opioid receptor desensitization by beta-arrestin-2 determines morphine tolerance but not dependence. Nature 2000;408(6813):720–723. [PubMed: 11130073]

Bohn LM, Gainetdinov RR, Sotnikova TD, Medvedev IO, Lefkowitz RJ, Dykstra LA, et al. Enhanced rewarding properties of morphine, but not cocaine, in beta(arrestin)-2 knock-out mice. J Neurosci 2003;23(32):10265–10273. [PubMed: 14614085]

Bohn LM, Lefkowitz RJ, Caron MG. Differential mechanisms of morphine antinociceptive tolerance revealed in (beta)arrestin-2 knock-out mice. J Neurosci 2002;22(23):10494–10500. [PubMed: 12451149]

Bohn LM, Lefkowitz RJ, Gainetdinov RR, Peppel K, Caron MG, Lin FT. Enhanced morphine analgesia in mice lacking beta-arrestin 2. Science 1999;286(5449):2495–2498. [PubMed: 10617462]

Bohn LM, Raehal KM. Opioid receptor signaling: relevance for gastrointestinal therapy. Curr Opin Pharmacol 2006;6(6):559–563. [PubMed: 16935560]

Breivogel CS, Lambert JM, Gerfin S, Huffman JW, Razdan RK. Sensitivity to delta9-tetrahydrocannabinol is selectively enhanced in beta-arrestin2 −/− mice. Behav Pharmacol 2008;19 (4):298–307. [PubMed: 18622177]

Charest PG, Oligny-Longpre G, Bonin H, Azzi M, Bouvier M. The V2 vasopressin receptor stimulates ERK1/2 activity independently of heterotrimeric G protein signalling. Cell Signal 2007;19(1):32–41. [PubMed: 16857342]

Chausmer AL, Katz JL. The role of D2-like dopamine receptors in the locomotor stimulant effects of cocaine in mice. Psychopharmacology (Berl) 2001;155(1):69–77. [PubMed: 11374338]

Claing A, Laporte SA. Novel roles for arrestins in G protein-coupled receptor biology and drug discovery. Curr Opin Drug Discov Devel 2005;8(5):585–589.

Conner DA, Mathier MA, Mortensen RM, Christe M, Vatner SF, Seidman CE, et al. beta-Arrestin1 knockout mice appear normal but demonstrate altered cardiac responses to beta-adrenergic stimulation. Circ Res 1997;81(6):1021–1026. [PubMed: 9400383]

Corne SJ, Pickering RW. A possible correlation between drug-induced hallucinations in man and a behavioural response in mice. Psychopharmacologia 1967;11(1):65–78. [PubMed: 5302272]

Corne SJ, Pickering RW, Warner BT. A method for assessing the effects of drugs on the central actions of 5-hydroxytryptamine. Br J Pharmacol Chemother 1963;20:106–120. [PubMed: 14023050]

Crespi D, Mennini T, Gobbi M. Carrier-dependent and Ca(2+)-dependent 5-HT and dopamine release induced by (+)-amphetamine, 3,4- methylendioxymethamphetamine, p-chloroamphetamine and (+)-fenfluramine. Br J Pharmacol 1997;121(8):1735–1743. [PubMed: 9283711]

Deshpande DA, Theriot BS, Penn RB, Walker JK. Beta-arrestins specifically constrain beta2-adrenergic receptor signaling and function in airway smooth muscle. Faseb J 2008;22(7):2134–2141. [PubMed: 18337459]

DeWire SM, Ahn S, Lefkowitz RJ, Shenoy SK. Beta-arrestins and cell signaling. Annu Rev Physiol 2007;69:483–510. [PubMed: 17305471]

Drake MT, Violin JD, Whalen EJ, Wisler JW, Shenoy SK, Lefkowitz RJ. beta-arrestin-biased agonism at the beta2-adrenergic receptor. J Biol Chem 2008;283(9):5669–5676. [PubMed: 18086673]

Ferrari SL, Pierroz DD, Glatt V, Goddard DS, Bianchi EN, Lin FT, et al. Bone response to intermittent parathyroid hormone is altered in mice null for {beta}-Arrestin2. Endocrinology 2005;146(4):1854–1862. [PubMed: 15705780]

