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Role for β-arrestin in mediating paradoxical β_2AR and PAR_2 signaling in asthma

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Abstract

G protein-coupled receptors (GPCRs) utilize (at least) two signal transduction pathways to elicit cellular responses including the classic G protein-dependent, and the more recently discovered β-arrestin-dependent, signaling pathways. In human and murine models of asthma, agonist-activation of β_2-adrenergic receptor (β_2AR) or Protease-activated-receptor-2 (PAR_2) results in relief from bronchospasm via airway smooth muscle relaxation. However, chronic activation of these receptors, leads to pro-inflammatory responses. One plausible explanation underlying the paradoxical effects of β_2AR and PAR_2 agonism in asthma is that the beneficial and harmful effects are associated with distinct signaling pathways. Specifically, G protein-dependent signaling mediates relaxation of airway smooth muscle, whereas β-arrestin-dependent signaling promotes inflammation. This review explores the evidence supporting the hypothesis that β-arrestin-dependent signaling downstream of β_2AR and PAR_2 is detrimental in asthma and examines the therapeutic opportunities for selectively targeting this pathway.

INTRODUCTION

Asthma is a chronic inflammatory disease characterized by airway inflammation, hyperresponsiveness and remodeling [1]. Airway hyperresponsiveness (AHR), a measure of bronchoconstrictor responsiveness, is associated with debilitating asthma signs and symptoms such as coughing, wheezing and shortness of breath. Beta-2-adrenergic receptor (β2-AR) agonist (β-agonist) administration is the mainstay therapy during bronchospastic episodes, providing significant relief to asthmatics [2]. However, chronic β-agonist therapy has also been associated with loss of asthma control, worsening of disease and increased morbidity and mortality (reviewed in [3]). Activation of Protease-activated receptor-2 (PAR_2), which promotes bronchorelaxation, has also been explored as a treatment for asthma. However, similar to the β_2-adrenergic receptor (β_2AR) paradox, murine studies have

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shown that PAR_2 activation can play diametrically opposed roles in allergic asthma providing both potent bronchorelaxation and increased inflammation [4]. Activation of dual independent signaling pathways by agonist binding to a single receptor may underlie these respective paradoxical effects. It is well known that signal transduction at the β_2AR and PAR_2 (as well as a multitude of other GPCRs) occurs through classic G-protein-dependent signaling, as well as via the more recently described β-arrestin-dependent signaling pathway (Fig. 1) [5,6]. Interestingly, the two pathways are also temporally separable, with β-arrestin signaling sometimes occurring earlier than the G-protein signaling pathway [7], and other times exhibiting a delayed and/or more prolonged signal [8]. Consistent with these distinct signaling pathway characteristics, we and others have shown that β_2AR and PAR_2 mediate bronchorelaxation through G protein-dependent signaling [4,9,10] and have accumulated evidence suggesting that β-arrestin-dependent signaling downstream of these receptors leads to a pro-inflammatory effect (Fig. 1) [4,11,12]. Because bronchorelaxation and inflammation are mediated via two independent signaling pathways, there is therapeutic potential in developing “biased” or “pathway-specific” ligands that preferentially activate or inhibit one signaling pathway over the other. Substantial murine and initial human data suggest that preferential activation of G-dependent, and inhibition of β-arrestin-dependent, signaling downstream of β_2AR or PAR_2 receptors would promote desirable effects for asthmatics such as bronchodilation while reducing associated pro-inflammatory effects (reviewed in [3]). This review examines how the dual signaling pathways activated by ligand binding at the β_2AR and PAR_2 give rise to both beneficial and detrimental effects in asthma and highlights β-arrestin-dependent signaling as the link underlying the parallels between the two.

