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PERSPECTIVE

How Can 1 + 1 = 3? β_2-Adrenergic and Glucocorticoid Receptor Agonist Synergism in Obstructive Airway Diseases

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ABSTRACT

For a long time it was believed that β_2-adrenergic receptor agonists used in the treatment of obstructive airway diseases worked primarily on airway smooth muscle cells, causing relaxation, whereas glucocorticoids primarily improved airway function via their anti-inflammatory action, indicating that their clinical synergism occurred at the organism rather than the cellular level. However, it is now becoming clear that both drug classes can affect airway function at multiple levels, including an integrated effect on several cell types. This article summarizes data on the molecular interaction between the two receptor systems, particularly with relevance to phenomena of β_2-adrenergic receptor desensitization and glucocorticoid insensitivity in the airways. These molecular interactions may contribute to the observed clinical synergism between both drug classes in the treatment of obstructive airway diseases.

Introduction

Obstructive airway diseases are a developing pandemic expected to become the world's third leading cause of death by 2020. Major drug classes used in obstructive airway diseases encompass β_2-adrenergic receptor (B2AR) agonists, including the long-acting B2AR agonists (LABAs), and the glucocorticoids, both typically being administered by inhalation. In many patients, the two drug classes are coadministered because their combination can be more effective than either monotherapy (Giembycz et al., 2008). However, a subset of patients with (severe) asthma is rather insensitive to glucocorticoid treatment, and this contributes considerably to the high morbidity and economic burden associated with asthma (Barnes, 2006a; Adcock and Barnes, 2008). Understanding of the underlying molecular mechanisms that contribute to the therapeutic benefit of coadministration of LABAs and glucocorticoids will further future pharmacotherapeutic strategies.

For a long time it was believed that B2AR agonists worked primarily on airway smooth muscle cells, causing relaxation, whereas glucocorticoids primarily improved airway function via their anti-inflammatory action, indicating that their clinical synergism occurred at the organism rather than the cellular level. However, it is now becoming clear that both drug classes can affect airway function at multiple levels. Thus, B2AR agonists can also inhibit immune function (Loza and Penn, 2010) and attenuate pulmonary fibrosis (Racke et al., 2008; Lamyel et al., 2011). Likewise, glucocorticoids can affect not only white blood cell but also airway smooth muscle and epithelial function (Kaur et al., 2008; Black et al., 2009). If both drug classes act on the same cell types in the treatment of airway disease, a possible interaction at the cellular level becomes important for the molecular understanding of their clinical synergism. Against this background, an article in the current issue of the journal reports on molecular mechanisms by which LABAs can reverse glucocorticoid insensitivity in the airways (Mercado et al., 2011).

β_2-Adrenergic Receptor Agonist Effects

The classic pathway of B2AR signaling includes binding of agonist-occupied receptor to a G_s protein, activation of adenylyl cyclases, and then cAMP effects via protein kinase A and other targets. Integration of cAMP signaling is supported by phos-

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ABBREVIATIONS: B2AR, β_2-adrenergic receptor; LABA, long-acting B2AR agonist; MAPK, mitogen-activated protein kinase; MKP-1, mitogen-activated protein kinase phosphatase-1; C/EBPα, CCAAT/enhancer binding protein α; NF-κB, nuclear factor-κB.

phodiesterases and A-kinase anchoring proteins that generate discrete gradients of cAMP at specific cellular sites (Giembycz and Newton, 2006). This signaling pathway can thereby culminate in modulation of gene transcription via cAMP-response elements. In recent years, two main additions to this concept have emerged. First, an agonist-occupied B2AR may signal not only via cAMP but also, secondary to arrestin binding, via other pathways (DeWire et al., 2007). Second, next to protein kinase A, cAMP can activate the exchange protein directly activated by cAMP (epac) (Grandoch et al., 2010) (Fig. 1). One or more of these signaling pathways lead to cellular responses including relaxation and inhibition of proliferation of airway smooth muscle, airway remodeling, inhibition of inflammatory mediator release from mast cells, survival of eosinophils, and inhibition of extracellular matrix release from airway fibroblasts (Giembycz and Newton, 2006; Black et al., 2009). Transcriptional activation of mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1), which dephosphorylates and inactivates both extracellular signal-regulated kinase and p38 MAPK, contributes to the relaxation of airway smooth muscle by B2AR agonists (Giembycz and Newton, 2006; Kaur et al., 2008).

