

Molecular mechanisms in allergy and clinical immunology

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Molecular mechanisms of β_2 -adrenergic receptor function, response, and regulation

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The human β_2 -adrenoceptor is a member of the 7-transmembrane family of receptors, encoded by a gene on chromosome 5, and widely distributed in the respiratory tract. Intracellular signaling after β_2 -adrenoceptor activation is largely affected through cyclic adenosine monophosphate and protein kinase A. Differences in the mechanism of interaction of short- and long-acting β_2 -agonists and the β_2 -receptor are reflected in the kinetics of airway smooth muscle relaxation and the onset and duration of bronchodilation in asthmatic patients. β -Adrenoceptor desensitization associated with prolonged β_2 -agonist activation differs depending on the cell type and is reflected in different profiles of clinical tolerance to chronic β_2 -agonist therapy. A number of genetic polymorphisms of the β_2 -receptor have been described that appear to alter the behavior of the receptor, including the response to β_2 -agonists. The synergy between the β_2 -receptor and the glucocorticoid receptor functions has implications for the combined use of β_2 -agonists and corticosteroids in the treatment of respiratory disease. (*J Allergy Clin Immunol* 2006;117:18-24.)

Key words: β_2 -Adrenergic receptor, β_2 -agonists, desensitization, polymorphisms

β_2 -ADRENOCEPTOR STRUCTURE AND DISTRIBUTION

The human β -adrenoceptor gene is situated on the long arm of chromosome 5 and codes for an intronless gene product of approximately 1200 base pairs.¹ The β -adrenoceptor is comprised of 413 amino acid residues of approximately 46,500 daltons. β -Adrenoceptors have been subdivided into at least 3 distinct groups, β_1 , β_2 , and β_3 , which are classically identified in cardiac, airway smooth

Abbreviations used

cAMP: Cyclic adenosine monophosphate
GR: Glucocorticoid receptor
GTP: Guanosine triphosphate
MAPK: Mitogen-activated protein kinase
PET: Positron emission tomography
PKA: Protein kinase A

muscle, and adipose tissue, respectively. There is a 65% to 70% homology between β_1 - β_3 and β_2 -receptors. This review focuses on the β_2 -adrenergic receptor with regard to its function and response to β_2 -agonists in the treatment of respiratory disease. The clinical implications of β_2 -receptor desensitization after chronic therapy and the potential therapeutic effect of genetic forms of the receptor are addressed.

Like all G protein-coupled receptors, the β_2 -receptor has 7 transmembrane-spanning α -helices.² There are 3 extracellular loops, with one being the amino-terminus, and 3 intracellular loops, with a carboxy-terminus (Fig 1). The receptor is N-glycosylated at amino acids 6, 15, and 187; these are important for insertion into the cell membrane and for agonist-induced receptor trafficking.³ At amino acid position 341, the cysteine of the human β_2 -receptor is palmitoylated, acting to anchor the carboxy-terminus to the membrane.⁴ The region between the seventh transmembrane-spanning domain and the palmitoylated cysteine is also an α -helix, sometimes denoted as the fourth intracellular loop.

Autoradiographic studies of human lung have suggested that β_2 -adrenoceptors are widely distributed, occurring not only in airway smooth muscle (30-40,000 per cell) but also on other cells in the lung, such as epithelial and endothelial cells, type II cells, and mast cells. However, the receptor density on the latter cells is substantially lower than in smooth muscle. Radioligand binding studies on lobectomy specimens have shown β_2 -receptor density to increase through the respiratory tract, with high levels in the central lung and the alveolar region. Computed tomographic scanning has confirmed this β_2 -receptor distribution in the airways.

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Positron emission tomography (PET) has now made possible the noninvasive quantification of β_2 -receptors *in vivo* and correlation with clinical response. Serial measurements have shown pulmonary β_2 -receptor density to be higher than in cardiac tissue. There was, however, no difference between healthy subjects and asthmatic patients, but an inverse relationship was reported between FEV₁ (percent predicted) and lung β_2 -receptor density.⁵ The number of mononuclear leukocyte β_2 -adrenergic receptors at rest and after exercise challenge was also determined in asthmatic children.⁶ A significant negative relationship was found between the number of receptors and maximum percentage decrease in FEV₁, and a significant positive relationship was found between the number of receptors and maximum volume of oxygen use per kilogram per minute. These results suggested that a reduced number of β_2 -receptors might contribute to the pathogenesis of exercise-induced asthma in children.