β-arrestins

β-arrestins are adaptor proteins that are recruited to GPCRs to promote receptor desensitization and internalization, but they can also promote G-protein-independent signals, leading to a diverse array of physiological responses [5,6]. The role for β-arrestins in G protein-dependent signal termination occurs on several levels. The first characterized role for β-arrestins was the uncoupling of GPCRs from their cognate heterotrimeric Gα subunit, leading to a decrease in the responsiveness of the receptor to further agonist stimulation. β-arrestins can also link receptors to clathrin-coated pits, facilitating their internalization. Finally, ubiquitination, and thus, degradation of internalized receptors is facilitated by β-arrestins. In this fashion, β-arrestins are thought to “arrest” the initial G-protein-triggered signal [13]. Over the last decade, a more extensive role for β-arrestins in GPCR signaling has become appreciated. β-arrestins can serve as scaffolds for signaling complexes that then promote G-protein-independent signals. Most of these signals are positive, in that they facilitate activation of the proteins they scaffold, but there are also examples of β-arrestin-dependent inhibition of the enzymatic activity of kinases and GTPases [7,14,15]. A common result of β-arrestin-dependent signaling is cell migration and actin reorganization, as well as transcription of specific genes not targeted by the G-protein pathway [13,16–18]. In some cases, these targets of β-arrestin-dependent inhibition are downstream of G-protein signaling pathways, providing yet another mechanism by which β-arrestins can turn off the G-protein signal. Both the desensitization and signaling roles for β-arrestins come into play in

physiological and pathological situations such as regulation of airway responsiveness and airway inflammation. While loss of β-arrestin-induced desensitization can result in uncontrolled G-protein signaling events, which can be pathological, other G-protein signaling events are protective and in the absence of β-arrestins, these protective pathways are enhanced. Furthermore, β-arrestins can promote inflammatory signals; thus in the absence of β-arrestin signaling downstream of a number of GPCRs, inflammation is abated [4,12,19]. This review focuses on two such receptors: β_2AR and PAR_2, highlighting recent studies that demonstrate the potential therapeutic advantage of developing biased agonists or antagonists that target these receptors.

β_2AR and PAR_2 signaling in asthma

Dual roles for β-arrestin and G protein signaling in mediating β_2AR effects in asthma

β_2ARs are ubiquitously expressed and modulate a wide range of cellular responses when activated by epinephrine, their only endogenous ligand [20]. In the airway smooth muscle (ASM) agonist-activated β_2ARs couple to Gαs resulting in stimulation of membrane bound adenylyl cyclase, cyclic adenosine monophosphate (cAMP) generation and activation of the cAMP-dependent protein kinase (PKA), which mediates relaxation through phosphorylation of cross-bridge cycling regulatory proteins. In addition to the $G\alpha_s$/cAMP second messenger system, β_2ARs also mediate cellular responses via $G\alpha_i$ – induced generation of cGMP and Ca^{2+}; however, cAMP/PKA is the predominant mechanism underlying ASM relaxation (for a more complete review see [21] and Pera and Penn this issue). Through activation of β_2AR coupling to Gas, beta-agonists oppose airway smooth muscle (ASM) constriction and inhibit the release of pro-contractile agents, chiefly vagally-released acetylcholine (reviewed in [21,22]). We have shown using multiple methods (*in vitro*, *ex vivo* and *in vivo*) that β-arrestin-2 constrains β_2AR-mediated G protein-dependent ASM relaxation [9] making β-arrestin-2 inhibition an attractive therapeutic strategy in asthma irrespective of its pro-inflammatory role.

