

Effect of β -agonists on inflammatory cells

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Although the major action of racemic β_2 -agonists on the airways is relaxation of airway smooth muscle, these drugs have several other effects mediated through β_2 -adrenergic receptors expressed on other cell types. These additional actions of β_2 -agonists may contribute to the efficacy of β_2 -agonists in relieving asthma symptoms. β_2 -agonists inhibit plasma exudation in the airways by acting on β_2 -receptors on postcapillary venule cells. They inhibit the secretion of bronchoconstrictor mediators from airway mast cells and inhibit effects on release of mediators from eosinophils, macrophages, T-lymphocytes, and neutrophils. In addition, β_2 -agonists may have an inhibitory effect on the release of neuropeptides from sensory nerves. The effect of β_2 -agonists on mediator release from structural cells in the airways such as epithelial cells is uncertain. Despite all of these inhibitory effects on inflammatory cells *in vitro*, β_2 -agonists do not appear to reduce the chronic inflammation of asthma. Desensitization is more readily seen in inflammatory cells than in airway smooth muscle cells and may account for this discrepancy. Corticosteroids may increase expression of β_2 -receptors in inflammatory cells to overcome the desensitization in response to chronic β_2 -agonist exposure. (*J Allergy Clin Immunol* 1999;104:S10-17.)

Key words: β_2 -adrenergic receptors, mast cell, eosinophil, T-lymphocyte, neutrophil, plasma exudation, sensory nerve

β_2 -agonists improve airway function largely by a direct relaxant effect of airway smooth muscle. However, β_2 -adrenergic receptors are also expressed on many other cell types in the airways, including inflammatory cells and cells that take part in the inflammatory response in asthma (Fig 1).¹ Whether the effects of inhaled β_2 -agonists on these other cells contribute to their beneficial effects in asthma is uncertain, however. Although several inhibitory effects of β_2 -agonists on inflammatory cells have been described *in vitro*, these are often not reflected by similar effects *in vivo*, possibly because of the rapid desensitization of β_2 -receptors on these cells.

There is a high level of β -receptor expression in animal and human lung, with a high density of receptors detected on radioligand binding and of messenger RNA (mRNA) by Northern blotting.^{2,3} Autoradiographic mapping techniques with radiolabeled antagonists has demonstrated the widespread distribution of β_2 -receptors in animal and human lungs.⁴⁻⁷ Studies with [³H]formoterol have demonstrated that high-affinity β_2 -receptors have a distribution similar to that in antagonist bind-

Abbreviations used

AP-1:	Activator protein-1
β -ARK:	β -adrenergic receptor kinase
CREB:	cyclic AMP response element binding protein
fMLP:	formyl-methionine-leucine-phenylalanine
NANC:	nonadrenergic, noncholinergic
RT-PCR:	reverse-transcription polymerase chain reaction

ing sites.⁸ *In situ* hybridization has demonstrated a similar localization of β_2 -receptor subtype mRNA.^{2,9-11} However, there are discrepancies between the relative density of mRNA and of β_2 -receptors in certain cells. Thus in airway smooth muscle there is a very high density of β -receptor mRNA, whereas the density of the β -receptors is relatively low; this suggests either that the rate of receptor synthesis is high, and there is a rapid turnover of receptors, or that the stability of mRNA is high. This may explain why it is difficult to downregulate β -receptors in airway smooth muscle and therefore to demonstrate desensitization to the bronchodilator action of β_2 -agonists. By contrast, in the alveolar walls there is a low level of mRNA but a very high receptor density, which may indicate a low receptor turnover, and this would be consistent with the fact that downregulation is readily produced in lung parenchyma.