β_2 -RECEPTOR ACTIVATION

There is now good evidence that β_2 -adrenoceptors oscillate between 2 forms, activated and inactivated, and that under resting conditions, these 2 forms are in equilibrium, with the inactivated state being predominant.² The β_2 -receptor is in the activated form when it is associated with the α -subunit of the Gs protein, together with a molecule of guanosine triphosphate (GTP). The replacement of the GTP by guanosine diphosphate dramatically reduces the affinity of the α -subunit for the receptor, causing dissociation and inducing the receptor to return to its low-energy inactivated form. It is probable that β_2 -agonists have their effects not through inducing a conformational change in the receptor but rather by binding to and temporarily stabilizing receptors in their activated state (ie, bound to Gs-GTP).⁷

Full agonists (see later discussion) might move the equilibrium completely in the direction of the activated form. Partial agonists either stabilize a different conformation of the receptor compared with full agonists or stabilize the same active conformation as a full agonist, although less frequently. Inverse agonists actually lower basal adenylate cyclase activity because they preferentially bind to the receptor in the inactive state.

The corollary is that β -antagonists bind with high affinity to the low-energy inactivated form of the β -adrenoceptor and thus shift the equilibrium away from the activated form. If this is the case, β -antagonists should not be considered as competing directly for the same receptor state but instead as binding to a different form of the β -adrenoceptor protein and moving the equilibrium in opposite directions. Although this would result in a competitive interaction, it is not competitive in the sense that β -agonist and β -antagonist molecules simultaneously compete for the same region of the β -adrenoceptor protein.

The oscillation of the β_2 -receptor between various conformations brings into question whether there is a single active state. Traditionally, this was defined as coupled to

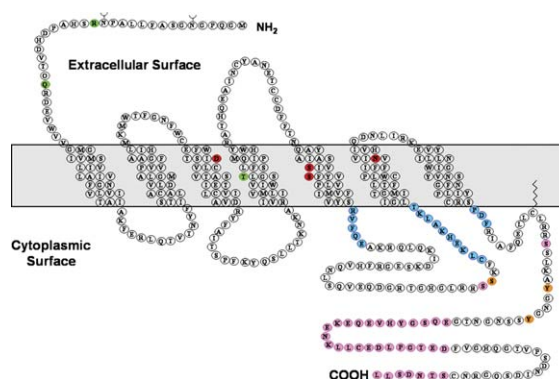


FIG 1. The structure of the human β_2 -receptor. The sites of polymorphic mutations of the receptor are shown in green. The 4 amino acid residues involved in β_2 -agonist binding are shown in red. Regions or specific domains involved in G protein coupling (blue), desensitization (pink), and downregulation (orange) are indicated.

Gs, leading to cyclic adenosine monophosphate (cAMP) production. However, it is now clear that multiple signals are promoted by β_2 -receptors and that the optimal conformation for one effector signal might not be the same as for another. For example, the β_2 -receptor activates the sodium-hydrogen exchanger regulatory factor through direct interaction with the carboxy-terminus domain, with no G protein involvement.⁸ It is conceivable that synthetic β_2 -agonists might be developed in the future that preferentially confine the receptor to a certain conformation of clinical importance (eg, coupling to Gs) while having a less stabilizing effect on unwanted conformations, such as those leading to phosphorylation and desensitization.

β_2 -RECEPTOR SIGNALING PATHWAYS

It has been the accepted dogma since the 1960s that β_2 -receptor activation is mediated by increased intracellular cAMP levels.⁹ This is the result of stimulation of adenylate cyclase, which catalyzes the conversion of adenosine triphosphate into cAMP. The coupling of the β_2 -receptor to adenylate cyclase is affected through a trimeric Gs protein,¹⁰ consisting of an α -subunit (which stimulates adenylate cyclase) and $\beta\gamma$ -subunits (which transduce other signals). cAMP levels are then regulated through the activity of phosphodiesterase isozymes/isoforms, which degrade it to 5'-AMP.

