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Bronchoprotection and bronchorelaxation in asthma: New targets, and new ways to target the old ones



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ABSTRACT

Despite over 50 years of inhaled beta-agonists and corticosteroids as the default management or rescue drugs for asthma, recent research suggests that new therapeutic options are likely to emerge. This belief stems from both an improved understanding of what causes and regulates airway smooth muscle (ASM) contraction, and the identification of new targets whose inhibition or activation can relax ASM. In this review we discuss the recent findings that provide new insight into ASM contractile regulation, a revolution in pharmacology that identifies new ways to “tune” G protein-coupled receptors to improve therapeutic efficacy, and the discovery of several novel targets/approaches capable of effecting bronchoprotection or bronchodilation.

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1. Introduction

Asthma is a prevalent disease. The World Health Organization estimates that 235 million people worldwide suffer from asthma, which is the most common noncommunicable disease among children (WHO,

2013). Unfortunately, multiple studies suggest up to 55% of asthmatics have suboptimal control of their disease (Peters et al., 2007), and concerns of asthma drug safety have persisted for decades (Donohue, 2008). These problems represent an obvious clarion call for the development of new, better and safer anti-asthma drugs. Although recent studies suggest certain asthmatic subpopulations may benefit from new antibody-based therapies that target IgE, specific cytokines, or their receptors (reviewed in (Mitchell et al., 2016)) these represent expensive and difficult-to-manage options for niche populations. For the vast majority of asthmatics, treatment options have changed little over the last 2 decades. Other than anti-cysteinyl leukotriene receptor antagonists, which have modest efficacy for most asthmatics, there have only been refinements of the mainstream asthma drugs – beta-agonists and anti-cholinergics – to improve their specificity and duration on action. Truly novel therapeutic approaches that overcome the

Abbreviations: ASM, airway smooth muscle; AHR, airway hyperresponsiveness; β_2 AR, beta 2 adrenergic receptor; BTR, bitter taste receptor; CysLT1R, cysteinyl leukotriene receptor 1; CaSR, calcium sensing receptor; EP, prostanoid EP receptor; GPCR, G protein-coupled receptor; M3 mAChR, cholinergic M3 receptor; OGR1, ovarian cancer G protein-coupled receptor 1; PAR, protease-activated receptor; PDE, phosphodiesterase; PKA, protein kinase A.

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limitations of current drugs (including efficacy, tolerance, ease of use, and safety) for obstructive airway diseases have not emerged.

Fortunately, recent advances in multiple fields of science now provide new direction that dramatically changes the prospects for asthma therapy. Most prominent among these advances are those in pharmacology, including increased efforts into the development of allosteric modulators, the introduction of “bi-functional” drugs that represent a fusion of two established drugs in one molecule, and a newfound appreciation of qualitative signaling that has stimulated the identification and development of “biased” ligands.

Moreover, recent studies which provide a better understanding of what dictates and controls airway smooth muscle (ASM) contraction have helped create an expanded list of therapeutic targets, including previously unappreciated GPCRs on ASM. This knowledge, coupled with drug discovery approaches that no longer focus solely on orthosteric ligands, has us poised to break the long drought in delivering new asthma therapeutics.

2. A brief and simplistic history of asthma treatment

Asthma management has historically focused on: 1) directly inhibiting ASM contraction to either prevent (bronchoprotection) or reverse bronchoconstriction (bronchorelaxation); and 2) controlling airway inflammation in order to limit the stimuli in the airway that cause ASM contraction. A secondary yet important benefit of controlling inflammation is the limiting of other factors, independent of ASM contractile state, that increase impedance to airflow (mucus, airway edema).

2.1. Airway inflammation control

Corticosteroids, either inhaled or oral, have been the drug of choice for managing airway inflammation (most commonly caused by inhaled allergen). Inhaled corticosteroids are the recommended choice for managing mild asthma. However, lack of direct bronchodilator activity and the time lag between inhalation of drug and resolution of inflammation does not permit them to be a rescue drug when rapid reversal of acute bronchospasm is required. Currently, the best-selling asthma drug is a combined inhaled formulation of the corticosteroid fluticasone and the long-acting beta-agonist salmeterol. However, due the slow onset of action of salmeterol, this drug is not suitable for rescue. Faster-acting beta-agonists, as either monotherapy or when combined with corticosteroids (formoterol/budesonide), are the drugs of choice for rescue from acute bronchospasm.

Other anti-inflammatory drugs used in asthma management include cromolyn sodium, prescribed for treatment of mild persistent asthma and presumed to work by stabilizing mast cells (Lane & Lee, 1996). Anti-IgE antibody treatment has recently emerged for the management of severe, corticosteroid-resistant asthma (Humbert et al., 2014). Other drugs that target specific inflammatory agents include anti-IL-13 antibodies which have shown promise in recent clinical trials (De Boever et al., 2014; Hodsman et al., 2013; Noonan et al., 2013), whereas results from trials testing anti-IL-5 antibodies have been mixed (Powell et al., 2015). Among those drugs with direct bronchodilator properties (discussed below), cysteinyl leukotriene type 1 receptor (CysLT1R) antagonists exhibit anti-inflammatory properties, which can be complementary to the anti-inflammatory effects of corticosteroids, in asthmatics (Currie & Lipworth, 2002; Davis et al., 2009; Laitinen et al., 2005; Maeba et al., 2005; Pizzichini et al., 1999; Ramsay et al., 2009; Sandrini et al., 2003). Anti-inflammatory effects of M3 mAChR antagonists have also been shown in animal models of asthma (Bos et al., 2007; Gosens et al., 2005; Ohta et al., 2010). The only study to address this phenomenon in humans paradoxically found that clinical benefits of tiotropium were associated with increased inflammation markers in sputum (Powrie et al., 2007). However, the potential concentrating effect of tiotropium on airway mucus (Powrie et al., 2007) as well as

other limitations (Soriano & Barnes, 2007) may be confounding these findings. Lastly, phosphodiesterase inhibitors, currently proposed as adjuncts or add-on therapy in asthma, have demonstrated anti-inflammatory properties in clinical studies (Fan, 2006).

The scope of this review precludes an in-depth analysis of the class of corticosteroid drugs; the reader is referred to (Barnes, 2005; Boardman et al., 2014; Ito et al., 2006a). Those corticosteroids currently prescribed vary modestly in their ability to control airway inflammation as assessed by clinical studies measuring various indices of inflammation (Westergaard et al., 2015). A wealth of literature exists detailing mechanisms by which corticosteroids suppress inflammation (Boardman et al., 2014; Newton, 2000); a topic well beyond the scope of this review. Since the first corticosteroid introduced to treat asthma, the refinement of these drugs has focused on improving potency and mode of delivery to reduce systemic side effects (Colice, 2006). Current efforts to develop better corticosteroids for asthma include attempts at dissociating the transactivating (gene transcription-inducing) from the transrepressive (transcription-suppressing) properties of the drug. This approach is based on the belief that the therapeutic benefit of corticosteroids is derived primarily from their ability to suppress, via either inhibition of transactivating transcription factors or direct binding to negative glucocorticoid response elements in gene promoters, transcription of pro-inflammatory genes (Westergaard et al., 2015). Whether this simplistic rationale will result in an improved corticosteroid is unclear. Of note, anti-inflammatory effects of GR-induced genes have been identified (King et al., 2013; Newton & Holden, 2007), suggesting that selective GR transactivation has therapeutic benefit and a more refined approach to gene regulation is desirable.

2.2. Bronchodilation/bronchoprotection

Most bronchodilators act through G protein-coupled receptor (GPCR) activation (G_s pathways) or inhibition (G_q pathways) at the receptor locus. We have previously espoused the concept of a dynamic or competitive balance of GPCR signaling in ASM as an important determinant of ASM contractile state, and thus, airway resistance in asthma and COPD (Humbert et al., 2014; Lane & Lee, 1996; Peters et al., 2007). Although airway resistance is admittedly influenced by additional factors including airway remodeling, airway architecture and tissue mechanics, and other phenomena contributing to impedance, agents that contract and relax airway smooth muscle have a dominant effect on airflow and largely work through ASM GPCRs. Allergic airway inflammation is typically associated with increased levels of numerous agonists for different pro-contractile (typically G_q -coupled) ASM GPCRs causing ASM contraction and increased airway resistance. One means of preventing or reversing this effect is to deliver to the airway a selective small molecule antagonist of a GPCR causing ASM contraction (for example M3 mAChR or CysLT1R antagonists). However, this strategy can be of limited effect, especially if multiple different receptors are contributing to bronchoconstriction. Alternatively, delivery of beta-agonists to the airway promotes broncho- protection/relaxation by antagonizing signaling from pro-contractile ASM GPCRs at multiple junctures including transmembrane receptor signaling, calcium flux through membrane channels and intracellular stores, and distal elements regulating myosin light chain phosphorylation (Fig. 1) through myosin light chain kinase regulation or myosin light chain phosphatase regulation (calcium sensitization). Thus, beta-agonists, as well as other agents (e.g., phosphodiesterase inhibitors and agonists of other G_s -coupled receptors discussed below and noted in Fig. 1) have the ability to broncho-relax/protect an airway exposed to numerous and multiple pro-contractile GPCR agonists.

Not surprisingly, the evolution of GPCR ligands as therapeutic bronchodilators has involved improving specificity of the ligands for the critical GPCR subtype target. These refinements have led to significant reductions in off-target/side effects that severely limited the usefulness of early drugs. For the M3 mAChR, progressive modification of the

nonspecific mAChR antagonist atropine has produced drugs with fewer side effects, especially when delivered by inhalation. The short-acting mAChR antagonist ipratropium was one such drug used for asthma (albeit more frequently for COPD) management for almost 30 years, ultimately supplanted by the long-acting mAChR antagonist tiotropium,

which possesses fast/slow dissociation kinetics for the M2 mAChR and M3 mAChR respectively, and exhibits a superior clinical profile to that of previous mAChR antagonists (Haddad et al., 1994). Current CysLT1R antagonists have not undergone refinement since their initial introduction as asthma therapies.

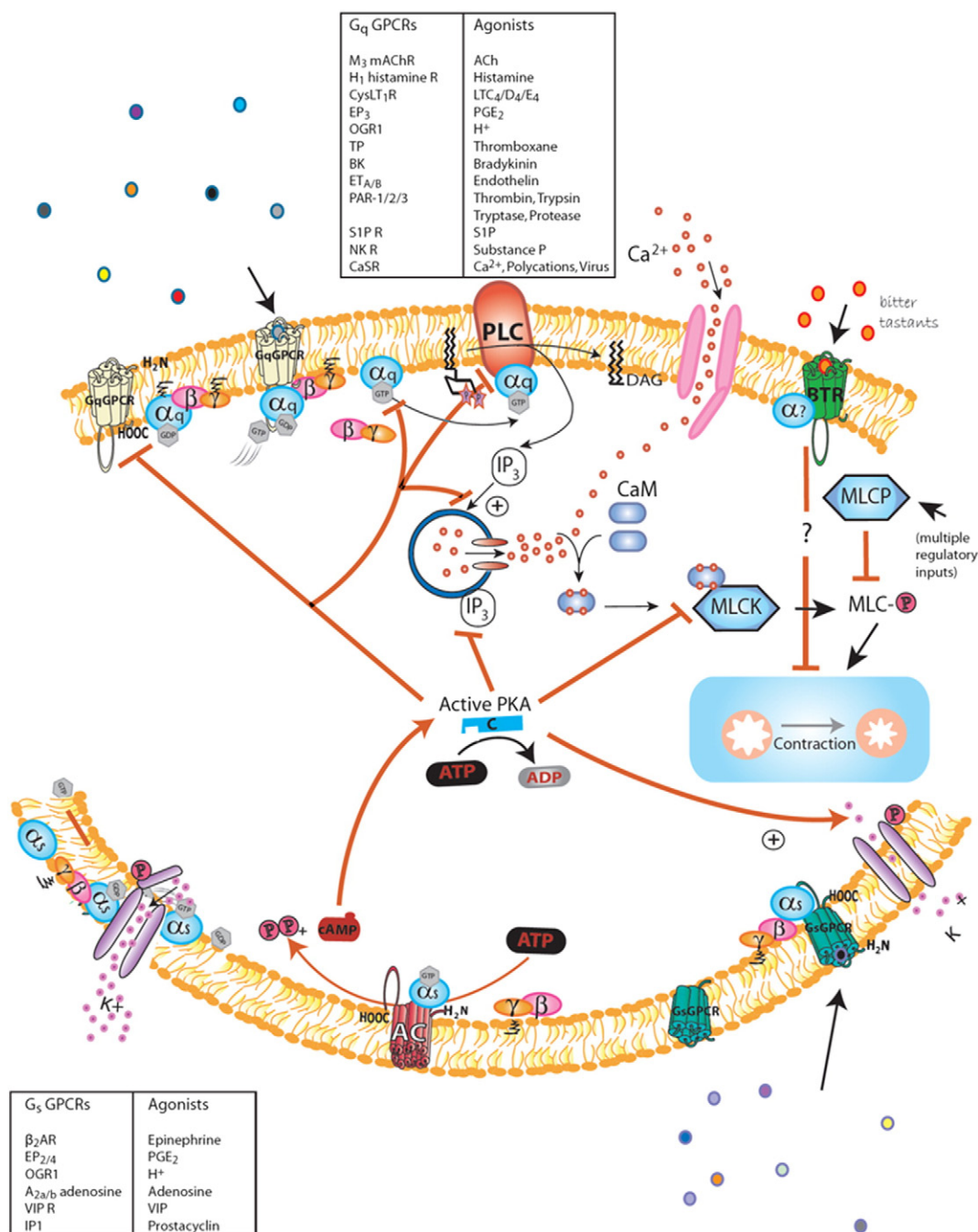


Fig. 1. GPCR control of airway smooth muscle contractility; competitive G_q and G_s signaling regulates ASM contractile state. Transmembrane signaling of GPCRs involves sequential activation of receptor, G protein, and effector. Upon agonist binding, the receptor undergoes a conformational change that facilitates its interaction with the C-terminus of a G-protein alpha subunit. G_q-coupled GPCR activation releases the Gα_q subunit to bind and activate PLC, which hydrolyzes PIP₂ into IP₃ and DAG. IP₃ induces Ca^{2+} release from intracellular stores via the IP₃R whereas DAG activates PKC (not shown). The initial intracellular Ca^{2+} contributes to a more sustained increase of intracellular Ca^{2+} , also promoted by additional extracellular Ca^{2+} influx through Ca^{2+} channels on the cell membrane. The rise in intracellular calcium promotes Ca^{2+} binding to calmodulin, and the formation of Ca^{2+} -calmodulin complexes to activate the myosin ATPase activity required for cross-bridge cycling and contraction. Activated PKC phosphorylates various substrates including calponin; PKC-mediated phosphorylation of calponin prevents calponin-mediated inhibition of actomyosin ATPase. In addition, multiple regulatory mechanisms, including PKC, inhibit myosin light chain phosphatase (MLCP) to promote Ca^{2+} -sensitization. G_s-coupled receptors on ASM are activated by endogenous agents such as circulating catecholamines, prostaglandins and iso-prostanoids, adenosine and VIP. G_s-coupled receptor activation of Gα_s and PKA antagonizes pro-contraction G_q signaling at multiple junctures. Released Gα_s binds to and activates membrane bound adenylyl cyclase which catalyzes the formation of cyclic AMP from ATP. cAMP binds to the regulatory subunits of the inactive PKA to release the active PKA catalytic subunits. PKA can phosphorylate GPCRs, Gα subunits, as well as other effectors (PLC, IP₃R, MLCK, HSP20, K⁺ Ca channels) to exert negative feedback (of G_s-coupled receptor signaling), inhibitory crosstalk (of G_q-coupled receptors) and ASM relaxation. To date, multiple ASM G_q and G_s-coupled receptors (listed in embedded tables) have been identified whose activation can affect ASM contractile state.

