

# Can the anti-inflammatory activities of $\beta$ 2-agonists be harnessed in the clinical setting?

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**Abstract:** Beta2-adrenoreceptor agonists ( $\beta$ 2-agonists) are primarily bronchodilators, targeting airway smooth muscle and providing critical symptomatic relief in conditions such as bronchial asthma and chronic obstructive pulmonary disease. These agents also possess broad-spectrum, secondary, anti-inflammatory properties. These are mediated largely, though not exclusively, via interactions with adenylyl cyclase-coupled  $\beta$ 2-adrenoreceptors on a range of immune and inflammatory cells involved in the immunopathogenesis of acute and chronic inflammatory disorders of the airways. The clinical relevance of the anti-inflammatory actions of  $\beta$ 2-agonists, although often effective in the experimental setting, remains contentious. The primary objectives of the current review are: firstly, to assess the mechanisms, both molecular and cell-associated, that may limit the anti-inflammatory efficacy of  $\beta$ 2-agonists; secondly, to evaluate pharmacological strategies, several of which are recent and innovative, that may overcome these limitations. These are preceded by a consideration of the various types of  $\beta$ 2-agonists, their clinical applications, and spectrum of anti-inflammatory activities, particularly those involving adenosine 3',5'-cyclic adenosine monophosphate-activated protein kinase-mediated clearance of cytosolic calcium, and altered gene expression in immune and inflammatory cells.

**Keywords:** adenylyl cyclase, corticosteroids, cyclic AMP, muscarinic receptor antagonists, neutrophils, phosphodiesterase inhibitors

## Introduction

Beta2-adrenergic agonists ( $\beta$ 2-agonists) are widely used in clinical practice to treat patients with obstructive airway disorders, such as asthma, chronic obstructive pulmonary disease (COPD) and bronchiolitis obliterans. These agents relax airway smooth muscle, resulting in bronchodilatation, via interaction with G-protein-coupled  $\beta$ 2-adrenoreceptors ( $\beta$ 2ARs), linked to adenylyl cyclase. The consequence is elevation of intracellular cyclic adenosine monophosphate (cAMP) concentrations and activation of protein kinase A (PKA).<sup>1</sup> In addition to their primary bronchodilatory effects,  $\beta$ 2-agonists have been shown to attenuate the proinflammatory activities of a range of immune and inflammatory cells in vitro, such as neutrophils, monocytes, mast cells, eosinophils, basophils, and lymphocytes, all of which contribute to the pathogenesis of various acute and chronic respiratory diseases.<sup>2</sup> In addition, these agents have demonstrated efficacy in animal models of experimental acute lung injury.<sup>3,4</sup> Clearly, the combination of bronchodilatory and anti-inflammatory activities is of considerable potential value in the pharmacotherapy of acute and chronic diseases of the airways, of both infective and noninfective origin. Disappointingly, however,  $\beta$ 2-agonists do not appear to possess significant anti-inflammatory activity in the clinical setting.

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The current review is focused on the cellular targets and mechanisms of anti-inflammatory activity of  $\beta_2$ -agonists, as well as on strategies, both current and future, that might enable these to be actualized in the clinical setting. This is preceded by a brief consideration of the current clinical applications and types of  $\beta_2$ -agonists.

## Types of $\beta_2$ -agonists

These agents are characterized according to their duration of action, the three categories being: short-acting beta-agonist (SABA), long-acting beta agonist (LABA) and ultra-LABA. Some commonly used examples of these are shown in Table 1,<sup>5,6-11</sup> together with their types of agonist activity, partition coefficients, and durations of action. The number of  $\beta_2$ ARs per cell on various immune and inflammatory cells, together with their dissociation constants, is summarized in Table 2.<sup>12-19</sup> In the case of LABAs, formoterol has a more rapid onset of action than salmeterol,<sup>5</sup> while both agents provide sustained bronchodilatation for at least 12 hours.<sup>20</sup> Although indacaterol is the only example shown of an ultra-LABA, several other such agents (abediterol, carmoterol, milveterol, olodaterol, vilanterol) are in the pipeline,<sup>5</sup> while another, vilanterol, has recently received US Food and Drug Administration approval for therapy of COPD.