Fiorica-Howells E, Hen R, Gingrich J, Li Z, Gershon MD. 5-HT(2A) receptors: location and functional analysis in intestines of wild-type and 5-HT(2A) knockout mice. Am J Physiol Gastrointest Liver Physiol 2008;282(5):G877–G893. [PubMed: 11960784]

Fong AM, Premont RT, Richardson RM, Yu YR, Lefkowitz RJ, Patel DD. Defective lymphocyte chemotaxis in beta-arrestin2- and GRK6-deficient mice. Proc Natl Acad Sci U S A 2002;99(11):7478–7483. [PubMed: 12032308]

Freedman NJ, Lefkowitz RJ. Desensitization of G protein-coupled receptors. Recent Prog Horm Res 1996;51:319–351. [PubMed: 8701085]discussion 352–313

Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ, Caron MG. Desensitization of G protein-coupled receptors and neuronal functions. Annu Rev Neurosci 2004;27:107–144. [PubMed: 15217328]

Glennon RA. Discriminative stimulus properties of the serotonergic agent 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). Life Sci 1976;39(9):825–830. [PubMed: 2943960]

Gonzalez-Maeso J, Weisstaub NV, Zhou M, Chan P, Ivic L, Ang R, et al. Hallucinogens recruit specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior. Neuron 2007;53(3):439–452. [PubMed: 17270739]

Groer CE, Tidgewell K, Moyer RA, Harding WW, Rothman RB, Prisinzano TE, et al. An opioid agonist that does not induce micro-opioid receptor—arrestin interactions or receptor internalization. Mol Pharmacol 2007;71(2):549–557. [PubMed: 17090705]

Gurevich EV, Gurevich VV. Arrestins: ubiquitous regulators of cellular signaling pathways. Genome Biol 2006;7(9):236. [PubMed: 17020596]

Hamdan FF, Audet M, Garneau P, Pelletier J, Bouvier M. High-throughput screening of G protein-coupled receptor antagonists using a bioluminescence resonance energy transfer 1-based beta-arrestin2 recruitment assay. J Biomol Screen 2005;10(5):463–475. [PubMed: 16093556]

Hedlund PB, Danielson PE, Thomas EA, Slanina K, Carson MJ, Sutcliffe JG. No hypothermic response to serotonin in 5-HT7 receptor knockout mice. Proc Natl Acad Sci U S A 2003;100(3):1375–1380. [PubMed: 12529502]

Heinricher, MM.; Morgan, MM. Supraspinal mechanisms of opioid analgesia. In: Stein, C., editor. Opioids in pain control: basic and clinical aspects. New York, NY: Cambridge University Press; 1999. p. 46-69.

Kenakin T. Collateral efficacy in drug discovery: taking advantage of the good (allosteric) nature of 7TM receptors. Trends Pharmacol Sci 2007;28(8):407–415. [PubMed: 17629960]

Kieffer BL. Opioids: first lessons from knockout mice. Trends Pharmacol Sci 1999;20(1):19–26. [PubMed: 10101958]

Kieffer BL, Gaveriaux-Ruff C. Exploring the opioid system by gene knockout. Prog Neurobiol 2002;66(5):285–306. [PubMed: 12015197]

Kim J, Zhang L, Peppel K, Wu JH, Zidar DA, Brian L, et al. Beta-arrestins regulate atherosclerosis and neointimal hyperplasia by controlling smooth muscle cell proliferation and migration. Circ Res 2008;103(1):70–79. [PubMed: 18519945]

Kohout TA, Lin FS, Perry SJ, Conner DA, Lefkowitz RJ. beta-Arrestin 1 and 2 differentially regulate heptahelical receptor signaling and trafficking. Proc Natl Acad Sci U S A 2001;98(4):1601–1606. [PubMed: 11171997]

LeBars D, Menetrey D, Besson JM. Effects of morphine upon the lamina V type cells activities in the dorsal horn of the decerebrate cat. Brain Res 1976;113(2):293–310. [PubMed: 182321]

