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GPCRs and arrestins in airways: implications for asthma

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Abstract

The obstructive lung disease asthma is treated by drugs that target, either directly or indirectly, G protein-coupled receptors (GPCRs). GPCRs coupled to G_q are the primary mediators of airway smooth muscle (ASM) contraction and increased airway resistance, whereas the G_s-coupled beta-2-adrenoceptor (β_2 AR) promotes pro-relaxant signaling in and relaxation of ASM resulting in greater airway patency and reversal of life-threatening bronchoconstriction. In additions, GPCR-mediated functions in other cell types, including airway epithelium and hematopoietic cells, are involved in control of lung inflammation that causes most asthma. The capacity of arrestins to regulate GPCR signaling, via either control of GPCR desensitization/resensitization, or via G protein-independent signaling, renders arrestins an intriguing therapeutic target for asthma and other obstructive lung diseases. This review will focus on the potential role of arrestins in those GPCR-mediated airway cell functions that are dysregulated in asthma.

Keywords

airway; arrestin asthma; beta-2-adrenoceptor; beta-agonist; beta-blocker; biased agonism; desensitization; epithelium; G protein-coupled receptor; G protein-coupled receptor kinase; inflammation; protease-activated receptor; tachyphylaxis; T cell

The lung is a highly complex organ system comprised of multiple cell types. Its primary function is to provide a conducting pathway (airways) and an interface (alveoli) to enable gas exchange-delivery of oxygen from ambient air to blood in exchange for carbon dioxide. Airways in the lung are the conduits of air flow. In the disease of asthma, airway conductance can be reduced (i.e., airway resistance is increased) by pathogenic mechanisms that cause constriction or blockage of the airway.

Lung inflammation caused by an exaggerated immune response to allergen is the most common cause of asthma. Numerous cells participate in allergic lung inflammation,

including airway epithelial cells and invading inflammatory blood cells. Airway smooth muscle (ASM) which surrounds conducting airways and whose contractile state influences airway diameter and thus airway conductance, is both a target and mediator of allergic lung inflammation.

Almost every cell in the lung has an important function that is regulated by G protein-coupled receptors (GPCRs). Thus arrestins, through either their capacity to regulate GPCR desensitization/sensitization, or mediate G protein-independent signaling, are likely to play an important role in influencing airway function. This review will focus on the potential role of arrestins in those GPCR-mediated airway cell functions that are dysregulated in asthma.

I. GPCRs play a prominent role in regulating airway resistance in normal physiology and in disease

Physiologic control of airway resistance

In health and under homeostatic conditions, ASM contractile state is an important determinant of airway resistance, and GPCRs tend to control this contractile state or “tone.” In both health and disease, members of the GPCR superfamily that activate the heterotrimeric G protein G_q are primarily responsible for the signaling events that promote ASM contraction, whereas GPCRs that activate G_s promote relaxation. Although ASM receives sympathetic and “nonadrenergic, noncholinergic” (van der Velden and Hulsmann, 1999; Widdicombe, 1998) neural input capable of modulating contractile state, release of acetylcholine from parasympathetic nerves, acting on G_q -coupled m3 muscarinic acetylcholine receptors (m3 mAChRs) on ASM, is the dominant physiologic regulator of the ASM tone (Canning and Fischer, 2001; Stephens, 2001) under normal conditions. Signaling events mediated by the m3 mAChR (and other G_q -coupled receptors) have been extensively characterized, and include activation of phospholipase C and production of inositol 1,4,5-trisphosphate (IP3), which binds to IP3 receptors on specialized intracellular stores to promote Ca^{2+} release from these stores. This flux combines with Ca^{2+} release from ryanodine-sensitive stores and influx through plasma membrane Ca^{2+} channels to elevate $[Ca^{2+}]_i$ to levels that stimulate calcium-calmodulin, myosin light chain kinase, and ultimately myosin ATPase, leading to cross bridge cycling and sarcomere shortening (reviewed in (An et al., 2007; Deshpande and Penn, 2006)). Under physiologic conditions, ASM tone is regulated primarily by the integration of afferent and efferent neural signals that determine parasympathetic release of acetylcholine (ACh); in contrast direct effects of (counterbalancing) endogenous G_s -coupled receptor agonists appear to play little if any role. Circulating catecholamines acting on ASM β_2 ARs have the potential to relax ASM, via multiple mechanisms including antagonism of pro-contractile signaling (Billington and Penn, 2003). However, in health such β_2 AR activity appears of little consequence, as administration of β_2 AR antagonists does not affect airway resistance in non-asthmatic subjects (Kiyangi et al., 1985; Vatrella et al., 2001). Thus, under baseline physiologic conditions, ASM tone is determined primarily by pro-contractile signaling and regulation of its stimuli, with little influence of competitive pro-relaxant stimuli. It is with the asthmatic condition where the signaling capacity and responsiveness of the β_2 AR to both endogenous