The mechanism by which cAMP induces, for example, airway smooth muscle cell relaxation is not fully understood, but it is believed that it catalyzes the activation of protein kinase A (PKA), which in turn phosphorylates key regulatory proteins involved in the control of muscle tone.¹⁰ cAMP also results in inhibition of calcium ion (Ca^{2+}) release from intracellular stores, reduction of membrane Ca^{2+} entry, and sequestration of intracellular Ca^{2+} , leading to relaxation of the airway smooth muscle. However, it has been suggested recently that some of the relaxant response to β_2 -agonists might be mediated through cAMP-independent mechanisms, involving direct

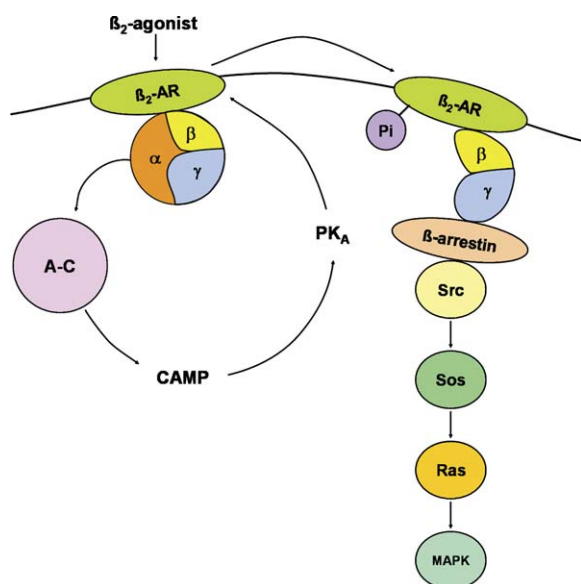


FIG 2. Intracellular signaling pathways for the β_2 -receptor. Activation of the β_2 -receptor by β_2 -agonists causes the α -subunit of the associated Gs protein to dissociate and couple with adenylyl cyclase (A-C), leading to enhanced production of cAMP and stimulation of PKA. PKA induces phosphorylation (Pi) of the receptor protein and uncoupling of Gs. The phosphorylated receptor, with its residual $\beta\gamma$ -subunits, then couples to Gi. After the binding of β -arrestin, which acts as an assembly scaffold for Src, SOS, and RAS, the MAPK pathway is activated. β_2 -AR, β_2 -Adrenergic receptor.

interaction of Gs with potassium channels, which are present in the airway smooth muscle cell membrane.

Although most of the actions of the β_2 -receptor appear to be mediated through Gs proteins and the cAMP-dependent PKA system, β_2 -receptors can also couple to Gi proteins,¹¹ resulting in stimulation of the extracellular signal-regulation kinase and p38 mitogen-activated protein kinase (MAPK) pathways. Activation of this pathway by the β_2 -receptor requires that the receptor be phosphorylated by PKA and is mediated by $\beta\gamma$ -subunits of the G protein acting as an assembly scaffold for a number of intracellular proteins, including nonreceptor tyrosine kinase cSrc, Raf, and RAS,¹¹ culminating in the stimulation of MAPK (Fig 2). This mechanism might serve to uncouple the β_2 -receptor from Gs and thus represent a means of terminating the β_2 -agonist/receptor signal and response. In addition, there is now evidence that β_2 -receptor-activated MAPK phosphorylates the glucocorticoid receptor (GR) at a number of proline-directed serine residues in the N-terminal region of the receptor, leading to a priming event, rendering the receptor more sensitive to steroid-dependent activation.¹² β_2 -Receptor activation also increases the translocation of the GR from the cell cytosol to the nucleus,¹³ a fundamental step in the anti-inflammatory activity of corticosteroids. This is mediated through activation of a CCAAT enhancer binding protein (C/EBP α).¹³ These mechanisms provide a molecular basis for synergy between the β_2 -receptor and the GR.