Lefkowitz RJ. G protein-coupled receptors. III. New roles for receptor kinases and beta-arrestins in receptor signaling and desensitization. J Biol Chem 1998;273(30):18677–18680. [PubMed: 9668034]

Lefkowitz RJ, Shenoy SK. Transduction of receptor signals by beta-arrestins. Science 2005;308(5721): 512–517. [PubMed: 15845844]

Lohse MJ, Andexinger S, Pitcher J, Trukawinski S, Codina J, Faure JP, et al. Receptor-specific desensitization with purified proteins. Kinase dependence and receptor specificity of beta-arrestin and arrestin in the beta 2-adrenergic receptor and rhodopsin systems. J Biol Chem 1992;267(12): 8558–8564. [PubMed: 1349018]

Lohse MJ, Benovic JL, Codina J, Caron MG, Lefkowitz RJ. beta-Arrestin: a protein that regulates beta-adrenergic receptor function. Science 1990;248(4962):1547–1550. [PubMed: 2163110]

Luttrell LM. Activation and targeting of mitogen-activated protein kinases by G-protein-coupled receptors. Can J Physiol Pharmacol 2002;80(5):375–382. [PubMed: 12056542]

Luttrell LM, Daaka Y, Lefkowitz RJ. Regulation of tyrosine kinase cascades by G-protein-coupled receptors. Curr Opin Cell Biol 1999;11(2):177–183. [PubMed: 10209148]

Luttrell LM, Roudabush FL, Choy EW, Miller WE, Field ME, Pierce KL, et al. Activation and targeting of extracellular signal-regulated kinases by beta-arrestin scaffolds. Proc Natl Acad Sci U S A 2001;98 (5):2449–2454. [PubMed: 11226259]

Masri B, Salahpour A, Didriksen M, Ghisi V, Beaulieu J-M, Gainetdinov RR, Caron MG. 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(36):13656–13661. [PubMed: 18768802]

Mailman RB. GPCR functional selectivity has therapeutic impact. Trends Pharmacol Sci 2007;28(8): 390–396. [PubMed: 17629962]

Mundell SJ, Loudon RP, Benovic JL. Characterization of G protein-coupled receptor regulation in antisense mRNA-expressing cells with reduced arrestin levels. Biochemistry 1999;38(27):8723–8732. [PubMed: 10393547]

Pitcher J, Lohse MJ, Codina J, Caron MG, Lefkowitz RJ. Desensitization of the isolated beta 2-adrenergic receptor by beta-adrenergic receptor kinase, cAMP-dependent protein kinase, and protein kinase C occurs via distinct molecular mechanisms. Biochemistry 1992;31(12):3193–3197. [PubMed: 1348186]

Premont RT, Gainetdinov RR. Physiological roles of G protein-coupled receptor kinases and arrestins. Annu Rev Physiol 2007;69:511–534. [PubMed: 17305472]

Premont RT, Inglese J, Lefkowitz RJ. Protein kinases that phosphorylate activated G protein-coupled receptors. Faseb J 1995;9(2):175–182. [PubMed: 7781920]

Przewlocka B, Sieja A, Starowicz K, Maj M, Bilecki W, Przewlocki R. Knockdown of spinal opioid receptors by antisense targeting beta-arrestin reduces morphine tolerance and allodynia in rat. Neurosci Lett 2002;325(2):107–110. [PubMed: 12044633]

Raehal KM, Walker JK, Bohn LM. Morphine side effects in beta-arrestin 2 knockout mice. J Pharmacol Exp Ther 2005;314(3):1195–1201. [PubMed: 15917400]

Ren XR, Reiter E, Ahn S, Kim J, Chen W, Lefkowitz RJ. Different G protein-coupled receptor kinases govern G protein and beta-arrestin-mediated signaling of V2 vasopressin receptor. Proc Natl Acad Sci U S A 2005;102(5):1448–1453. [PubMed: 15671180]

Schmid CL, Raehal KM, Bohn LM. 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(3):1079–1084. [PubMed: 18195357]