β_1 -Receptor mRNA is also detected in animal and human lung tissue, although it is less abundant than β_2 -receptor mRNA.¹² β_1 -Receptor mRNA is localized to submucosal glands and alveolar walls, as predicted by autoradiographic receptor mapping.^{6,12} β_3 -Receptor mRNA has not been detected in human or animal lung by Northern blotting or *in situ* hybridization^{12,13} nor by the more sensitive technique of reverse transcription polymerase chain reaction (RT-PCR).¹⁴

PLASMA EXUDATION

Exudation of plasma from postcapillary venules is an important component of inflammation. β_2 -receptors are present on postcapillary venular endothelial cells, and racemic β_2 -agonists inhibit plasma exudation by preventing separation of endothelial cells in postcapillary venules.¹⁵ In this way β_2 -agonists may exert anti-inflammatory and anti-edema effects in the airways. In addition, the long-acting β_2 -agonist formoterol reduces the adhesion of neutrophils and eosinophils to venular endothelial cells and thus inhibits the trafficking of granulocytes into the airway wall.¹⁶ Although intravenously administered β -agonists are ineffective in inhibiting plasma exudation in guinea pigs,¹⁷ they are effective in inhibiting the leakage induced by inhaled mediators

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when given by the aerosol route, indicating that high local concentrations may be useful in inhibiting exudation of plasma from postcapillary venules.¹⁸⁻²¹ Whether these effects of inhaled β -agonists are relevant to their anti-asthma actions is not yet certain because studies of plasma exudation in the lower airways is difficult to measure in human beings. Terbutaline applied topically to the nose significantly reduces albumin concentrations in nasal lavage fluid after allergen challenge, suggesting an inhibitory effect on plasma exudation, although this could also be explained by an inhibitory effect on mast cell mediator release.²²

MAST CELLS

Racemic β -agonists inhibit the release of histamine from chopped human lung and dispersed human lung mast cells through β_2 -receptors, but this effect is often variable between preparations.²³⁻²⁶ Both short-acting and long-acting β_2 -agonists are effective in vitro, and the release of histamine and cysteinyl-leukotrienes is inhibited.^{27,28} The inhibitory effect of β_2 -agonists is mediated by a sustained increase in cAMP.²⁹ In some human mast cell preparations albuterol acts as a partial agonist, having less effect than the full agonist isoproterenol, and in these preparations there is evidence for receptor reserve, whereas in other preparations there appears to be no receptor reserve.²⁶ The reasons for these differences are not yet understood, but it may indicate that there are different effects among patients.

Inhaled albuterol inhibits the increase in plasma histamine induced by allergen exposure in asthmatic patients,³⁰ but there are some doubts about the interpretation of plasma histamine measurements. Urinary LTE₄ excretion may be a more accurate reflection of airway mediator release after allergen exposure, but the effects of inhaled β -agonists are relatively small and transient.³¹ Functional evidence suggests that inhaled β -agonists may have an effect on mast cells in vivo because a nebulized β -agonist has a significantly greater effect on AMP-induced bronchoconstriction than on histamine- or methacholine-induced bronchoconstriction.^{32,33} This increased protective effect is also seen after the normal therapeutic dose of β -agonist from a metered-dose inhaler.³³ The increased protection against AMP challenge compared with the directly acting constrictors may reflect an additional effect on airway mast cells because AMP-induced bronchoconstriction in asthmatic subjects is reduced by an antihistamine³⁴ and adenosine-induced constriction of asthmatic bronchi in vitro is inhibited by histamine and leukotriene antagonists.³⁵ Salmeterol does not have an additional protective effect against AMP challenge compared with histamine challenge, suggesting that this partial agonist does not have a mast cell stabilizing effect in vivo.³⁶

EOSINOPHILS

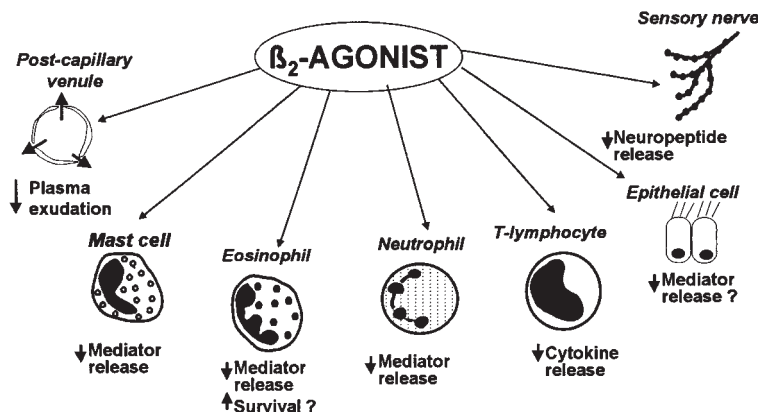
Radioligand binding studies demonstrate the presence of β_2 -receptors on human and guinea pig eosinophils,