Beta-agonists as asthma therapy have a long and illustrious history. Since the introduction of epinephrine use for asthma in the early 1900s and the later use of isoproterenol in the 1940s, beta-agonists have undergone continuous refinement in terms of increased specificity for the beta-2 (β_2)-adrenoceptor (AR) (versus β_1 AR and α AR) subtype, and development of increasingly longer acting compounds (Walker et al., 2011). Albuterol (salbutamol), introduced in 1969, was the first widely used inhaled β_2 AR-selective drug, and remains the drug of choice today for rescue from acute bronchospasm.

Beta-agonists confer bronchoprotection and cause bronchodilation primarily via activation of the cAMP-dependent protein kinase *aka* protein kinase A (PKA). This principal mechanism of action was the long-held dogma based not on direct empirical evidence, but on the observation that most agents known to stimulate intracellular cAMP accumulation caused relaxation. In 2011 Zieba et al. (2011) challenged this notion asserting Epac as the cAMP effector mediating the relaxant effect of cAMP-inducing agents in ASM, providing evidence that Epac-selective activators were sufficient to cause ASM relaxation. However, our group recently demonstrated that direct inhibition of PKA in human ASM cells or murine airways *ex vivo* inhibited the vast majority of the relaxant effect of beta-agonist, demonstrating that PKA is indeed the principal effector of beta-agonist-mediated ASM relaxation (Morgan et al., 2014).

3. Limitations of asthma therapy

Although asthma drugs undoubtedly save thousands of lives each year, they are not perfect. Numerous clinical studies assessing various measures of clinical efficacy report that a high percentage (in one study up to 55%) of asthmatics have suboptimal control (Joyce & McIvor, 1999). Because asthma is a syndrome with multiple contributing pathogenic mechanisms that can vary among sufferers, this fact is not too surprising, particularly when one considers the ever present problem of treatment adherence. Beyond efficacy issues, related safety concerns for certain asthma drugs have also existed for years. Below we will discuss the various issues related to the limitations of several asthma drugs.

3.1. Limitations of bronchodilator drugs

3.1.1. Beta-agonist efficacy, safety

Despite inhaled short acting beta-agonists being the drug of choice for relief from acute asthmatic attacks while long acting beta-agonists (LABAs) combined with corticosteroids are the most frequently prescribed asthma control medication, beta-agonist efficacy and safety have been the subject of ongoing debate for decades. Several studies have linked chronic beta-agonist use with adverse patient outcomes such as functional β_2 AR tachyphylaxis (Lands et al., 1967; Waldeck, 2002), deterioration of asthma control (Nelson et al., 2006; Salpeter et al., 2006), and death (Salpeter et al., 2006). Although chronic beta-agonist treatment is not always associated with adverse events (Walters et al., 2007), there is clearly a dearth of mechanistic understanding of the effects of beta-agonists on airway physiology and asthma pathology.

3.1.2. Loss of drug efficacy with continued use

Drug tolerance or tachyphylaxis is a well-appreciated concept that applies to many drugs. Multiple clinical studies have documented a loss of the bronchoprotective effect of inhaled beta-agonist that occurs with repeated beta-agonist use (Cates & Cates, 2008; Salpeter et al., 2006). Compelling data do not exist to support a loss of the bronchodilatory effect of beta-agonists, as assessed by the reversal of the drop in FEV1 after methacholine challenge (Rosenthal et al., 1999). However, the safety concerns associated with long-acting beta-agonist (LABA) use (discussed below) suggest that under certain conditions beta-agonist-mediated bronchodilation might be compromised.

3.1.3. Safety concerns

Initial safety concerns over beta-agonist treatment of asthma related to the nonselective nature of early β -agonists such as epinephrine (α - and β adrenoceptor-selective) and isoproterenol (β adrenoceptor-selective) which resulted in numerous cardiovascular-related side effects including tachycardia, arrhythmia, tremor, and headache. However, a progressive understanding of adrenoceptor subtypes (Ahlquist, 1948; Lands et al., 1967) facilitated drug discovery efforts that led to the development of the β_2 AR-selective albuterol and terbutaline (Waldeck, 2002). Despite the relative β_2 AR-selectivity of the widely used short-acting beta-agonists (SABAs) and LABAs in the treatment of asthma, there is considerable variability among these drugs, and patient sensitivity for experiencing cardiovascular side effects varies as well.

The more pressing beta-agonist safety issue for over two decades now relates to mortality concerns. A history of the various “epidemics” associated with use of SABAs and LABAs (not in combination with corticosteroids) as asthma drugs is detailed in Ortega and Peters (Ortega & Peters, 2010). Increasing concern over beta-agonist safety reached a crescendo following termination in 2003 of the Salmeterol Multicentre Asthma Research Trial (SMART), a prospective study of salmeterol initiated by Glaxo Smith Kline in 1996. Termination of the trial was prompted when an interim analysis demonstrated a 4.4-fold increase in death in asthmatics receiving salmeterol versus placebo (Nelson et al., 2006). A subsequent meta-analysis of multiple LABA trials reported significantly increased odds ratios for life-threatening exacerbations and asthma-related deaths (Salpeter et al., 2006). Shortly after termination of the SMART trial the FDA issued a black box warning issued for LABA (including salmeterol and formoterol) use in the United States, which has since been updated multiple times.

Although considerable debate continues over the interpretation of the SMART study and the Salpeter meta-analysis, and of the appropriateness of the FDA black box warning, concerns of LABA safety with asthma are a reality that currently impacts asthma management, basic and clinical research in beta-agonist efficacy and pharmacology, and current drug discovery efforts.

3.1.4. Why? What mechanisms drive safety and efficacy issues associated with beta-agonist use?

Although the basis for the loss of the clinical effect of beta-agonist and the current safety concerns is unknown, desensitization of the beta-2-adrenoceptor, the transducer of beta-agonist effects on airway smooth muscle, has been assumed to underlie this loss of functional effect (Billington & Penn, 2003; Deshpande & Penn, 2006; Walker et al., 2011).

Mechanisms effecting β_2 AR desensitization, including those specifically in ASM, have been reviewed elsewhere (Billington & Penn, 2003; Penn et al., 2000; Reiter & Lefkowitz, 2006), but will be briefly summarized here. Activation of the β_2 AR in any cell sets in motion both rapid and more slowly induced feedback mechanisms that shut down signaling. Rapid mechanisms involve receptor phosphorylation by kinases that serve to uncouple the β_2 AR from the G_s protein. The agonist-occupied receptor is rapidly phosphorylated by GPCR kinases (GRKs) which not only diminish the ability of the β_2 AR to couple to G_s , but also promote the association of arrestin molecules with the receptor; this arrestin- β_2 AR association serves many purposes (discussed below), one of which is to sterically inhibit β_2 AR- G_s association. Other kinases, including PKA and PKC can also phosphorylate the β_2 AR to promote uncoupling, without the β_2 AR necessarily being occupied by agonist. More extended mechanisms of β_2 AR desensitization include receptor internalization (mediated by arrestins for the agonist-occupied receptor) which sequesters the receptor from G protein, and receptor downregulation (loss of total cellular β_2 AR protein) which can occur over the course of hours when internalized receptors traffic to lysosomes for degradation.

Another important rapid feedback mechanism is the rapid activation of phosphodiesterases (PDEs) that occurs as a result of intracellular PKA activation. PDEs break down cAMP thus removing the stimuli for PKA activation; PDE inhibitors sustain β_2 AR-mediated (or that by any other GPCR or agent that can activate adenylyl cyclase) induction of cellular cAMP levels, resulting in sustained intracellular PKA activity.

We have demonstrated the relative importance of the different β_2 AR negative feedback mechanisms in ASM, and have established their (negative) impact on the ability of beta-agonists to relax ASM in vitro, ex vivo, and in vivo. Knockdown of GRK2/3 or β -arrestin-2 augments beta-agonist-stimulated cAMP accumulation and PKA activation in human ASM cells, and knockout of β -arrestin-2 also augments β_2 AR signaling and the ability of beta-agonists to relax methacholine-stimulated murine airways ex vivo and bronchoconstriction in vivo (Deshpande et al., 2008; Pera et al., 2015). Inhibition of PKA via heterologous expression of a PKI inhibitory peptide also significantly increases beta-agonist-stimulated cAMP accumulation, but inhibits beta-agonist-mediated relaxation of airways ex vivo due to the role of PKA as the principal effector of β_2 AR-mediated relaxation of ASM (Morgan et al., 2014). Collectively, these studies strongly suggest that β_2 AR desensitization does occur in ASM and does limit the therapeutic utility of beta-agonist in obstructive lung diseases.

Also likely contributing to the limitations of beta-agonists as asthma drugs is the increasing realization that despite providing symptomatic relief, beta-agonists do little to address the underlying pathogenic mechanisms of asthma. Increasing evidence now supports beta-agonists having no anti-inflammatory effect, and perhaps possessing a pro-inflammatory effect (Callaerts-Vegh et al., 2004; Loza et al., 2007; Nguyen et al., 2009; Thanawala et al., 2013). Despite some early studies suggesting that beta-agonists can suppress pro-inflammatory properties of certain cells (Ammit et al., 2002; Hallsworth et al., 2001), beta-agonists do not appear capable of suppressing airway/lung inflammation, and appear to be important in promoting airway mucus production (Thanawala et al., 2013). Indeed, editorials published shortly after the SMART trial controversy suggested the symptomatic relief provided by beta-agonists has a masking effect on uncontrolled airway inflammation (Salpeter et al., 2006). Although this interpretation at first seemed to lose traction due to a lack of supportive research, recent studies suggest it may indeed be fairly accurate (see below).

Additionally, beta-agonists (as well as corticosteroids) fail to address the pathogenic progression of airway remodeling (including increases in ASM/mesenchymal cell mass, matrix deposition, basement membrane thickening) that occurs in many asthmatics to increase the severity of their disease with age (and likely contributes to the prevalence and severity of asthma in the elderly (Hanania et al., 2011)). Although beta-agonists have been shown to inhibit mitogen-stimulated proliferation of ASM cells in culture (Yan et al., 2011), the effect is rather modest, and there is no evidence from animal or clinical studies suggesting that beta-agonists deter airway remodeling in vivo. The apparent inability of current asthma drugs to address airway remodeling has emerged as an important topic, yet resolving this problem will continue to be a significant challenge until regulatory and economic forces become favorable for drugs that target slow-developing (and age-related) mechanisms of disease.

Other bronchodilators drugs do not suffer from the significant safety concerns plaguing beta-agonists, but do have limitations with respect to efficacy, and side effects. Muscarinic receptor antagonists are not considered as efficacious in bronchodilating as beta-agonists (particularly for those asthmatics in which non-cholinergic factors contribute to bronchoconstriction) (Baigelman & Chodosh, 1977; Rebuck et al., 1983), and can produce dry mouth in some patients (Kesten et al., 2006; Tashkin et al., 2008). CysLT1R agonists can also have limited efficacy relative to beta-agonists (Ducharme et al., 2006). Limitations of PDE inhibitors are discussed below.

3.2. Limitations of corticosteroids and other anti-inflammatory treatments

3.2.1. Corticosteroids

Although corticosteroids tend to be extremely effective drugs in suppressing allergic lung inflammation, they suffer from limitations with respect to: 1) side effects; and 2) limited efficacy in specific asthma populations or with specific asthma triggers (Cavkaytar et al., 2015; Cooper et al., 2015; Fuhlbrigge & Kelly, 2014).

Chronic corticosteroid therapy is associated with significant and serious local and systemic side effects. Systemic side effects of oral corticosteroid use include suppressed hypothalamic–pituitary–adrenal (HPA)-axis function, reduced mineral bone density and retarded growth velocity in children (Xu et al., 2009). Although systemic side effects are reduced by local delivery of corticosteroids by inhalation, the drug retains systemic bioavailability through ingestion of particles which do not reach the lung, as well as absorption through the pulmonary circulation. High dose inhaled corticosteroid use has been linked to transient reduction in growth velocity, which can negatively influence treatment compliance in children (Boulet, 1998; Xu et al., 2009). The local side effects are caused by the deposition of corticosteroids in the mouth and throat during inhalation and include pharyngitis, cough and oropharyngeal infections (Dahl, 2006).

Although inhaled corticosteroids are effective in most asthmatics, there are subgroups of patients which do not respond sufficiently in what is referred to as corticosteroid resistant asthma. In particular, severe asthma, presence of a COPD-like asthma phenotype and current cigarette smoker status are associated with steroid resistance (Nyenhuis et al., 2010). There are several potential mechanisms of steroid insensitivity. Under inflammatory conditions, in particular through the actions of the cytokines IL-2, IL-4 and IL-13 the GR α may undergo increased phosphorylation by p38 MAP kinase (Irusen et al., 2002), rendering it unable to bind GRE promoter sites in the nucleus. In addition, proinflammatory cytokines including IL-2, IL-4 and IL-13 as well as TNF α and IL-1 β upregulate the expression of GR β (Bamberger et al., 1995; Webster et al., 2001). GR β is a homolog of GR α which does not bind corticosteroids or promote GRE-mediated gene transcription, while retaining its ability to bind GRE sequences and thus has been proposed to act as an endogenous inhibitor of GR-signaling. Importantly, increased numbers of inflammatory cells (primarily T-cells) expressing GR β have been found in steroid resistant and fatal asthma (Christodoulopoulos et al., 2000; Leung et al., 1997). Other mechanisms include activation of transcription factors (Adcock et al., 1995) AP1 and IRF-1 which inhibit GRE binding either by direct interaction with GR α or by scavenging the GR transcriptional co-activator GR interacting protein 1 (GRIP-1) (Bhandare et al., 2010; Tliba et al., 2008), respectively. Decreased HDAC2 expression in severe asthma and in smokers with asthma may also contribute to steroid insensitivity, as GR-mediated increase in HDAC2 expression is a mechanism by which steroids inhibit proinflammatory gene expression (Ito et al., 2000, 2006b). For additional detail regarding mechanisms mediating corticosteroid resistance, the reader is referred to the following reviews (Adcock & Barnes, 2008; Boardman et al., 2014).

3.2.2. CysLT1R antagonists

In addition to their ability to inhibit contraction mediated by CysLT1Rs on ASM, CysLT1R antagonists inhibit cysteinyl leukotriene-mediated inflammatory events effected by various inflammatory cells. Because these events are but a subset of the inflammatory gestalt in the airway, the effectiveness of CysLT1R antagonists can be limited, and those patients effectively managed on CysLT1R antagonists may have inflammation (or bronchoconstriction) that is largely cysteinyl leukotriene-mediated. Similarly, the efficacy of other asthma drugs with potential anti-inflammatory properties (e.g., M3 mAChR antagonists, cromolyn), is limited due to their restricted modes of action.

With respect to other treatment options intended to address lung inflammation, the most currently discussed option is anti-IgE therapy. However, the high cost and (high maintenance) of anti-IgE therapy render it an option primarily for poorly- or un-controlled severe asthmatics. The strengths and limitations of other anti-inflammatory treatments for asthma are reviewed elsewhere (Chung, 2015; Durham et al., 2015; Humbert et al., 2014; Olin & Wechsler, 2014; Schuligoi et al., 2010).