Schmidt CJ, Black CK, Taylor VL, Fadayel GM, Humphreys TM, Nieduzak TR, et al. The 5-HT2 receptor antagonist, MDL 28,133A, disrupts the serotonergic-dopaminergic interaction mediating the neurochemical effects of 3,4-methylenedioxymethamphetamine. Eur J Pharmacol 1992;220(2–3): 151–159. [PubMed: 1425989]

Shenoy SK, Drake MT, Nelson CD, Houtz DA, Xiao K, Madabushi S, et al. beta-arrestin-dependent, G protein-independent ERK1/2 activation by the beta2 adrenergic receptor. J Biol Chem 2006;281(2): 1261–1273. [PubMed: 16280323]

Shenoy SK, Lefkowitz RJ. Seven-transmembrane receptor signaling through beta-arrestin. Sci STKE 2005;2005(308)cm10

Sibley DR, Benovic JL, Caron MG, Lefkowitz RJ. Regulation of transmembrane signaling by receptor phosphorylation. Cell 1987;48(6):913–922. [PubMed: 3030559]

Sommer W, Hyytia P, Kiianmaa K. The alcohol-preferring AA and alcohol-avoiding ANA rats: neurobiology of the regulation of alcohol drinking. Addict Biol 2006;11(3–4):289–309. [PubMed: 16961760]

Sorensen SM, Kehne JH, Fadayel GM, Humphreys TM, Ketteler HJ, Sullivan CK, et al. Characterization of the 5-HT2 receptor antagonist MDL 100907 as a putative atypical antipsychotic: behavioral, electrophysiological and neurochemical studies. J Pharmacol Exp Ther 1993;266(2):684–691. [PubMed: 8102646]

Su Y, Raghuwanshi SK, Yu Y, Nanney LB, Richardson RM, Richmond A. Altered CXCR2 signaling in beta-arrestin-2-deficient mouse models. J Immunol 2005;175(8):5396–5402. [PubMed: 16210646]

Tohgo A, Pierce KL, Choy EW, Lefkowitz RJ, Luttrell LM. beta-Arrestin scaffolding of the ERK cascade enhances cytosolic ERK activity but inhibits ERK-mediated transcription following angiotensin AT1a receptor stimulation. J Biol Chem 2002;277(11):9429–9436. [PubMed: 11777902]

Urban JD, Clarke WP, von Zastrow M, Nichols DE, Kobilka B, Weinstein H, et al. Functional selectivity and classical concepts of quantitative pharmacology. J Pharmacol Exp Ther 2007;320(1):1–13. [PubMed: 16803859]

Violin JD, Lefkowitz RJ. Beta-arrestin-biased ligands at seven-transmembrane receptors. Trends Pharmacol Sci 2007;28(8):416–422. [PubMed: 17644195]

von Degenfeld G, Wehrman TS, Hammer MM, Blau HM. A universal technology for monitoring G-protein-coupled receptor activation in vitro and noninvasively in live animals. Faseb J 2007;21(14): 3819–3826. [PubMed: 17942828]

Walker JK, Fong AM, Lawson BL, Savov JD, Patel DD, Schwartz DA, et al. Beta-arrestin-2 regulates the development of allergic asthma. J Clin Invest 2003;112(4):566–574. [PubMed: 12925697]

Wang Q, Zhao J, Brady AE, Feng J, Allen PB, Lefkowitz RJ, et al. Spinophilin blocks arrestin actions in vitro and in vivo at G protein-coupled receptors. Science 2004;304(5679):1940–1944. [PubMed: 15218143]

Wang Y, Tang Y, Teng L, Wu Y, Zhao X, Pei G. Association of beta-arrestin and TRAF6 negatively regulates Toll-like receptor-interleukin 1 receptor signaling. Nat Immunol 2006;7(2):139–147. [PubMed: 16378096]

Whistler JL, von Zastrow M. Morphine-activated opioid receptors elude desensitization by beta-arrestin. Proc Natl Acad Sci U S A 1998;95(17):9914–9919. [PubMed: 9707575]

Yaksh TL. Pharmacology and mechanisms of opioid analgesic activity. Acta Anaesthesiol Scand 1997;41 (1 Pt 2):94–111. [PubMed: 9061092]