although the density is relatively low.³⁷ β_2 -Agonists have an inhibitory effect on the oxidative burst and the release of thromboxane and LTC₄.³⁷⁻⁴⁰ R-Albuterol has an inhibitory effect similar to RS-albuterol, and S-albuterol is inactive.⁴¹ In human eosinophils, RS- and R-albuterol inhibit IL-5-induced superoxide anion release, whereas S-albuterol significantly enhances superoxide production.⁴² No effects on eosinophil degranulation (measured by eosinophil-derived neurotoxin) are seen. The inhibitory effect of formoterol and procaterol is greater than for the albuterol, possibly because the former are fuller agonists. Salmeterol, which has even less agonist activity than albuterol, even acts as a competitive antagonist in guinea pig and human eosinophils.^{43,44} β -Agonists also have a weak inhibitory effect on immunoglobulin-induced degranulation of human eosinophils, but only in the presence of a phosphodiesterase inhibitor,⁴⁵ although fMLP-induced degranulation is more potently inhibited by albuterol.³⁹ The nonselective antagonist propranolol has a low affinity, however, indicating that the β -receptors on eosinophils may be atypical (β_3 -receptors?). Longer incubation with β -agonists results in a loss of inhibitory effect, indicating the rapid development of tachyphylaxis.³⁷ Recent studies have demonstrated that although β_2 -agonists do not directly affect eosinophil survival, they block the inhibitory effects of corticosteroids on survival and may thus perpetuate eosinophil survival in the airways.⁴⁶ Although regular treatment with albuterol has no effect on the number of eosinophils in induced sputum of asthmatic patients,⁴⁷ there is an increased number of activated eosinophils in induced sputum after allergen challenge.⁴⁸ Albuterol pretreatment does not inhibit the influx of eosinophils induced by segmental allergen challenge.⁴⁹ In contrast to these studies, animal studies indicate that salmeterol has an inhibitory effect on eosinophil recruitment into the skin.⁵⁰

MACROPHAGES

Although β -receptors have been demonstrated on human alveolar macrophages by radioligand binding and racemic β_2 -agonists increase cAMP concentrations in isolated macrophages in vitro,^{51,52} β_2 -agonists do not appear to have a significant inhibitory effect on macrophage mediator secretion in vitro⁵³ and no effect on phagocytosis.⁵⁴ It is possible that β -agonists may have some other action on macrophage function, however. There is some evidence that alveolar macrophages from patients with asthma have a reduced cAMP response to β -agonists, although this may involve a postreceptor mechanism.⁵⁵ β_2 -Receptors on alveolar macrophages are markedly desensitized by oral and inhaled β_2 -agonist treatment,^{56,57} and this is not prevented by inhaled corticosteroid therapy.⁵⁸

By contrast, peripheral blood monocytes appear to respond to β -agonists, suggesting that β -adrenergic responsiveness may be lost as monocytes mature into macrophages in the lung. Thus β -agonists have an inhibitory effect on the secretion of the cytokines TNF- α and GM-CSF from human peripheral blood monocytes.^{58,59}

FIG 1. Anti-inflammatory effects of β_2 -agonists.

LYMPHOCYTES

Peripheral blood lymphocytes express β_2 -receptors, and β -agonists increase cAMP concentrations^{60,61} and are expressed equally on both B- and T-lymphocytes.⁶² Helper (CD4⁺) T cells are reported to have a lower density of β_2 -receptors than suppressor (CD8⁺) T cells.⁶³ β_2 -Agonists inhibit synthesis of IL-2 and IL-2 receptor expression^{64,65} and the release of GM-CSF, IFN- γ , and IL-3 but have no effect on expression of IL-4.⁶⁶ The β -receptor on lymphocytes is rapidly tachyphylactic, however, and therefore any effect on lymphocyte function may not be relevant in vivo.⁶⁰ There is some evidence that β -receptor function in circulating lymphocytes may be impaired in asthmatic patients after allergen challenge,⁶⁷ possibly as a result of inflammatory mediator or cytokine release.