However, long-term agonist exposure can lead to B2AR desensitization (Johnson, 2006). Actually, B2ARs are the prototypical receptor used to establish the mechanisms involved in agonist-induced desensitization of G-protein-coupled receptors (Lefkowitz, 1998). Such desensitization can involve multiple mechanisms and has been demonstrated in various cell types, including airway smooth muscle cells and circulating white blood cells (Penn, 2008). Actually, B2AR expression on circulating blood cells has repeatedly been shown to correlate with that in solid tissues such as heart or myometrium (Brodde et al., 1986; Michel et al., 1989), although such correlation may not hold up under all conditions (Brodde et al., 1989). Thus, circulating white blood cells have been used as models to longitudinally monitor human B2AR regulation in vivo (Brodde et al., 1988), although they may be more sensitive to down-regulation than airway smooth muscle cells. The down-regulation of the B2ARs involves multiple mechanisms, including a sequestration to intracellular compartments (Cheung et al., 1990), which may lead to recycling to the cell surface but also to receptor degradation. Moreover, a reduced de novo synthesis of receptor protein (Bouvier et al., 1989), due at least in part to a reduced mRNA stability, can also contribute to B2AR down-regulation (Mak et al., 1995a). Up-regulation of cAMP-specific phosphodiesterases and down-regulation of G_s can also contribute to persistent B2AR desensitization (Giembycz and Newton, 2006).

Glucocorticoid Effects

Glucocorticoids modulate the transcription of many inflammatory mediators in several cell types and are therefore of great use in the treatment of chronic inflammatory diseases (Barnes, 2006b; Black et al., 2009). The 90-kDa heat shock protein is required for agonist binding to and nuclear translocation of the cytoplasmic glucocorticoid receptor, a ligand-inducible transcription factor. Subsequently, glucocorticoid receptor dimers bind to glucocorticoid recognition elements to activate or to inhibit corticoid-sensitive genes via mechanisms known as *trans*-activation and *trans*-repression (Barnes, 2006b). Activated glucocorticoid receptors recruit