Zhang J, Ferguson SS, Barak LS, Bodduluri SR, Laporte SA, Law PY, 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(12):7157–7162. [PubMed: 9618555]

Table 1

Enhanced Drug Responsiveness in βarrestin1-KO and βarrestin2-KO mice

Proposed Target	Drug/Challenge	Model System	Phenotype	Reference
β_2 Adrenergic receptor	Isoproterenol	βarr1-KO mice	Stimulated increase in cardiac ejection fraction	Conner et al., 1997
	Albuterol	βarr2-KO mice	Increased bronchodilation	Deshpande et al., 2008
CB1 cannabinoid Receptor	Δ9-THC	βarr2-KO mice	Enhanced antinociception and hypothermia	Brievogel et al., 2008
CXCR2	CXCL1	βarr2-KO mice	Increased neutrophil migration into air pouches	Su et al., 2005
	Excisional punch wounds	βarr2-KO mice	Increased neutrophil migration into wound bed	
			Increased rate of wound re-epithelialization	
Mu opioid receptor	Morphine	βarr2-KO mice	Enhanced and prolonged antonocieption and hypothermia	Bohn et al., 1999; 2000; 2004b
			Enhanced drug reinforcement	Bohn et al., 2003
			Reduced antinociceptive tolerance	Bohn et al., 2000; 2002
		βarr2-anti-sense (rat)	Reduced antinociceptive tolerance	Przewlocka et al., 2002
	Heroin	βarr2-KO mice	Enhanced and prolonged antinociception	Bohn et al., 2004b
Parathyroid receptor 1	Parathyroid hormone	βarr2-KO mice	Disrupted increase in bone mineral content and trabecular bone parameters and increased osteoclast number	Ferrari et al., 2005
Toll-like receptor 4	LPS and D-galactosamine	βarr2-KO mice	Increased susceptibility to endotoxin shock and enhanced expression of proinflammatory cytokines	Wang et al., 2006

THC: tetrahydrocannabinol; LPS: Lipopolysaccharides

Table 2

Decreased Drug Responseiveness in βarrestin 1-KO and βarrestin2-KO mice

Proposed Target	Drug/Challenge	Model System	Phenotype	Reference
α_2 Adrenergic receptor	UK 14,304	βarr2-KO mice	Disrupted increase in sedation	Wang et al., 2004
Chemokine receptors	Airway challenge	βarr2-KO mice	Reduced T lymphocyte accumulation and asthmatic response	Walker et al., 2003
Dopamine receptors (direct)	Apomorphine	βarr1-KO mice	Reduced climbing behavior	Gainetdinov et al., 2004
		βarr2-KO mice	Reduced climbing behavior	Gainetdinov et al., 2004
			Reduced hyperlocomotor activity	Beaulieu et al., 2005
Dopamine receptors (indirect)	Amphetamine	βarr2-KO mice	Reduced hyperlocomotor activity	Beaulieu et al., 2005
	Lithium	βarr2-KO mice	Disrupted reduction in locomotor activity and anti-depressant-like behaviors	Beaulieu et al., 2008
GABA receptors	Ethanol	βarr2-KO mice	Reduced ethanol intake and preference and decreased ethanol-induced locomotion	Bjork et al., 2008
LPA, Protease-activated & S1P receptors	High fat diet	βarr2-KO mice	Reduced aortic atherosclerosis and decreased prevalence of atheroma smooth muscle cells	Kim et al., 2008
Mu opioid receptor	Morphine	βarr2-KO mice	Reduced hyperlocomotor activity	Bohn et al., 2003
			Reduced constipation and respiratory suppression	Raehal et al., 2005
	Loperamide	βarr2-KO mice	Reduced constipation	Raehal et al., 2005
Serotonin 2A receptor	5-hydroxy-L-tryptophan	βarr2-KO mice	Reduced head twitch response	Schmid et al., 2008

LPA: Lysophosphotidic Acid; S1P: sphingosine-1-phosphate; UK14,304: 5-Bromo-6-(2-imidazolin-2-ylamino)quinoxaline.

