Contrasting properties of albuterol stereoisomers

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Racemic albuterol is composed of an equimolar mixture of stereoisomers. For asthma therapy, (R)-albuterol is the eutomer and (S)-albuterol is the distomer. By interacting with β_2-adrenoceptors, (R)-albuterol has bronchodilator, bronchoprotective, anti-edematous properties and inhibits activation of mast cells and eosinophils. (S)-albuterol does not activate β_2-adrenoceptors and does not modify activation of β_2-adrenoceptors by (R)-albuterol so that for many years it was presumed to be biologically inert. Recently, it has been established that regular and excessive use of racemic albuterol induces paradoxical reactions in some subjects with asthma. Because such effects cannot be accounted for by activation of β_2-adrenoceptors, the pharmacologic profile of (S)-albuterol has been more carefully defined. (S)-albuterol has distinctive pharmacologic properties that are unrelated to activation of β_2-adrenoceptors. Thus, (S)-albuterol intensifies bronchoconstrictor responses of sensitized guinea pigs and induces hypersensitivity of asthmatic airways; it also promotes the activation of human eosinophils in vitro. These actions of (S)-albuterol may explain why racemic albuterol can intensify allergic bronchospasm and promote eosinophil activation in asthmatic airways. The capacity of (S)-albuterol to elevate intracellular Ca^{2+} would account for the paradox because this action will oppose, or even nullify, the consequences of adenylyl cyclase activation by (R)-albuterol. Because (S)-albuterol is metabolized more slowly than (R)-albuterol and is retained preferentially within the airways, paradoxical effects become more prominent during regular and excessive use of racemic albuterol. Because (S)-albuterol has detrimental effects in asthmatic airways, levalbuterol [homochiral (R)-albuterol] should have advantages over racemic albuterol in therapy for asthma. (J Allergy Clin Immunol 1999;104:S31-41.)

Key words: *Racemic albuterol, levalbuterol, enantiomers, asthma, bronchodilation, bronchoprotection, airway hyperreactivity, eosinophils, β-adrenoceptor, calcium ions*

Abbreviations used
cAMP: 3',5'-cyclic adenosine monophosphate
$[Ca^{2+}]_i$: intracellular Ca^{2+}
MDI: metered-dose inhaler
TDI: toluene di-isocyanate

In human airways, bronchoconstrictor responses are countered by (R)-epinephrine, which is released from storage sites within the suprarenal glands. Endogenous (R)-epinephrine resolves airway obstruction by acting on both α-adrenoceptors and β-adrenoceptors. Hence synthetic compounds that mimic these actions of (R)-epinephrine might be expected to resolve obstruction of diseased airways. However, although activation of α-adrenoceptors contributes to the resolution of airway obstruction (eg, by reducing mucosal edema), the consequences of activation of these receptors elsewhere (eg, cardiac tissues) are too pronounced to be acceptable. For this reason, bronchodilator sympathomimetics have been selected for the singular property of activating β-adrenoceptors and, as such, have proved especially effective in asthma therapy.

Like (R)-epinephrine, synthetic β-sympathomimetics activate adenylyl cyclase and thereby elevate intracellular concentrations of 3',5'-cyclic adenosine monophosphate (cAMP). In the airways, increased concentrations of cAMP relax bronchial smooth muscle and preempt contraction of hyperresponsive airways; increased concentrations of cAMP also inhibits the release of inflammatory mediators from mast cells and eosinophils. In heart tissue, increased concentrations of cAMP will have chronotropic and inotropic actions. In part, these cardiac effects of β-sympathomimetics are the result of activation of β_1-adrenoceptors. Hence, because activation of β_1-adrenoceptors does not contribute to resolution of airway obstruction in asthma, selective activation of β_2-adrenoceptors has been preferred when sympathomimetics have been chosen for development as bronchodilator therapies.

Stereoisomerism and β_2-adrenoceptor activation

In common with many other neurohormones and neurotransmitters, (R)-epinephrine (Fig 1) activates cells by binding to a specific protein that spans the plasmalemma. For bronchodilator effects, (R)-epinephrine interacts with the β_2-adrenoceptor, a protein whose 413 amino acid residues fold into α-helixes that traverse back and forth across the cell membrane. By use of site-directed mutagenesis, it has been established that particular amino acid residues are critical for binding between the β_2-adrenoceptor and (R)-epinephrine, its physiologic ligand. The residues that are essential for high-affinity binding are widely separated along the primary sequence of the β_2-adrenoceptor protein, being located within the third, fifth and sixth loops of the α-helixes. In spanning the cell membrane, these α-helixes must be constrained into a particular orientation if critical amino acid residues are to

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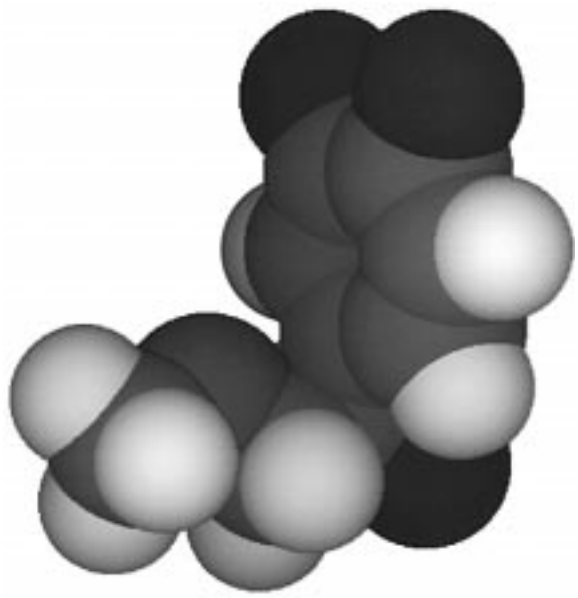