Shenoy et al. were the first to show that β_2ARs can utilize a G-protein-independent, β-arrestin-dependent signaling pathway to exert physiological effects [23]. Since then, several pieces of evidence, when taken together, suggest that β_2AR-mediated β-arrestin-dependent signaling is pro-inflammatory in asthma. Firstly, β-arrestins have been implicated as regulators of inflammation in a variety of diseases including asthma, sepsis, rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis and atherosclerosis [24,25]. Lung β-arrestin expression is up-regulated in murine models of asthma [10] and is similarly dynamically regulated in other inflammatory diseases [24]. Results from multiple murine studies have shown that the asthma phenotype is strikingly diminished in β-arrestin-$2^{-/-}$ mice irrespective of the method of induction of allergic airway disease [4,12,19]. Although development of the asthma phenotype is likely mediated by multiple GPCRs that signal through the β-arrestin-dependent pathway, β_2ARs are distinguished as being extremely important. Results from murine studies have shown that the asthma phenotype is significantly suppressed when either β_2AR expression or epinephrine synthesis is genetically abrogated [26,27]. Furthermore, blockade of β_2AR with select β_2AR antagonists (nadolol) can significantly diminish asthma severity in murine models [26,28] and reduce airway

sensitivity to methacholine in human trials [29]. Finally, chronic dosing with β-agonists can restore the asthma phenotype in mice that lack epinephrine or exacerbate it in mice that are replete with epinephrine [10,27,30] strongly suggesting that the deleterious effects associated with chronic β_2AR agonism in asthma result from β-arrestin-2-dependent signaling.

Like β_2ARs, β-arrestin proteins are also widely expressed. Thus, presently it is unknown in which cell type(s) β_2AR-mediated β-arrestin-dependent signaling contributes to the development of asthma. Recent evidence points to airway epithelial cells as playing a key role [31]. Airway epithelial expression of membrane-bound β_2AR and cytosolic β-arrestin-2 is significantly elevated relative to that in ASM cells [9,10] or T lymphocytes (unpublished data), two additional cell types that also figure prominently in asthma pathogenesis, and the magnitude of impairment of the mucin phenotype in $\beta_2AR^{-/-}$ mice is noticeably greater than that of the inflammatory cell or AHR phenotypes [11]. However, data from β-arrestin-$2^{-/-}$ bone marrow transplant chimeric mice suggest that βarr-mediated signal transduction in hematopoietic cells, in addition to that in lung structural cells, is required for full development of the asthma phenotype [19]. Consistent with this notion, both PAR_2- and C-C chemokine receptor 4 (CCR4)-mediated inflammatory cell chemotaxis is β-arrestin-dependent [4,12] in murine asthma models.

Dual roles for β-arrestin and G-protein signaling in mediating PAR_2 effects in asthma

PAR_2 is widely expressed on inflammatory cells, airway epithelium, smooth muscle and vascular endothelium, where it is activated by serine proteases that cleave the extracellular N-terminus to unveil a tethered ligand (human: SLIGKV/mouse: SLIGRL)[32,33]. This tethered ligand then binds to and activates the receptor, triggering downstream signaling events. Recently, high-affinity and stable agonists, such as 2-furoyl-LIGRL-O (aka 2fAP), have been developed and used both *in vivo* and *in vitro* to activate PAR_2 [34–36]. Although PAR_2 has been implicated in a number of inflammatory disorders, and has been heavily investigated as a putative therapeutic target for asthma, the nature of its involvement remains highly controversial. In favor of a protective role for PAR_2 in the airway, $PAR_2^{-/-}$ mice demonstrate heightened bronchial smooth muscle cell contraction and airway constriction, and administration of a PAR_2 peptide agonist promotes smooth muscle relaxation in isolated bronchioles and abrogates LPS-mediated inflammation [32,37,38]. In contrast, in an OVA-induced model of airway inflammation, cytokine production and infiltration of leukocytes into the bronchioles is impaired in $PAR_2^{-/-}$ mice, suggesting a pro-inflammatory role for PAR_2 [39–42], and intranasal administration of SLIGRL or 2fAP exacerbates these effects [4,43,44].