4. New targets

Aided by the increasing power of molecular biology and genetic approaches, screening strategies, and animal models of asthma, the last decade has witnessed the discovery of numerous players that function as asthma mediators and/or therapeutic targets. Below we describe both previously unappreciated GPCRs and non-GPCRs that have potential as useful therapeutic targets, focusing on those capable of direct effects on ASM contractile state (Table 1).

4.1. Bitter taste receptors (BTRs)

Initial studies by the Liggett lab identified multiple mRNA species of the BTR family in a transcriptome analysis of human ASM cultures. Deshpande et al. (2010) subsequently demonstrated that various BTR ligands, including saccharin, chloroquine, and quinine stimulated a calcium flux in ASM cells. Despite promoting calcium mobilization, the bitter tastants decrease ASM cell stiffness, relax contracted ASM tissue from mice and humans, and decrease airway resistance in a murine model of asthma. The exact mechanism for this relaxant effect has been subject of debate (Deshpande et al., 2010; Tan & Sanderson, 2014; Zhang et al., 2013b), but bitter tastant receptors have nevertheless emerged as intriguing new therapeutic targets, with considerable efforts underway to synthesize new, more potent TAS2R agonists (Behrens & Meyerhof, 2013; Brockhoff et al., 2010; Levit et al., 2014).

4.2. OGR1/GPR68

OGR1 *aka* GPR68 was a poorly understood orphan receptor until the discovery in Ludwig et al. that lowering extracellular pH (i.e. acidifying cell culture media), with the proton as the presumed “agonist”, could stimulate the receptor (Ludwig et al., 2003). This discovery contributed the characterization of a GPCR subfamily of “proton-sensing” receptors, comprised of OGR1, GPR4, TDAG8 (GPR65) and G2A (GPR132), with diverse functions including the regulation of cell/survival growth under oncogenic conditions (Seuwen et al., 2006). OGR1 was found to be expressed in vascular smooth muscle (Tomura et al., 2005) and later in ASM (Ichimonji et al., 2010; Saxena et al., 2012). Modest reductions in extracellular pH (acidification; pH 8.0–pH 6.8) contract ASM cells and tissue, and multiple OGR1-dependent signals including calcium mobilization, cAMP accumulation and PKA activation, and p42/p44

MAPK activation was elicited in response to reduced extracellular pH (Saxena et al., 2012). Interestingly, a significant proportion of the OGR1-mediated PKA activation was G_q -independent, indicating OGR1 has the capacity to couple to both G_q and G_s . This raises the possibility of exploiting qualitative signaling properties of OGR1 to bias it towards a G_s -mediated, pro-relaxant function (see below).

However, given the nature of its apparent cognate ligand- the proton- OGR1 appears to be an intractable target or at least an extremely difficult receptor to study and manipulate. Fortunately, a large collaborative effort headed by the Roth lab recently identified a subset of benzodiazepines that function as allosteric modulators of OGR1, (Huang et al., 2015) thus providing a critical tool for characterizing the properties of this receptor. Huang et al. recently demonstrated that certain desmethyl benzodiazepines can stimulate OGR1 to promote calcium mobilization, cAMP accumulation, and elicit the same phosphoprotein induction caused by decreased extracellular pH (Huang et al., 2015). Moreover, insight provided by structure of OGR1 enabled the synthesis of novel OGR1 ligand capable with demonstrable signaling and function effects in a behavioral model (OGR1 is highly expressed in brain). Future studies assessing the effects of these benzodiazepines on ASM function will be interesting, and offer the hope that OGR1 can be manipulated to function primarily as a G_s -coupled GPCR mediating bronchodilation.

4.3. Calcium sensing receptors (CaSRs)

Although we tend to focus on *intracellular* calcium as the bad boy causing ASM contraction and bronchoconstriction, extracellular calcium is now known to effect ASM contraction by acting as a ligand for CaSRs expressed on ASM. CaSRs are best known for being expressed on the parathyroid gland, serving as a homeostatic sensor of blood calcium levels by controlling the release of parathyroid hormone. In ASM, the G_q -coupled CaSR transduces the extracellular calcium signal into an intracellular calcium signal to regulate ASM contractile state (Yarova et al., 2015). Interestingly, the CaSR also responds to polyvalent cations, certain amino acids, and virus elements all of which can be present in the airway under different pathological conditions. Yarova et al. (2015) recently demonstrated the relevance of multiple CaSR activators as stimuli for ASM contraction, and demonstrated the contributory roles of CaSRs in the development of pathology in a murine model of asthma. Inhaled small molecule CaSR antagonists termed calcilytics were effective in reversing both AHR as well as lung inflammation. Interestingly, CaSR expression in ASM appears greater in asthmatic versus non-asthmatic humans (Yarova et al., 2015). The potential for calcilytics as asthma therapy (calcilytics are currently in trials for treatment of autosomal dominant hypocalcemia (“A Study to Determine the Effects of NPSP795 on the Calcium-sensing Receptor in Subjects With Autosomal Dominant Hypocalcemia as Measured by PTH Levels and Blood Calcium Concentrations <https://clinicaltrials.gov/ct2/show/NCT02204579?term=npSP795&rank=1>,”) has been recently discussed (Penn, 2015).

Table 1
Overview of current and future/potential therapeutic targets and ligands for bronchodilation.

	Target	Current Therapeutic ligand	Future/potential Therapeutic ligand
G_q	M3 mAChR	Ipratropium, tiotropium, glycopyrronium, aclidinium	
	H1R	Loratidine, desloratidine	
	CysLT1R	Montelukast, zafirlukast, pranlukast	
	CaSR		NPS89636, NPS2143, Calhex 231, NPSP795
G_s	PAR2		GB83, GB88, G protein-biased agonist
	β 2AR	Albuterol, formoterol, salmeterol, indacaterol, olodaterol	Nadolol, G_s -biased agonist
	OGR1		Lorazepam, Sulazepam
	EP2/EP4		ONO-AE1-259, ONO-AE1-329
Other	PDE	Theophylline, roflumilast	RPL554
	BTR		Chloroquine, quinine, saccharin, flufenamic acid

5. Not-so-new targets but with new life

Several potential asthma therapy targets have been recognized for years but for various reasons, effective drugs targeting them were either never developed or never advanced through clinical trials. Recent studies providing clarity in their role in asthma pathology or how they might be better targeted, or simply improved medicinal chemistry have brought these targets back into play. Some of these are discussed below.

5.1. EP receptors

Although recognized as regulators of ASM contractile state for some time, interest in targeting EP receptors as asthma therapy waned once initial clinical trials identified substernal burning and cough as significant side effects of inhaling the endogenous EP receptor ligand PGE₂ (Walters & Davies, 1982). However, recent studies have shed light on the specific actions of the different EP receptor subtypes in various resident and infiltrating lung cell types, and suggest that recently-developed subtype selective ligands may limit such side effects and possibly serve as effective bronchodilators. The Belvisi and Birrell labs recently demonstrated the EP3 receptor being the EP receptor that mediates cough (Maher et al., 2009). The same groups also recently demonstrated striking species differences in the roles of various EP receptor subtypes in mediating relaxation/contraction of ASM. Using subtype-selective ligands in human airway tissue, the EP4 receptor was shown to mediate the relaxant effect of PGE₂. In contrast, the EP2 receptor mediated relaxation of murine and guinea pig smooth muscle. Alternatively, the EP3 receptor has been shown to mediate contractions and pro-contractile signaling in both murine and human ASM (Maher et al., 2009; Tilley et al., 2003) (also Penn, unpublished observations). With respect to EP receptor function in the inflammation process, studies of EP receptor knockout mice are somewhat conflicting and attribute a predominate anti-inflammatory role to either the EP4 (Birrell et al., 2015) or EP2 (Zaslona et al., 2013) receptor. Our understanding of EP receptor subtype function in human inflammatory cells and (human) allergic inflammation is relatively poor. Studies from Feng et al. (2006) implicate the EP3 receptor as pro-inflammatory and the EP2 receptor as anti-inflammatory in human mast cells, and both EP2 and EP4 inhibiting the hyperosmolarity-induced histamine release in human mast cells.

Collectively, the data point to EP4 and possibly EP2/4 agonists as putative asthma therapeutics, for both their direct effects on ASM and their anti-inflammatory potential.

5.2. Protease-activated receptor 2 (PAR2)

Protease-activated receptors are activated via an irreversible mechanism in which proteases cleave the receptor's extracellular N-terminus to create a new N-terminus that functions as a tethered ligand activating the receptors. There are 4 members of PAR family (PAR1–4), coupled primarily to G_q and G_i, and different complements of the receptor subtypes are expressed in multiple cell types including endothelium, epithelium, various immune cells and ASM. PAR expression in cells important in allergic lung inflammation and ASM contraction has been known for some time (Knight et al., 2001) but like the EP receptor family, targeting PARs in asthma has been problematic given the promiscuity (i.e. ability to engage a variety of G proteins) of numerous receptor subtypes and an abundance of agents (thrombin, trypsin, tryptase, and various proteolytic allergens/fungi) capable of activating them. However, recent studies have demonstrated an important role for PAR2 in mediating both allergic lung inflammation and associated AHR (Asaduzzaman et al., 2015; Ebeling et al., 2005; Ramachandran et al., 2012; Schmidlin et al., 2002; Takizawa et al., 2005). Studies using PAR2 knockout mice have demonstrated a requirement for PAR2 in allergen-induced leukocyte recruitment (and allergen-challenged PAR2 knockout mice have a significantly

attenuated asthma phenotype), whereas transgenic mice overexpressing PAR2 have an exaggerated inflammatory phenotype (Schmidlin et al., 2002). Administration of a PAR-2 activating peptide (Ebeling et al., 2005; McGuire et al., 2004) enhanced allergen-induced AHR and airway inflammation in mice, while an anti-PAR(2) blocking antibody delivered during the sensitization phase to cockroach extract inhibited airway inflammation and decreased AHR (Arizmendi et al., 2011). Collectively, these and other studies (Cocks & Moffatt, 2001) implicate PAR2 in the development of allergic inflammation and the asthma phenotype (de Boer et al., 2014).

Yet not all actions of PAR2 are pro-asthmatic/inflammatory, due in part to actions of COX-derived PGE₂ induced via PAR2 activation. Administration of PAR2 agonists can promote prostanoid-induced relaxation of mouse, rat, guinea-pig and human airways, and bronchoconstriction is elevated in PAR2^{−/−} mice (Cocks & Moffatt, 2001). COX-dependent PGE₂ production is also known to inhibit eosinophil migration and degranulation (Cocks et al., 1999; Ebeling et al., 2007; Gatti et al., 2006; Moffatt et al., 2002; Nichols et al., 2012; Schmidlin et al., 2001).

These mixed actions of PAR2 have the potential to limit the therapeutic utility of any strategy for antagonizing PAR2. However, Nichols et al. recently demonstrated that the disparate effects of PAR2 could be separated into arrestin-dependent and -independent actions of the receptor (Nichols et al., 2012). Using an ovalbumin sensitization/challenge model, repeated insufflation of a PAR2 agonist 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. However, PAR2-mediated ASM relaxation ex vivo as well as bronchodilation in vivo was similar in WT and β -arrestin2 knockout mice, as were BAL levels of PGE₂. PAR2-dependent PGE₂ production and release were dependent upon nuclear p42/p44 MAPK activation and PI3K activity, both of which have been shown to be inhibited by chelation of intracellular Ca²⁺, G α_q knockdown or pharmacological inhibition of PLC β . Thus, it is likely that the protective effects of PAR2 in the airway are mediated downstream of G α_q . In contrast, PAR2-dependent activation of β -arrestin-dependent events, such as activation of actin cytoskeletal events and cell migration, was independent of G α_q , G α_i and G α_{12} signaling, consistent with the hypothesis that the pro-inflammatory effects of PAR2 in the airway are independent of G-protein coupling. Collectively, these findings posit PAR2 as an intriguing GPCR for which biased ligand pharmacology or biasing strategies might enable optimal therapeutic targeting of the receptor. Towards that end, ongoing efforts to develop novel PAR2 agonists and antagonists have been made. Recently a number of high affinity “tethered” PAR2 peptidomimetic agonists and antagonists, effective both in vivo and in vitro, have been developed (Boitano et al., 2014). Further investigation of the potential bias of these compounds may lead to the identification of PAR2 targeted peptides with sufficient pathway specificity to inhibit the inflammatory but not the protective effects of PAR2 in the airway (Boitano et al., 2015; Flynn et al., 2013; Sherwood et al., 2014).

5.3. GABA receptors

The role of GABA receptors in neurotransmission has been long appreciated, yet their function in non-neuronal tissues remains poorly understood. Early studies discounted a role for GABA in the direct modulation of airway smooth muscle function, in part because of confounding effects of GABA receptor function in neural control of ASM, but also due to a failure to appreciate the antithetical effects of GABA_A and GABA_B receptors on ASM.

GABA_B receptors are G_i-coupled GPCRs and have recently been shown to be expressed on ASM and represent one of the few G_i-coupled GPCRs capable of promoting ASM contraction (Mizuta et al., 2008). Moreover, GABA_B receptor activation augments pro-contractile signaling and contraction stimulated by ASM G_q-coupled receptors

(Osawa et al., 2006). Alternatively, GABA_A receptors are ion channels, composed of multiple subunits, that conduct inward chloride currents that serve to hyperpolarize the cell and limit depolarization. GABA_A channels in the brainstem regulate cholinergic transmission to the lung (Haxhui et al., 1986; Moore et al., 2004), and both GABA_A channels and GABA_B receptors reside on lung postganglionic parasympathetic nerves of the lung and serve to regulate cholinergic nerve activity (Chapman et al., 1991; Tamaoki et al., 1987; Tohda and Kubo, 1998).

The Emala lab has demonstrated the GABA_A receptors are expressed in ASM (Mizuta et al., 2008), that selective GABA_A activation with muscimol can relax contracted ASM (Gleason et al., 2009), and also potentiate the relaxant effect of beta-agonists on ASM (Gallos et al., 2008). Because ASM expresses primarily the $\alpha 4$ and $\alpha 5$ subunits of the GABA_A, medicinal chemistry efforts to develop specific $\alpha 4$ or $\alpha 5$ activators have been undertaken. Most recently, the Emala and Cook laboratories have demonstrated that specific targeting of $\alpha 5$ GABA_A subunits can relax contracted airway ex vivo as well as lung slices, and a specific $\alpha 4$ allosteric modulator of the GABA_A channel relaxed human ASM ex vivo and reduced respiratory resistance on a murine asthma model (Gallos et al., 2012a, 2015; Yocum et al., 2015). These latter studies suggest that precise targeting of ASM GABA_A subunits represents a promising means of regulating bronchomotor tone while avoiding potentially confounding off-target effects (in epithelia, or the nervous system, for example) of nonspecific GABA_A activation.