FIG 1. The three-dimentional molecular conformation of (R)-epinephrine.

be aligned with complementary sites on the epinephrine molecule (Fig 2). For this reason, high-affinity binding to (R)-epinephrine will have an absolute requirement for a particular three-dimensional conformation of the β_2-adrenoceptor protein. In accord with this conclusion, disruption of cysteine bonds effectively eliminates high-affinity binding by the β_2-adrenoceptor protein.[1]

It has long been recognized that multiple points of attachment between drug molecules and receptor sites could provide a basis for specificity of drug-receptor interaction. Stereochemistry is implicit in such interaction and came to the fore when a three-point attachment between ligand and acceptor molecules was proposed to explain drug specificity.[2] Although this hypothesis anteceded precise chemical definition of receptor structure by more than 50 years, it adequately accounts for the marked stereoselectivity of β-adrenoceptor activation. Involvement of a chiral center to facilitate binding is critical to the proposal of Easson and Stedman[2] and accommodates all agonists or antagonists that are structural analogues of epinephrine.[3] By suggesting that orientation of the benzylic β-hydroxyl group ensures close interaction between (R)-epinephrine and β_2-adrenoceptors, an equivalent interaction between that group and (S)-epinephrine is automatically precluded, leaving only two of the three points of attachment available for (S)-epinephrine. The biological consequence of this restriction is a high eudismic ratio for β-sympathomimetics [ie, biological activities of (R)- or (R,R)-enantiomers exceeding those of (S)- or (S,S)-enantiomers by >100-fold].

More recently, x-ray crystallography has verified the interpretation of Easson and Stedman. When the β_2-adrenoceptor is occupied by (R)-epinephrine, sites of close apposition include a serine residue (Ser 413) on helix 4 that interacts closely with the hydroxyl group of the β-carbon (the chiral center of epinephrine).[4] Because the enantiomers of epinephrine are not congruent, the β-hydroxyl group of (S)-epinephrine cannot appose to this serine site when the phenyl ring of the ligand is being held between the paired phenyl rings (Phe 517 and Phe 508) of the β_2-adrenoceptor in helix 5 (Fig 2). Analogous considerations apply to other synthetic sympathomimetics, including racemic albuterol.

β_2-Adrenoceptor activation by (R)-albuterol

As predicted by the Easson-Stedman hypothesis, effects of (R)-albuterol may be readily distinguished from effects of (S)-albuterol in assays that depend on selective binding to β_2-adrenoceptors. In early studies of these enantiomers, distinction was achieved by measuring mechanical responses of tissues, as when defining dose-effect relations of individual enantiomers on isolated heart or airway smooth muscle.[5,6] More recently, displacement of a β_2-selective ligand ([^{3}H] ICI 118,551) has been used to reveal that binding of racemic albuterol to adrenoceptors on membrane preparations from guinea pig lung is accounted for predominantly by (R)-albuterol.[7] This finding has now been extended to intact cells, which have been transfected to express cloned adrenoceptors of human origin. In such cells, (R)-albuterol exhibited greater affinity (90 to 100-fold) than did (S)-albuterol for either β_1- or β_2-adrenoceptors; also, it increased intracellular cAMP to an extent (23-fold) that was indistinguishable from the racemate (24-fold) and demonstrated a degree of intrinsic activity (45%) that was indistinguishable from the racemate (46%).[8] From these observations, it may be concluded that activation of β_2-adrenoceptors is largely determined by (R)-albuterol. As a corollary, any contribution to activation of β_2-adrenoceptors from (S)-albuterol must be insubstantial. Indeed, because of the high eudismic ratio, it is uncertain whether (S)-albuterol makes any direct contribution to the activation of β_2-adrenoceptors. Activation of β_2-adrenoceptors by preparations of (S)-albuterol achieves no more than 0.1% to 1% of responses to corresponding concentrations of (R)-albuterol. Because preparations of (S)-albuterol may be composed of up to 1% of (R)-albuterol, estimation of the contribution of (R)-albuterol to the eudismic ratio is impractical.[9] In this respect, findings from studies of receptor binding are in close agreement with results from studies with isolated airway smooth muscle.[10]

The Easson-Stedman hypothesis explains how analogues of (R)-epinephrine can bind selectively to β_2-adrenoceptors and why eutomers of sympathomimetics [eg, (R)-albuterol] will be far more potent in promoting receptor activation than distomers [eg, (S)-albuterol]. The consequences of activation of β_2-adrenoceptors have been documented extensively. Interaction between (R)-albuterol and β_2-adrenoceptors promotes activation of membrane-bound adenylyl cyclase to cause an increased concentration of intracellular cAMP. Binding of cAMP to the regulatory subunit of cAMP-dependent protein

