PERSPECTIVE

How Can 1 + 1 = 3? β2-Adrenergic and Glucocorticoid Receptor Agonist Synergism in Obstructive Airway Diseases

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ABSTRACT

For a long time it was believed that β2-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 β2-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 β2-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).

β2-Adrenergic Receptor Agonist Effects

The classic pathway of B2AR signaling includes binding of agonist-occupied receptor to a G protein, activation of adenyl cyclases, and then cAMP effects via protein kinase A and other targets. Integration of cAMP signaling is supported by phos-
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 (Broder et al., 1989). Thus, circulating white blood cells have been used as models to longitudinally monitor human B2AR regulation in vivo (Broder 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 $G_o$ 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 Gs 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<sup>WAF1/CIP1</sup>. 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<sup>KIP2</sup> 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. Phosphorylation 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 pro-
inflammatory 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 acetylation activity and gene expression
(Barnes, 2006b; Black et al., 2009) (Fig. 1).

In addition to the potent-inflammatory effects of glucocorti-
coïds, in vitro studies have indicated that they inhibit prolifer-
ation 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 air-
ways 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 benefi-
cial glucocorticoid effects on obstructed airway function. How-
ever, as noted, asthma symptoms are not adequately controlled by
glucocorticoids in a subset of patients with particularly se-
vere asthma, indicating glucocorticoid insensitivity (Barnes,
2006a; Adcock and Barnes, 2008). In glucocorticoid-sensitive
control subjects, glucocorticoids activate histone acetylas-
les, 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 nu-
clear translocation of the glucocorticoid receptor, thereby caus-
ing glucocorticoid insensitivity (Barnes, 2006a,b; Adcock
and Barnes, 2008). In addition, glucocorticoids enhance the trans-
cription 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 proinflam-
matory 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 glu-
corticoid-insensitive patients (Irusen et al., 2002; Barnes,
2006a; Bhavsar et al., 2008).

Interaction between β2-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 con-
tributes 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 subse-
quent phosphorylation of the glucocorticoid receptor alters its
nuclear translocation and modifies glucocorticoid responsive-
ness (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 ele-
ment-dependent transcription, LABAs synergistically en-
hanced glucocorticoid recognition element-dependent transcrip-
tion 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 glucocorti-
coid insensitivity (Roth et al., 2002). Although not yet thor-
oughly studied in the airways, the proapoptotic protein Bim
can 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 glucocor-
ticoids increase mRNA expression of the B2AR (Haddock 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 myome-
trium (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 β2-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 concomi-
tantly up-regulates lymphocyte B2ARs (Brodde et al., 1988).
Glucocorticoids also can increase the efficacy of B2AR/Gs
coupling and reduce the production of interleukin-1β, 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 traf-
ficking pattern of the B2AR driven by distinct agonists seems to
determine the spatiotemporal characteristics of tissue re-
ponses 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.

References