5.4. Chloride channels, transporters

In addition to GABA_A channels, other types of chloride channels exist including the glycine channel (Yim et al., 2011) and calcium-activated chloride channels (CaCCs) (Huang et al., 2012; Zhang et al., 2013a), both of which have been shown to be important in ASM relaxation. The effects of chloride flux in or out of ASM can be complicated, and likely relate to the difference in the subcellular localization of chloride channels in ASM cells and their effects on: 1) membrane potential; or 2) the regulation of other ion fluxes, including calcium fluxes, at either the plasma membrane or the sarcoplasmic reticulum (Gallos et al., 2012b). Whereas GABA_A channel activation appears to relax ASM via an inward chloride current that hyperpolarizes the plasma membrane, the glycine channel and CaCCs can mediate a chloride efflux, which contributes to membrane depolarization during calcium increases (Huang et al., 2009). Numerous studies have demonstrated that various chloride channel blockers can inhibit agonist- or depolarization- mediated contraction of multiple species of ASM ex vivo or in vivo, with the efficacy of different blockers dependent upon the nature of induced contraction (Danielsson et al., 2015; Gallos et al., 2013). Only recently has the TMEM 16 family been identified as the true molecular identity of calcium-activated chloride channels expressed on ASM (Huang et al., 2009; Rock et al., 2008) and selective TMEM16 antagonists have been shown to inhibit contraction of guinea pig (Gallos et al., 2012a), mouse and human ASM (Huang et al., 2012), and reduce airway hyperactivity in a murine asthma model (Huang et al., 2012; Zhang et al., 2013a). Collectively, these studies identify chloride channels as an intriguing therapeutic target for asthma. Moreover, results from these studies shed a new (positive) light into the regulatory role of membrane depolarization in ASM contraction. Early studies demonstrating a lack of effect of the L-type calcium channel blocker verapamil (Fish, 1984) led to an abandoning of strategies for manipulating membrane potential to control ASM contraction. Yet with the complexity of ion channel function and its role in calcium handling in ASM starting to be revealed, such strategies are clearly back in play and offer the promise of completely novel approaches to regulating bronchomotor tone.

5.5. Rho kinase inhibitors

Various G_q-coupled GPCRs activate Rho kinase in ASM to augment contractile responsiveness of ASM tissues. Rho kinase augments

contraction by inhibiting myosin light chain phosphatase (MLCP), a process referred to as “calcium sensitization”. The role of Rho kinase in ASM contraction has been well documented (Gosens et al., 2006). Rho kinase inhibitors have been shown to inhibit ASM contraction both ex vivo (including human tissues) (Gosens et al., 2004a, 2004b; Iizuka et al., 1999; Schaafsma et al., 2005; Yoshii et al., 1999) and in vivo (animals) (Iizuka et al., 2000; Tokuyama et al., 2002). Of note, the contribution of Rho kinase to ASM contraction is increased in various animal models of allergen sensitization (Chiba et al., 2001; Hashimoto et al., 2002; Schaafsma et al., 2004), indicating an important role for this enzyme under disease conditions. However, despite a plethora of encouraging findings from animal studies, including effective bronchodilation by inhaled Rho kinase inhibitors (Schaafsma et al., 2008), inhalation studies in human subjects have not been published to date. The only currently clinically available Rho kinase inhibitor, Fasudil, has been found to effectively lower pulmonary arterial pressure when administered to PAH patients by inhalation (Fujita et al., 2010), suggesting that inhalation is a viable method of delivery for this drug. Given the profound efficacy of Rho kinase inhibitors to relax contracted ASM under a wide range of conditions and systems, interest in these drugs remains high, and ultimate clinical application may depend on overcoming the hypotension associated with their use (Kuroda et al., 2015), perhaps through development of improved (airway) targeting strategies.

5.6. PDE inhibitors

The nonspecific PDE inhibitor theophylline has been used to treat COPD for decades (Giembycz & Newton, 2015; Matera et al., 2014; Page, 2014). Its use has been constrained however, by its narrow therapeutic window, and significant side effects including gastrointestinal stress. Xanthine PDE inhibitors, other than theophylline, are not used to treat asthma; current interest in testing existing or newly developed PDE4 (BinMahfouz et al., 2015) subtype inhibitors as asthma drugs appear focused on using such drugs as adjuncts, in combination with glucocorticoids or direct bronchodilators. Although both nonspecific PDE and PDE4 inhibition in human airway smooth muscle cells can increase intracellular cAMP (Billington et al., 2007; Horvat et al., 2012), nonselective PDE or PDE4 inhibitors are poor direct bronchodilators, and do not reverse acute bronchoconstriction. PDE concentrations required for ASM relaxation by far exceed those required for anti-inflammatory effects and would lead to intolerable adverse effects (Nicholson et al., 1995). Recent studies suggest the PDE4 inhibitors as monotherapy can improve lung function in COPD (Boswell-Smith & Spina, 2007) and recently, in asthma (Bateman et al., 2015), largely due to anti-inflammatory actions rather than direct bronchodilator properties.

A reasonable conceptual basis exists for supporting PDE inhibitors as part of a combination therapy. PDE inhibition appears to produce some beneficial effects that are non-overlapping with those of corticosteroids, and by increasing cAMP/PKA activity should similarly induce mechanisms of cooperativity demonstrated between beta-agonists and corticosteroids (Rider et al., 2011), including synergistic effects on gene transcription (BinMahfouz et al., 2015; Holden et al., 2011). Moreover, cAMP/PKA signaling also appears to augment glucocorticoid receptor nuclear translocation and signaling (Eickelberg et al., 1999; Usmani et al., 2005) through mechanisms discussed below. Finally, an interesting property of theophylline appears to be its ability to regulate histone acetylation which is believed important in steroid resistance (Ito et al., 2000, 2006b).

6. New ways to treat old targets

6.1. Combination therapies

The idea of combination therapies is not new, but new combinations of various drugs are being considered and coming to the market. Two

types of combination therapies exist, one being the combined delivery of 2 drugs, and the other being “bi-functional” molecules.

The rationale underlying combination therapy is that 2 (or possibly more) drugs are better than one; equal or greater therapeutic efficacy might be achieved at lower drug doses due to additive or synergistic effects between the two components. Particularly if the drugs address non-overlapping features of the disease, simply inhibiting more mechanisms of disease is likely to better mitigate symptoms and manage the disease. For the combination therapy of inhaled long-acting beta-agonist and corticosteroids, these drugs provide both direct bronchodilation and inflammation control; enabling both acute symptomatic relief while addressing underlying mechanism translates into better asthma control. Moreover, an argument can be made that bronchoconstriction per se plays a pathogenic role (Gosens & Grainge, 2015; Grainge et al., 2011), thus beta-agonist-mediated bronchorelaxation also addresses, to some degree, mechanism of disease. For combinations involving two direct bronchodilator drugs (e.g., a muscarinic receptor antagonist plus β_2 AR agonist such as Combivent), two distinct mechanisms effecting bronchodilation are targeted.

Beyond the concept of addressing multiple disease mechanisms (as beneficial), some studies have also suggested that beta-agonists and corticosteroids cooperate at a cellular/molecular level to augment each other's effect (Giembycz & Newton, 2015; Holden et al., 2011). In addition to studies demonstrating beta-agonists can induce ligand-independent translocation of the glucocorticoid receptor in cell-based assays (Eickelberg et al., 1999; Roth et al., 2002). Usmani et al. (2005) reported that combination therapy (inhaled salmeterol plus fluticasone propionate) in seven asthmatics augmented glucocorticoid receptor translocation in vivo to greater extent than either salmeterol or fluticasone treatment alone. On the flip side, steroids are also believed to enhance responsiveness to beta-agonists by upregulating β_2 AR expression which has been shown in the lung (Mano et al., 1979), presumably by GR transactivation of the ADBR gene (Malbon & Hadcock, 1988). Moreover, dexamethasone was shown to inhibit beta-agonist-induced β_2 AR downregulation in rat lung (Mak et al., 1995). The Panetierri group also reported that acute (1 h) pre-treatment of precision cut human lung slices with dexamethasone completely prevented the loss of albuterol-mediated ASM relaxation; an effect not attributed to changes in β_2 AR expression (Cooper & Panetierri, 2008). Thus, multiple mechanisms appear to exist that cause positive cross-talk between GR and β_2 AR signaling to create the observed therapeutic cooperativity of beta-agonists and corticosteroids.

Currently several LAMA/LABA combinations including tiotropium/olodaterol, glycopyrronium/indacaterol, umeclidinium/vilanterol and aclidinium/formoterol, as well as the LABA/ICS vilanterol/fluticasone are being evaluated as treatments for COPD (Bateman et al., 2014; Ramadan et al., 2015), but currently not for asthma.

A new approach to combination therapy has been the development of bifunctional molecules; two pharmacophores linked covalently so as to form a single molecule with the ability to engage two therapeutic targets (dual pharmacology). One example of such a bifunctional molecule is GS-5759, a molecule with a (long-acting) beta agonist and PDE4 inhibitor moiety, designed for inhaled delivery (Tannheimer et al., 2014). The compound retains both bronchodilator and anti-inflammatory properties in vitro (Tannheimer et al., 2014) and in vivo in animals (Salmon et al., 2014) suggesting a use for this approach as treatment of disease. In addition to the potential benefits of potentiation of beta agonist effectiveness by concomitant inhibition of phosphodiesterases and the resultant increase in cAMP levels (Seldon et al., 2005; Tannheimer et al., 2012a, 2012b), a number of advantages to using bi-functional molecules (as opposed to two separate molecular entities formulated together) have been discussed (Matera et al., 2011). These include simplified formulation for inhaled delivery, a single pharmacokinetic profile and an easier route to regulatory approval as opposed to two combined pharmacological agents. However, for all these

perceived potential benefits of bifunctional agonists, their success will ultimately depend on their effectiveness in patients.

Other bifunctional molecule approaches include the combined muscarinic antagonism/ β_2 -agonism (MABA) activity such as the GSK961081 (Bateman et al., 2013). These types of compounds would also facilitate the use of triple therapy (LAMA/LABA/ICS) as combining three drugs in one inhaler may be technically challenging and could pose an issue with variable pharmacokinetic profiles of the different components. Tiotropium as an add-on to LABA/ICS has been shown to be more effective than LABA/ICS alone in both COPD (van Noord et al., 2010), and severe asthma (Kerstjens et al., 2011, 2012) indicating an added benefit of triple therapy. An additional benefit of tiotropium as an add-on to LABA/ICS is its potential corticosteroid-sparing effect in severe asthma (Fardon et al., 2007); lowering the ICS dose lowers the risk of ICS related side-effects.

6.2. “Tuning” receptors: qualitative signaling, functional selectivity, and the promise of biased ligand pharmacology

The philosophy guiding most drug development over the last several decades has focused on improving existing drugs to make them: 1) more selective and possibly more potent for their intended target, with fewer off-target side effects; and 2) longer-acting, which would presumably improve patient adherence. Related to this latter goal are those attempts to improve adherence by making drugs easier to take—for example, by making a drug in an oral formulation patient adherence to their drug regimen is more likely. Yet these goals can be competing, given oral drugs provide systemic delivery which increases the likelihood of off-target effects, whereas although inhaled drugs improve targeting specificity to the lung, effective drug delivery as well as adherence can suffer.

An underlying premise of drug discovery efforts to date is that GPCRs have simplistic linear signaling properties that promote or thwart disease, and that GPCR ligands either turn their target receptor “on” or “off”, that antagonists serve to turn them off, agonists turn them on, and the goal is to make drugs that are best at these functions. This premise is reflected in the high throughput screening strategies for many candidate GPCR ligands, which often entail multi-well (96, 384, or more) plate assays for assessing the ability of candidates to stimulate or inhibit an intracellular signal (Ca^{2+} or cAMP) in cells (often artificial expression systems) expressing the receptor of interest. Later the “passed” drug candidates are tested in more integrative, physiologically-relevant systems.

The emerging concept of *biased agonism*, also referred to as *functional selectivity* or *qualitative signaling*, suggests that most GPCRs have a much more diverse signaling capacity than previously appreciated, which effectively renders traditional definitions of “agonism” and “antagonism” if not obsolete, certainly oversimplistic. Although many receptors are known to be able to couple to more than one G protein, and thereby stimulate more than one signaling pathway, it is now known that the diversity of GPCR signaling extends much further, and includes the ability to signal through G protein-independent pathways. Due to such pleiotropic capabilities of the receptor, it is clear that the characterization of any ligand or modulator must take into account its ability to regulate the multiple signals produced. Indeed, certain ligands have been shown to engage the same receptor to act as an agonist of one signaling pathway and inverse agonist or antagonist of another. For example, the 5-HT₂CR ligand SB 242084 functions as an agonist for 5-HT₂cR-mediated PLC activation (Cussac et al., 2002) yet is an inverse agonist for 5-HT₂c R-stimulated arachidonic acid (AA) release by phospholipase A₂ (PLA₂) (De Deurwaerdere et al., 2004). In addition, ligands exhibit differences in their ability to induce receptor internalization or desensitization that is dissociated from their signaling efficacy (Urban et al., 2007).

G protein-independent signaling by GPCRs has been frequently attributed to arrestin proteins. Arrestins, were originally characterized

as important regulators of GPCR internalization and desensitization. It is now clear they can also bind to GPCRs and serve as scaffolds for the binding of various proteins which initiate signaling independent of heterotrimeric G proteins (Lefkowitz & Shenoy, 2005). Numerous studies demonstrate the capacity of ligands to promote arrestin-dependent p42/p44 MAPK activation that is independent of their efficacy in activating G proteins. Azzi et al. (2003) demonstrated that ICI 118,551 and propranolol, which function as inverse agonists for β_2 AR-mediated G_s activation, are partial agonists for β_2 AR-mediated, arrestin-dependent p42/p44 MAPK activation. Unlike with the β_2 AR agonist isoproterenol, ICI 118,551- and propranolol- stimulated p42/p44 MAPK activation occurred in the absence of either G_{α_s} or G_{α_i} . Similar findings of dissociated G protein- and arrestin-dependent signaling for other GPCRs are plentiful (Penn, 2008).

Collectively, these findings suggest that ligands can differ in their ability to induce various receptor conformations that preferentially activate one pathway over another. The diversity with which ligands can induce different receptor conformations linked to different events is portrayed in Wisler et al. (2007), in which an analysis of 16 different β_2 AR ligands revealed a significant range of efficacies for β_2 AR-mediated G_s - and arrestin- dependent signaling, receptor phosphorylation, and receptor internalization. Carvedilol- traditionally known as a “beta-blocker” used in the treatment of heart failure, demonstrated unique properties in its: 1) negative efficacy for G_s -mediated adenylyl cyclase activation; 2) modest ability to promote β_2 AR phosphorylation and internalization; 3) ability to recruit arrestin; and 4) stimulation of arrestin-dependent, G protein-independent p42/p44 MAPK activity.