and exogenous beta-agonists is of importance in countering increased pro-contractile signaling and ASM tone.

Dysregulated airway resistance: pathologic ASM tone

Asthma reflects increased ASM tone. One cause of this increased tone is an exaggerated presentation of G_q -coupled receptor agonists causing increased pro-contractile signaling. Airway inflammation associated with both early and late phase allergic reactions promotes increased levels of pro-contractile agonists, acting primarily on ASM G_q -coupled receptors, that promote most asthmatic attacks. Many of these agonists (including histamine, leukotrienes, serotonin (5-HT), and some prostanoids) are secreted from numerous cell types in the airway including both infiltrating blood cells and resident airway cells. In addition, increased parasympathetic release of ACh has been shown to occur as a consequence of a loss of negative feedback inhibition by ACh acting on pre-junctional m2 mAChRs (Jacoby et al., 1993). Numerous agonists capable of activating G_i -coupled receptors are also elevated in the airway during inflammation. Although some of these agonists have direct effects on ASM, many have an indirect effect on ASM by functioning as chemokines for various cell types in the orchestration of the time-dependent inflammatory response (see below).

Unlike in the healthy lung, β_2 AR activity stimulated by endogenous catecholamines does appear to be important in the asthmatic subject, as administration of β AR antagonists is not well tolerated in many asthmatic subjects (Paterson et al., 1984). Thus, under conditions of excess G_q -coupled receptor activation and increased pro-contractile signaling, ASM contractile state acquires a greater sensitivity to β_2 AR activity.

Although airway inflammation is also associated with an increase in certain G_s -coupled receptor agonists in the airway, this upregulation does not appear to effectively counterbalance the increase in pro-contractile stimuli. In certain asthmatics, elevation of the COX product PGE₂ can mitigate augmented ASM pro-contractile signaling (cyclooxygenase inhibition tends to precipitate asthma attacks in sensitive patients (Morwood et al., 2005)), however the induction of G_q -coupled receptor agonists in the airway appears to dominate.

Most therapeutic approaches to asthma are effective in their ability to restore a competitive balance of pro- and anti- contractile signaling

Glucocorticoids, for example, reduce the induction of pro-contractile agonists in the airway by inhibiting the influx/survival of inflammatory cells, and by inhibiting the secretory actions of both inflammatory and mesenchymal airway cells. Muscarinic ACh and cysteinyl leukotriene receptor antagonists block specific G_q -coupled receptor signaling.

However, for over a century, the widely accepted first line treatment for asthma has been β -adrenoceptor agonists (actually, the use of the indirect acting sympathomimetic, ephedrine, dates back to over 5,000 years ago, but produced mixed results, and in general, the discovery of adrenaline is recognized as the first widely accepted treatment for an asthma exacerbation.) Inhaled beta-agonist activates ASM β_2 ARs to stimulate PKA activity which antagonizes G_q -coupled receptor signaling at multiple junctures (reviewed in (Billington and Penn, 2003; Kotlikoff and Kamm, 1996)). Under most conditions, inhaled beta-agonist is effective in preventing or reversing bronchoconstriction otherwise induced by exaggerated