NEUTROPHILS

β_2 -Receptors have been detected on circulating neutrophils, and their stimulation results in a rise in intracellular cAMP concentration.⁶⁸ Functionally racemic β_2 -agonists have an inhibitory effect on mediator release, although relatively high concentrations are required.⁶⁹ β_2 -Agonists decrease adhesion of neutrophils to human airway epithelial cells through an increase in intracellular cAMP, resulting in reduced expression of the adhesion molecule Mac-1.⁷⁰ β -Receptors on human neutrophils are rapidly downregulated.⁶⁸ Salmeterol has inhibitory effects on the oxidative burst on neutrophils that are not blocked by propranolol and appear to be the result of a membrane-stabilizing effect.⁷¹

AIRWAY EPITHELIAL CELLS

There is a very high density of β_2 -receptors on human airway epithelial cells,^{6,72,73} and although β_2 -agonists increase ciliary beating,^{74,75} it is not certain whether other functions are affected. Airway epithelial cells are able to secrete many inflammatory mediators in asthma, including nitric oxide, cytokines (IL-6, TNF- α , GM-CSF, IL-11), chemokines (IL-8, MIP-1 α , RANTES, eotaxin), and growth factors.⁷⁶ It is not certain whether β_2 -agonists affect the secretion of these mediators, how-

ever. Formoterol has been shown to inhibit the expression of IFN- γ -induced ICAM-1 in human airway epithelial cells.⁷⁷ β_2 -Receptors on human airway epithelial cells are rapidly desensitized in vitro⁷⁸ and in vivo.⁵⁷

NEUROGENIC INFLAMMATION

β -Agonists may also have effects on sensory nerves. β -Agonists inhibit excitatory nonadrenergic noncholinergic (NANC) bronchoconstrictor responses in guinea pig bronchi in vitro at concentrations that do not block equivalent tachykinin-induced responses.^{79,80} This modulatory effect is mediated by β_2 -receptors on capsaicin-sensitive sensory nerves in the airways.⁸⁰ There is also evidence for an inhibitory effect of β_3 -receptor agonists, suggesting the presence of modulatory β_3 -receptors.^{81,82} Whether β -receptors modulate sensory nerves in human airways is not certain. Some evidence that suggests that β_2 -receptors may be modulatory is provided by the inhibitory action of albuterol on cough responses.⁸³ However, an inhaled β_2 -agonist, even in a high dose, has no additional protective effect on inhaled metabisulfite (which is believed to act on airway sensory nerves) than on methacholine challenge.⁸⁴

EFFECTS ON AIRWAY INFLAMMATION

The effect of β -agonists on airway inflammation is controversial, partly because of the differing understanding of what inflammation involves. Because β -agonists are clearly capable of inhibiting plasma exudation in the airways in response to inflammatory mediators they must be considered to be anti-inflammatory agents. β -Agonists also inhibit the release of histamine and leukotrienes from mast cells, which participate in the acute allergic inflammatory response. The fact that clinically used doses of terbutaline have a greater inhibitory effect on AMP-induced bronchoconstriction than on methacholine-induced bronchoconstriction provides indirect evidence for a mast cell-stabilizing effect,³³ although the long-acting β_2 -agonist salmeterol has little effect on the urinary excretion of LTE₄ after allergen