If a receptor is capable of diverse signaling events that are associated with distinct physiological consequences, and ligands can “bias” signaling and therefore function, the opportunity exists to “tune” the receptor and optimize the most therapeutically beneficial signaling profile. In heart failure, a belief that G_s /cAMP signaling in cardiac myocytes is pathological while arrestin-dependent signaling is beneficial has been used

to explain the efficacy of carvedilol relative to other “beta-blockers”. In asthma, the roles of these two signaling pathways appear reversed. We have demonstrated that the G_s /cAMP/PKA pathway mediates the relaxant effect of beta-agonists on ASM, whereas beta-arrestin 2 serves to not only constrain the efficacy of beta-agonists but is also critical to the development of inflammation induced by allergen (Walker et al., 2003). These findings suggest that beta-agonists stimulate both pathogenic and therapeutic signals through β_2 ARs in the lung (Fig. 2). A series of studies by the Bond lab supports this notion, and the profile of effects of various beta-agonists suggests that the bias properties of these β_2 AR ligands influence the asthma phenotype. Early studies demonstrated that either ADBR gene ablation or systemic delivery of the inverse agonist nadolol greatly reduced both allergen-induced inflammation and AHR in murine model, suggesting that the sum of beta-agonist effects in the lung is deleterious (Nguyen et al., 2009). Yet certain “beta-blockers” worked better in preventing the allergen-induced asthma phenotype, and these differences could not be explained by the inverse agonism properties as initially proposed (Wisler et al., 2007). As the bias properties of numerous β_2 AR ligands were revealed in multiple studies (Walker et al., 2011), a more consistent interpretation emerged in which β_2 AR-mediated arrestin signaling drove pathogenic features (mucus production, inflammation) of asthma and the capacity of any β_2 AR ligand to stimulate or block arrestin-dependent signaling was critical to its effect. Thus, ligands such as nadolol which blocked epinephrine-stimulated arrestin signaling, and lacked the ability to stimulate arrestin-dependent signaling on their own, were effective in inhibiting allergic lung inflammation despite failing to stimulate (while also blocking epinephrine-stimulated) cAMP/PKA signaling. Alternatively, “beta-blockers” such as carvedilol which can stimulate β_2 AR-mediated arrestin signaling, failed to antagonize allergic lung inflammation in wild type mice and promoted inflammation and AHR in mice lacking endogenous epinephrine (Thanawala et al., 2015). Similarly, the failure of propranolol to improve the asthma phenotype in a

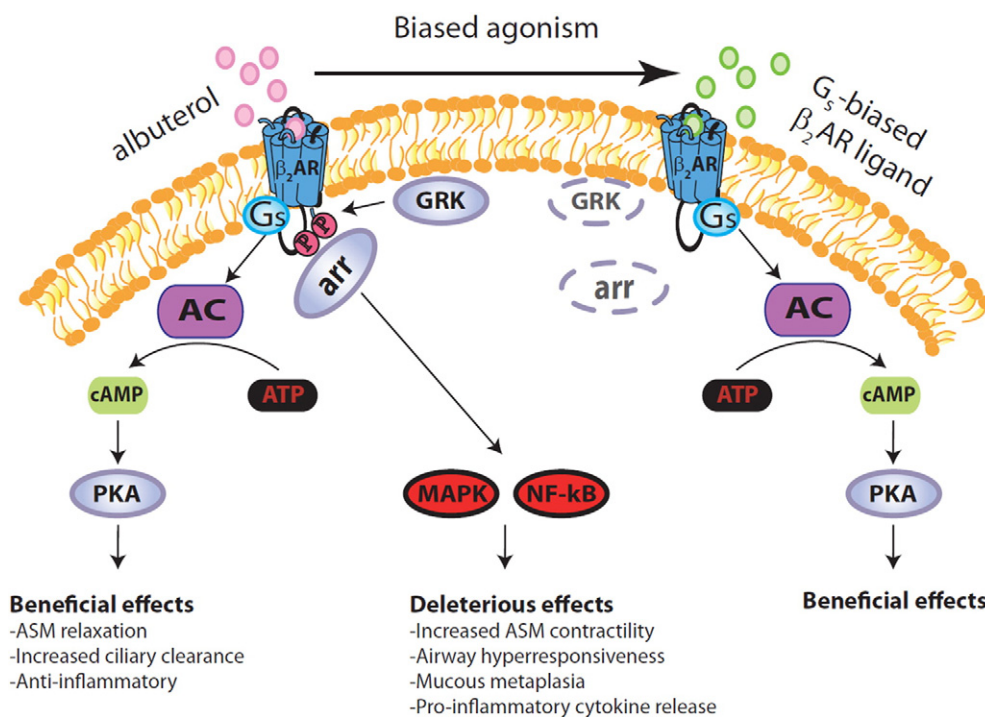


Fig. 2. Proposed model of beneficial and deleterious β_2 AR signaling, and therapeutic potential of a biased β_2 AR ligand. Upon agonist-induced activation of the β_2 AR (e.g. with the current asthma drug albuterol), the G_s protein activates the enzyme adenylyl cyclase (AC) which converts ATP to cAMP. Subsequently, cAMP activates protein kinase A (PKA) which mediates the beneficial effects of beta-agonists. However, shortly after activation, the intracellular domain of the β_2 AR is phosphorylated by a G protein-coupled receptor kinase (GRK), promoting arrestin (arr) binding which leads to deleterious β_2 AR signaling, including the activation of mitogen activated protein kinase (MAPK) and NF- κ B pathways. A biased ligand, which functions as an agonist for G_s signaling but as a null or inverse agonist for arrestin-dependent signaling, would invoke only beneficial signaling and functional outcomes, and be therapeutically superior.

recent human trial can be attributed to its ability to stimulate arrestin-dependent signaling (Penn, 2014).

The impressive pre-clinical results of nadolol have prompted the current clinical trial sponsored by the National Institute of Allergy and Infectious Diseases ("<https://clinicaltrials.gov/ct2/show/NCT01804218?term=NCT01804218&rank=1>,"), assessing the effect of nadolol on airway hyperresponsiveness, airway inflammation, and asthma control in mild asthmatics. Should nadolol prove effective, the possibility remains that an even more effective biased β_2 AR ligand (capable of both blocking barrestin-signaling and stimulating canonical G_s /cAMP/PKA signaling) could emerge. Alternatively, a therapeutic approach designed to skew signaling towards the G_s /cAMP/PKA pathway (e.g., augmenting cAMP/PKA with PDE4 inhibitors while maintaining inhibition of arrestin signaling with nadolol) also represents a potentially superior strategy.

Of course, the application of biased ligand pharmacology to asthma is not limited to β_2 AR ligands. As noted above, PAR2 signaling in the airway can be parsed into (beneficial) G protein-dependent and (pathogenic) arrestin-dependent signaling and functional consequences. Strategies to bias the signaling of PAR2, be they biased orthosteric ligands or allosteric modulators, have the potential to overcome past difficulties in targeting this receptor for asthma.

For a more in-depth analyses of functional selectivity and its impact on models of GPCR activation and drug discovery, the reader is referred to Penn (Penn et al., 2014) and references therein.

7. What we can expect in the near (and not-so-near) future

Forces impacting asthma drug development and drug approval/application today include not only the research findings in asthma, airway cell biology, structural biology, and pharmacology, but also the economic forces that dictate priorities and areas of investment.

Although the research detailed in this review suggests multiple, exciting opportunities for new asthma drugs, the reality is that it is extremely difficult to advance any conceptually appealing and empirically supported therapy into an approved and used asthma drug. Given the current significant cost of new drug development as well as regulatory hurdles in getting any new drug approved (Barnes et al., 2015), it is more likely that drugs that represent modest variations of existing drugs, or new combinations of existing drugs, are the most likely to supplant those drugs used currently. The reduced cost of development and preclinical/clinical validation of repurposed drugs, combined with less onerous regulatory hurdles, also renders repurposed drugs more likely to advance to the market. Indeed, this reality likely prompted recent programmatic efforts of the NIH to employ a crowdsourcing strategy to establish collaboration between industry and academia supported by specific funding mechanism for research into repurposed drugs and their application (Colvis & Austin, 2014), in addition to the past and ongoing efforts of the National Center for Advancing Translational Sciences (<https://ncats.nih.gov>).

However, one would hope that basic, preclinical, and clinical research efforts will prevail despite current funding levels and economic forces, and accurately identify those drugs that are either superior, or at least non-inferior, to currently prescribed asthma drugs. Layer in the global interest in personalized medicine and existing asthma clinical network efforts to categorize specific asthma populations based on their underlying pathology (Levy et al., 2015), and it is not difficult to envision an expanded repertoire of asthma drugs, or drug combinations tailored to better manage specific asthmatic subpopulations. Perhaps most encouraging (for the treatment of all diseases) is the promise of biased ligand pharmacology, which now has a strong foundation built on multiple basic science disciplines and will likely find wide spread clinical application once industry expands its platforms and strategies to accommodate the reality that ligands have much greater capacity to manipulate GPCRs than previously thought.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

- Adcock, I. M., & Barnes, P. J. (2008). Molecular mechanisms of corticosteroid resistance. *Chest* 134, 394–401.
- Adcock, I. M., Lane, S. J., Brown, C. R., Lee, T. H., & Barnes, P. J. (1995). Abnormal glucocorticoid receptor-activator protein 1 interaction in steroid-resistant asthma. *J Exp Med* 182, 1951–1958.
- Ahlquist, R. P. (1948). A study of the adrenotropic receptors. *Am J Phys* 153, 586–600.
- Ammit, A. J., Lazaar, A. L., Irani, C., O'Neill, G. M., Gordon, N. D., Amrani, Y., ... Panettieri, R. A., Jr. (2002). Tumor necrosis factor- α -induced secretion of RANTES and interleukin-6 from human airway smooth muscle cells: modulation by glucocorticoids and beta-agonists. *Am J Respir Cell Mol Biol* 26, 465–474.
- Arizmendi, N. G., Abel, M., Mihara, K., Davidson, C., Polley, D., Nadeem, A., ... Vliagoftis, H. (2011). Mucosal allergic sensitization to cockroach allergens is dependent on proteinase activity and proteinase-activated receptor-2 activation. *J Immunol* 186, 3164–3172.
- Asaduzzaman, M., Nadeem, A., Arizmendi, N., Davidson, C., Nichols, H. L., Abel, M., ... Vliagoftis, H. (2015). Functional inhibition of PAR2 alleviates allergen-induced airway hyperresponsiveness and inflammation. *Clin Exp Allergy* 12, 1844–1855.
- Azzi, M., Charest, P. G., Angers, S., Rousseau, G., Kohout, T., Bouvier, M., & Pineyro, G. (2003). Beta-arrestin-mediated activation of MAPK by inverse agonists reveals distinct active conformations for G protein-coupled receptors. *Proc Natl Acad Sci U S A* 100, 11406–11411.
- Baigelman, W., & Chodosh, S. (1977). Bronchodilator action of the anticholinergic drug, ipratropium bromide (Sch 1000), as an aerosol in chronic bronchitis and asthma. *Chest* 71, 324–328.
- Bamberger, C. M., Bamberger, A. M., de Castro, M., & Chrousos, G. P. (1995). Glucocorticoid receptor beta, a potential endogenous inhibitor of glucocorticoid action in humans. *J Clin Invest* 95, 2435–2441.
- Barnes, P. J. (2005). Molecular mechanisms and cellular effects of Glucocorticosteroids. *Immunol Allergy Clin N Am* 25, 451–468.
- Barnes, P. J., Bonini, S., Seeger, W., Belvisi, M. G., Ward, B., & Holmes, A. (2015). Barriers to new drug development in respiratory disease. *Eur Respir J* 45, 1197–1207.
- Bateman, E. D., Bousquet, J., Aubier, M., Bredenbröker, D., & O'Byrne, P. M. (2015). Roflumilast for asthma: efficacy findings in non-placebo-controlled comparator and dosing studies. *Pulm Pharmacol Ther* 35, S11–S19.
- Bateman, E. D., Kornmann, O., Ambery, C., & Norris, V. (2013). Pharmacodynamics of GSK961081, a bi-functional molecule, in patients with COPD. *Pulm Pharmacol Ther* 26, 581–587.
- Bateman, E. D., Mahler, D. A., Vogelmeier, C. F., Wedzicha, J. A., Patalano, F., & Banerji, D. (2014). Recent advances in COPD disease management with fixed-dose long-acting combination therapies. *Expert Rev Respir Med* 8, 357–379.
- Behrens, M., & Meyerhof, W. (2013). Bitter taste receptor research comes of age: from characterization to modulation of TAS2Rs. *Sem Cell Dev Biol* 24, 215–221.
- Bhandare, R., Damera, G., Banerjee, A., Flammer, J. R., Kessler, S., Rogatsky, I., ... Tliba, O. (2010). Glucocorticoid receptor interacting protein-1 restores glucocorticoid responsiveness in steroid-resistant airway structural cells. *Am J Respir Cell Mol Biol* 42, 9–15.
- Billington, C. K., & Penn, R. B. (2003). Signaling and regulation of G protein-coupled receptors in airway smooth muscle. *Respir Res* 4, 2.
- Billington, C. K., Le, J. I., Young, K. W., & Hall, I. P. (2007). A major functional role for phosphodiesterase 4D5 in human airway smooth muscle cells. *Am J Respir Cell Mol Biol* 38, 1–7.
- BinMahfouz, H., Borthakur, B., Yan, D., George, T., Gienbycz, M. A., & Newton, R. (2015). Superiority of combined phosphodiesterase PDE3/PDE4 inhibition over PDE4 inhibition alone on glucocorticoid- and long-acting β_2 -adrenoceptor agonist-induced gene expression in human airway epithelial cells. *Mol Pharmacol* 87, 64–76.
- Birrell, M. A., Maher, S. A., Dekkak, B., Jones, V., Wong, S., Brook, P., & Belvisi, M. G. (2015). Anti-inflammatory effects of PGE2 in the lung: role of the EP4 receptor subtype. *Thorax* 70, 740–747.
- Boardman, C., Chachi, L., Gavrilu, A., Keenan, C. R., Perry, M. M., Xia, Y. C., ... Sharma, P. (2014). Mechanisms of glucocorticoid action and insensitivity in airways disease. *Pulm Pharmacol Ther* 29, 129–143.
- Boitano, S., Hoffman, J., Flynn, A. N., Asiedu, M. N., Tillu, D. V., Zhang, Z., ... Price, T. J. (2015). The novel PAR2 ligand C391 blocks multiple PAR2 signalling pathways in vitro and in vivo. *Br J Pharmacol* 172, 4535–4545.
- Boitano, S., Hoffman, J., Tillu, D. V., Asiedu, M. N., Zhang, Z., Sherwood, C. L., ... Vagner, J. (2014). Development and evaluation of small peptidomimetic ligands to protease-activated receptor-2 (PAR(2)) through the use of lipid tethering. *PLoS One* 9, e99140.