presentation of pro-contractile agonists in the airway, demonstrating the strong regulatory capacity of the signaling events mediated by β_2 ARs. Interestingly, other G_s -coupled receptors in ASM demonstrate as good if not better regulatory control of ASM contraction relative to the β_2 AR. The PGE₂-activated EP2 (and possibly EP4) receptor is expressed in ASM; multiple ASM cell and tissue studies demonstrate that PGE₂ is actually more efficacious than beta-agonist in stimulating intracellular cAMP accumulation and PKA activation, and in relaxing ASM tissue ex vivo. In vivo, the ability of PGE₂ to prevent/reverse bronchoconstriction appears compromised by the existence of other EP receptor subtypes (some serving bronchoconstriction) in the lung, although a strong bronchorelaxant effect of EP2 receptor activation can be discerned from studies of EP1-4 receptor knockout mice (Tilley et al., 2003). Thus, EP2 or EP4 receptors represent a useful therapeutic target pending the development of selective receptor-subtype ligands. Presently, however, beta-agonists remain the gold standard of bronchodilators. In addition beta-agonists may also decrease pro-contractile agents by mechanisms such as inhibiting the release of histamine from mast cells and inhibiting Ach release by activation of pre-junctional inhibitory β_2 ARs from cholinergic nerves. Despite all of these advantages, β AR-agonists also have adverse events now termed the 'β-paradox', and recent developments in this story will be more fully described below.

Importance of GPCR-mediated functions in cell types other than ASM in asthma pathogenesis

Although the dominance of ASM in determining airway resistance renders it an obvious target of asthma therapies, the potential for developing effective asthma therapies that target GPCR-mediated functions in other cell types appears high. As will be discussed below, GPCRs that mediate allergic inflammation via either the capacity to induce inflammatory agents (in resident or invading cells) or promote inflammatory cell chemotaxis represent prime candidates for targeting.

II. Regulation of GPCR responsiveness in airway function, roles of GRKs and arrestins in effecting GPCR desensitization and the functional consequences

Whether regulation of GPCR responsiveness in ASM affects disease state or the response to therapy has been speculated for other 30 years. Appreciating the role of β AR agonism in regulating airway resistance, Szentinavyi (Szentivanyi, 1968) originally hypothesized that asthma constituted an intrinsic defect in β_2 AR function. It is now appreciated that exaggerated presentation of pro-contractile agents (discussed above) and sensitization to the calcium signal induced by these agents, and not a failure of the β_2 AR to respond to endogenous catecholamines is what drives most asthma. However, the discovery of variation in β_2 AR gene (ADRB2) coding sequence prompted many to speculate that differing susceptibility to receptor desensitization could influence asthma prevalence, or possibly the response to inhaled beta-agonist therapy. With respect to the former, studies to date paint a mixed picture; although some data appear to suggest ADRB2 variation may influence asthma disease progression/severity, the collective evidence indicates the more common

ADRB2 polymorphisms are not clear risk factors for asthma per se. In an analysis of 8018 asthmatics, Hall et al. (Hall et al., 2006) concluded that although ADRB2 polymorphisms might predict a small component of the long-term prognosis in childhood asthma, they are not important determinants of asthma incidence or prevalence in the British population. A more recent consortium-based genome-wide association study of 10,265 asthmatics and 16,111 nonasthmatics did not find a genome wide significant signal for the ADRB2 locus (Moffatt et al., 2010). With respect to response to therapy, the clinical relevance of any influence of ADRB2 variation appears minimal. Several studies (reviewed in Sayers and Hall (Sayers and Hall, 2005)) suggest asthmatics homozygous for the Arg16 variation have lower forced expired volumes (FEV1) and peak flow rates in response to regular inhaled albuterol, a short acting beta-agonists. However, patients treated with long acting beta-agonists, the more commonly prescribed maintenance asthma therapy, exhibited no genotype specific effects on efficacy (Bleecker et al., 2007).