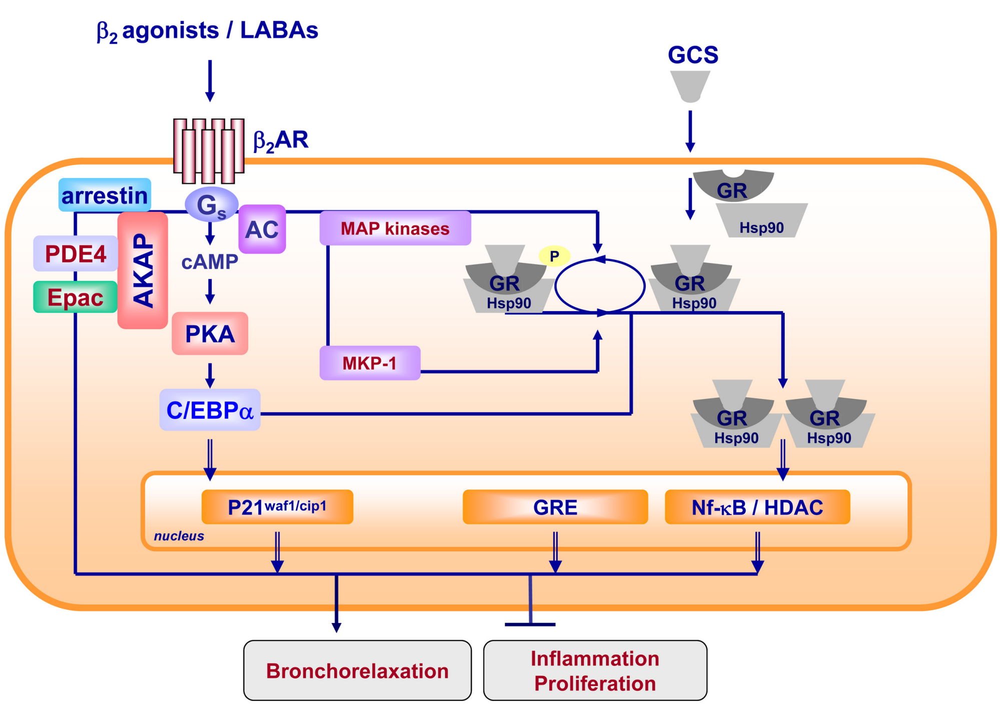

Fig. 1. Proposed model of B2AR adrenergic and glucocorticoid receptor (GR) agonist synergism in obstructive airway disease. Activation of the B2AR by agonists, including the LABAs, leads to the activation of adenylyl cyclase (AC) via coupling to G_s and subsequent generation of cAMP. Modulation of gene transcription via cAMP-response elements are mediated by the C/EBPα, leading to the transcription of the cell cycle inhibitor $p21^{waf1/cip1}$. Concerted action of several signaling pathways induced by the B2AR leads to the modulation of cellular responses (see text for further details). Upon 90-kDA heat shock protein (Hsp90)-dependent ligand binding to the GR, GR dimers bind to glucocorticoid recognition elements (GRE) to modulate gene transcription of corticoid-sensitive genes including $p57^{kip2}$ and MKP-1. Recruitment of histone deacetylase 2 (HDAC2) modulates transcription of NF-κB. Heterodimers of the GR and C/EBPα contribute to the B2 agonist and glucocorticoid synergism. Phosphocycling of the GR driven by mitogen-activated protein kinases and MKP-1 adds another level of complexity. See text for further details. AKAP, A-kinase anchor protein; Epac, exchange protein directly activated by cAMP; PDE4, phosphodiesterase E4; PKA, protein kinase A.

histone deacetylase 2 and reverse histone acetylation of proinflammatory genes such as the nuclear factor NF-κB. Interaction of the glucocorticoid receptor with the cAMP-response element binding protein inhibits (*trans*-represses) the NF-κB-associated histone acetylase activity and gene expression (Barnes, 2006b; Black et al., 2009) (Fig. 1).

In addition to the potent-inflammatory effects of glucocorticoids, in vitro studies have indicated that they inhibit proliferation of airway smooth muscle cells upon acceleration of the nuclear translocation of the glucocorticosteroid receptor and CCAAT/enhancer binding protein α (C/EBPα) and subsequent increases in the expression of the cell cycle inhibitor p21$^{waf1/cip1}$ (Roth et al., 2002) (Fig. 1). Down-regulation of growth factor-induced increases in cyclin D1 expression and retinoblastoma protein phosphorylation also contribute to inhibition of airway smooth muscle cell proliferation by glucocorticoids (Black et al., 2009). In addition to their antimitogenic effects, glucocorticoids inhibit airway smooth muscle α-actin expression (Goldsmith et al., 2007), indicating that they can also alter airway smooth muscle contractile properties. Indeed, glucocorticoids inhibit airway smooth muscle remodeling in a model of allergic asthma (Bos et al., 2007). Additional glucocorticoid effects in the airways may relate to increased eosinophil apoptosis and to reduced production of extracellular matrix proteins (Barnes, 2006a; Black et al., 2009).

The above mechanisms form the molecular basis for beneficial glucocorticoid effects on obstructed airway function. However, as noted, asthma symptoms are not adequately controlled by glucocorticoids in a subset of patients with particularly severe asthma, indicating glucocorticoid insensitivity (Barnes, 2006a; Adcock and Barnes, 2008). In glucocorticoid-sensitive control subjects, glucocorticoids activate histone acetylases, particularly histone deacetylase 2, and thereby inhibit gene transcription driven by transcription factors such as NF-κB. This process is largely diminished under oxidative and nitrative stress, which inactivate histone deacetylase 2 and impair nuclear translocation of the glucocorticoid receptor, thereby causing glucocorticoid insensitivity (Barnes, 2006a,b; Adcock and Barnes, 2008). In addition, glucocorticoids enhance the transcription of MKP-1, which inhibits p38 MAPK (Barnes, 2006b) (Fig. 1). Pro-inflammatory stimuli typically induce rapid and transient expression of MKP-1 mRNA, a process known to involve p38 MAPK and the nuclear factor NF-κB. Because MKP-1 is regulated by the same pathways that it suppresses, it forms part of a classic negative feedback loop to limit proinflammatory cellular responses (Barnes, 2006b; Clark et al., 2008). Persistent activation of p38 MAPK and reduced apoptosis of eosinophils have been described in clinical samples from glucocorticoid-insensitive patients (Irusen et al., 2002; Barnes, 2006a; Bhavsar et al., 2008).