Recent evidence suggests that there might be cross-talk between β_2 -receptors and other G protein-coupled

receptor systems. Under experimental conditions in which the β_2 -receptor was either ablated or overexpressed, responses to Gq-coupled contractile agonists were inhibited and enhanced, respectively.¹⁴ Intracellular inositol-1,4,5-trisphosphate production and phospholipase C levels were decreased in the absence of the β_2 -receptor and increased in the presence of high β_2 -receptor density. There is therefore an interaction between β_2 -receptors and phospholipase C, termed *antithetic regulation*,¹⁴ that might cross-regulate stimulatory and inhibitory cell responses. This is in addition to the negative cross-talk between muscarinic M_2 receptors and β_2 -receptors in airway smooth muscle, where M_2 activation leads to attenuated cAMP accumulation.¹⁵

β_2 -RECEPTOR/AGONIST INTERACTIONS

The regions of the β_2 -adrenoceptor protein important for β_2 -agonist binding and G protein coupling have been identified by using site-directed mutagenesis. The active site of the receptor with which β_2 -agonists must interact to exert their biologic effects is located approximately one third of the way (15Å) into the receptor core (Fig 1). It is generally agreed that the residues of critical importance with respect to agonist binding to the active site are aspartate residue 113 (counted from the extracellular or N-terminus end) in the third domain; 2 serine residues, 204 and 207, which are both on the fifth domain; and asparagine 293, in the sixth domain.² Thus a model has emerged for the agonist binding site of the β_2 -adrenoceptor, in which the ligand is bound within the hydrophobic core of the protein in the transmembrane helices and anchored by specific molecular interactions between amino acid residues in the receptor and functional groups on the ligand. Aspartate forms an ion pair with the amino nitrogen, whereas the 2 serine residues interact with the hydroxyl groups on the phenyl ring of the β_2 -adrenoceptor agonist molecule. The β -hydroxy group in the β_2 -agonist side chain binds to asparagine 293.²

The molecular structure of a β_2 -agonist determines the manner in which it interacts with the β_2 -adrenoceptor. Short-acting β_2 -agonists, such as albuterol, which are hydrophilic in nature, access the active site of the β_2 -adrenoceptor directly from the extracellular aqueous compartment.¹⁶ There is therefore a rapid onset of action. However, these drugs rapidly re-equilibrate, their residency time at the receptor active site is limited, and the resulting duration of action is short (4-6 hours). The long-acting β_2 -agonists have different mechanisms of action. Formoterol is moderately lipophilic, and it is taken up into the cell membrane in the form of a depot, from where it progressively leaches out to interact with the active site of the β_2 -receptor.¹⁶ The size of the depot is determined by the concentration or dose of formoterol applied. The onset of action of formoterol is somewhat delayed compared with that of albuterol, and the duration of activity is longer and concentration dependent.¹⁶ This profile has been confirmed clinically in asthmatic patients in whom

bronchodilation was observed for 8, 10, and 12 hours after doses of 6, 12, and 24 μ g, respectively.¹⁷

Salmeterol, on the other hand, is more than 10,000 times more lipophilic than albuterol.¹⁶ The molecule partitions rapidly (<1 minute) into the cell membrane and then diffuses laterally to approach the activity site of the β_2 -adrenoceptor through the membrane,¹⁶ with this process appearing to be slow (>30 minutes). The onset of action of salmeterol in airway smooth muscle is therefore slower than that of other β_2 -agonists, such as albuterol and formoterol. The mechanism of action of salmeterol involves the interaction of the side chain of the molecule with an auxiliary binding site (exosite), a domain of highly hydrophobic amino acids within the fourth domain of the β_2 -adrenoceptor. When the side chain is in association with the exosite, the molecule is prevented from dissociating from the β_2 -adrenoceptor, but the head can freely engage and disengage the active site by means of the Charnière (hinge) principle, with flexion being about the O atom in the side chain. Salmeterol appears to be inherently long acting, in that its effects are independent of dose as a result of exosite binding.¹⁶

The affinity of a ligand is a measure of the avidity of its binding to its receptor. Few β_2 -agonists have been shown to have much higher affinity than isoproterenol, and indeed, albuterol has a relatively low affinity for β_2 -adrenoceptors. In contrast, salmeterol and formoterol have 3- to 4-fold higher affinities for the β_2 -adrenoceptor than albuterol.