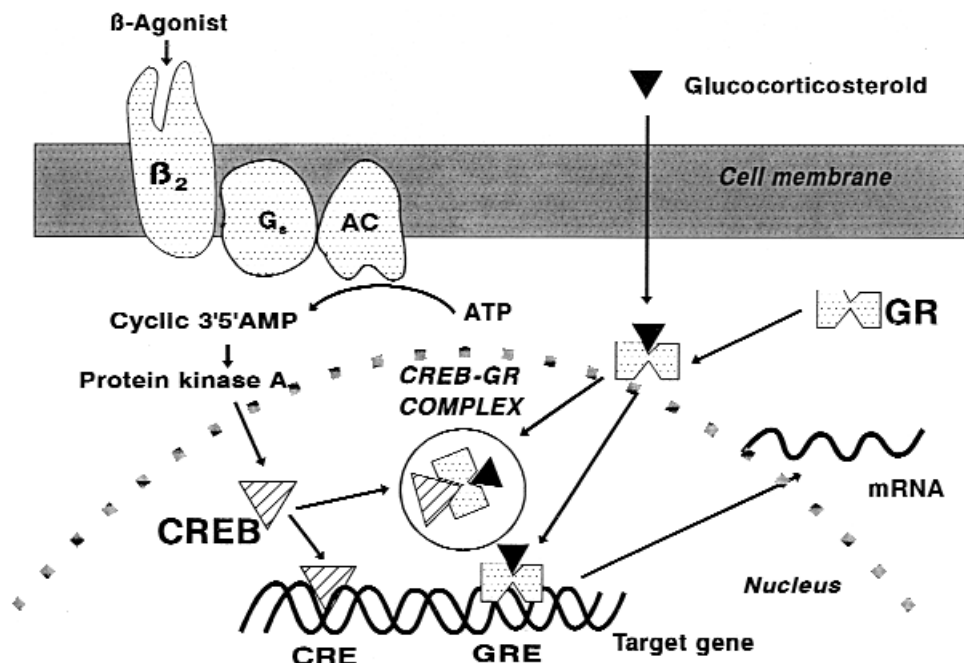


FIG 2. Interaction between β_2 -agonists and glucocorticoids. High concentrations of β_2 -agonists activate the transcription factor CREB (cyclic AMP response element binding protein), which interacts with activated glucocorticoid receptors (GR), thus potentially interfering with the anti-inflammatory actions of corticosteroids.

challenge, indicating a small effect.³¹ β -Agonists have little or no effect on the chronic inflammatory response that underlies airway hyperresponsiveness and chronic asthma. This is most clearly demonstrated by biopsy studies that show that regular treatment with racemic β -agonists, including salmeterol, fail to resolve the inflammatory process, as judged by the presence of activated mast cells, eosinophils, and macrophages.⁸⁵⁻⁸⁸

DESENSITIZATION

Desensitization or loss of responsiveness to racemic β_2 -agonists after repeated dosing may occur rapidly (tachyphylaxis) or over a long-term period (tolerance). Because β_2 -agonists, and particularly long-acting inhaled β_2 -agonists, are used repeatedly in asthma treatment, this is an important clinical issue. Although there is little evidence for loss of bronchodilator responses to inhaled β_2 -agonists, there is some loss of their protective effect against bronchoconstrictor challenges. Inhaled β_2 -agonists have a greater protective effect against AMP-induced bronchoconstriction than against methacholine-induced constriction, suggesting that there is some extra protective effect on mast cell mediator release, as discussed.³³ After 1 week of regular inhaled β_2 -agonist (500 μ g terbutaline qid) in mildly asthmatic subjects, there is no evidence for tachyphylaxis of the bronchodilator response to terbutaline, a slight reduction in protection against methacholine, and a marked reduction in protective effect against AMP.³² This indicates preferential desensitization of the mast cell-inhibitory effect of β -ago-

nists. A similar loss of protection by albuterol on allergen challenge has also been reported,⁸⁹ suggesting that desensitization of mast cells occurs more readily than in airway smooth muscle cells. Desensitization is readily produced in human mast cells in vitro,⁹⁰ although there is variability between different preparations that appears to correspond to differences in receptor reserve.²⁶ After regular treatment with the long-acting partial agonist salmeterol, there is no loss of protective effect against AMP challenge, whereas there is a reduction of protection against methacholine, suggesting that salmeterol may not desensitize β_2 -receptors on mast cells.⁹¹ Similarly, eosinophils and T-lymphocytes are also rapidly desensitized, so that any anti-inflammatory effect of β_2 -agonists may be lost. In animal studies the inhibitory effect of β_2 -agonists on plasma exudation is also desensitized, although this response is not completely lost.⁹²