Although ADRB2 genotype may have minimal effect on β_2 AR responsiveness in asthma, there is considerable evidence, from both clinical and basic science studies, that β_2 AR desensitization is of consequence in asthma. Tachyphylaxis, defined as a loss of functional response at a more integrative level, is observed for the β_2 AR in asthma as loss of the bronchoprotective effect (reviewed in (Billington and Penn, 2003; Deshpande and Penn, 2006; Walker et al., 2011)) in asthmatics, which can be recapitulated in murine models of asthma through chronic oropharyngeal insufflation of albuterol (Lin et al., 2012). Loss of bronchoprotection has long been cited as evidence of desensitization of the β_2 AR in the airway, presumably ASM β_2 ARs. Moreover, chronic use of inhaled beta-agonists by asthmatics is associated with a loss of disease control (reviewed in^{56, 59, 65}), and even mortality^{36, 47, 61, 63}. These events have resulted in a black box warning for products containing long-acting beta-agonists (LABAs).

Whether these poor clinical outcomes associated with chronic beta-agonist use are a consequence of β_2 AR desensitization is unclear. To more directly pursue the question of agonist-induced β_2 AR desensitization in ASM, and the roles of GRKs and arrestins, we undertook a series of studies. Initial studies demonstrated a clear loss of β_2 AR signaling capacity, that was enhanced by GRK2 overexpression, in cultured human ASM cultures following brief pretreatment with beta-agonist (Penn et al., 1998). Similarly, overexpression of β -arrestin1 or β -arrestin2 attenuated beta-agonist-signaling in ASM (Penn et al., 2001). We subsequently demonstrated that agonist-specific desensitization of the β_2 AR, but not of the EP2 receptor, in ASM cultures could be reversed by siRNA-mediated knockdown of GRK2/3, or via expression of the (inhibitory) GRK2/3 mini-gene (Kong et al., 2008).

We subsequently employed a more comprehensive approach to explore the physiological relevance of GRK/arrestin-dependent desensitization of ASM β_2 ARs. In Deshpande et al. (Deshpande et al., 2008) we demonstrated the capacity of siRNA-mediated knockdown of β -arrestin1/2 (in human ASM cultures) and or genetic deletion of β -arrestin2 (in ASM cultures derived from β -arrestin2 knockout mice) to specifically augment β_2 AR signaling (Fig. 1A,B). In analyses of ASM contraction, the ability of beta-agonist to relax contracted ASM *ex vivo* was greater in airways excised from β -arrestin2 knockout mice compared to airways from wild type mice (Fig. 1C). Finally, beta-agonist (but not PGE₂) was more effective in

reducing the increase in airway resistance *in vivo* caused by methacholine challenge (Fig. 1D). Interestingly, we observed no evidence of desensitization of mAChRs; arrestin knockdown or knockout affected neither methacholine-stimulated signaling, nor methacholine-stimulated contraction of ASM *ex vivo* or *in vivo*. Although previous studies have demonstrated GRK- and arrestin-mediated desensitization of endogenous m3mAChRs (in HEK293 cells)(Luo et al., 2008), in ASM no evidence of such at a signaling or functional level was evident. Whether this reflects a lack of m3mAChR modification, or simply a large receptor reserve in ASM, is unknown.

An important observation that emerges from the above studies is the greater capacity of the β_2 AR, relative to other GPCRs, to be subject to GRK/arrestin-dependent desensitization in ASM. The EP2 receptor, which in ASM is more efficacious than the β_2 AR in signaling and functional antagonism of contraction, exhibits minimal agonist-specific desensitization (Deshpande et al., 2008;Kong et al., 2008;Yan et al., 2011) that is unaffected by either overexpression or inhibition of GRKs/arrestins(Deshpande et al., 2008;Kong et al., 2008;Penn et al., 2001). Similarly, neither the m3mAChR nor the cysteinyl leukotriene type 1 receptor (Deshpande et al., 2007;Naik et al., 2005) (arguably the 2 most clinically relevant mediators of bronchoconstriction) are subject to GRK/arrestin-dependent desensitization in ASM. Clinically, the relative selectivity of GRKs/arrestins for the β_2 AR suggests that therapies targeting GRKs or arrestins in ASM may be useful, given they would preferentially augment β_2 AR (broncho-relaxant) while failing to enhance Gq-coupled receptor (pro-contractile) function (Fig. 2).