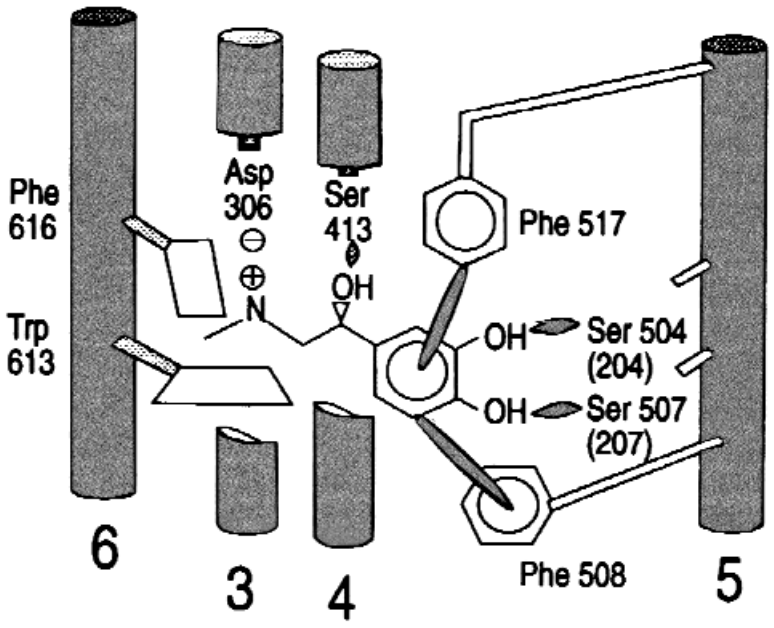

FIG 2. Schematic representation of the relation between key amino acid residues of the β_2-adrenoceptor and (R)-epinephrine. (From Anderson GP, Linden A, Rabe KF. Why are long-acting beta-adrenoceptor agonists long acting? Eur Respir J 1994;7:569-78. Copyright European Respiratory Society Journals Ltd.)

kinase activates the catalytic subunit of this enzyme, which phosphorylates substrates that regulate intracellular Ca^{2+} ions. The net effect of this intracellular biochemical cascade is a reduced concentration of intracellular Ca^{2+} $[Ca^{2+}]_i$. Mechanisms that are susceptible to cAMP when determining $[Ca^{2+}]_i$ include extrusion of Ca^{2+} (Na^+/K^+ ATPase, Na^+/Ca^{2+} antiporter), intracellular sequestration of Ca^{2+} (Ca^{2+} ATPase, phosphoinositide metabolism), and influx of Ca^{2+} (Ca^{2+} ion channel, voltage-dependent ion channel).[11] In smooth muscle cells, reduced $[Ca^{2+}]_i$ will promote relaxation of contracted cells; additionally, it will oppose activation of these cells by contractile stimuli. In neurons, it will diminish extrusion of neurotransmitters. In vascular endothelium, it will reduce the frequency of separation of vascular endothelial cells and thereby diminish vascular permeability. In mast cells, it will impair release or generation of smooth muscle spasmogens, and in eosinophils, it will impair the release of reactive oxygen. Consequently, there is consensus that interaction between (R)-albuterol and β_2-adrenoceptors adequately explains the ability of racemic albuterol to reduce airway obstruction in asthma.

Anomalous effects of racemic albuterol

Despite an attractive preclinical pharmacologic profile, racemic albuterol (Fig 3) has distinctive shortcomings. Resolution of airway obstruction and suppression of hyperresponsiveness are impressive. By way of contrast, anti-inflammatory and anti-allergic actions have proved modest, so that contributions from these pharmacologic properties are usually discounted when accounting for therapeutic efficacy of racemic albuterol. Furthermore, substantial loss of protective efficacy becomes evident when racemic albuterol is used regularly to control the symptoms of asthma. For ambulant subjects with asthma who use racemic albuterol regularly, this phenomenon leads inevitably to a foreshortening of the interval between metered-dose inhaler (MDI) actuation.[12] It seems likely that doses of racemic albuterol have been selected for regular use from the plateau of the dose-effect relations for bronchodilation and bronchoprotection to circumvent this limitation. It is usual to recommend inhalation from MDIs of two doses (2×100 μg per actuation) in close succession or to use nebulized albuterol (2.5 mg) at hourly intervals. Hence supramaximal doses in clinical settings are not exceptional, and excessive use of racemic albuterol is widespread. For a drug that provides a mainstay of therapy for one of the most common diseases in industrialized societies, routine overdose should be viewed as a misuse of health care resources. This analysis is justified by clinical experience because the empirical procedure of halving the dose of racemic albuterol per MDI actuation did not diminish control of asthma in an outpatient population.[13] Use of racemic albuterol in amounts that exceed the maximally effective dose is not merely an issue of health economics. Overdose will favor the emergence of effects that are associated with activation of extrathoracic β_2-adrenoceptors and more ominously with loss of efficacy as an asthma medication. It is not especially surprising, therefore, that epidemiologic studies should have detected an association between death or near death from asthma and the excessive use of racemic albuterol.[14] Viewed in isolation, correlation between death and increased use of racemic albuterol cannot establish whether excessive use of the