Several molecular mechanisms are involved in desensitization of β -receptors.⁹³ Short-term desensitization involves phosphorylation of the receptor, which results in uncoupling from the stimulatory G-protein G_s by two kinases, protein kinase A and β -adrenergic receptor kinase (β ARK)/G-protein receptor kinase-2 (GRK2).⁹⁴ Longer-term mechanisms include downregulation of surface receptor number, a process that involves internalization of the receptor and its subsequent degradation. The uncoupled internalized receptor may return to the cell membrane once the β -agonist is removed (resensitization). More long-term effects of β -agonists involve changes in β -receptor synthesis with increased degradation of β_2 -receptor mRNA that may involve a specific

TABLE I. β -Adrenoceptors on inflammatory cells

Cell type	Subtype	Function
Mast cells	β_2	Reduced mediator release
Macrophages	β_2	No effect?
Eosinophils	β_2	Reduced mediator release Increased survival?
T-lymphocytes	β_2	Reduced cytokine release
Epithelium	β_2	Increased ion transport Secretion of inhibitory factor? Increased ciliary beating Inhibition of mediator release?
Sensory nerves	β_2/β_3	Reduced neuropeptide release Reduced activation?
Endothelial cells	β_2	Reduced plasma extravasation

protein that selectively binds to β_2 -receptor mRNA and is upregulated by β_2 -agonists.^{95,96} Long-term exposure to β_2 -agonists results in decreased transcription of the β_2 -receptor gene as the result of decreased activity of the transcription factor CREB (Fig 2, Table I).^{10,11} Inflammatory cells may be more susceptible to desensitization for several reasons. First, they may have a low rate of transcription, so that resynthesis of β_2 -receptors is slow. They may also have less receptor reserve, so that when receptors are lost or uncoupled there is a concomitant loss of functional response. Finally, there may be increased expression of β ARK, so that desensitization is greater. Thus mast cells have a much greater expression of β ARK than does airway smooth muscle.⁹⁷

Glucocorticoids increase the transcription of the β_2 -receptor gene in rat and human lung and may prevent desensitization.^{98,99} Corticosteroids also prevent desensitization of human lung mast cells in vitro.¹⁰⁰ In asthmatic patients, desensitization to the protective effects of terbutaline against AMP challenge were not reversed by a single high dose of an inhaled corticosteroid.¹⁰¹

Inflammation itself may have effects on β_2 -receptor desensitization, and this may be more marked on inflammatory cells than on airway smooth muscle cells. Protein kinase C is capable of phosphorylating both β -receptors and the α -subunit of G_s , resulting in either downregulation or uncoupling of the receptor.¹⁰² This mechanism may be contributory to the impaired β -adrenergic responsiveness in circulating lymphocytes after allergen exposure in asthmatic patients.¹⁰² Whereas in more controlled asthma it is unlikely that β -receptors are dysfunctional, it is possible that in the inflammatory milieu airway and inflammatory cell β -receptors may be more susceptible to downregulation. Direct measurements of airway β -receptors have recently been made after an inhaled β_2 -agonist and demonstrate substantial downregulation and uncoupling of β_2 -receptors in epithelial cells and macrophages in normal subjects.⁵⁷ Desensitization of β_2 -receptors on airway epithelial cells is also seen in asthmatic patients after treatment with an inhaled β_2 -agonist.¹⁰³ After segmental allergen challenge in asthmatic patients there is a small but insignificant reduction in β -agonist-induced cAMP production.¹⁰⁴

INTERACTION BETWEEN β_2 -RECEPTORS AND CORTICOSTEROIDS

High concentrations of β_2 -agonists activate the transcription factor CREB (cAMP response element binding protein), and there are interactions that have recently been recognized between CREB and other transcription factors, such as activator protein-1 (AP-1) and glucocorticoid receptors.^{105,106} This has potentially important clinical implications because activation of CREB by exposure to β_2 -agonists may directly interact with activated glucocorticoid receptors and therefore inhibit the anti-inflammatory actions of steroids.¹⁰⁷ This blocking effect of corticosteroids has been demonstrated in rat lung¹⁰⁸ and in human T-lymphocytes,¹⁰⁹ but this has not been seen in human monocytes.⁵⁹ The final functional outcome will depend on the net balance between the number of glucocorticoid receptors, the amount of CREB, the density of β_2 -receptors, and the presence of other proinflammatory cytokines and will therefore differ from cell to cell.

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