In summary, studies suggest that the β_2 AR is subject to significant agonist-specific desensitization in ASM, and that inhibition of arrestins can improve β_2 AR signaling and the bronchodilation effect of beta-agonist. Thus, given their ability to constrain the physiological actions of endogenous and exogenous activators of the β_2 AR on ASM, arrestins likely have a role in influencing the effectiveness of asthma therapy, and possibly a role in asthma occurrence/severity, via their ability to mediate β_2 AR desensitization.

III. The critical role for arrestins in allergic lung inflammation

As noted above, numerous cell types, other than ASM, participate in the development of allergic inflammation in the lung, which is the pathologic event that causes most asthma. Therefore, via their role in regulating various GPCR-dependent functions in these cell types, arrestins have the potential to influence allergic lung inflammation, and thus, the asthma phenotype.

In 2003 Walker et al. (Walker et al., 2003) provided insight into the role of arrestins in mediating allergic lung inflammation using a murine model in which ovalbumin sensitization and challenge (OVA S/C) was performed. Despite clear evidence of OVA sensitization, the asthma phenotype, as assessed by airway inflammation and hyperresponsiveness (Fig. 3a,b) and BAL fluid cytokine levels, was strikingly absent in OVA S/C β -arrestin2 knockout mice. β -arrestin-mediated regulation of ASM contraction or general immune function was ruled out by demonstrating comparable, significant elevations

in airway inflammation and hyperresponsiveness in response to LPS inhalation in both WT and β -arrestin2 knockout mice.

Numerous inflammatory processes in the lung involve GPCR-dependent functions in numerous cell types. Among the most obvious is chemotaxis of inflammatory cells from the vascular compartment and into the airway. Once in, these cells cooperate with other invading cells, and with resident cells, to promote the inflammatory phenotype of asthma, and in turn promote AHR. Given the early and important role for T cells in the asthma inflammatory cascade, further investigation revealed that T cell migration to the airways was significantly reduced in OVA S/C β -arrestin2 knockout mice (Fig. 3d) mice and this finding was supported by in vitro data showing that T cells devoid of β -arrestin2 exhibit a significant reduction in CCL22-mediated T cell chemotaxis (Fig. 3c). Additionally, in vitro work using naïve CD4⁺ T cells has shown that β -arrestin2 regulates neither polarization to, nor proliferation of, Th1 or Th2 cells (data not shown). Interestingly, chemotaxis of CD4⁺ T cells devoid of β -arrestin2 is only impaired, not abrogated; thus, it will be interesting to determine if aspects of Th2 cell function, once arriving in the airway, is also regulated by β -arrestin2.

In a follow-up study designed to explore if cell types other than CD4⁺ T cells are required for β -arrestin2-dependent allergic inflammation, Hollingsworth et al. (Hollingsworth et al., 2010) generated bone marrow chimera mice. OVA S/C treatment of β -arrestin2 bone marrow chimeric mice showed that β -arrestin2 expression in either hematopoietic and non-hematopoietic cells was insufficient to fully rescue the asthma phenotype. In response to IL-13 airway challenge, which can induce the signs of allergic inflammatory airway disease independent of T cells, lung eosinophilia was significantly impaired in chimeric mice that lacked β -arrestin2 expression in hematopoietic cells. Although not measured directly, these data suggest that in addition to T cell chemotaxis, β -arrestin2 may also regulate eosinophil chemotaxis in asthma.