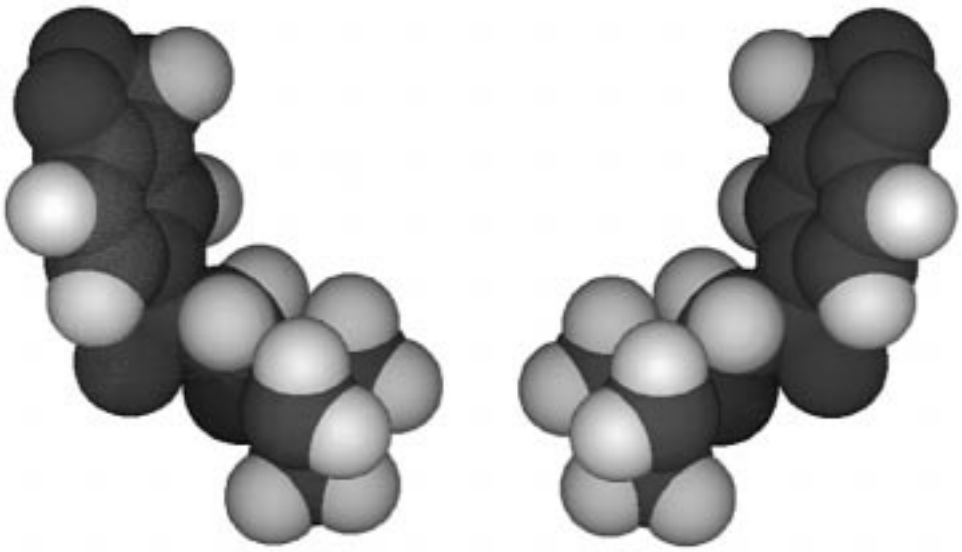

FIG 3. Three-dimensional molecular conformation of (S)-albuterol and (R)-albuterol (also called levalbuterol).

drug is a cause or a consequence of disease exacerbation. It is therefore particularly significant that when apparently trivial asthma has led to death in children, this too can be associated with the use of β-agonists.[15,16]

In laboratory studies of isolated cells, the phenomenon of β_2-adrenoceptor desensitization is a well-defined consequence of excessive exposure to β_2-adrenoceptor agonists. Because there is a trend toward excessive use of β_2-adrenoceptor agonists during disease exacerbation, it is understandable that tachyphylaxis or desensitization (downregulation) of β_2-adrenoceptors has been promulgated as an explanation for diminished protective efficacy. However, more detailed consideration of the properties of racemic albuterol reveal that other processes might equally contribute to or even exclusively determine this phenomenon. For instance, in addition to impaired activation of β_2-adrenoceptors as a result of tachyphylaxis or desensitization, reduced efficacy may simply reflect a diminished concentration of (R)-albuterol. Additionally or alternatively, it may reflect loss of activation efficiency if (S)-albuterol were to oppose signal transduction by the ligand/receptor complex or intracellular consequences of β_2-adrenoceptor activation. A number of processes might contribute to a reduced concentration of (R)-albuterol, including chiral inversion, interaction between (R)-albuterol and (S)-albuterol during binding to functional β_2-adrenoceptors, and competition between these two enantiomers at sites of metabolic breakdown.

As an alternative to reduced activation of β_2-adrenoceptors, diminished therapeutic efficacy of racemic albuterol during regular use might stem from unrelated pharmacologic actions that oppose the consequences of β_2-adrenoceptor activation.[17] Opposing actions are manifest in cells as diverse as neurons, smooth muscle cells, and inflammatory leukocytes and hence may reflect an intracellular action common to these cell types. Intracellular concentration of calcium ions is an obvious candidate, especially as it is well known that $[Ca^{2+}]_i$ can be increased by racemic isoproterenol.[18] More recently, this finding has been extended by the recognition that $[Ca^{2+}]_i$ of smooth muscle cells can be elevated by (S)-isoproterenol[19] and by demonstration that this pharmacologic activity is expressed also by (S)-albuterol.[20] It would seem prudent, therefore, to recognize that the pharmacologic effects of racemic albuterol may reflect the sum of opposing actions of the constituent stereoisomers on $[Ca^{2+}]_i$. During acute exposure to racemic albuterol, effects of β_2-adrenoceptor activation must predominate; otherwise, racemic albuterol would not be spasmolytic and would not have been developed as a bronchodilator. On acute administration, any attenuation of the fall of $[Ca^{2+}]_i$ caused by actions of (S)-albuterol could not readily be distinguished from attenuation that would result from receptor desensitization. Similarly, during regular use of racemic albuterol, any progressive reduction of $[Ca^{2+}]_i$ could be attributed to receptor desensitization. On the other hand, when $[Ca^{2+}]_i$ becomes elevated, this cannot be accounted for by β_2-adrenoceptor activation, and the activation of neurons and inflammatory and smooth muscle cells is therefore paradoxical. Because elevation of $[Ca^{2+}]_i$ cannot arise as a consequence of tachyphylaxis or desensitization, an action of the distomer (S)-albuterol becomes the only plausible mechanism that might account for this anomalous effect of racemic albuterol.

Mechanisms contributing to loss of efficacy

Desensitization

Reduced responsiveness can arise when tissues are exposed disproportionately to an agonist, an effect termed tachyphylaxis (after acute exposure) or desensitization (after repetitive exposure).[21] Whether caused by excessive concentrations or by excessive exposure, the

outcome is the same: diminished protection from inflammatory or spasmogenic stimuli. Loss of protection might be determined by a reduced incidence of receptors at the cell surface or by an impaired transduction of the activation signal. Either effect would be revealed as a reduced level of intracellular cAMP and less effective opposition of $[Ca^{2+}]_i$ elevation when cells are activated by spasmogenic or other stimuli.