## $\beta_2$ -adrenoceptor agonists and therapy of respiratory airway disorders

SABAs are commonly used as rescue bronchodilator therapy to provide symptomatic relief for patients with exacerbations of asthma or COPD. Longer-term control of airway inflammation in asthma is usually achieved using inhaled corticosteroids (ICS). Significantly, LABAs in combination with ICS, currently play an important role in the management

**Table 1** Types of commonly used  $\beta_2$ -agonists: their activity, partition coefficients, and duration of action

Types	Agonist activity	Partition coefficient (Log P)	Duration of action (hours)
Short acting			
Salbutamol <sup>5-7,11</sup>	Partial	0.34–0.6	4–6
Terbutaline <sup>7,11</sup>	Partial	0.44–0.55	4–8
Fenoterol <sup>5,7,8,11</sup>	Full	1.36–1.47	6–8
Long acting			
Salmeterol <sup>5-7,11</sup>	Partial	3.61–3.82	12
Formoterol <sup>5-7,11</sup>	Full	1.06–1.91	12
Ultra-long acting			
Indacaterol <sup>5,7,9-11</sup>	Full	3.26–3.31	24

**Table 2** The number of receptors per cell and dissociation constants of  $\beta_2$ -agonists in various immune and inflammatory cells

Cell type	Number of receptors/cell ( $B_{max}$ )	Dissociation constant ( $K_d$ ) (pM)
Neutrophils <sup>12</sup>	20878±2470 (females)	
	7331±3179 (males)	
Macrophages <sup>13</sup>	5643±942	29±9
Monocytes <sup>14</sup>	1000–3000	38±29
Mast cells <sup>15</sup>	Variable btw preparations	40±10
Epithelial cells <sup>16</sup>	9908±1127 (Calu-3 cells)	
	6423±895 (16HBE14o(-) cells)	
Eosinophils <sup>17</sup>	4333±318	25.3±1.4
T-cells <sup>18,19</sup>	776±183*	19.7±1.6

**Note:** The results are expressed as the mean ± standard error of the mean or \*standard deviation.

**Abbreviation:** btw, between.

of chronic persistent asthma.<sup>21</sup> Both types of  $\beta_2$ -agonists, as well as the more recently introduced ultra-LABAs, are generally considered to have good safety profiles, and these, as well as the cost-effectiveness of bronchodilator therapies, have been covered extensively in a recent review.<sup>1</sup>

There has been some concern that LABAs may mask ongoing airway inflammation in asthma and, accordingly, these agents should not be used as monotherapy in this condition.<sup>22</sup> Beta-agonists are not recommended as monotherapy in asthma, as these agents may increase airway hyper-responsiveness, and increase the risk of death in patients with chronic asthma.<sup>23,24</sup> In contradistinction to asthma, long-term use of LABAs as monotherapy in patients with COPD appears to be safe and effective.<sup>25</sup> Importantly, however, the combination of low-dose ICS with a LABA has been shown to be more effective than high doses of ICS.<sup>26</sup> This is supported by the findings of a study which demonstrated that the control of airway inflammation, determined by means of bronchial biopsies, was not compromised when asthmatic patients on high doses of ICS were switched to a combination of low-dose ICS and LABA.<sup>27</sup> This is consistent with the well-documented anti-inflammatory interactions of combinations of LABAs and ICS, as discussed later in this review. Current guidelines for asthma therapy incorporate ICS/LABA combinations for patients not controlled on low doses of ICS.<sup>21,22</sup>

The role of LABAs in the management of COPD has been confirmed in a number of large randomized controlled trials, demonstrating improved pulmonary function tests, reduced frequency of exacerbations, and apparent slowing of the rate of decline of forced respiratory volume in 1 second.<sup>28</sup> Patients with symptomatic COPD may be given long-acting anti-cholinergics or LABAs as first-line therapy. In addition, some studies have shown LABAs to attenuate airway inflammation in COPD, and to decrease systemic markers of inflammation.<sup>29</sup>

Large clinical trials have indicated that LABAs are safe in COPD patients, as mentioned above.<sup>30,31</sup> However, in patients with COPD, the use of ICS may be associated with an increased risk of pneumonia, which should be considered when using ICS/LABA combinations in this setting.<sup>5</sup>

Bronchiolitis obliterans is a chronic fibrotic disorder of small airways and bronchioles which results in progressive and usually fixed airflow obstruction. Adjunctive therapy with a combination of a LABA and ICS has been shown to provide symptomatic relief and significant improvement in airflow obstruction, in patients with this condition.<sup>32,33</sup>

Acute lung injury (ALI) is a common and serious disorder, characterized by alveolar flooding, neutrophil accumulation in the lungs, and markedly impaired gaseous exchange. Beta2-agonists have been reported to enhance fluid clearance from alveoli, to attenuate both neutrophil recruitment and proinflammatory activity, and to decrease endothelial permeability in experimental ALI.<sup>3,4,34</sup> Consequently,  $\beta_2$ -agonists were considered potentially useful in ALI, a contention which was supported by data from limited human trials.<sup>35</sup> However, recent, larger, randomized controlled trials have not shown improved outcomes for patients with ALI, treated with aerosolized or intravenous albuterol/salbutamol.<sup>34,36,37</sup> Although  $\beta_2$ AR-desensitization may contribute to the lack of efficacy of intravenous salbutamol in the clinical setting of ALI, it is unlikely to be the only mechanism; others include the partial agonist properties of this agent, rendering it less potent than full agonists, with respect to the anti-inflammatory mechanisms described below.