IV. Further evidence arrestins promote allergic inflammation in the lung; honing in on the receptors, and the potential role of G protein-independent signaling

Further mechanistic insight into how arrestins mediate allergic lung inflammation is provided by a recent study by Nichols et al. (Nichols et al., 2012) which identifies the importance of protease activated receptor 2 (PAR2) in mediating allergic lung inflammation and distinguishes protective and pathogenic roles, respectively, for PAR2 G protein and arrestin – dependent signaling. To assess the role for arrestin-dependent PAR2 signaling in mediating the asthma phenotype, repeated insufflation of a PAR2 agonist was combined with the OVA S/C model. PAR2 agonist administration exacerbated airway inflammation and mucin production in WT mice, but not in β -arrestin2 knockout mice, suggesting that the pro-inflammatory effect of PAR2 activation requires β -arrestin2. In contrast, PAR2-mediated ASM relaxation (both in vivo and ex vivo), was similar in WT and β -arrestin2 knockout mice, as were BAL levels of PGE₂, suggesting that the G protein-dependent

signaling pathway mediates the anti-contractile effect of PAR2 activation through release of PGE₂.

These findings exemplify the concept of “qualitative signaling”, functional selectivity” or “biased agonism” that has emerged over the last 15 years since the discovery that arrestins could function as scaffolds and initiate signaling events distinct and independent of those promoted by heterotrimeric G proteins (reviewed in (Luttrell and Gesty-Palmer, 2010)). Indeed, the mounting evidence that arrestin-mediated G protein-independent signaling by numerous GPCRs, the importance of such signaling in normal physiologic processes and disease, and the potential to selectively manipulate receptor signaling pathways via biased ligand approaches (see Kenakin (EDITOR-insert ref) in this volume), has ushered in a new era of GPCR biology and likely, a new generation of clinical pharmacology. While the studies of the DeFea lab suggest the development of PAR2 biased ligands have the potential to differentially regulate pro- and anti-asthmatic processes, recent studies by the Bond lab raise similar possibilities for a biased ligand approach for β_2 ARs in the treatment of asthma.

Because as stated above, β_2 AR agonists are the mainstay of asthma therapy and involved in all steps of asthma management, their history and potential mechanisms merit greater detail. In 1900 adrenaline was discovered, and by 1907 had become the first widely accepted treatment for asthma (Barnes, 2006). Eventually, following seminal studies by Ahlquist in 1948 (AHLQUIST, 1948a; AHLQUIST, 1948b), subdividing adrenergic receptors into alpha- and beta-subtypes, and then Lands (Lands et al., 1967) in 1967 subdividing beta-receptors into β_1 ARs and β_2 ARs, adrenaline was identified as the endogenous ligand for the β_2 AR. In the 1950's inhaled isoproterenol was introduced as a highly effective bronchodilator with less potential for cardiovascular events like hypertension than adrenaline. Shortly after the debut of isoproterenol began the highly controversial suggestion that the β_2 AR also mediated deleterious adverse effects in asthma therapy. The first β_2 AR agonist ‘epidemic’ was reported was an increase in mortality associated with the use of high dose isoproterenol in England and Wales (Stolley, 1972; Stolley and Schinnar, 1978). The scientific cause for this increase mortality was attributed to its non-selective agonism of both β_1 ARs and β_2 ARs, combined with its high efficacy, producing excessive cardiac stimulation. The next β_2 AR agonist epidemic occurred in Australia and New Zealand (Grainger et al., 1991; Pearce et al., 1995) and was associated with the use of the β_2 AR agonist, fenoterol, and despite fueling the ‘ β -agonist paradox’ controversy because of the failure to establish a causal effect of fenoterol, the observed spike in asthma mortality decreased following its withdrawal from the market. Fenoterol was another fairly high-efficacy drug and perhaps continued to support the desensitization hypothesis as the cause of adverse effects. However, there were also sporadic epidemiological reports correlating the amounts of use of the rescue β_2 AR agonist, albuterol (a much lower efficacy agonist), and increased mortality. In turn these results were explained by the fact it was much more likely it was people with severe asthma who were likely to need more frequent use of rescue inhalers (Sears et al., 1990).