For racemic albuterol, as for other sympathomimetics, it has long been recognized that desensitization of airway smooth muscle is difficult to achieve in vitro. Consistent with this experience is the clinical finding that the amplitude of bronchodilator responses does not decline when asthmatic subjects use racemic albuterol regularly (4×200 μg/d).[22] In animals that have been exposed to substantially higher doses of racemic albuterol (200 μg/kg per day by continuous infusion), sensitivity and responsivity of the airways to bronchodilator actions of racemic albuterol remain unchanged, prominent hyperresponsiveness notwithstanding.[23] Resistance of airway smooth muscle to desensitization is now widely accepted and has been attributed to a high receptor reserve.[1] In support of this opinion, maximal relaxation of airway smooth muscle can be demonstrated in vitro in the absence of detectable elevation of cAMP or receptor occupancy.[24] It is unlikely, therefore, that desensitization to the bronchorelaxant effects of racemic albuterol will contribute appreciably to loss of bronchoprotection.

Leukocytes, on the other hand, readily reveal β_2-adrenoceptor desensitization. This was recognized because of the proposal that β_2-adrenoceptor subsensitivity is pathognomonic in asthma. With the use of leukocytes to test this proposition, it became necessary to exclude the possibility that subsensitivity in these cells might have resulted from sympathomimetic therapy. Such studies established that elevation of cAMP in response to racemic isoproterenol was markedly diminished in lymphocytes collected from the peripheral blood of asthmatic subjects during regular treatment by racemic albuterol.[25] Diminished β_2-adrenoceptor activation has also been reported for polymorphonuclear leukocytes[26] and eosinophils.[27] It seems likely that a corresponding loss of responsiveness may be manifest in mast cells and basophils, but direct evidence is lacking. Because leukocytes are a major source of inflammatory and allergic mediators, including smooth muscle spasmogens, it seems likely that β_2-adrenoceptor desensitization of leukocytes may contribute to the substantial loss of protection from allergic bronchospasm that has been noted during regular use of racemic albuterol both in animals and human beings.[28,29]

Desensitization is common to all sympathomimetics and may be a homeostatic response to β_2-adrenoceptor activation. In the case of racemic albuterol, it has been established that activation of β_2-adrenoceptors is determined exclusively by (R)-albuterol.[7-9] Hence stereoisomerism would not be expected to influence the various processes that contribute to tachyphylaxis and desensitization. However, because this topic has yet to be investigated experimentally, it may be prudent to reserve judgment until experimental data become available.

Chiral inversion

Chiral inversion is the interconversion of enantiomers. Obviously, if (R)-albuterol were to be transformed into (S)-albuterol, the concentration of (R)-albuterol would fall and β_2-adrenoceptor activation would be diminished accordingly. However, both enantiomers of albuterol are highly stable in physiologic solution, making interconversion improbable, and there has been no gross indication of chiral inversion [eg, loss of activity of (R)-albuterol with time] from in vitro studies. More recently, it has become possible to exclude chiral inversion in vivo because highly sensitive assays are now available for detection of individual enantiomers of albuterol in body fluids. Such assays have not detected chiral inversion either in animals or human beings.[30,31] Implicit in the perception of biological effects of racemic albuterol as being exclusive to the eutomer is an absence of chiral conversion.[10,32] This presumption is valid, for there is no evidence that suggests chiral inversion as being contributory to the pharmacologic activity of either enantiomer of albuterol.

Physiologic interactions and metabolism. (R)-albuterol and (S)-albuterol share chemical identity and, with the exception of the –OH group of the chiral β-carbon, binding sites to acceptor molecules involved in cellular activation or metabolism are common to both enantiomers. The possibility arises, therefore, that (S)-albuterol might influence binding of (R)-albuterol to specific acceptor sites, as when (R)-albuterol binds to β_2-adrenoceptors during cell activation or to enzymatic sites during catabolism.

Closely similar binding affinities and intrinsic efficacy have been observed when (R)-albuterol has been compared with racemic albuterol.[8] On this basis, it may be concluded that equimolar concentrations of (S)-albuterol do not appreciably influence β_2-adrenoceptor activation by (R)-albuterol. Whether this conclusion holds when (S)-albuterol is in substantial excess is less well documented. Studies of isolated airway smooth muscle have revealed that preparations in which (S)-albuterol predominates (>100-fold excess) can mimic effects of (R)-albuterol.[5,6,8] Whether this should be attributed exclusively to undiminished effects of traces of (R)-albuterol is not known. On the basis of available evidence, it seems unlikely that (S)-albuterol will substantially impair activation of β_2-adrenoceptors by (R)-albuterol. Even so, studies of enantiomer interaction when (S)-albuterol is in substantial excess (ie, >10-fold) continue to be of interest because concentrations of (S)-albuterol may exceed those of (R)-albuterol during regular use of racemic albuterol in asthma by more than 10-fold.[32,33]

The chemical identity of (S)-albuterol and (R)-albuterol ensures that both enantiomers follow the same pathways of metabolic elimination. This raises the possibility of competitive interaction between stereoisomers during biochemical transformation. Interaction between

enantiomers of albuterol has been demonstrated during catabolism of racemic albuterol, a process whose initial step is sulfate conjugation.[34] Sulfate conjugation of albuterol has been detected in preparations of human liver, intestinal wall, platelets, and airway epithelium and, as for activation of β_2-adrenoceptors, is markedly enantioselective.[35,36] In preparations of human liver cytosol, the rate of sulfate conjugation of (R)-albuterol is markedly diminished when (S)-albuterol is present.[37] The extent to which metabolic interaction between the two enantiomers contributes to the asymmetrical pharmacokinetics of racemic albuterol has yet to be determined.