β_2 -Agonist potency, however, is a function not only of receptor affinity but also of efficacy. A *full* agonist will have a high efficacy, whereas a pure antagonist will have low or zero efficacy. The majority of β_2 -adrenoceptor agonists have an intermediate efficacy, and if receptor numbers permit, they will behave as full agonists, but if receptor density is too low or receptor coupling is inadequate, the β_2 -agonist might behave in a *partial* manner; that is, it will be incapable of achieving the same maximum effect as an agonist of higher efficacy, and it might even behave as an antagonist. Examples of compounds of high efficacy (*full* agonists) are isoproterenol, fenoterol, and formoterol, whereas albuterol and terbutaline are of moderate efficacy (*partial* agonists). Salmeterol has an efficacy at β_2 -adrenoceptors in airway smooth muscle lower than that of albuterol. Low efficacy in a β_2 -adrenoceptor agonist does not, however, compromise clinical performance as a bronchodilator drug but might limit its effectiveness in restoring airway caliber under conditions of acute severe bronchospasm.

Pharmacologically, a *partial* agonist will be required to occupy a higher proportion of receptors than a *full* agonist to exert its maximum effect. *Ex vivo* studies in asthmatic patients have shown salmeterol at clinical doses to occupy only 4% of the available pulmonary β_2 -receptors.¹⁶ This represents a receptor reserve of 96% and is reflected in a normal bronchodilator response to albuterol, when used as rescue therapy, in patients chronically treated with salmeterol.¹⁶

All β_2 -adrenoceptor agonists have an asymmetric center because of the presence of a β -OH group. The presence

of an asymmetric center results in the molecule existing as a pair of optical isomers (or mirror images), referred to as the R- and S-enantiomers (or [−] and [+]), in a racemic mixture (eg, R- and S-albuterol and R- and S-salmeterol). In fact, some agonists, such as fenoterol, formoterol, and procaterol, have 2 asymmetric centers, and there are 4 enantiomers, RR, SS, RS, and SR, present. It is a feature of most biologic systems that they are stereo-specific, and this is true of β_2 -agonist/receptor interactions. It is clear that the activity lies predominantly in the R-enantiomer, probably as a result of an optimal interaction between the down orientation of the β -OH group and key amino acids in the receptor. In albuterol, for example, the R-enantiomer is at least 100-fold more potent as a β_2 -agonist than the S-enantiomer,¹⁸ whereas this difference is greater than 1000-fold for the RR and SS forms of formoterol. Although there have been reports of S-albuterol having different and possibly detrimental pharmacologic properties compared with the R-form, clinical studies have suggested either no adverse effects or a small negative effect on lung function.¹⁹ In the case of salmeterol, the S-enantiomer is only 40-fold less potent than the R-form and 15-fold weaker than the racemic mixture. Interestingly, both the R- and S-enantiomers of salmeterol are long acting.¹⁸ There is no evidence of the S-isomer of salmeterol antagonizing the effects of the corresponding R-form or of the S-enantiomer having pharmacologic effects different to those of the racemic mixture.¹⁸

β_2 -RECEPTOR DESENSITIZATION

Associated with β_2 -adrenoceptor activation is the autoregulatory process of receptor desensitization. This process operates as a safety device to prevent overstimulation of receptors in the face of excessive β_2 -agonist exposure. Desensitization occurs in response to the association of the receptor with the agonist molecule. The mechanisms by which desensitization can occur consist of 3 main processes: (1) uncoupling of the receptors from adenylate cyclase, (2) internalization of uncoupled receptors, and (3) phosphorylation of internalized receptors. The extent of desensitization depends on the degree and duration of the β_2 -adrenoceptor/ β_2 -agonist response.

The principal mechanism of homologous, short-term, β_2 -agonist-promoted desensitization of the β_2 -adrenoceptor is phosphorylation of the receptor by PKA, β -adrenoceptor kinase, or other closely related G protein-coupled receptor kinases.²⁰ Phosphorylation can occur at multiple serine and threonine residues in the third intracellular loop and in the proximal cytoplasmic tail (Fig 1). Such phosphorylation ultimately results in β -arrestin binding and partial uncoupling of the agonist-occupied form of the receptor from the stimulatory Gs protein, thereby limiting receptor function. Alternatively, the scaffolding action of β -arrestins might bring other proteins, such as phosphodiesterase IV, into the receptor microenvironment. This then acts to metabolize locally generated cAMP and terminate the effects of receptor activation.