Then in 1993 the Serevent® Surveillance Trial results showed that, while not reaching significance, a new longer-acting β_2 AR agonist (LABA), salmeterol, that was administered twice daily, also produced higher mortality than the placebo group which used as needed albuterol for rescue (Castle et al., 1993). These last results prompted the FDA to ask the

makers of salmeterol to begin a post-marketing surveillance trial that began in 1998 (Nelson et al., 2006). This trial was a very large multi-center trial of ~13,000 patients receiving salmeterol twice daily, and another 13,000 in the placebo group (which consisted of as needed rescue with albuterol). This trial was prematurely stopped due to excess mortality in the salmeterol group, and began a series of FDA labeling requirements each causing further restrictions on the use of LABAs, including their removal as add-on therapy to as needed short-acting β_2 AR agonist rescue. Thus while clearly the most effective bronchodilators ever produced, and eminently useful in reversing bronchoconstriction and saving countless lives, β_2 AR agonists were shadowed by reports that constant, chronic use may negatively adverse asthma control and negatively impact morbidity and mortality.

It was against this background that in 2001 we formulated a hypothesis that asthma and congestive heart failure (CHF), while different in many ways, may be similar with regards to the chronic use and outcomes of β -adrenoceptor ligands (Bond, 2001). In CHF, as in asthma, the use of β -agonists was associated with symptomatic improvement, and as in asthma, β -blockers were contraindicated. However, evidence was accumulating in CHF that chronic treatment with certain β -blockers resulted in decreased mortality. Finally, in 1997, the FDA approved the first β -blocker, carvedilol, for the treatment of CHF. To our knowledge, this marked the first time in history that a drug had moved from a contraindicated drug, to the drug of choice for decreasing mortality in the disease it was once contraindicated.

Our studies to date show a requirement of β_2 AR signaling for development of the asthma phenotype in murine asthma models (reviewed in (Walker et al., 2011)). To date we have shown that chronic treatment with certain β -blockers attenuates AHR, inflammation, mucin overproduction, and the increase in Th2 cytokines (Callaerts-Vegh et al., 2004; Lin et al., 2012). We have demonstrated that the effect is mediated via the β_2 AR, as treatment with the highly selective β_2 AR antagonist, ICI-118,551 (Nguyen et al., 2008), or ablation of ADRB2 (Nguyen et al., 2009), prevents the development of all the measured parameters of the asthma phenotype. Furthermore, two small, proof-of-concept clinical trials have shown that chronic treatment with the β -blocker nadolol, attenuates AHR (as measured by the PC20 methacholine) in mild asthmatics (Hanania et al., 2007; Hanania et al., 2010). These results have now led to an ongoing NIH sponsored double blind, placebo controlled, multi-center trial using nadolol in mild asthmatics (NIMA trial; ClinicalTrials.gov).

Several critical questions arise from these findings; perhaps most importantly, what is the signaling pathway(s), and in what cell type(s) are they necessary to allow the pro-inflammatory allergic response? Whereas the pro-inflammatory actions of the PAR2 receptor appear dependent on G protein-independent arrestin signaling (and PAR2 dependent (indirect) activation of Gs appears protective), the β_2 AR-mediated signaling events that promote inflammation and the asthma phenotype are unclear. In ASM, Gs/PKA signaling is implicated in bronchodilation, and in the inhibition of ASM growth, caused by either beta-agonists or by EP2 receptor activation. As noted, EP2 receptors do not recruit arrestins and appear resistant to GRK/arrestin-mediated desensitization (Deshpande et al., 2008; Kong et al., 2008). Only a handful of inflammatory cytokines (e.g., IL-6) are known to be positively regulated by Gs/PKA signaling (reviewed in (Billington et al., 2012)). With respect to β_2 AR-mediated augmentation of mucin production in airway epithelia, Gray et al.

(Gray et al., 2004) reported that IL-1 β and PGE₂-stimulated Muc5a secretion in human airway epithelial cells was blocked by either cyclooxygenase inhibitors and PKA inhibitors, respectively. However, an early study suggested that cyclic AMP production stimulated by beta-agonist is dissociated from mucin secretion in rat submandibular acinar cells (Bradbury et al., 1990).