- Bos, I. S., Gosens, R., Zuidhof, A. B., Schaafsma, D., Halayko, A. J., Meurs, H., & Zaagsma, J. (2007). Inhibition of allergen-induced airway remodelling by tiotropium and budesonide: a comparison. *Eur Respir J* 30, 653–661.
- Boswell-Smith, V., & Spina, D. (2007). PDE4 inhibitors as potential therapeutic agents in the treatment of COPD-focus on roflumilast. *Int J Chron Obstruct Pulmon Dis* 2, 121–129.
- Boulet, L.-P. (1998). Perception of the role and potential side effects of inhaled corticosteroids among asthmatic patients. *Chest* 113, 587–592.
- Brockhoff, A., Behrens, M., Niv, M. Y., & Meyerhof, W. (2010). Structural requirements of bitter taste receptor activation. *Proc Natl Acad Sci U S A* 107, 11110–11115.
- Callaerts-Vegh, Z., Evans, K. L., Dudekula, N., Cuba, D., Knoll, B. J., Callaerts, P. F., ... Bond, R. A. (2004). Effects of acute and chronic administration of beta-adrenoceptor ligands on airway function in a murine model of asthma. *Proc Natl Acad Sci U S A* 101, 4948–4953.
- Cates, C. J., & Cates, M. J. (2008). Regular treatment with salmeterol for chronic asthma: serious adverse events. *Cochrane Database Syst Rev*, CD006363-CD006363.
- Cavkaytar, O., Vurali, D., Arık Yılmaz, E., Buyuktiyaki, B., Soyer, O., Sahiner, U., ... Sekerel, B. (2015). Evidence of hypothalamic-pituitary-adrenal axis suppression during moderate-to-high-dose inhaled corticosteroid use. *Eur J Pediatr*, 1–11.
- Chapman, R. W., Danko, G., Rizzo, C., Egan, R. W., Mauser, P. J., & Kreutner, W. (1991). Prejunctional GABA-B inhibition of cholinergic, neurally-mediated airway contractions in guinea-pigs. *Pulm Pharmacol* 4, 218–224.
- Chiba, Y., Sakai, H., & Misawa, M. (2001). Augmented acetylcholine-induced translocation of RhoA in bronchial smooth muscle from antigen-induced airway hyperresponsive rats. *Br J Pharmacol* 133, 886–890.
- Christodouloulopoulos, P., Leung, D. Y. M., Elliott, M. W., Hogg, J. C., Muro, S., Toda, M., ... Hamid, Q. A. (2000). Increased number of glucocorticoid receptor- β -expressing cells in the airways in fatal asthma. *J Allergy Clin Immunol* 106, 479–484.
- Chung, K. F. (2015). Targeting the interleukin pathway in the treatment of asthma. *Lancet* 386, 1086–1096.
- Cocks, T. M., & Moffatt, J. D. (2001). Protease-activated receptor-2 (PAR2) in the airways. *Pulm Pharmacol Ther* 14, 183–191.
- Cocks, T. M., Fong, B., Chow, J. M., Anderson, G. P., Frauman, A. G., Goldie, R. G., ... Moffatt, J. D. (1999). A protective role for protease-activated receptors in the airways. *Nature* 398, 156–160.
- Colice, G. L. (2006). New developments in inhaled corticosteroids. *Allergy Asthma Proc* 27, 332–340.
- Colvis, C. M., & Austin, C. P. (2014). The NIH-industry new therapeutic uses pilot program: demonstrating the power of crowdsourcing. *DRRR* 1, 15–16.
- Cooper, P. R., & Panettieri, R. A., Jr. (2008). Steroids completely reverse albuterol-induced β_2 -adrenergic receptor tolerance in human small airways. *J Allergy Clin Immunol* 122, 734–740.
- Cooper, V., Metcalf, L., Versnel, J., Upton, J., Walker, S., & Horne, R. (2015). Patient-reported side effects, concerns and adherence to corticosteroid treatment for asthma, and comparison with physician estimates of side-effect prevalence: a UK-wide, cross-sectional study. *NPJ Prim Care Respir Med* 25, 15026.
- Currie, G. P., & Lipworth, B. J. (2002). Bronchoprotective effects of leukotriene receptor antagonists in asthma*: a meta-analysis. *Chest* 122, 146–150.
- Cussac, D., Newman-Tancredi, A., Duqueyroux, D., Pasteau, V., & Millan, M. J. (2002). Differential activation of Gq/11 and Gi(3) proteins at 5-hydroxytryptamine(2C) receptors revealed by antibody capture assays: influence of receptor reserve and relationship to agonist-directed trafficking. *Mol Pharmacol* 62, 578–589.
- Dahl, R. (2006). Systemic side effects of inhaled corticosteroids in patients with asthma. *Respir Med* 100, 1307–1317.
- Danielsson, J., Perez-Zoghbi, J., Bernstein, K., Barajas, M. B., Zhang, Y., Kumar, S., ... Emala, C. W. (2015). Antagonists of the TMEM16A calcium-activated chloride channel modulate airway smooth muscle tone and intracellular calcium. *Anesthesiology* 123, 569–581.
- Davis, B. E., Illamperuma, C., Gauvreau, G. M., Watson, R. M., O'Byrne, P. M., Deschesnes, F., ... Cockcroft, D. W. (2009). Single-dose desloratadine and montelukast and allergen-induced late airway responses. *Eur Respir J* 33, 1302–1308.
- de Boer, J. D., van't Veer, C., Stroo, I., van der Meer, A. J., de Vos, A. F., van der Zee, J. S., ... van der Poll, T. (2014). Protease-activated receptor-2 deficient mice have reduced house dust mite-evoked allergic lung inflammation. *Innate Immun* 20, 618–625.
- De Boever, E. H., Ashman, C., Cahn, A. P., Locantore, N. W., Overend, P., Pouliquen, I. J., ... Wenzel, S. E. (2014). Efficacy and safety of an anti-IL-13 mAb in patients with severe asthma: a randomized trial. *J Allergy Clin Immunol* 133, 989–996.e984.
- Deurwaerdere, P., Navailles, S., Berg, K. A., Clarke, W. P., & Spampinato, U. (2004). Constitutive activity of the serotonin2C receptor inhibits in vivo dopamine release in the rat striatum and nucleus accumbens. *J Neurosci* 24, 3235–3241.
- Deshpande, D. A., & Penn, R. B. (2006). Targeting G protein-coupled receptor signaling in asthma. *Cell Signal* 18, 2105–2120.
- Deshpande, D. A., Theriot, B. S., Penn, R. B., & Walker, J. K. (2008). β -Arrestins specifically constrain β 2-adrenergic receptor signaling and function in airway smooth muscle. *FASEB J* 22, 2134–2141.
- Deshpande, D. A., Wang, W. C., McIlmoyle, E. L., Robinett, K. S., Schillinger, R. M., An, S. S., ... Liggett, S. B. (2010). Bitter taste receptors on airway smooth muscle bronchodilate by localized calcium signaling and reverse obstruction. *Nat Med* 16, 1299–1304.
- Donohue, J. F. (2008). Safety and efficacy of β agonists. *Respir Care* 53, 618–624.
- Ducharme, F. M., Lasserson, T. J., & Cates, C. J. (2006). Long-acting beta2-agonists versus anti-leukotrienes as add-on therapy to inhaled corticosteroids for chronic asthma. *Cochrane Database Syst Rev*, CD003137.
- Durham, A. L., Caramori, G., Chung, K. F., & Adcock, I. M. (2015). Targeted anti-inflammatory therapeutics in asthma and chronic obstructive lung disease. *Transl Res* 167, 192–203.
- Ebeling, C., Forsythe, P., Ng, J., Gordon, J. R., Hollenberg, M., & Vliagoftis, H. (2005). Proteinase-activated receptor 2 activation in the airways enhances antigen-mediated airway inflammation and airway hyperresponsiveness through different pathways. *J Allergy Clin Immunol* 115, 623–630.
- Ebeling, C., Lam, T., Gordon, J. R., Hollenberg, M. D., & Vliagoftis, H. (2007). Proteinase-activated receptor-2 promotes allergic sensitization to an inhaled antigen through a TNF-mediated pathway. *J Immunol* 179, 2910–2917.
- Eickelberg, O., Roth, M., Lörx, R., Bruce, V., Rüdiger, J., Johnson, M., & Block, L. -H. (1999). Ligand-independent activation of the glucocorticoid receptor by β_2 -adrenergic receptor agonists in primary human lung fibroblasts and vascular smooth muscle cells. *J Biol Chem* 274, 1005–1010.
- Fan, C. K. (2006). Phosphodiesterase inhibitors in airways disease. *Eur J Pharmacol* 533, 110–117.
- Fardon, T., Haggart, K., Lee, D. K. C., & Lipworth, B. J. (2007). A proof of concept study to evaluate stepping down the dose of fluticasone in combination with salmeterol and tiotropium in severe persistent asthma. *Respir Med* 101, 1218–1228.
- Feng, C., Beller, E. M., Bagga, S., & Boyce, J. A. (2006). Human mast cells express multiple EP receptors for prostaglandin E(2) that differentially modulate activation responses. *Blood* 107, 3243–3250.
- Fish, J. E. (1984). Calcium channel antagonists in the treatment of asthma. *J Asthma* 21, 407–418.
- Flynn, A. N., Hoffman, J., Tillu, D. V., Sherwood, C. L., Zhang, Z., Patek, R., ... Boitano, S. (2013). Development of highly potent protease-activated receptor 2 agonists via synthetic lipid tethering. *FASEB J* 27, 1498–1510.
- Fuhlbrigge, A. L., & Kelly, H. W. (2014). Inhaled corticosteroids in children: effects on bone mineral density and growth. *Lancet Respir Med* 2, 487–496.
- Fujita, H., Fukumoto, Y., Saji, K., Sugimura, K., Demachi, J., Nawata, J., & Shimokawa, H. (2010). Acute vasodilator effects of inhaled fasudil, a specific Rho-kinase inhibitor, in patients with pulmonary arterial hypertension. *Heart Vessel* 25, 144–149.
- Gallos, G., Yim, P., & Emala, C. W. (2012b). Chloride in airway smooth muscle: the ignored anion no longer? *Am J Physiol Lung Cell Mol Physiol* 302, L733–L735.
- Gallos, G., Yim, P., Chang, S., Zhang, Y., Xu, D., Cook, J. M., ... Emala, C. W., Sr. (2012a). Targeting the restricted alpha-subunit repertoire of airway smooth muscle GABAA receptors augments airway smooth muscle relaxation. *Am J Physiol Lung Cell Mol Physiol* 302, L248–L256.
- Gallos, G., Gleason, N. R., Zhang, Y., Pak, S. -W., Sonett, J. R., Yang, J., & Emala, C. W. (2008). Activation of endogenous GABA(A) channels on airway smooth muscle potentiates isoproterenol-mediated relaxation. *Am J Physiol Renal Physiol* 295, L1040–L1047.
- Gallos, G., Remy, K. E., Danielsson, J., Funayama, H., Fu, X. W., Chang, H. -Y. S., ... Emala, C. W. (2013). Functional expression of the TMEM16 family of calcium-activated chloride channels in airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 305, L625–L634.
- Gallos, G., Yocum, G. T., Siviski, M. E., Yim, P. D., Fu, X. W., Poe, M. M., ... Emala, C. W. (2015). Selective targeting of the α_5 -subunit of GABAA receptors relaxes airway smooth muscle and inhibits cellular calcium handling. *Am J Physiol Lung Cell Mol Physiol* 308, L931–L942.
- Gatti, R., Andre, E., Amadesi, S., Dinh, T. Q., Fischer, A., Bunnett, N. W., ... Trevisani, M. (2006). Protease-activated receptor-2 activation exaggerates TRPV1-mediated cough in guinea pigs. *J Appl Phys* 101, 506–511.
- Giembycz, M. A., & Newton, R. (2015). Potential mechanisms to explain how LABAs and PDE4 inhibitors enhance the clinical efficacy of glucocorticoids in inflammatory lung diseases. *F1000Prime Rep* 7, 16.
- Gleason, N. R., Gallos, G., Zhang, Y., & Emala, C. W. (2009). The GABAA agonist muscimol attenuates induced airway constriction in guinea pigs in vivo. *J Appl Physiol* 106, 1257–1263.
- Gosens, R., & Grainge, C. (2015). Bronchoconstriction and airway biology: potential impact and therapeutic opportunities. *Chest* 147, 798–803.
- Gosens, R., Schaafsma, D., Grootte Bromhaar, M. M., Vrugt, B., Zaagsma, J., Meurs, H., & Nelemans, S. A. (2004a). Growth factor-induced contraction of human bronchial smooth muscle is Rho-kinase-dependent. *Eur J Pharmacol* 494, 73–76.
- Gosens, R., Schaafsma, D., Meurs, H., Zaagsma, J., & Nelemans, S. A. (2004b). Role of Rho-kinase in maintaining airway smooth muscle contractile phenotype. *Eur J Pharmacol* 483, 71–78.
- Gosens, R., Bos, I. S., Zaagsma, J., & Meurs, H. (2005). Protective effects of tiotropium bromide in the progression of airway smooth muscle remodeling. *Am J Respir Crit Care Med* 171, 1096–1102.
- Gosens, R., Schaafsma, D., Nelemans, S. A., & Halayko, A. J. (2006). Rho-kinase as a drug target for the treatment of airway hyperresponsiveness in asthma. *Mini Rev Med Chem* 6, 71–89.
- Grainge, C. L., Lau, L. C. K., Ward, J. A., Dulay, V., Lahiff, G., Wilson, S., ... Howarth, P. H. (2011). Effect of bronchoconstriction on airway remodeling in asthma. *N Engl J Med* 364, 2006–2015.
- Haddad, E. B., Mak, J. C., & Barnes, P. J. (1994). Characterization of [^{3} H]Ba 679 BR, a slowly dissociating muscarinic antagonist, in human lung: radioligand binding and autoradiographic mapping. *Mol Pharmacol* 45, 899–907.
- Hallsworth, M. P., Twort, C. H., Lee, T. H., & Hirst, S. J. (2001). Beta(2)-adrenoceptor agonists inhibit release of eosinophil-activating cytokines from human airway smooth muscle cells. *Br J Pharmacol* 132, 729–741.
- Hanania, N. A., King, M. J., Brame, S. S., Saltoun, C., Wise, R. A., Enright, P., ... Ledford, D. K. (2011). Asthma in the elderly: current understanding and future research needs—a report of a National Institute on Aging (NIA) workshop. *J Allergy Clin Immunol* 128, S4–S24.
- Hashimoto, K., Peebles, R., Sheller, J., Jarzecka, K., Furlong, J., Mitchell, D., ... Graham, B. (2002). Suppression of airway hyperresponsiveness induced by ovalbumin sensitization and RSV infection with Y-27632, a Rho kinase inhibitor. *Thorax* 57, 524–527.
- Haxhui, M. A., Deal, E. C., Jr., Norcia, M. P., Van Lunten, E., Mitra, J., & Cherniack, N. S. (1986). Medullary effects of nicotine and GABA on tracheal smooth muscle tone. *Respir Physiol* 64, 351–363.