Recent studies have provided a plausible answer to this conundrum of the PAR_2 role in asthma. First, cytoskeletal reorganization and chemotaxis, which are the main cellular processes underlying the pro-inflammatory effects of PAR_2, do not require $G\alpha q/Ca^{2+}$ signaling, but rather utilize β-arrestins [7,14,45,46]. β-arrestins can signal to various actin assembly pathways to promote chemotaxis, but a major player in PAR_2/β-arrestin signaling is the actin filament severing protein, cofilin [7,47]. Conversely, other studies have demonstrated that PAR_2-induced smooth muscle relaxation, which initiates the protective

effects in the airway, is mediated by prostaglandin E_2 (PGE_2), derived from epithelial cells. PGE_2 production requires Gαq-induced mobilization of intracellular Ca^{2+}, activation of PI3K and phosphorylation of Akt (which leads to release of PGE_2), and nuclear ERK1/2 activation (which leads to expression of cyclooxygenase-1 and -2) [37,48]. Solidifying the importance of the PAR_2/β-arrestin signaling axis to inflammation in a modified mouse OVA-induced asthma model are the observations that PAR_2-induced recruitment of leukocytes to the airways, mucus production and cytokine release are abolished in β-arrestin-$2^{-/-}$ mice, while PGE_2 release and smooth muscle relaxation remain intact [4]. These studies suggest that two different signaling pathways may account for the contrasting effects of PAR_2: β-arrestin-dependent leukocyte chemotaxis and Gαq-dependent PGE_2 production.

Just as β_2ARs are ubiquitously expressed, so too is PAR_2, being found in the airway epithelium as well as the invading leukocytes (neutrophils, eosinophils, lymphocytes and macrophages) [37,49–51]. The protective effects of PAR_2 have been shown to be epithelium-dependent, as PGE_2 production and smooth muscle relaxation in bronchiolar rings in response to PAR_2 agonists is abolished when the epithelial cells are removed [48,51]. PAR_2-induced inflammation in mice is significantly reduced when wild type mice are transplanted with $PAR_2^{-/-}$ bone marrow, suggesting a crucial role for PAR_2 expressed on the infiltrating leukocytes [4]. However, $PAR_2^{-/-}$ mice transplanted with wild type bone marrow also showed reduced leukocyte infiltration, pointing to a role of epithelial PAR_2 in the progression of inflammation as well as the protective PGE_2 release. Together these data suggest that β-arrestins expressed within the structural and epithelial cells of the lungs, as well as invading leukocytes, contribute to the inflammatory responses during asthma.

Therapeutic Opportunities

Therapeutic targeting of GPCRs has, until very recently, been focused entirely on modulation of responses thought to be mediated by G protein-dependent signaling pathways. With the discovery that GPCRs also utilize β-arrestin-dependent signaling to cause physiological (and pathophysiological) effects, comes the opportunity to therapeutically regulate a second signaling pathway. Specifically, the now established concept of *dual independent GPCR signaling pathways* allows us to consider the possibility that one signaling pathway can be selectively manipulated to promote events that are therapeutic and avoid, or even inhibit, those that are harmful. For example, the superior clinical efficacy of carvedilol over other β-blockers, which inhibit classic G protein adrenoceptor signaling, in treating heart failure is attributed to carvedilol's activation of the β-arrestin-dependent signaling pathway [52]. Whereas β–arrestin-dependent signaling is beneficial in heart failure, it appears deleterious in several other diseases (cancer, atherosclerosis, chronic inflammation) including asthma. Substantial murine, and initial human, data suggest that asthma treatment would be significantly improved by preferential activation of β_2AR- and PAR_2–mediated G protein-dependent bronchodilation and/or inhibition of β-arrestin-dependent pro-inflammatory signaling.