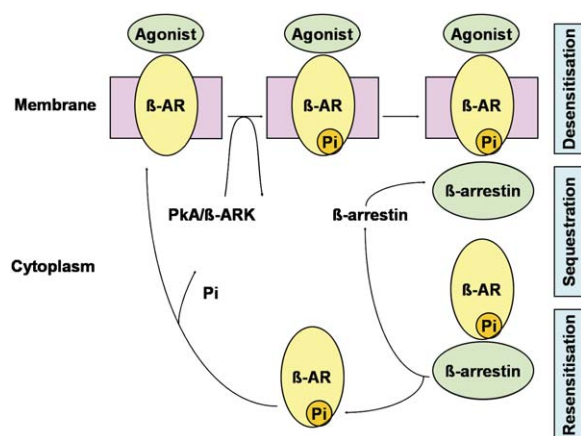


FIG 3. β_2 -Adrenoceptor trafficking. After β_2 -agonist binding, PKA, β -adrenoceptor kinase, or both phosphorylate the β_2 -receptor, leading to desensitization. β -Arrestin binding is stimulated, and the resulting β_2 -receptor/ β -arrestin complex is sequestered from the cell surface and internalized. The receptor is then dephosphorylated, β -arrestin dissociates, and the β_2 -receptor is recycled back to the membrane. β_2 -AR, β_2 -Adrenergic receptor; Pi, phosphorylation.

This simple form of desensitization is a transient process and might be reversed within minutes of removal of the agonist.

After more prolonged agonist exposure, an internalization of receptors occurs that results in some loss from the cell surface. This process, termed *sequestration*, might play a major role in short-term regulation of the receptor, because while sequestered, dephosphorylation of the receptor occurs (Fig 3).²⁰ Internalization takes longer to reverse than uncoupling, but full reversal normally occurs within hours. Receptor trafficking as part of the overall process of receptor desensitization has now been investigated in the form of kinetic analysis of internalization and recycling of the human β_2 -receptor. Under resting conditions, recycling of the receptor reflects 1-phase exponential kinetics with a half-life of 7.5 minutes.²⁰ In the presence of a β -agonist (isoproterenol), the total transit time for the receptor cycling between the cell surface and the endocytic compartment was increased.

After hours of agonist exposure, a net loss of cellular receptors occurs (denoted *downregulation*) through several mechanisms that are independent of receptor phosphorylation. Ubiquitination of the β_2 -receptor occurs (Fig 1) through an E3 ligase, activating the process of receptor degradation.²¹ Transcription at the β_2 -receptor gene level and posttranslation conversion of mRNA to protein is now required to restore the membrane complement of β_2 -receptors. It is now well appreciated that in addition to desensitization processes that negatively regulate the function of the β_2 -receptor protein itself, β_2 -receptor/agonists, acting through the cAMP pathway, also modulate β_2 -receptor gene expression. For example, exposure of cells to high concentrations of isoproterenol resulted in a significant decrease (50%) in β_2 -receptor transcripts.²² In contrast, cells treated with salmeterol showed no downregulating effect on β_2 -receptor gene

expression. These data are consistent with the hypothesis that the long-acting characteristics of salmeterol might be due, at least in part, to the ability of this agonist to maintain a population of functional β_2 -receptors through persistent increase of gene transcription, despite a prolonged low-level exposure to the agonist.