Marked enantioselective metabolism of racemic albuterol by human tissue anticipates differences in enantiomer disposition when racemic albuterol is administered during asthma therapy. Differences in clearance rates are quite pronounced and can be expected to amplify interactions between (S)-albuterol and (R)-albuterol. Thus clearance of (R)-albuterol (0.62 ± 0.18 L/h per kilogram) from serum is rapid by comparison with (S)-albuterol (0.39 ± 0.12 L/h per kilogram) in healthy human volunteers after intravenous or oral dosing with racemic albuterol.[33] More recently, it has been shown that after administration of racemic albuterol (2.5 mg) or (R)-albuterol (1.25 mg) by nebulization, maximal or cumulative (area under curve) concentrations of (R)-albuterol in peripheral blood were closely comparable after either single or repetitive administrations of either formulation. However, (R)-albuterol (t_{max} circa 1 ng/mL at 20 minutes) was more rapidly absorbed and more rapidly eliminated than (S)-albuterol (t_{max} circa 1.8 ng/mL at 60 minutes). As a consequence, the proportion of the distomer, (S)-albuterol, increases progressively until the concentration of (R)-albuterol in peripheral blood is severalfold less than the concentration of (S)-albuterol.[33]

Mechanisms contributing to paradoxical effects

Calcium ion influx. Relaxation of airway smooth muscle by sympathomimetics is attributed to a reduction of $[Ca^{2+}]_i$; yet it has been recognized for some time that $[Ca^{2+}]_i$ may be paradoxically increased by exposure to sympathomimetic drugs.[18] More recently, the possible contribution of enantiomers to this anomaly has been investigated experimentally. Measurement of the fluorescence of a Ca^{2+} indicator within dispersed tracheal smooth muscle cells has confirmed that (R)-albuterol (>5 nmol) reduces $[Ca^{2+}]_i$. Conversely, (S)-albuterol (EC_{50} 3.3 nmol) and racemic albuterol (EC_{50} 12.9 nmol) induced dose-related elevation of $[Ca^{2+}]_i$ in these smooth muscle cells, whether at rest or during contractile responses.[20] By increasing $[Ca^{2+}]_i$, (S)-albuterol would not be expected to contribute to spasmolysis; on the contrary, it might even induce contractile responses. Thus higher concentrations of (S)-albuterol (1 to 10 μmol) induced oscillations of $[Ca^{2+}]_i$ in a proportion (10% to 25%) of cells and caused a shortening (circa 10%) of all smooth muscle cells. This capacity of (S)-albuterol (10 μmol) to increase $[Ca^{2+}]_i$ was associated with increased (>200%) phospatidyl-inositol turnover, an effect blocked by nimodipine (an antagonist of L-type Ca^{2+} channels) but not by ICI 118,551 (a β_2-adrenoceptor antagonist). Because this response to (S)-albuterol was abolished by atropine or by 4-DAMP (a selective muscarinic receptor antagonist), it has been concluded that elevation of $[Ca^{2+}]_i$ was determined by muscarinic M_3 receptors at the cell surface.[20] Increased $[Ca^{2+}]_i$ in response to (S)-albuterol could explain the occurrence, latency, and spasmogen specificity of hyperresponsiveness of airways exposed regularly to racemic albuterol. However, modulation of responses to airway spasmogens would seem a more likely outcome than direct contraction of airway smooth muscle because overt contractile responses to (S)-albuterol have not previously been reported after exposure of intact airway smooth muscle to (S)-albuterol either in vitro or in vivo.[5,8,38]

An alternative mechanism whereby albuterol may elevate $[Ca^{2+}]_i$ is suggested from studies of dispersed smooth muscle cells from guinea pig teania caeci. In such cells, $[Ca^{2+}]_i$ increased substantially (by up to 60%) when cells were depolarized in the presence of low concentrations of racemic isoproterenol (EC_{50} 40 nmol). This potentiation of voltage-dependent influx of Ca^{2+} was not determined by changes of cAMP/adenylyl cyclase or protein kinase C and was not diminished by racemic propranolol.[39] The enantiomers of isoproterenol were equipotent in promoting such influx of Ca^{2+}, as were enantiomers of epinephrine and norepinephrine.[40] Closely comparable potencies of L-dopa, dopamine, and catechol justify suggestion that influx of Ca^{2+} into these smooth muscle cells is determined by a receptor for the catechol moiety that lacks stereoselectivity.[40] Elevation of $[Ca^{2+}]_i$ was not accompanied by increased tension of isolated muscle strips, nor were spontaneous contractions modified. Even so, it is possible that such elevation of $[Ca^{2+}]_i$ might facilitate responses to certain contractile stimuli and thereby provide a basis for hyperresponsiveness in airway smooth muscle. For the guinea pig, a catechol receptor on airway smooth muscle cells provides an attractive rationale for hyperresponsiveness: It has been shown that hyperresponsiveness of the guinea pig airways develops in reaction to infusion of either enantiomer of isoproterenol as well as to infusion of norepinephrine or dopamine.[41] Enantiomers of albuterol have yet to be studied in preparations of guinea pig teania caeci. However, because potentiation of voltage-dependent influx of Ca^{2+} into this tissue was unrelated to β_2-adrenoceptor activation and because racemic albuterol (EC_{50} 0.4 to 4 μmol) caused potentiation of voltage-dependent Ca^{2+} influx, a capacity of (S)-albuterol to promote Ca^{2+} influx would not be unexpected.