- Hodsmann, P., Ashman, C., Cahn, A., De Boever, E., Locantore, N., Serone, A., & Pouliquen, I. (2013). A phase 1, randomized, placebo-controlled, dose-escalation study of an anti-IL-13 monoclonal antibody in healthy subjects and mild asthmatics. *Br J Pharmacol* 175, 118–128.
- Holden, N. S., Bell, M. J., Rider, C. F., King, E. M., Gaunt, D. D., Leigh, R., ... Newton, R. (2011). $\beta(2)$ -Adrenoceptor agonist-induced RGS2 expression is a genomic mechanism of bronchoprotection that is enhanced by glucocorticoids. *Proc Natl Acad Sci U S A* 108, 19713–19718.
- Horvat, S. J., Deshpande, D. A., Yan, H., Panettieri, R. A., Codina, J., DuBose, T. D., Jr., ... Penn, R. B. (2012). A-kinase anchoring proteins regulate compartmentalized cAMP signaling in airway smooth muscle. *FASEB J* 26, 3670–3679.
- Huang, X. -P., Karpiak, J., Kroeze, W. K., Zhu, H., Chen, X., Moy, S. S., ... Roth, B. L. (2015). Allosteric ligands for the pharmacologically dark receptors GPR68 and GPR65. *Nature* 527, 477–483.
- Huang, F., Rock, J. R., Harfe, B. D., Cheng, T., Huang, X., Jan, Y. N., & Jan, L. Y. (2009). Studies on expression and function of the TMEM16A calcium-activated chloride channel. *Proc Natl Acad Sci U S A* 106, 21413–21418.
- Huang, F., Zhang, H., Wu, M., Yang, H., Kudo, M., Peters, C. J., ... Rock, J. R. (2012). Calcium-activated chloride channel TMEM16A modulates mucin secretion and airway smooth muscle contraction. *Proc Natl Acad Sci U S A* 109, 16354–16359.
- Humbert, M., Busse, W., Hanania, N. A., Lowe, P. J., Canvin, J., Erpenbeck, V. J., & Holgate, S. (2014). Omalizumab in asthma: an update on recent developments. *J Allergy Clin Immunol Pract* 2(525–536), e521.
- Ichimonji, I., Tomura, H., Mogi, C., Sato, K., Aoki, H., Hisada, T., ... Okajima, F. (2010). Extracellular acidification stimulates IL-6 production and Ca^{2+} mobilization through proton-sensing OGR1 receptors in human airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 299, L567–L577.
- Iizuka, K., Shimizu, Y., Tsukagoshi, H., Yoshii, A., Harada, T., Dobashi, K., ... Mori, M. (2000). Evaluation of Y-27632, a Rho-kinase inhibitor, as a bronchodilator in guinea pigs. *Eur J Pharmacol* 406, 273–279.
- Iizuka, K., Yoshii, A., Samizo, K., Tsukagoshi, H., Ishizuka, T., Dobashi, K., ... Mori, M. (1999). A major role for the rho-associated coiled coil forming protein kinase in G-protein-mediated Ca^{2+} sensitization through inhibition of myosin phosphatase in rabbit trachea. *Br J Pharmacol* 128, 925–933.
- Irusen, E., Matthews, J. G., Takahashi, A., Barnes, P. J., Chung, K. F., & Adcock, I. M. (2002). p38 mitogen-activated protein kinase-induced glucocorticoid receptor phosphorylation reduces its activity: role in steroid-insensitive asthma. *J Allergy Clin Immunol* 109, 649–657.
- Ito, K., Chung, K. F., & Adcock, I. M. (2006a). Update on glucocorticoid action and resistance. *J Allergy Clin Immunol* 117, 522–543.
- Ito, K., Yamamura, S., Essilfie-Quaye, S., Cosio, B., Ito, M., Barnes, P. J., & Adcock, I. M. (2006b). Histone deacetylase 2-mediated deacetylation of the glucocorticoid receptor enables NF- κ B suppression. *J Exp Med* 203, 7–13.
- Ito, K., Barnes, P. J., & Adcock, I. M. (2000). Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits interleukin-1 β -induced histone H4 acetylation on lysines 8 and 12. *Mol Cell Biol* 20, 6891–6903.
- Joyce, D. P., & McIvor, R. A. (1999). Use of inhaled medications and urgent care services. *Study of Canadian asthma patients. Can Fam Physician* 45, 1707–1713.
- Kerstjens, H. A. M., Disse, B., Schröder-Babo, W., Bantje, T. A., Gahlemann, M., Sigmund, R., ... van Noord, J. A. (2011). Tiotropium improves lung function in patients with severe uncontrolled asthma: a randomized controlled trial. *J Allergy Clin Immunol* 128, 308–314.
- Kerstjens, H. A. M., Engel, M., Dahl, R., Paggiaro, P., Beck, E., Vandewalker, M., ... Bateman, E. D. (2012). Tiotropium in asthma poorly controlled with standard combination therapy. *N Engl J Med* 367, 1198–1207.
- Kesten, S., Jara, M., Wentworth, C., & Lanes, S. (2006). Pooled clinical trial analysis of tiotropium safety. *Chest* 130, 1695–1703.
- King, E. M., Chivers, J. E., Rider, C. F., Minnich, A., Gienbycz, M. A., & Newton, R. (2013). Glucocorticoid repression of inflammatory gene expression shows differential responsiveness by transactivation- and transrepression-dependent mechanisms. *PLoS One* 8, e53936.
- Knight, D. A., Lim, S., Scaffidi, A. K., Roche, N., Chung, K. F., Stewart, G. A., & Thompson, P. J. (2001). Protease-activated receptors in human airways: upregulation of PAR-2 in respiratory epithelium from patients with asthma. *J Allergy Clin Immunol* 108, 797–803.
- Kuroda, S., Tashiro, H., Kimura, Y., Hirata, K., Tsutada, M., Mikuriya, Y., ... Ohdan, H. (2015). Rho-kinase inhibitor targeting the liver prevents ischemia/reperfusion injury in the steatotic liver without major systemic adversity in rats. *Liver Transpl* 21, 123–131.
- Laitinen, A., Lindqvist, A., Halme, M., Altraja, A., & Laitinen, L. A. (2005). Leukotriene E4-induced persistent eosinophilia and airway obstruction are reversed by zafirlukast in patients with asthma. *J Allergy Clin Immunol* 115, 259–265.
- Lands, A. M., Luduena, F. P., & Buzzo, H. J. (1967). Differentiation of receptors responsive to isoproterenol. *Life Sci* 6, 2241–2249.
- Lane, S. J., & Lee, T. H. (1996). Mast cell effector mechanisms. *J Allergy Clin Immunol* 98, S67–S71 (discussion S71–62).
- Lefkowitz, R. J., & Shenoy, S. K. (2005). Transduction of receptor signals by β -arrestins. *Science* 308, 512–517.
- Leung, D. Y. M., Hamid, Q., Vottero, A., Szeffer, S. J., Surs, W., Minshall, E., ... Klemm, D. J. (1997). Association of glucocorticoid insensitivity with increased expression of glucocorticoid receptor β . *J Exp Med* 186, 1567–1574.
- Levit, A., Nowak, S., Peters, M., Wiener, A., Meyerhof, W., Behrens, M., & Niv, M. Y. (2014). The bitter pill: clinical drugs that activate the human bitter taste receptor TAS2R14. *FASEB J* 28, 1181–1197.
- Levy, B. D., Noel, P. J., Freemer, M. M., Cloutier, M. M., Georas, S. N., Jarjour, N. N., ... Erzurum, S. C. (2015). Future research directions in asthma: an NHLBI working group report. *Am J Respir Crit Care Med*.
- Loza, M. J., Peters, S. P., Foster, S., Khan, I. U., & Penn, R. B. (2007). Beta-agonist enhances type 2 T-cell survival and accumulation. *J Allergy Clin Immunol* 119, 235–244.
- Ludwig, M. G., Vanek, M., Guerini, D., Gasser, J. A., Jones, C. E., Junker, U., ... Seuwen, K. (2003). Proton-sensing G-protein-coupled receptors. *Nature* 425, 93–98.
- Maeba, S., Ichijima, T., Ueno, Y., Makata, H., Matsubara, T., & Furukawa, S. (2005). Effect of montelukast on nuclear factor κ B activation and proinflammatory molecules. *Ann Allergy Asthma Immunol* 94, 670–674.
- Maher, S. A., Birrell, M. A., & Belvisi, M. G. (2009). Prostaglandin E(2) mediates cough via the EP(3) receptor: implications for future disease therapy. *Am J Respir Crit Care Med* 180, 923–928.
- Mak, J. C., Nishikawa, M., Shirasaki, H., Miyayasu, K., & Barnes, P. J. (1995). Protective effects of a glucocorticoid on downregulation of pulmonary beta 2-adrenergic receptors in vivo. *J Clin Invest* 96, 99–106.
- Malbon, C. C., & Hadcock, J. R. (1988). Evidence that glucocorticoid response elements in the 5'-noncoding region of the hamster beta 2-adrenergic receptor gene are obligate for glucocorticoid regulation of receptor mRNA levels. *Biochem Biophys Res Commun* 154, 676–681.
- Mano, K., Akbarzadeh, A., & Townley, R. G. (1979). Effect of hydrocortisone on beta-adrenergic receptors in lung membranes. *Life Sci* 25, 1925–1930.
- Matera, M. G., Page, C. P., & Cazzola, M. (2011). Novel bronchodilators for the treatment of chronic obstructive pulmonary disease. *Trends Pharmacol Sci* 32, 495–506.
- Matera, M. G., Page, C., & Cazzola, M. (2014). PDE inhibitors currently in early clinical trials for the treatment of asthma. *Expert Opin Investig Drugs* 23, 1267–1275.
- McGuire, J. J., Saifedine, M., Triggle, C. R., Sun, K., & Hollenberg, M. D. (2004). 2-Furoyl-LIGRL-amide: a potent and selective proteinase-activated receptor 2 agonist. *J Pharmacol Exp Ther* 309, 1124–1131.
- Mitchell, P. D., El-Gammal, A. I., & O'Byrne, P. M. (2016). Emerging monoclonal antibodies as targeted innovative therapeutic approaches to asthma. *Clin Pharmacol Ther* 99, 38–48.
- Mizuta, K., Xu, D., Pan, Y., Comas, G., Sonett, J. R., Zhang, Y., ... Emala, C. W., Sr. (2008). GABA A receptors are expressed and facilitate relaxation in airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 294, L1206–L1216.
- Moffatt, J. D., Jeffrey, K. L., & Cocks, T. M. (2002). Protease-activated receptor-2 activating peptide SLIGRL inhibits bacterial lipopolysaccharide-induced recruitment of polymorphonuclear leukocytes into the airways of mice. *Am J Respir Cell Mol Biol* 26, 680–684.
- Moore, C. T., Wilson, C. G., Mayer, C. A., Acquah, S. S., Massari, V. J., & Haxhiu, M. A. (2004). A GABAergic inhibitory microcircuit controlling cholinergic outflow to the airways. *J Appl Phys* 96, 260–270.
- Morgan, S. J., Deshpande, D. A., Ties, B. C., Misior, A. M., Yan, H., Hershfild, A. V., ... Penn, R. B. (2014). Beta-agonist-mediated relaxation of airway smooth muscle is protein kinase A-dependent. *J Biol Chem* 289, 23065–23074.
- Nelson, H. S., Weiss, S. T., Blecker, E. R., Yancey, S. W., & Dorinsky, P. M. (2006). The Salmeterol Multicenter Asthma Research Trial: a comparison of usual pharmacotherapy for asthma or usual pharmacotherapy plus salmeterol. *Chest* 129, 15–26.
- Newton, R. (2000). Molecular mechanisms of glucocorticoid action: what is important? *Thorax* 55, 603–613.
- Newton, R., & Holden, N. S. (2007). Separating transrepression and transactivation: a distressing divorce for the glucocorticoid receptor? *Mol Pharmacol* 72, 799–809.
- Nguyen, L. P., Lin, R., Parra, S., Omoluabi, O., Hanania, N. A., Tuvim, M. J., ... Bond, R. A. (2009). Beta2-adrenoceptor signaling is required for the development of an asthma phenotype in a murine model. *Proc Natl Acad Sci U S A* 106, 2435–2440.
- Nichols, H. L., Saifedine, M., Theriot, B. S., Hegde, A., Polley, D., El-Mays, T., ... DeFea, K. A. (2012). β -Arrestin-2 mediates the proinflammatory effects of proteinase-activated receptor-2 in the airway. *Proc Natl Acad Sci U S A* 109, 16660–16665.
- Nicholson, C. D., Shahid, M., Bruin, J., Barron, E., Spiers, I., de Boer, J., van Amsterdam, R. G., Zaagsma, J., Kelly, J. J., Dent, G., et al. (1995). Characterization of ORG 20241, a combined phosphodiesterase IV/III cyclic nucleotide phosphodiesterase inhibitor for asthma. *J Pharmacol Exp Ther* 274, 678–687.
- Noonan, M., Korenblat, P., Mosesova, S., Scheerens, H., Arron, J. R., Zheng, Y., ... Matthews, J. G. (2013). Dose-ranging study of lebrikizumab in asthmatic patients not receiving inhaled steroids. *J Allergy Clin Immunol* 132, 567–574.e512.
- Nyenhuis, S. M., Schwantes, E. A., Evans, M. D., & Mathur, S. K. (2010). Airway neutrophil inflammatory phenotype in older subjects with asthma. *J Allergy Clin Immunol* 125, 1163–1165.
- Ohta, S., Oda, N., Yokoe, T., Tanaka, A., Yamamoto, Y., Watanabe, Y., ... Adachi, M. (2010). Effect of tiotropium bromide on airway inflammation and remodelling in a mouse model of asthma. *Clin Exp Allergy* 40, 1266–1275.
- Olin, J. T., & Wechsler, M. E. (2014). Asthma: pathogenesis and novel drugs for treatment. *BMJ* 349, g5517.
- Ortega, V. E., & Peters, S. P. (2010). Beta-2 adrenergic agonists: focus on safety and benefits versus risks. *Curr Opin Pharmacol* 10, 246–253.
- Osawa, Y., Xu, D., Sternberg, D., Sonett, J. R., D'Armiento, J., Panettieri, R. A., & Emala, C. W. (2006). Functional expression of the GABA B receptor in human airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 291, L923–L931.
- Page, C. P. (2014). Phosphodiesterase inhibitors for the treatment of asthma and chronic obstructive pulmonary disease. *Int Arch Allergy Immunol* 165, 152–164.
- Penn, R. B. (2008). Embracing emerging paradigms of G protein-coupled receptor agonism and signaling to address airway smooth muscle pathobiology in asthma. *Naunyn-Schmiedeberg's Arch Pharmacol* 378, 149–169.
- Penn, R. B. (2014). Far from “disappointing”. *Am J Respir Crit Care Med* 189, 360.
- Penn, R. B. (2015). Physiology. Calcilytics for asthma relief. *Science* 348, 398–399.
- Penn, R. B., Bond, R. A., & Walker, J. K. (2014). GPCRs and arrestins in airways: implications for asthma. *Handb Exp Pharmacol* 219, 387–403.
- Penn, R. B., Pronin, A. P., & Benovic, J. L. (2000). Regulation of G protein-coupled receptor kinases. *Trends Cardiovasc Med* 10, 81–89.