Interaction between β_2-Adrenergic Receptor Agonists and Glucocorticoids. B2AR agonists including the LABAs can activate MAP kinase signaling and thereby drive the development of airway remodeling that significantly contributes to asthma pathophysiology (Adcock et al., 2002; Pelaia et al., 2005). In the context of airway remodeling, deposition of extracellular matrix from human airway cells is reduced by LABAs, but this effect requires the presence of glucocorticoids (Todorova et al., 2006; Black et al., 2009). Indeed, it has been reported that LABA-dependent activation of MAPK and subsequent phosphorylation of the glucocorticoid receptor alters its nuclear translocation and modifies glucocorticoid responsiveness (Eickelberg et al., 1999; Adcock et al., 2002). Studies in human airway cells demonstrated that although glucocorticoids had no effect on B2AR agonist-induced cAMP-response element-dependent transcription, LABAs synergistically enhanced glucocorticoid recognition element-dependent transcription by glucocorticoids, including that of the cell cycle kinase inhibitor p57^{kip2} and the MAPK phosphatase MKP-1 (Kaur et al., 2008). In airway smooth muscle cells, glucocorticoids and LABAs also synergize to accelerate nuclear translocation of the glucocorticoid receptor and C/EBPα, resulting in the synergistic activation of the cell cycle inhibitor p21$^{waf1/cip1}$ (Roth et al., 2002) and in a faster and longer activation of p21$^{waf1/cip1}$ and inhibition of airway smooth muscle proliferation. A decreased expression level of C/EBPα in human airway smooth cells from patients with asthma correlates with the severity of glucocorticoid insensitivity (Roth et al., 2002). Although not yet thoroughly studied in the airways, the proapoptotic protein Bim may confer glucocorticoid sensitivity and act as a convergence point for the induction of apoptosis by glucocorticoids and long-acting B2AR agonists (Zhang and Insel, 2004). Together, these studies provide insights into the molecular mechanisms that underlie the superior clinical efficacy of LABA/glucocorticoid combination therapies in the treatment of obstructive airway diseases.

Moreover, B2AR agonists and glucocorticoids can regulate cellular sensitivity to each other. Early work in DDT1 MF-2 hamster smooth muscle cells has demonstrated that glucocorticoids increase mRNA expression of the B2AR (Hadcock and Malbon, 1988), apparently by increasing its gene transcription rather than improving mRNA stability (Mak et al., 1995a). Such observations have later been extended to several other tissues, including the lung (Mak et al., 1995b) or the myometrium (Herman-Gnjidic et al., 1994). In particular, the LABA-dependent inhibition of inflammatory mediator release from mast cells is maintained by this glucocorticoid action (Black et al., 2009). The presence of glucocorticoid response elements in the β_2-adrenergic receptor gene promoter is the molecular basis of this increase. This interaction also occurs in humans in vivo: subjects receiving long-term B2AR agonist treatment have a reduced expression of lymphocyte B2ARs, and treatment with a glucocorticoid not only improves their airway function, as assessed by forced expiratory volume in 1 s, but also concomitantly up-regulates lymphocyte B2ARs (Brodde et al., 1988). Glucocorticoids also can increase the efficacy of B2AR/G_s coupling and reduce the production of interleukin-1b, which is known to uncouple B2ARs from its downstream effectors (Black et al., 2009). However, recent studies provided evidence for the notion that in some instances, glucocorticoids do not rescue the effect of B2AR agonists (Black et al., 2009; Cooper et al., 2011). Thus, it has been reported that the glucocorticoid budesonide prevented desensitization induced by the LABA formoterol but not that induced by another LABA, salmeterol. A distinct trafficking pattern of the B2AR driven by distinct agonists seems to determine the spatiotemporal characteristics of tissue responses and thereby the ability of glucocorticoids to rescue B2AR desensitization. Budesonide seems to primarily promote post-transcriptional mechanisms such as the stabilization of B2AR mRNA and effects on endosome trafficking (Cooper et al., 2011). Endosomes, now being recognized as an essential site of cellular signaling, may provide a novel platform for treatment of diseases (Murphy et al., 2009), including obstructive airway diseases. Using peripheral blood leukocytes, an article in this issue of the journal (Mercado et al., 2011) provides molecular information on the opposite mechanisms (i.e., restoration of glucocorticoid sensitivity by LABAs). This B2AR-mediated rescue of glucocorticoid receptor function occurs by a mechanism involving the p38 MAPK (Mercado et al., 2011) (Fig. 1). The ability of BAR agonists to activate p38 has long been recognized (e.g., in cardiomyocytes) (Sabri et al., 2000).

Conclusions

Taken together, these findings demonstrate that LABAs and glucocorticoids, which for a long time have been considered to act in parallel, rather work in concert, and each can alleviate the other's shortcomings. This interaction often is synergistic and may even create a self-enhancing cycle at the level of B2AR and glucocorticoid receptor expression. The frequent coadministration of glucocorticoids and LABAs may explain why the clinical relevance of agonist-induced B2AR down-regulation remains under debate. However, it should be noted that B2AR agonists and glucocorticoids do not always have similar downstream effects in the airways, in that the former can increase eosinophil survival whereas the latter have the opposite effect (Black et al., 2009). The importance of clinical synergism between LABAs and glucocorticoids in many patients, therefore, makes additional studies into the mechanism of this interaction necessary.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Schmidt and Michel.

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