Appreciation for the impact of β-arrestin-dependent signaling on drug discovery is rapidly emerging in the quest for biased ligands, allosteric receptor modulators and β-arrestin

inhibitor compounds. Recently developed cell based assays demonstrate that endogenous GPCR ligands are unbiased, in that they activate both the G protein- and β-arrestin-dependent signaling pathways at functionally relevant levels (Fig. 2a). Using these assays, β_2AR or PAR_2 ligands that favor G signaling over β-arrestin signaling could be found, or designed, and used to treat asthma (Fig. 2b). As an alternative approach, the signaling effects of an unbiased ligand could be shifted to favor Gs-dependent signaling over β-arrestin-dependent signaling by addition of a second drug that, through allosteric modulation, moves the receptor into a biased signaling conformation (Fig. 2c) (see Thanawala this issue). Along the same lines, an unbiased ligand could give rise to G protein-biased signaling if β-arrestin was pharmacologically prevented from participating in signal transduction (Fig. 2d). Yet another angle by which unbiased ligand-induced G protein-dependent signaling might be favored over that mediated by β-arrestin is to develop a drug that inhibits β-arrestin-mediated constraint of G protein-dependent bronchorelaxation (Fig. 2e). Such a drug may allow a normal bronchodilation effect to be generated by a much lower dose of β-agonist/PAR_2-agonist which in turn would only weakly activate the pro-inflammatory β-arrestin-dependent signaling pathway.

Concluding remarks

Unbiased agonists acting at either β_2AR or PAR_2 elicit paradoxical responses in asthma. Evidence suggests that ligand-induced protective bronchodilation and deleterious pro-inflammation are mediated through separate G protein- and β-arrestin-dependent signaling pathways, respectively. Selective promotion of the protective signaling pathway or inhibition of the inflammatory one, will lead to therapeutic advances in the treatment of asthma.

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Highlights

1. β_2AR or PAR_2 activation is both protective and pro-inflammatory in asthma
2. β_2AR and PAR_2 G protein-dependent signaling is protective in asthma
3. β_2AR and PAR_2 β-arrestin-dependent signaling is pro-inflammatory in asthma
4. Biased activation of PAR_2 and β_2AR G protein signaling may improve asthma treatment.
5. Biased inhibition of PAR_2 and β_2AR β-arrestin signaling may improve asthma treatment.