The process of desensitization can differ markedly from cell to cell and tissue to tissue. It is clear, for example, that human lymphocytes desensitize very rapidly on exposure to β_2 -adrenoceptor agonists, whereas human bronchial smooth muscle is singularly resistant. The level of β -adrenoceptor kinase and G protein-coupled receptor kinase activity in airway smooth muscle cells was only 10% to 20% of that in mast cells for example. This predicts that airway smooth muscle β_2 -receptors would undergo minimal short-term agonist-promoted desensitization compared with the β_2 -receptor expressed on mast cells. In response to isoproterenol, mast cell cAMP reached maximum levels after 90 seconds and did not further increase over time, which is indicative of receptor desensitization in this cell. In contrast, cAMP levels of airway smooth muscle cells did not plateau, increasing at a constant rate consistent with little desensitization over the study period.²³ This might explain the clinical observation that repetitive administration of β_2 -agonists to asthmatic subjects appears to result in tolerance to bronchoprotective responses against provocative stimuli²⁴ thought to be mediated largely by pulmonary mast cell β_2 -receptors but not the bronchodilatory response of β_2 -receptors expressed on bronchial smooth muscle. This type of difference might also be manifested in the well-documented decrease in the side effects associated with β_2 -adrenoceptor agonist therapy (eg, tachycardia and physiologic tremor) in asthmatic patients with the maintenance of bronchodilation despite regular treatment for prolonged periods.¹⁶

Two weeks of albuterol treatment (4 mg orally twice daily and 200 μ g 4 times daily by means of inhalation) resulted in a decrease in β_2 -receptor density, as assessed by means of PET scanning, of 22% in the lung.²⁵ This was associated with a reduction in bronchodilator response to albuterol. Corticosteroids have facilitative effects on the β_2 -adrenoceptor, increasing β_2 -receptor gene transcription and regulating both the numbers of the receptors and the coupling of the receptor to adenylate cyclase. Systemic corticosteroids have been shown to reverse β_2 -adrenoceptor downregulation in healthy subjects and asthmatic subjects who have been exposed to β_2 -agonists. It is of interest, however, that an inhaled corticosteroid does not apparently prevent tolerance to the bronchoprotective effects of a long-acting β_2 -agonist, such as formoterol or salmeterol.²⁶

POLYMORPHISMS OF THE β_2 -ADRENOCEPTOR

A number of polymorphisms of the β_2 -receptor have recently been described that alter the behavior of the receptor after agonist exposure. The main clinical interest

in these polymorphisms lies in the possibility that they might determine the extent to which the receptor downregulates in the airways and as such might modify bronchodilator responses. There are 2 genes for the β_2 -adrenoceptor, and therefore an individual can be homozygous or heterozygous for a given polymorphism.

Studies on the β_2 -adrenoceptor identified a total of 9 different polymorphisms. All of these differed from the accepted wild-type sequence by a single base change at different positions in the coding sequence of the gene. Because of redundancy in the amino acid code, a number of these polymorphisms are clinically silent. However, 4 polymorphisms, resulting from single base changes, altered the amino acid sequence of the receptor protein.²⁷ Three of these polymorphisms (Fig 1) appear to alter the functional properties of the receptor, such that the airways of individuals with these forms of the receptor might be expected to behave differently when exposed to circulating catecholamines or exogenously applied β_2 -agonists.

The initial studies²⁷ focused on amino acid 16, which can be either arginine (Arg) or glycine (Gly), and the data suggest that the Gly 16 receptor downregulates after exposure to an agonist to a much greater extent than the Arg 16 form in both transfected cell systems and in primary cultured human airway smooth muscle cells. Two clinical studies have supported the possibility that the Gly 16 form of the receptor is associated with markers of more severe asthma. Preliminary data from Dutch families with asthma suggest that Gly 16 might be associated with airway hyperreactivity.²⁸ In addition, patients with significant nocturnal worsening of their asthma were more likely to have the Gly 16 form of the receptor than asthmatic patients without nocturnal decreases in peak flow rate.²⁹ The allelic frequencies for Arg 16 and Gly 16 are 35% and 65%, respectively.