- Pera, T., Hegde, A., Deshpande, D. A., Morgan, S. J., Tiegs, B. C., Theriot, B. S., ... Penn, R. B. (2015). Specificity of arrestin subtypes in regulating airway smooth muscle G protein-coupled receptor signaling and function. *FASEB J* 29, 4227–4235.
- Peters, S. P., Jones, C. A., Haselkorn, T., Mink, D. R., Valacer, D. J., & Weiss, S. T. (2007). Real-world evaluation of asthma control and treatment (REACT): findings from a national web-based survey. *J Allergy Clin Immunol* 119, 1454–1461.
- Pizzichini, E., Leff, J., Reiss, T., Hendeles, L., Boulet, L., Wei, L., ... Hargreave, F. (1999). Montelukast reduces airway eosinophilic inflammation in asthma: a randomized, controlled trial. *Eur Respir J* 14, 12–18.
- Powell, C., Milan, S. J., Dwan, K., Bax, L., & Walters, N. (2015). Mepolizumab versus placebo for asthma. *Cochrane Database Syst Rev* 7, CD010834.
- Powrie, D. J., Wilkinson, T. M. A., Donaldson, G. C., Jones, P., Scrine, K., Viel, K., ... Wedzicha, J. A. (2007). Effect of tiotropium on sputum and serum inflammatory markers and exacerbations in COPD. *Eur Respir J* 30, 472–478.
- Ramachandran, R., Noorbakhsh, F., DeFea, K., & Hollenberg, M. D. (2012). Targeting proteinase-activated receptors: therapeutic potential and challenges. *Nat Rev Drug Discov* 11, 69–86.
- Ramadan, W. H., Kabbara, W. K., El Khoury, G. M., & Al Assir, S. A. (2015). Combined bronchodilators (tiotropium plus olodaterol) for patients with chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis* 10, 2347–2356.
- Ramsay, C. F., Sullivan, P., Gazycki, M., Wang, D., Swern, A. S., Barnes, N. C., ... Jeffery, P. K. (2009). Montelukast and bronchial inflammation in asthma: a randomised, double-blind placebo-controlled trial. *Respir Med* 103, 995–1003.
- Rebuck, A. S., Gent, M., & Chapman, K. R. (1983). Anticholinergic and sympathomimetic combination therapy of asthma. *J Allergy Clin Immunol* 71, 317–323.
- Reiter, E., & Lefkowitz, R. J. (2006). GRKs and β -arrestins: roles in receptor silencing, trafficking and signaling. *Trends Endocrinol. Metab.* 17, 159–165.
- Rider, C. F., King, E. M., Holden, N. S., Giembycz, M. A., & Newton, R. (2011). Inflammatory stimuli inhibit glucocorticoid-dependent transactivation in human pulmonary epithelial cells: rescue by long-acting β 2-adrenoceptor agonists. *J Pharmacol Exp Ther* 338, 860–869.
- Rock, J. R., Futtner, C. R., & Harfe, B. D. (2008). The transmembrane protein TMEM16A is required for normal development of the murine trachea. *Dev Biol* 321, 141–149.
- Rosenthal, R. R., Busse, W. W., Kemp, J. P., Baker, J. W., Kalberg, C., Emmett, A., & Rickard, K. A. (1999). Effect of long-term salmeterol therapy compared with as-needed albuterol use on airway hyperresponsiveness. *Chest* 116, 595–602.
- Roth, M., Johnson, P. R. A., Rüdiger, J. J., King, G. G., Ge, Q., Burgess, J. K., ... Black, J. L. (2002). Interaction between glucocorticoids and β 2 agonists on bronchial airway smooth muscle cells through synchronised cellular signalling. *Lancet* 360, 1293–1299.
- Salmon, M., Tannheimer, S. L., Gentzler, T. T., Cui, Z., -H., Sorensen, E. A., Hartsough, K. C., ... Wright, C. D. (2014). The in vivo efficacy and side effect pharmacology of GS-5759, a novel bifunctional phosphodiesterase 4 inhibitor and long-acting β (2)-adrenoceptor agonist in preclinical animal species. *Pharmacol Res Perspect* 2, e00046.
- Salpeter, S. R., Buckley, N. S., Ormiston, T. M., & Salpeter, E. E. (2006). Meta-analysis: effect of long-acting beta-agonists on severe asthma exacerbations and asthma-related deaths. *Ann Intern Med* 144, 904–912.
- Sandrini, A., Ferreira, I. M., Gutierrez, C., Jardim, J. R., Zamel, N., & Chapman, K. R. (2003). Effect of montelukast on exhaled nitric oxide and nonvolatile markers of inflammation in mild asthma*. *Chest* 124, 1334–1340.
- Saxena, H., Deshpande, D., Tiegs, B., Yan, H., Battafarano, R., Burrows, W., ... Penn, R. B. (2012). The GPCR OGR1 (GPR68) mediates diverse signalling and contraction of airway smooth muscle in response to small reductions in extracellular pH. *Br J Pharmacol* 166, 981–990.
- Schaafsma, D., Bos, I. S. T., Zuidhof, A. B., Zaagsma, J., & Meurs, H. (2008). The inhaled Rho kinase inhibitor Y-27632 protects against allergen-induced acute bronchoconstriction, airway hyperresponsiveness, and inflammation. *Am J Physiol Lung Cell Mol Physiol* 295, L214–L219.
- Schaafsma, D., Gosens, R., Bos, I. S., Meurs, H., Zaagsma, J., & Nelemans, S. A. (2004). Allergic sensitization enhances the contribution of Rho-kinase to airway smooth muscle contraction. *Br J Pharmacol* 143, 477–484.
- Schaafsma, D., Gosens, R., Bos, I. S., Meurs, H., Zaagsma, J., & Nelemans, S. A. (2005). Role of contractile prostaglandins and Rho-kinase in growth factor-induced airway smooth muscle contraction. *Respir Res* 6, 85.
- Schmidlin, F., Amadesi, S., Dabbagh, K., Lewis, D. E., Knott, P., Bunnett, N. W., ... Stevens, M. E. (2002). Protease-activated receptor 2 mediates eosinophil infiltration and hyperactivity in allergic inflammation of the airway. *J Immunol* 169, 5315–5321.
- Schmidlin, F., Amadesi, S., Vidil, R., Trevisani, M., Martinet, N., Caughey, G., ... Bunnett, N. (2001). Expression and function of proteinase-activated receptor 2 in human bronchial smooth muscle. *Am J Respir Cell Mol Biol* 164, 1276–1281.
- Schuligoi, R., Sturm, E., Luschniig, P., Konya, V., Philipose, S., Sedei, M., ... Heinemann, A. (2010). CRTH2 and D-type prostanoid receptor antagonists as novel therapeutic agents for inflammatory diseases. *Pharmacology* 85, 372–382.
- Seldon, P. M., Mejia, K. K., & Giembycz, M. A. (2005). Rolipram, salbutamol and prostaglandin E2 suppress TNF α release from human monocytes by activating type II cAMP-dependent protein kinase. *Pulm Pharmacol Ther* 18, 277–284.
- Seuwen, K., Ludwig, M. G., & Wolf, R. M. (2006). Receptors for protons or lipid messengers or both? *J Recept Signal Transduct Res* 26, 599–610.
- Sherwood, C. L., Daines, M. O., Price, T. J., Vagner, J., & Boitano, S. (2014). A highly potent agonist to protease-activated receptor-2 reveals apical activation of the airway epithelium resulting in Ca²⁺-regulated ion conductance. *Am J Physiol Cell Physiol* 307, C718–C726.
- Soriano, J. B., & Barnes, N. C. (2007). Eppure si muove, or COPD treatment success and inflammation. *Eur Respir J* 30, 409–410.
- Takizawa, T., Tamiya, M., Hara, T., Matsumoto, J., Saito, N., Kanke, T., ... Hattori, Y. (2005). Abrogation of bronchial eosinophilic inflammation and attenuated eotaxin content in protease-activated receptor 2-deficient mice. *J Pharmacol Sci* 98, 99–102.
- Tamaoki, J., Graf, P. D., & Nadel, J. A. (1987). Effect of gamma-aminobutyric acid on neurally mediated contraction of guinea pig trachealis smooth muscle. *J Pharmacol Exp Ther* 243, 86–90.
- Tan, X., & Sanderson, M. J. (2014). Bitter tasting compounds dilate airways by inhibiting airway smooth muscle calcium oscillations and calcium sensitivity. *Br J Pharmacol* 171, 646–662.
- Tannheimer, S. L., Sorensen, E. A., Haran, A. C., Mansfield, C. N., Wright, C. D., & Salmon, M. (2012b). Additive anti-inflammatory effects of beta 2 adrenoceptor agonists or glucocorticosteroid with roflumilast in human peripheral blood mononuclear cells. *Pulm Pharmacol Ther* 25, 178–184.
- Tannheimer, S., Wright, C., & Salmon, M. (2012a). Combination of roflumilast with a beta-2 adrenergic receptor agonist inhibits proinflammatory and profibrotic mediator release from human lung fibroblasts. *Respir Res* 13, 28.
- Tannheimer, S. L., Sorensen, E. A., Cui, Z., -H., Kim, M., Patel, L., Baker, W. R., ... Salmon, M. (2014). The in vitro pharmacology of GS-5759, a novel bifunctional phosphodiesterase 4 inhibitor and long acting β 2-adrenoceptor agonist. *J Pharmacol Exp Ther* 349, 85–93.
- Tashkin, D. P., Celli, B., Senn, S., Burkhart, D., Kesten, S., Menjoge, S., ... Investigators, U. S. (2008). A 4-year trial of tiotropium in chronic obstructive pulmonary disease. *N Engl J Med* 359, 1543–1554.
- Thanawala, V. J., Forkuo, G. S., Al-Sawalha, N., Azzegagh, Z., Nguyen, L. P., Eriksen, J. L., ... Bond, R. A. (2013). beta2-Adrenoceptor agonists are required for development of the asthma phenotype in a murine model. *Am J Respir Cell Mol Biol* 48, 220–229.
- Thanawala, V. J., Valdez, D. J., Joshi, R., Forkuo, G. S., Parra, S., Knoll, B. J., ... Bond, R. A. (2015). β -Blockers have differential effects on the murine asthma phenotype. *Br J Pharmacol* 172, 4833–4846.
- Tilley, S. L., Hartney, J. M., Erikson, C. J., Jania, C., Nguyen, M., Stock, J., ... Koller, B. H. (2003). Receptors and pathways mediating the effects of prostaglandin E2 on airway tone. *Am J Physiol Lung Cell Mol Physiol* 284, 599–606.
- Tilba, O., Damera, G., Banerjee, A., Gu, S., Baidouri, H., Keslacy, S., & Amrani, Y. (2008). Cytokines induce an early steroid resistance in airway smooth muscle cells: novel role of interferon regulatory factor-1. *Am J Respir Cell Mol Biol* 38, 463–472.
- Tohda, Ohkawa, Kubo, Muraki, Fukuoka, & Nakajima (1998). Role of GABA receptors in the bronchial response: studies in sensitized guinea-pigs. *Clin Exp Allergy* 28, 772–777.
- Tokuyama, K., Nishimura, H., Iizuka, K., Kato, M., Arakawa, H., Saga, R., ... Morikawa, A. (2002). Effects of Y-27632, a Rho/Rho kinase inhibitor, on leukotriene D-4 and histamine-induced airflow obstruction and airway microvascular leakage in guinea pigs in vivo. *Pharmacology* 64, 189–195.
- Tomura, H., Wang, J. Q., Komachi, M., Damirin, A., Mogi, C., Tobo, M., ... Okajima, F. (2005). Prostaglandin I(2) production and cAMP accumulation in response to acidic extracellular pH through OGR1 in human aortic smooth muscle cells. *J Biol Chem* 280, 34458–34464.
- Urban, J. D., Clarke, W. P., von, Z. M., Nichols, D. E., Kobilka, B., Weinstein, H., ... Mailman, R. B. (2007). Functional selectivity and classical concepts of quantitative pharmacology. *J Pharmacol Exp Ther* 320, 1–13.
- Usmani, O. S., Ito, K., Maneechotesuwan, K., Ito, M., Johnson, M., Barnes, P. J., & Adcock, I. M. (2005). Glucocorticoid receptor nuclear translocation in airway cells after inhaled combination therapy. *Am J Respir Crit Care Med* 172, 704–712.
- van Noord, J. A., Aumann, J. L., Janssens, E., Smeets, J. J., Zaagsma, J., Mueller, A., & Cornelissen, P. J. G. (2010). Combining tiotropium and salmeterol in COPD: effects on airflow obstruction and symptoms. *Respir Med* 104, 995–1004.
- Waldeck, B. (2002). Beta-adrenoceptor agonists and asthma—100 years of development. *Eur J Pharmacol* 445, 1–12.
- Walker, J. K., Fong, A. M., Lawson, B. L., Savov, J. D., Patel, D. D., Schwartz, D. A., & Lefkowitz, R. J. (2003). Beta-arrestin-2 regulates the development of allergic asthma. *J Clin Invest* 112, 566–574.
- Walker, J. K., Penn, R. B., Hanania, N. A., Dickey, B. F., & Bond, R. A. (2011). New perspectives regarding beta(2)-adrenoceptor ligands in the treatment of asthma. *Br J Pharmacol* 163, 18–28.
- Walters, E., & Davies, B. (1982). Dual effect of prostaglandin E2 on normal airways smooth muscle in vivo. *Thorax* 37, 918–922.
- Walters, E. H., Gibson, P. G., Lasserson, T. J., & Walters, J. A. (2007). Long-acting beta2-agonists for chronic asthma in adults and children where background therapy contains varied or no inhaled corticosteroid. *Cochrane Database Syst Rev*, CD001385.
- Webster, J. C., Oakley, R. H., Jewell, C. M., & Cidlowski, J. A. (2001). Proinflammatory cytokines regulate human glucocorticoid receptor gene expression and lead to the accumulation of the dominant negative β isoform: a mechanism for the generation of glucocorticoid resistance. *Proc Natl Acad Sci U S A* 98, 6865–6870.
- Westergaard, C. G., Porsbjerg, C., & Backer, V. (2015). Emerging corticosteroid agonists for the treatment of asthma. *Expert Opin Investig Drugs*, 1–10.
- WHO (2013). *Fact sheet no. 307 vol. 2015*.
- Wisler, J. W., DeWire, S. M., Whalen, E. J., Violin, J. D., Drake, M. T., Ahn, S., ... Lefkowitz, R. J. (2007). A unique mechanism of beta-blocker action: carvedilol stimulates beta-arrestin signaling. *Proc Natl Acad Sci U S A* 104, 16657–16662.
- Xu, J., Sabarinath, S. N., & Derendorf, H. (2009). Cortisol suppression as a surrogate marker for inhaled corticosteroid-induced growth retardation in children. *Eur J Pharm Sci* 36, 110–121.
- Yan, H., Deshpande, D. A., Misior, A. M., Miles, M. C., Saxena, H., Riemer, E. C., ... Penn, R. B. (2011). Anti-mitogenic effects of beta-agonists and PGE2 on airway smooth muscle are PKA dependent. *FASEB J* 25, 389–397.
- Yarova, P. L., Stewart, A. L., Sathish, V., Britt, R. D., Thompson, M. A., P. Lowe, A. P., ... Riccardi, D. (2015). Calcium-sensing receptor antagonists abrogate airway hyperresponsiveness and inflammation in allergic asthma. *Sci Transl Med* 7, 284ra260.

- Yim, P. D., Gallos, G., Xu, D., Zhang, Y., & Emala, C. W. (2011). Novel expression of a functional glycine receptor chloride channel that attenuates contraction in airway smooth muscle. *FASEB J* 25, 1706–1717.
- Yocum, G. T., Gallos, G., Zhang, Y., Jahan, R., Stephen, M. R., Varagic, Z., ... Emala, C. W. (2015). Targeting the GABAA receptor $\alpha 4$ subunit in airway smooth muscle to alleviate bronchoconstriction. *Am J Respir Cell Mol Biol*.
- Yoshii, A., Iizuka, K., Dobashi, K., Horie, T., Harada, T., Nakazawa, T., & Mori, M. (1999). Relaxation of contracted rabbit tracheal and human bronchial smooth muscle by Y-27632 through inhibition of Ca^{2+} sensitization. *Am J Respir Cell Mol Biol* 20, 1190–1200.
- Zasłona, Z., Okunishi, K., Bourdonnay, E., Domingo-Gonzalez, R., Moore, B. B., Lukacs, N. W., ... Peters-Golden, M. (2013). Prostaglandin E2 suppresses allergic sensitization and lung inflammation by targeting the E prostanoid 2 receptor on T cells. *J Allergy Clin Immunol* 133, 379–387.e371.
- Zhang, C. -H., Li, Y., Zhao, W., Lifshitz, L. M., Li, H., Harfe, B. D., ... ZhuGe, R. (2013a). The transmembrane protein 16A Ca^{2+} -activated Cl^- channel in airway smooth muscle contributes to airway hyperresponsiveness. *Am J Respir Crit Care Med* 187, 374–381.
- Zhang, C. -H., Lifshitz, L. M., Uy, K. F., Ikebe, M., Fogarty, K. E., & ZhuGe, R. (2013b). The cellular and molecular basis of bitter tastant-induced bronchodilation. *PLoS Biol* 11, e1001501.
- Zieba, B. J., Artamonov, M. V., Jin, L., Momotani, K., Ho, R., Franke, A. S., ... Somlyo, A. V. (2011). The cAMP-responsive Rap1 guanine nucleotide exchange factor, Epac, induces smooth muscle relaxation by down-regulation of RhoA activity. *J Biol Chem* 286, 16681–16692.