Bronchoconstrictor responses

In asthmatic subjects, the most prominent pharmacologic effect of racemic albuterol is relaxation of airway smooth muscle, a property that underlies the extensive use of racemic albuterol in asthma therapy. Even so, iso-

lated human airway smooth muscle cannot be relaxed fully by racemic albuterol and is unresponsive to concentrations of racemic albuterol in vitro that are demonstrably bronchodilatory in vivo during asthma therapy.[42,43] Thus during intravenous infusion of racemic albuterol (0.5 μg/kg per minute), substantial bronchodilation is achieved by serum concentrations of racemic albuterol that are substantially less than 100 nmol.[44] The low sensitivity of isolated human airway smooth muscle to racemic albuterol has yet to be explained. Insensitivity is not a result of impairment of β_2-adrenoceptor activation by (S)-albuterol. Racemic albuterol and (R)-albuterol are effectively equipotent relaxants of isolated strips of human airway smooth muscle. Preparations of (S)-albuterol are without obvious effect, except at high concentrations when relaxation becomes evident, owing to the presence of traces of (R)-albuterol.[45] No contractile response is observed when either (R)-albuterol or (S)-albuterol is applied to isolated strips of human airway smooth muscle.[45] However, preincubation of human bronchus with a relatively high concentration of (S)-albuterol (100 μmol) has been observed to exaggerate contractile responses to increasing doses of histamine or LTC_4 (but not methacholine), whereas, after preincubation with (R)-albuterol (100 μmol), contractile responses to both histamine and methacholine were reduced.[45] Other investigators have failed to detect pharmacologic actions of sympathomimetic distomers when using isolated airway smooth muscle preparations.[46] By reference to this rather limited evidence, it has been asserted that these substances are inert, but such opinion disregards the implications of in vivo evidence of spasmogen selectivity when animal or human airways develop hyperresponsiveness in reaction to regular racemic albuterol.[47] Recent recognition that hyperresponsiveness is spasmogen-selective accommodates the synergy between hyperresponsiveness produced by sympathomimetic drugs and allergic reactions.[48] This interaction, which has been observed repeatedly, implies that allergic mediators may be more sensitive indicators of altered sensitivity in isolated tissues, as has been demonstrated for intact airways responding to racemic albuterol.[29]

In the guinea pig, intravenous injection of bombesin produces a sustained bronchoconstrictor response, which facilitates detection and measurement of spasmolytic effects of sympathomimetics in intact airways. In this preparation, racemic albuterol produced a near maximal bronchodilation (78%) that was dose-related (ID_{50} 385 ng/kg).[23] Maximal bronchodilation (82%) and sensitivity (ID_{50} 363 ng/kg) were undiminished in animals that had been exposed continuously to high bronchodilator doses of racemic albuterol (200 μg/kg per day).[23] Comparable sensitivity (ID_{50} 480 ng/kg) has been defined by use of histamine[49] in confirmation of an earlier estimate (ID_{50} 120 ng/kg).[50] This spasmolytic effect of racemic albuterol may be attributed exclusively to (R)-albuterol because, by comparison with racemic isoproterenol, (R)-albuterol (2.93-fold) and racemic albuterol (3.75-fold) were only slightly less effective as inhibitors of bronchoconstrictor responses to acetylcholine, whereas (S)-albuterol (112-fold) was considerably less effective.[5] Bronchodilator responses to (S)-albuterol resembled those of (R)-albuterol in being sensitive to inhibition by pronethalol and therefore most probably stem from traces of (R)-albuterol in preparations of (S)-albuterol.[5]

An unexpected finding was made when the bronchodilator potency of racemic albuterol was determined in airways that had been made hyperresponsive by infusion of platelet activating factor. In such animals, sensitivity to inhibitory effects of albuterol (ID_{50} 0.44 ng/kg) was increased by 273-fold by comparison with equivalent assay in normal animals (ID_{50} 120 ng/kg).[50] A similar increased sensitivity to racemic albuterol has subsequently been observed in animals whose hyperresponsiveness was induced by intravenous injection of immune complexes. In this circumstance, bronchospasm caused by bombesin was 125-fold more sensitive to suppression by racemic albuterol (ID_{50} 8 ng/kg) than equivalent obstruction in normal animals (ID_{50} 1000 ng/kg).[51] It might reasonably be presumed that these very high potencies of racemic albuterol after an allergic reaction are manifestations of β_2-adrenoceptor activation by (R)-albuterol. However, no experimental data are yet available to test that presumption, and detailed experimental analysis would be instructive.