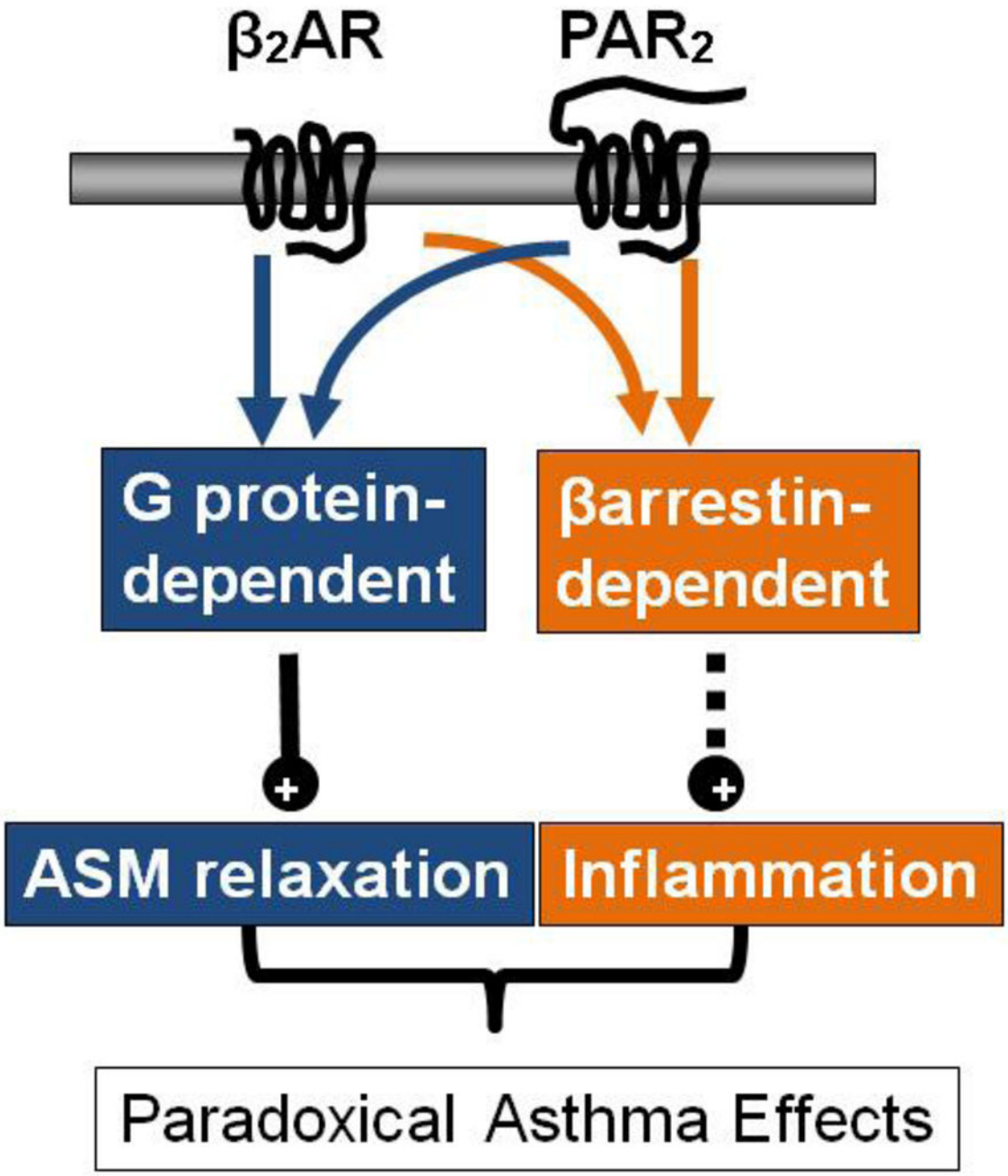

Figure 1. Dual signaling pathways and paradoxical response to bronchodilators

Activation of β_2AR or PAR_2 leads to both β-arrestin-dependent and G protein-dependent signaling. Activation of the G protein-dependent pathway leads to bronchorelaxation whereas activation of the β-arrestin-2-dependent signaling pathway is pro-asthmatic. (β_2AR, beta-2-adrenergic receptor; PAR_2, protease-activated-receptor-2; ASM, airway smooth muscle.

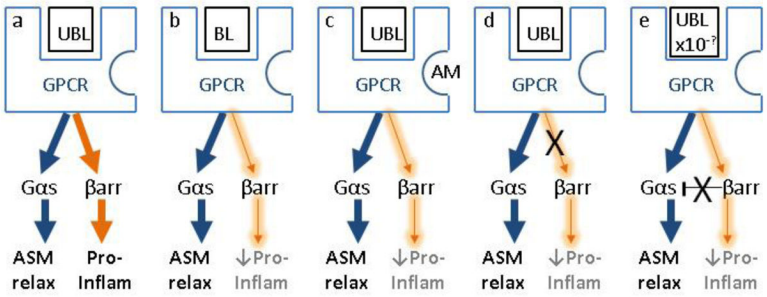

Figure 2. Impact of β-arrestin-dependent signaling on drug discovery for asthma treatment

a) Unbiased GPCR ligand functionally activates both the G protein- and Barrestin-dependent signaling pathways leading to bronchorelaxation and inflammation, respectively; b) Biased ligand favors G signaling over β-arrestin signaling; c) Allosteric modulation of the receptor facilitates biased signaling even though the ligand is unbiased; d) Intracellular inhibition of β-arrestin-dependent signaling; e) Low dose unbiased agonist results in normal ASM relaxation due to intracellular inhibition of β-arrestin-mediated constraint of G protein signaling. UBL, unbiased ligand; BL, biased ligand; AM, allosteric modulator; GPCR, G protein-coupled receptor, ASM relax, airway smooth muscle relaxation; Pro-inflam, pro-inflammatory; βarr, β-arrestin.