## Anti-inflammatory activities of $\beta_2$ -agonists

Of the various types of immune and inflammatory cells involved in the immunopathogenesis of bronchial asthma and COPD, all have been reported to express  $\beta_2$ ARs. Seemingly, neutrophils have the highest level of expression, albeit at lower density than airway smooth muscle cells (Table 2). Beta2-agonists have been reported to suppress the proinflammatory activities of all of these cell types (monocytes/macrophages, basophils, eosinophils, mast cells, neutrophils, T lymphocytes), as well as those of airway structural cells (epithelial cells, fibroblasts, smooth muscle cells) by several distinct mechanisms. These are broadly categorized as being either cAMP-dependent or cAMP-independent, with LABAs (especially formoterol) being most effective, while little is known about the anti-inflammatory potential of ultra-LABAs. The effects of  $\beta_2$ -agonists on various immune and inflammatory cells and their mediators are summarized in Table 3.<sup>2,38,39</sup>

**Table 3** The effects of  $\beta_2$ -agonists on various immune and inflammatory cells

<b>Neutrophils<sup>2,38,39</sup></b>
↓ O <sub>2</sub> <sup>-</sup>
↓ Elastase release
↓ LTB <sub>4</sub>
↓ CR3
↓ IL-8
↓ Chemotaxis
↑ Ca <sup>2+</sup> clearance from cytosol
↓ Adhesion to bronchial epithelial cells
↓ Bacterial permeability-increasing protein
↑ Membrane-stabilizing effects
<b>Monocytes/macrophages<sup>2,38,39</sup></b>
↓ iNOS
↓ ERK 1/2
↓ LTB <sub>4</sub> , PGE <sub>2</sub> , TBX B <sub>2</sub>
↓ GM-CSF, TNF- $\alpha$ , IL-1 $\beta$
↓ IL-2, IL-6, IL-8, IL-12, IL-18
↑ IL-10
<b>Mast cells<sup>2,38,39</sup></b>
↓ Histamine release
↓ IL-5, TNF- $\alpha$ , GM-CSF, MIP-1 $\alpha$
↓ LTC <sub>4</sub> , LTD <sub>4</sub> and PGD <sub>2</sub>
<b>Epithelial cells<sup>39</sup></b>
↓ GM-CSF, VEGF
↓ IL-8, RANTES, eotaxin, IP-10
↓ ICAM-1, VCAM-1
<b>Eosinophils<sup>2,36,37</sup></b>
↓ O <sub>2</sub> <sup>-</sup>
↓ Eosinophil cationic protein
↓ Eosinophil peroxidase release
↓ LTC <sub>4</sub>
↓ Chemotaxis
↓ Adherence to fibronectin-coated plates
↓ Eosinophil-derived neurotoxin
<b>Basophils<sup>2</sup></b>
↓ Histamine release
↓ IL-4, IL-13
<b>T-cells<sup>2,38,39</sup></b>
↓ Proliferation in response to anti-CD3 Ab
↓ IL-2, IL-3, IL-4, IL-5, IL-13
↓ IFN- $\gamma$ , GM-CSF, TNF- $\alpha$
↑ IL-10

**Abbreviations:** O<sub>2</sub><sup>-</sup>, superoxide anion; iNOS, inducible nitric oxide synthase; ERK, extracellular-regulated kinase; LTB<sub>4</sub>, leukotriene B<sub>4</sub>; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; TBX B<sub>2</sub>, thromboxane B<sub>2</sub>; GM-CSF, granulocyte-macrophage colony-stimulating factor; TNF- $\alpha$ , tumor necrosis factor alpha; IL-1 $\beta$ , interleukin-1 beta; IL-; interleukin-; MIP-1 $\alpha$ , macrophage inflammatory protein 1 alpha; LTD<sub>4</sub>, leukotriene D<sub>4</sub>; PGD<sub>2</sub>, prostaglandin D<sub>2</sub>; VEGF, vascular endothelial growth factor; RANTES, regulated upon activation normal T cell expressed and secreted; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion protein 1; anti-CD3 Ab, anti-CD3 monoclonal antibody; IFN- $\gamma$ , interferon gamma; CR3, complement receptor 3; LTC<sub>4</sub>, leukotriene C<sub>4</sub>; IP-10, interferon  $\gamma$  inducible protein.