Some, but not all, studies have shown a relationship between Arg-Gly 16 polymorphisms and clinical responses to β_2 -agonists. In 10 of 14 subjects with nonresistant genotypes (Gly/Gly 16 and Gly/Arg 16), there was a significant reduction in pulmonary β_2 -adrenoceptors, as assessed by means of PET scanning, after 2 weeks of dosing with albuterol. Four subjects who were heterozygous for the glutamate 27 polymorphism were resistant to downregulation of pulmonary β_2 -receptors.³⁰ However, when compared with children who were homozygous for Gly 16, homozygotes for Arg 16 were 5.3 and heterozygotes for Arg/Gly 16 were 2.3 times more likely to respond to albuterol, respectively.³¹ Similarly, homozygous Gly 16 was significantly more prone to bronchodilator tolerance than Arg 16 after administration of formoterol (24 μ g twice daily) for 4 weeks.³²

However, other studies have shown the converse, in that patients with the resistant Arg-Arg (16) polymorphic form of the receptor exhibited a progressive reduction in bronchodilator response to regular administration of albuterol and an improvement in lung function when the β_2 -agonist was withdrawn.³³ Gly 16 homozygotes showed no such responses. Similarly, the presence of Arg-Arg, but not Gly-Gly, was associated with an increased frequency of

asthma exacerbations in patients receiving albuterol.³⁴ This was not observed with salmeterol treatment.

The second polymorphism is at position 27, which exists as either glutamine (Gln) or as glutamate (Glu). The allelic frequency for Gln 27 and Glu 27 is 55% and 45%, respectively.²⁹ In contrast to Gly 16, the Glu 27 form of the receptor appears to protect against downregulation. Using primary cultured human airway smooth muscle cells, after prolonged exposure to β_2 -agonists, the Glu 27 form downregulated to a much lesser extent than the Gln 27 receptor, as assessed by changes in receptor number.²⁹ In addition, a similar relative resistance to downregulation was observed using β_2 -agonist-mediated cAMP formation as an end point for receptor coupling. In a group of subjects with mild-to-moderate asthma, individuals with the Glu 27 form of the receptor had 4 times less reactive airways than those with Gln 27 when assessed with methacholine challenge. Heterozygotes had an intermediate mean PD₂₀ value.²⁸ No association was found between the 27 polymorphism and response to albuterol in children.³¹ Where heterozygous Glu 27, which is predicted to protect against receptor desensitization, is combined with homozygous Gly 16, the effects of Gly 16 are dominant.

The third polymorphism is at amino acid 164, which can either be threonine (Thr) or isoleucine (Ile). This polymorphism is much rarer than that at amino acid 16 or 27, with an allelic frequency of about 1%,²⁹ but is potentially interesting in that amino acid 164 is situated in the fourth transmembrane-spanning domain of the receptor and is adjacent to serine 165, which has been predicted to interact with the OH groups of adrenergic ligands. This polymorphism has been studied in a transfected cell system and has been shown to alter the agonist-binding properties of the receptor. Cells expressing Ile 164 were found to have approximately 4 times less ligand affinity. This alteration in binding affinity was reflected in a reduced capacity for the receptor to activate adenylate cyclase, relative to the wild-type (Thr 164) form of the receptor.³⁵ There are few clinical data available to assess the importance of this receptor polymorphism.

Of interest is a recent observation of a highly significant interaction between a coding nonsynonymous polymorphism (Ile 772 Met) of adenylate cyclase 9 and bronchodilator response to albuterol in those pediatric asthmatic patients taking inhaled budesonide.³⁶ This suggests that genotype might influence β_2 -receptor signaling when β_2 -agonists and inhaled corticosteroids are coadministered.

Given that most individuals will be heterozygous and that Arg-Gly 16 and Gln-Glu 27 polymorphisms might be in linkage disequilibrium, it is likely that large populations will have to be studied to determine the importance of β_2 -adrenoceptor polymorphisms to the asthma phenotype.

CONCLUSION

β_2 -Adrenergic agonists are used widely as bronchodilators and also in combination therapy with inhaled

corticosteroids in the treatment of respiratory diseases, such as asthma and chronic obstructive pulmonary disease. Knowledge of the function, response, and regulation of the β_2 -receptor is important to the clinician in interpreting patient response to both short- and long-acting β_2 -agonists. Similarly, an understanding of the mechanisms of receptor desensitization that might lead to tolerance to maintenance and rescue therapy is useful. The presence of a particular genetic form of the β_2 -receptor, which might influence clinical efficacy of regular β_2 -agonists, is of increasing interest in predicting good and poor responders. Finally, in the future, it might be possible to design drug molecules that interact with the β_2 -receptor in a specific manner that overcomes the problems of desensitization and genetic variability.

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