Table 3

No Difference in Drug Response in βarr1-KO and βarr2-KO mice

Proposed Target	Drug/Challenge	Model System	Normal Phenotype	Reference
Adenosine 1 receptor	R-PIA	βarr2-KO mice	Sedation	Wang et al., 2004
CB1 cannabinoid receptor	CP 55940 Methanandamide	βarr2-KO mice	Antinociception and hypothermia	Brievogel et al., 2008
Chemokine receptor	LPS	βarr2-KO mice	Airway inflammatory response	Walker et al., 2003
Dopamine receptors (indirect)	Cocaine	βarr1-KO mice	Locomotor activity	Gainetdinov et al., 2004
		βarr2-KO mice	Locomotor activity	Bohn et al., 2003;
			Locomotor sensitization	Gainetdinov et al., 2004
			Drug Reinforcement	Bohn et al., 2003
Mu opioid receptor	Morphine	βarr1-KO mice	Antinociception	Bohn et al., 2004b
		βarr2-KO mice	Antagonist-precipitated withdrawal	Bohn et al., 2000
	Etorphine, Fentanyl, Methadone	βarr2-KO mice	Antinociception	Bohn et al., 2004b
Muscarinic acetylcholine receptors	Methacholine	βarr2-KO mice	Airway constriction	Walker et al., 2003
			Bronchoconstriction	Deshpande et al., 2008
Parathyroid receptor 1	Parathyroid hormone	βarr2-KO mice	Osteoblast number and activity; serum calcium and phosphate levels	Ferrari et al., 2005
Prostaglandin E_2 receptors	Prostaglandin E_2	βarr2-KO mice	Bronchodilation	Deshpande et al., 2008
Serotonin 2A receptors	DOI	βarr2-KO mice	Head twitch response	Schmid et al., 2008

R-PIA: R-phenylisopropyladenosine; CP 55940: 5-(1,1-Dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]phenol; LPS: Lipopolysaccharides: DOI: (±)-2,5-Dimethoxy-4-iodoamphetamine hydrochloride.

Table 4

Baseline Phenotypes Compared to WT Littermates

Genotype	Biological Measure	Phenotype	Reference
β3arr1-KO	Blood chemistry	No differences in hemoglobin, hemocrit, white blood cell counts and red blood cell counts	Conner et al., 1997
	Spleen	No differences in numbers of T or B lymphocytes or macrophages	Conner et al., 1997
	Heart	No differences in resting mean blood pressure, heart rate or ejection fraction	Conner et al., 1997
	Locomotor activity	No difference in activity in a novel environment	Gainetdinov et al., 2004
βarr2-KO	Body composition (homozygous breeding*)	***Lower* body weight, total body percent fat and total body bone mineral content**	Ferrari et al., 2005
	Body temperature	No differences in rectal temperature	Bohn et al., 1999
	Food consumption	No differences in grams consumed	Raehal et al., 2005
	Antinociception (hot-plate)	No differences in response latency	Bohn et al., 2002
	Antinociception (tail-flick)	***Increased* response latencies**	Bohn et al., 2002
	Locomotor activity	***Decreased* activity in a novel environment**	Bohn et al., 2003
	Spleen	No differences in numbers of T or B lymphocytes in spleen	Fong et al., 2002
	Respiration	No differences in resting breathing frequency	Raehal et al., 2005

*Homozygous breeding refers to studies where WT were compared to KO mice derived from homozygous breeding (WT×WT compared to KO×KO mice); these are not comparisons between littermates and differences could reflect an impact of inbreeding rather than a result of the genetic deletion.

Table 5

Functional Selectivity Demonstrated in βarrestin2-KO Mice

Proposed Target	Drug/Challenge	Behavior Phenotype	Reference
Mu opioid receptor	Morphine Heroin	Enhanced and prolonged antinociception	Bohn et al., 1999; 2000; 2004b
	Etorphine, Fentanyl, Methadone	No differences in antinociception	Bohn et al., 2004b
Serotonin 2A receptor	5-hydroxy-L-tryptophan	Reduced head twitch response	Schmid et al., 2008
	DOI	No difference in head twitch response	Schmid et al., 2008

DOI: (±)-2,5-Dimethoxy-4-iodoamphetamine hydrochloride