## cAMP-dependent anti-inflammatory mechanisms

Intracellular cAMP has two major targets, PKA and guanine nucleotide exchange protein directly activated by cAMP-1 (Epac1), both of which regulate the proinflammatory

activities of various cell types, with the former mechanism being the best characterized. The major cellular targets of PKA are  $\text{Ca}^{2+}$  mobilization and clearance mechanisms,<sup>40–50</sup> and regulation of the expression of genes encoding anti-inflammatory/proinflammatory proteins at both the transcriptional and translational levels.<sup>51–74</sup> Epac1 also appears to modulate gene expression.<sup>75–77</sup>

## Regulation of cytosolic $\text{Ca}^{2+}$ fluxes by cAMP/PKA

Several distinct, albeit interactive, mechanisms have been described, by which cAMP/PKA downregulates the  $\text{Ca}^{2+}$ -dependent proinflammatory activities of immune and inflammatory cells, as well as structural cells. These mechanisms, which are best characterized in activated human neutrophils, are regulated by various types of cAMP-elevating agents, including  $\beta_2$ -agonists, agonists of subtype A2A adenosine receptors, and inhibitors of cAMP phosphodiesterases (PDEs), especially PDE4.<sup>40–43</sup>

In the case of neutrophils, receptor-mediated stimulation of these cells is accompanied by activation of phospholipase C $\beta_3$  (PLC), which, in turn, cleaves membrane phosphatidylinositol to generate inositol triphosphate (IP3). This second messenger interacts with  $\text{Ca}^{2+}$ -mobilizing IP3 receptors on intracellular  $\text{Ca}^{2+}$  stores (calciosomes, endoplasmic reticulum), resulting in an abrupt, transient increase in cytosolic  $\text{Ca}^{2+}$ . This is a critical event, which precedes, and is a prerequisite for, activation of most of the proinflammatory activities of these cells, including: i) generation of both reactive oxygen species and the eicosanoid, leukotriene B4 (LTB<sub>4</sub>); ii) mobilization of cytosolic granules; and iii)  $\beta_2$ -integrin-mediated firm adhesion to vascular endothelium.<sup>42</sup>

To counter neutrophil hyper-reactivity, these events are stringently regulated by several cAMP/PKA-dependent mechanisms, which interact to restore  $\text{Ca}^{2+}$  homeostasis. These are: i) phosphorylative inactivation of PLC by PKA;<sup>44</sup> ii) upregulation of the activity of the  $\text{Ca}^{2+}$ -resequestering endomembrane  $\text{Ca}^{2+}$  ATPase (adenosine triphosphatase; probably via phosphorylation of serine (Ser)16 on the phospholamban regulatory subunit);<sup>41</sup> iii) inactivation of the IP3 receptor;<sup>45</sup> iv) inhibition of influx of extracellular  $\text{Ca}^{2+}$ , via regulation of store-operated  $\text{Ca}^{2+}$  channels;<sup>46</sup> and v) phosphorylative inactivation of p38 mitogen-activated protein kinase (MAPK), resulting in failure of activation of 5'-lipoxygenase (5-LO), with resultant attenuation of an autocrine, LTB<sub>4</sub>-mediated secondary wave of  $\text{Ca}^{2+}$  influx;<sup>47</sup> in addition, PKA-mediated phosphorylation of 5-LO inhibits the nuclear import of this enzyme.<sup>48</sup>

In this setting of restoration of  $\text{Ca}^{2+}$  homeostasis to activated neutrophils, the autocrine interaction of adenosine with G-protein/adenylyl cyclase-coupled A2A receptors appears to be the primary mechanism for generation of cAMP;<sup>49,50</sup> which is complemented by  $\beta_2$ -agonists and PDE4 inhibitors.<sup>43</sup>

## PKA modulation of inflammatory/anti-inflammatory gene expression

Cyclic AMP/PKA-dependent, gene-targeted, anti-inflammatory mechanisms are also operative at several levels, including: i) antagonism of transcription factors;<sup>51,52</sup> ii) induction of synthesis of the anti-inflammatory cytokine, interleukin (IL)-10;<sup>3,53–57</sup> and iii) potentiation of glucocorticoid (GC)/glucocorticoid receptor (GR)-activated gene transcription, which contributes to the beneficial therapeutic interactions of LABAs with ICS.<sup>58–74</sup>

## Antagonism of transcription factors by PKA

Activation of PKA results in phosphorylation of the transcription factor, cAMP response binding protein (CREB), which, in turn, competes with proinflammatory transcription factors, such as nuclear factor kappa B (NF- $\kappa$ B), and activator protein-1, for limited binding sites on the transcriptional coactivator, with intrinsic histone acetyl transferase activity and CREB-binding protein. This mechanism, which has been described in epithelial cells and macrophages, results in decreased expression of genes encoding a range of proinflammatory proteins.<sup>51,52</sup>

## Modulation of IL-10 gene expression by PKA

The promoter region of the IL-10 gene contains a cAMP response element. Gene transcription results, in turn, from PKA-mediated phosphorylation of CREB, a known transcription factor for IL-10 promoter activation. This anti-inflammatory mechanism is operative in several cell types, such as dendritic cells and macrophages,<sup>3,53–56</sup> and may be particularly important in controlling neutrophilic inflammation, as these cells respond to, but do not produce, IL-10.<sup>57</sup>