Alternatively, arrestin dependent signaling is capable of activating several pathways (e.g., p44/p42, Akt, NF- κ B, NFAT) important in inducing inflammatory gene expression in multiple cells types. Similar to that observed for the PAR2 receptor, β_2 AR-mediated arrestin signaling may also play a role in promoting the inflammatory cell migration mechanism dependent on arrestin. An intriguing hypothesis suggested by the collective findings to date is that arrestin-dependent signaling, mediated by various GPCRs in multiple cells, is an important effector of multiple features of asthma, whereas G protein signaling, specifically that which results in PKA activation, has a counterbalancing effect (Fig. 4).

To date however, limited evidence exists specifically implicating arrestin signaling in asthma pathogenesis associated with β_2 AR agonism. The subset of β -blockers that possesses β_2 AR inverse agonist properties, such as ICI-118,551 and nadolol, has been shown to be effective in attenuation of the development of allergic inflammation in murine models. However this same subset is effective in antagonizing both Gs and arrestin-dependent signaling by the β_2 AR, and thus no insight into which specific β_2 AR signaling pathway promotes AHR is afforded by these studies. This dual inhibitory property of the β -blockers for both pathways also prevents us from determining if one of the pathways may be protective. Future studies employing genetic approaches in murine models, as well as testing different β_2 AR ligands of varying “bias”, should clarify the role of β_2 AR-mediated arrestin signaling in asthma pathogenesis. Should a contributory role be discerned, either global targeting of arrestins in the lung, or more selective targeting of β_2 AR-mediated arrestin signaling with biased ligands that fail to promote arrestin signaling, would represent innovative asthma drugs.

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Figure 1. β -arrestin2 specifically regulates the β_2 AR signaling and function in ASM β_2 AR (A) but not m3 mAChR or EP2 receptor (B and not shown) signaling is augmented ASM cultures derived from ASM tissue from β -arrestin2 knockout mice. This increase in β_2 AR is associated with an increased ability to relax carbachol-contracted ASM ex vivo (C) and in vivo (reflected in change in airway resistance; D). Data from Deshpande et al. (Deshpande et al., 2008) and reproduced with permission from *FASEB Journal*.

Figure 2. Arrestin control of ASM signaling that regulates ASM contraction

β -arrestin2, and possibly β -arrestin1, limits β_2 AR signaling and the capacity of beta-agonists to antagonize Gq-mediated ASM contraction. Conversely, Gq-coupled receptor (e.g., m3 mAChR or CysLT1R) signaling is minimally/not affected by arrestins. Thus, global or selective arrestin targeting has the potential to preferentially increase β_2 AR signaling throughput and shift the balance of signaling resulting in reduced contractile tone.

Figure 3. β-arrestin2 is required for development of the Asthma Phenotype

OVA-treated Wild type mice (black bars) displayed a significant increase in (A) total BAL lung cells, eosinophils and lymphocytes as well as (B) airway hyperresponsiveness relative to saline-treated WT and β-arrestin2-KO mice (light and dark gray bars, respectively) and compared to OVA—treated β-arrestin2-KO mice (white bars). (C) Chemotaxis of β-arrestin2-KO T lymphocytes to macrophage-derived chemokine (MDC) was significantly impaired as was (D) the accumulation of lung CD3+ and CD4+ T cells. Data from Walker et al. (Walker et al., 2003) and reproduced with permission of *J Clin Invest*.

Figure 4. Pro- and anti-asthmatic effects linked to specific G protein-independent and dependent signaling, respectively

Proposed model by which qualitative signaling influences asthma pathogenesis. Based on studies by the Walker, DeFea, and Bond labs, G protein-dependent signaling mediated by either PAR2 or β_2 AR receptors in various airway cell types (including ASM, airway epithelium, or invading hematopoietic cells) can result in inhibition of multiple indices of inflammation or direct relaxation of ASM, thus attenuating the asthma phenotype. Conversely, G protein-independent signaling mediated by arrestins promotes inflammation and associated airway hyperresponsiveness.