## Potentiation of glucocorticoid receptor-activated gene transcription by cAMP/PKA

Cyclic AMP/PKA-activating agents, particularly LABAs, have well documented enhancing actions, both direct and indirect, on the GR/glucocorticoid response element

(GRE) dependent transactivation of genes encoding anti-inflammatory proteins.<sup>58</sup> These effects are complex, and are achieved primarily via positive, site-specific phosphorylation of serine residues situated in the regulatory N-terminal region of the GR, either by PKA directly, or indirectly, via PKA-mediated phosphorylation of other kinases, such as cyclin-dependent kinases.<sup>59,60</sup> These events have been described in a variety of cell types, including airway epithelial cells, smooth muscle cells, fibroblasts, and monocytes/macrophages, as well as hippocampal progenitor cells. They result in the following: i) increased stability and nuclear translocation of the GR, both ligand-dependent and ligand-independent; and ii) increased transcriptional efficiency, via enhanced GRE binding of the activated GR, apparently via recruitment of transcription-promoting coregulatory proteins.<sup>58-69</sup> The consequence is activation of genes encoding a series of anti-inflammatory proteins. One of these, MAPK phosphatase-1, also promotes stabilization of the GR via dephosphorylation of p38 MAPK and c-jun-N-terminal kinase (JNK), both of which phosphorylate the GR at position Ser226, inhibiting translocation and GRE binding.<sup>69</sup> Inactivation of these kinases by MAPK phosphatase-1 also attenuates their ability to activate several proinflammatory transcription factors.

These GR-targeted activities of LABAs are complemented by several other cAMP/PKA-dependent mechanisms, including: i) increased expression of functional GRs by stabilization of GR messenger (m)RNA;<sup>58</sup> ii) phosphorylation of phosphatidylinositol-3 kinase (PI3 K)/protein kinase B (Akt) signaling, which prevents inactivation of histone deacetylase 2, increasing the efficiency of GR-mediated repression of genes encoding proinflammatory proteins;<sup>70,71</sup> and iii) interference with  $LTB_4$  production, thereby antagonizing the antiapoptotic/proinflammatory effects of GR activation on neutrophils and monocytes, which are mediated via upregulation of expression of the  $LTB_4$  receptor 1, BTL1.<sup>72</sup>

As well as underpinning the improved efficacy of ICS in patients receiving combination therapy with LABAs, these various cAMP/PKA-mediated mechanisms may also attenuate the development of corticosteroid (CS) resistance.<sup>26,58,69,71,73,74</sup>

## Epac I

Cyclic AMP has also been reported to suppress the proinflammatory activities of several cell types, including macrophages, microglia, and epithelial cells, by a PKA-independent mechanism. In this setting, the alternative target of cAMP

is Epac1, the substrate of which is the Ras-like, small guanosine triphosphatase, Rap1.<sup>75</sup> Notwithstanding effects on the cytoskeleton,<sup>76</sup> Epac1, acting via Rap1, modulates the proinflammatory activities of NF- $\kappa$ B and glycogen synthase kinase-3 $\beta$ , resulting in decreased synthesis of tumor necrosis factor by target cells.<sup>77</sup>

These various mechanisms of cAMP-mediated anti-inflammatory activity are summarized in Table 4.

## Cyclic AMP/PKA-independent mechanisms of $\beta_2$ -agonist-mediated anti-inflammatory activity

Although the anti-inflammatory mechanisms of  $\beta_2$ -agonists are mediated predominantly by cAMP/PKA-dependent mechanisms, the existence of alternative mechanisms has been described in various cell types, including bronchial epithelial cells and macrophages.<sup>3,78-80</sup> Perhaps the most significant of these was described in a recent study, in which formoterol was found to cause  $\beta_2$ AR-independent activation of the serine/threonine phosphatase, PP2A.<sup>80</sup> Activation of PP2A, in turn, reversed JNK-mediated phosphorylation of the GR at Ser226, thereby increasing CS sensitivity.

**Table 4** cAMP-mediated mechanisms of anti-inflammatory activity

Target	Effect
<b>Protein kinase A-mediated</b>	
<ul style="list-style-type: none"> <li>Regulation of <math>Ca^{2+}</math> mobilization and cytosolic clearance in activated immune and inflammatory cells</li> </ul>	Downregulation of $Ca^{2+}$ -dependent proinflammatory activities <sup>40-48</sup>
<ul style="list-style-type: none"> <li>Antagonism of proinflammatory transcription factors (NF<math>\kappa</math>B, AP-1, others)</li> </ul>	Decreased expression of genes encoding proinflammatory proteins <sup>51,52</sup>
<ul style="list-style-type: none"> <li>Interaction of CREB with the promoter region of the IL-10 gene</li> </ul>	Activation of synthesis of IL-10 <sup>53-56</sup>
<ul style="list-style-type: none"> <li>Potential of GR/GRE-mediated gene transcription (<i>trans</i>-activation)</li> </ul>	Activation of synthesis of other anti-inflammatory proteins <sup>58-69</sup>
<ul style="list-style-type: none"> <li>Inhibition of 5'-lipoxygenase</li> </ul>	Decreased production of proinflammatory leukotrienes, and antagonism of glucocorticoid-mediated anti-apoptotic effects on neutrophils <sup>47,48,72</sup>
<b>Epac I-mediated</b>	
<ul style="list-style-type: none"> <li>Antagonism of NF<math>\kappa</math>B and glycogen synthase kinase</li> </ul>	Decreased production of TNF <sup>75,77</sup>

**Abbreviations:** cAMP, cyclic adenosine monophosphate; NF $\kappa$ B, nuclear factor kappa B; AP-1, activator protein-1; CREB, cAMP response element-binding protein; IL-10, interleukin 10; GR, glucocorticoid receptor; GRE, glucocorticoid response element; TNF, tumor necrosis factor; Epac1, guanine nucleotide exchange protein directly activated by cAMP-1.

## Mechanisms which restrict the anti-inflammatory efficacy of $\beta_2$ -agonists

Beta2-agonists, especially LABAs, are ineffective, possibly harmful, when used as monotherapy in bronchial asthma, administered intravenously, or used in aerosols in patients with ALI.<sup>36,37,81</sup> This seems surprising, given the broad range of cellular targets and inflammatory mediators which are negatively regulated by cAMP.

Notwithstanding differences in molecular structure, agonist activity, and duration of action, the following mechanisms, operative in airway smooth cells, are also likely to undermine the anti-inflammatory efficacy of  $\beta_2$ -agonists: i) homologous receptor desensitization;<sup>26,73,81,82</sup> and ii) induction of cAMP-specific PDE4.<sup>82,73,83</sup> These mechanisms are compounded by the lower numbers of  $\beta_2$ ARs on immune and inflammatory cells, relative to those present on airway smooth muscle.<sup>38</sup>

### Receptor desensitization

Homologous receptor desensitization and downregulation of receptor expression, following extended use of  $\beta_2$ -agonists, are considered to be major limitations of these agents, with respect to anti-inflammatory activity.<sup>2,26,73,81</sup> In this setting, prolonged activation of the  $\beta_2$ AR results in sequential uncoupling of the receptor from adenylyl cyclase, internalization and phosphorylation by  $\beta$ AR kinases, PKA and G-protein-coupled receptor kinases, with resultant desensitization.<sup>73</sup> Extended exposure to  $\beta_2$ -agonists also results in decreased  $\beta_2$ AR gene transcription and receptor expression.<sup>73</sup> These mechanisms, compounded by the relatively low numbers of  $\beta_2$ ARs on immune and inflammatory cells,<sup>38</sup> are likely to restrict not only the anti-inflammatory potential of  $\beta_2$ -agonists, but also that of endogenous catecholamines.

In addition, prolonged exposure to  $\beta_2$ -agonists has also been reported to cause heterologous receptor desensitization (cross-desensitization) in airway smooth muscle cells, via the aforementioned mechanisms.<sup>82,84</sup> Although it is speculative, heterologous receptor desensitization, if operative *in vivo*, may attenuate other types of endogenous, receptor-mediated, cAMP-dependent, anti-inflammatory mechanisms, such as those involving activation of adenosine A2A receptors.<sup>85</sup>

### Induction of cAMP-specific PDE4

Prolonged exposure of airway smooth muscle cells has also been reported to result in increased transcription of the gene encoding PDE4D5, the predominant isoform

present in these cells, by mechanisms involving PKA and extracellular-regulated kinase 1/2.<sup>82,84,86</sup> The consequence is markedly decreased levels of intracellular cAMP and loss of responsiveness to  $\beta_2$ -agonists, which can be prevented by pretreatment of the cells with selective and nonselective inhibitors of PDE4.<sup>82,84,86</sup>

### $\beta_2$ -adrenoreceptors on immune and inflammatory cells

Because they express lower numbers of  $\beta_2$ ARs, immune and inflammatory cells are likely to be particularly prone to loss of sensitivity to  $\beta_2$ -agonists by the aforementioned mechanisms. The probable consequence is attenuation of the cAMP-dependent anti-inflammatory mechanisms described above.

## Pharmacological strategies which potentiate the anti-inflammatory activities of $\beta_2$ -agonists

Several pharmacological strategies, some well-established and others currently in the preclinical and clinical phases of evaluation, have been described that may counter  $\beta_2$ AR desensitization and increased activity of PDEs.

### Combining LABAs with CS

As described above, combining LABAs with CS into a single inhaler device has proven to be a major innovation in the therapy of bronchial asthma.<sup>26,73</sup> The magnitude of improvement in asthma control resulting from a LABA/ICS combination is superior to that achieved with high doses of ICS alone.<sup>87</sup> Similarly, for patients with COPD, the clinical efficacy of a LABA/ICS combination is significantly greater than with either agent used as monotherapy.<sup>88</sup> Commonly used LABA/ICS combinations, together with their times of onset of action, efficacies, and adverse effects are shown in Table 5.

It is generally assumed that the primary role of the LABA is to augment the anti-inflammatory actions of the CS, by the mechanisms described in the preceding sections.<sup>83</sup> However, CS can also augment the anti-inflammatory actions of LABAs, consistent with synergistic interactions between the two components. These mechanisms include CS-mediated increases in: i) the numbers of  $\beta_2$ ARs, by potentiating receptor gene transcription; and ii) the efficacy of coupling between the  $\beta_2$ AR and its Gs-protein subunit. The consequence is enhanced and sustained cellular responses to  $\beta_2$ -agonists.<sup>73</sup>

**Table 5** Inhaled bronchodilator therapies that may be used alone or in combination for the management of chronic stable COPD

Class of agent	Onset of action	Therapeutic efficacy	Adverse effects
<b>LABA*</b>			
Formoterol <sup>98-111</sup>	10 minutes	↑ Lung functions	Tremor
Salmeterol <sup>97,112</sup>	3 hours	↑ Quality of life ↓ Symptoms	Headache Cough Cardiac toxicity
<b>LABA + ICS*</b>			
Formoterol/budesonide <sup>98,100,103,113</sup>	As above for LABAs	↑ Lung functions ↑ Quality of life ↓ Symptoms ↓ Exacerbations	Tremor Headache Cough Cardiac toxicity Pneumonia
Salmeterol/fluticasone <sup>97,104,106,108,109,114</sup>			
<b>LAMA</b>			
Tiotropium <sup>97,107,110,112,113,115-120</sup>	3-4 hours	↑ Lung functions ↑ Quality of life ↓ Symptoms ↓ Exacerbations	Dry mouth Hoarseness Cardiac toxicity Pneumonia
<b>LABA + LAMA</b>			
Formoterol + tiotropium <sup>121-124,125</sup>	As above	As above	As above
Salmeterol + tiotropium <sup>126,127</sup>	As above	As above	As above
<b>LABA + LAMA + ICS<sup>126,128</sup></b>	As above	As above	As above
<b>Ultra-LABA</b>			
Indacaterol <sup>99,129,130</sup>	5 minutes	↑ Lung functions ↑ Quality of life ↓ Symptoms ↓ Exacerbations	Cough Nasopharyngitis Worsening COPD
<b>Experimental agents</b>			
Various Ultra-LABAs <sup>129</sup>	To be established	See Ultra-LABA	See Ultra-LABA
New LAMAs <sup>127</sup>		See LAMA	See LAMA
Glycopyrronium	To be established		
GSK-573719			
BEA-2180BR			
Acclidinium bromide			
Darotroprum bromide			

**Notes:** \*Randomized placebo-controlled trials of duration >24 weeks have been selected, as this duration allows the best assessment of treatment effects. Data from Decramer et al.<sup>111</sup>

**Abbreviations:** COPD, chronic obstructive pulmonary disease; LABA, long-acting beta agonist; ICS, inhaled corticosteroid; LAMA, long-acting muscarinic agent.

## Combining LABAs with inhibitors of PDE4

Clearly, pharmacological strategies that target PDEs (the enzymes that inhibit the hydrolysis of cyclic nucleotides) are attractive for potentiating the anti-inflammatory actions of cAMP-elevating agents, including  $\beta_2$ -agonists. One such agent, roflumilast, is a selective inhibitor of type 4 PDE, the predominant type in human neutrophils, and which is also present in other types of immune and inflammatory cells. This agent has been approved for clinical application in COPD, while several other such agents are in the pipeline.<sup>83,89,90</sup> Interestingly, roflumilast has been reported to enhance the potentiating action of formoterol on CS-mediated gene transcription in human airway epithelial cells in vitro.<sup>91</sup> However, drug intolerance due to gastrointestinal and central nervous system side-effects may be limiting, while therapeutic

efficacy has also been questioned by some.<sup>92</sup> To counter these problems, inhaled formulations of roflumilast, for use in combination with ICS/LABAs, are currently under development, as are other novel PDE4 inhibitors, for both oral and inhaled administration, with apparently improved windows of therapeutic efficacy.<sup>89</sup> Moreover, given that PDE4 is not the only isoform present in immune and inflammatory cells (with one or both of PDE3 and PDE7 also being present, both of which hydrolyze cAMP), there is an increasing realization that simultaneous targeting of several PDE isoforms may be a more effective strategy.<sup>90</sup>

In this respect, it is noteworthy that montelukast, an antagonist of type 1 cysteinyl leukotriene receptors (CysLT1Rs), which is used primarily in the therapy of exercise-induced asthma (and as add-on therapy to ICS/LABAs in severe asthma, as well as in some cases of

moderate-to-severe COPD), has also been reported to possess secondary, nonspecific PDE-inhibitory activity.<sup>93</sup> Exposure of human neutrophils to montelukast alone, but especially in combination with formoterol, was found to suppress the proinflammatory activities of these cells by a CysLT1R-independent, apparently cAMP-mediated, mechanism.<sup>43,93,94</sup> Interestingly, montelukast has recently been reported to prevent salbutamol-mediated  $\beta$ 2AR desensitization in airway smooth muscle cells, via direct inhibition of PDE4D5.<sup>86</sup>

Other agents which possess this combination of CysLT1R-antagonism and nonspecific PDE inhibitory activity include ibudilast (also known as KC-404, AV-411 and MN-166) and CR3465. Ibudilast is currently undergoing clinical evaluation as a treatment for chronic neuropathic pain and multiple sclerosis, while CR3465 is under early assessment as a potential therapy for bronchial asthma and interstitial cystitis.<sup>93</sup> Clearly, all three of these agents have the potential to augment the anti-inflammatory activities of LABAs in the clinical setting, with supporting evidence, albeit limited, in the case of montelukast.<sup>95,96</sup>

## Combinations of anticholinergics with $\beta$ 2-agonists

Anticholinergic agents induce bronchodilation by antagonism of muscarinic receptors on smooth muscle cells. The short-acting anti-muscarinic agent, ipratropium bromide provides short-term symptomatic relief for patients with airflow obstruction, while longer-acting muscarinic agents (LAMAs), such as tiotropium, maintain bronchodilatation for a sustained period.<sup>97</sup> A number of new-generation LAMAs are currently being developed, as shown in Table 5. Because LAMAs and LABAs act via separate mechanisms, the combination of these two agents may induce greater bronchodilatation than either agent alone. Clinical trials support the safety and efficacy of LABA/LAMA combinations for the management of COPD.<sup>97</sup>

Triple therapy with a LABA/LAMA/ICS combination has been used for patients with severe COPD who do not respond adequately to other therapeutic regimens. There does appear to be some additional benefit to be gained by following this strategy of triple therapy for selected patients.<sup>97</sup>

In addition to its bronchodilating actions, tiotropium also possesses anti-inflammatory activities, albeit by mechanisms that remain to be conclusively established.<sup>130</sup> Given their potential complementary bronchodilatory and anti-inflammatory actions, several recent studies have addressed the therapeutic potential of combinations of tiotropium with either LABAs or ultra-LABAs only, or with ICS/LABAs, in the clinical setting of COPD. Improvements in several

clinical and inflammatory indices were observed.<sup>131–134</sup> Likewise, combining tiotropium as add-on therapy to ICS/LABAs in patients with severe, uncontrolled asthma was found to improve lung function over 24 hours.<sup>135</sup> Although promising, firm recommendations on the clinical utility of combining tiotropium with either LABAs/ultra-LABAs, or as a single bifunctional molecule,<sup>136</sup> with or without CS, in the therapy of COPD or asthma cannot be made on the basis of the limited data currently available.<sup>131–132</sup> This needs to be reinforced, not only by additional clinical trial data, but also by the acquisition of compelling mechanistic data on the possible additive/synergistic anti-inflammatory interactions of tiotropium with  $\beta$ 2-agonists and CS.

## Conclusion

LABAs are used in the management of obstructive airways diseases, primarily for their very effective bronchodilator activity.<sup>1</sup> They are an established therapy for COPD, often as single agents, and are considered to be safe and effective in this condition.<sup>9,11,12</sup> However, since their introduction, controversy has surrounded the use of LABAs in asthma with concerns that their use as monotherapy is associated with adverse outcomes, including an increase in asthma exacerbations and mortality.<sup>137</sup> Accordingly, they are always used in combination with a conventional anti-inflammatory agent, most commonly ICS.<sup>22,26</sup> However, the improved control of asthma, together with reduced airway responsiveness to allergen challenge, achieved by combining LABAs with an ICS (as opposed to simply increasing the dose of the ICS) has been interpreted by some as evidence for anti-inflammatory activity of  $\beta$ 2-agonists.<sup>137</sup> A better understanding of the mechanisms which both underpin and counteract the anti-inflammatory potential of  $\beta$ 2-agonists may facilitate the design of pharmacological strategies to optimize the clinical benefit of these agents.

## Disclosure

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