Airway hyperresponsiveness

In experimental animals that have been exposed repeatedly to sympathomimetics, increased sensitivity of the airways to spasmogens or antigen has been detected at regular intervals over the last 60 years.[48] Being both unexpected and inexplicable, these observations were discounted until it became apparent that airway obstruction and death in asthmatic patients could be associated with the use of racemic isoproterenol and possibly secondary to airway hyperresponsiveness. Observations of direct relevance to this clinical problem were reported but not understood or incorrectly interpreted.[48] For instance, although increased lethality of inhaled histamine was evident in animals after regular injection of racemic albuterol, the possibility that death might have been caused by an effect on the airways was not contemplated.[52] This is a surprising omission because the lung is the target organ for lethal doses of histamine in this species.[52] Failure to recognize this property of racemic albuterol was unfortunate because more than a decade was to lapse before hyperresponsiveness was recognized as a direct consequence of regular exposure to racemic isoproterenol: Even then, the phenomenon was attributed (incorrectly) to β-adrenoceptor desensitization.[53]

The capacity of sympathomimetics to induce hyperresponsiveness was rediscovered inadvertently during studies of platelet activating factor in the guinea pig.[54] Stereoisomerism was implicated when studies to determine the role β-adrenoceptors in development of airway hyperresponsiveness used (S)-isoproterenol as an appropriate control for (R)-isoproterenol and detected substantial hyperresponsiveness.[41] In these studies, the response

TABLE I. Comparison of pharmacologic actions of enantiomers of albuterol

Pharmacologic activity	(R)-albuterol	(S)-albuterol	Eudismic ratio	Reference
Binding to β_2-adrenoceptors	Binds with high affinity	Binding is insignificant	90-100	7
Activation of β_2-adrenoceptors	Promotes adenylyl cyclase activation	Marginal effect on adenylyl cyclase	23	8
Determination of intracellular Ca^{2+}	Reduced	Elevated	<<1	20
Contraction of isolated human bronchus	Suppressed	Enhanced	<<1	45
Bronchodilation	Effective	Ineffective	>>100	74
Airway responsiveness	Reduced	Enhanced	<<1	38, 66, 74
Eosinophil activation	Diminished	Promoted	<<1	59
Metabolic clearance	(0.62 ± 0.18 L/h per kilogram)	(0.39 ± 0.12 L/h per kilogram)	1.6	32, 33
Acute intravenous lethality in rat (LD_{50})	75-100 mg/kg	25-50 mg/kg	2-3	75

Eudismic ratio is the geometric ratio between the activity of (R)-albuterol and of (S)-albuterol.

to racemic isoproterenol had been shown to have three distinctive characteristics that made it anomalous (ie, not attributable to activation of β-adrenoceptors). First, airway hyperresponsiveness was neither preempted nor reversed by racemic propranolol at a dose that fully suppressed bronchodilator actions of racemic isoproterenol. Second, such hyperresponsiveness did not follow infusion of forskolin when used in amounts sufficient to evoke bronchodilation comparable to that evoked by racemic isoproterenol. Third, hyperresponsiveness was preempted by sectioning both vagal nerves before infusion of racemic isoproterenol, even though bronchodilator responses to racemic isoproterenol were unaffected by this procedure. From these findings, it may be inferred that induction of airway hyperresponsiveness by racemic albuterol is not determined by β-adrenoceptor activation and might be determined by a pharmacologic action of (S)-isoproterenol. Infusion of (S)-isoproterenol confirmed this prediction by producing an increased responsiveness of guinea pig airways to intravenous histamine, which, in the absence of confounding effects of bronchodilation, became evident within 15 to 30 minutes.[55]

These properties of (S)-isoproterenol are shared by (S)-albuterol, which also induces hyperresponsiveness of guinea pig airways during intravenous infusion. The capacity of (S)-albuterol to induce hyperresponsiveness is also not blocked by infusion of racemic propranolol yet is preempted when both vagal nerves are sectioned before infusion of (S)-albuterol.[38] It is not known whether (R)-albuterol also has the capacity to induce hyperresponsiveness. In the eye, topical application of racemic albuterol will reduce intraocular pressure, and this property is shared equally by both enantiomers,[56] and both enantiomers of propranolol exhibit a capacity to induce hyperresponsiveness in the airways of the guinea pig.[57] The possibility that (R)-albuterol might induce hyperresponsiveness of the guinea pig airway therefore merits consideration, especially as both (R)-isoproterenol and (S)-isoproterenol produced comparable hyperresponsiveness on intravenous infusion, with the effect of (R)-isoproterenol becoming manifest when the bronchodilator action had waned.[55] Because racemic albuterol produces a bronchodilator response in the guinea pig that is much more protracted than racemic isoproterenol, use of acute infusion of racemic albuterol may obscure any hyperresponsiveness. Resolution of this issue may depend on detection of indirect effects of (R)-albuterol, such as exaggeration of airway responsiveness caused by racemic propranolol[58] or intensification of allergic bronchospasm.[38]

As well as producing hyperresponsiveness, racemic albuterol can mimic another aspect of the allergic response that is equally unexpected. Thus regular treatment with racemic albuterol not only increased the amplitude of allergic bronchospasm in subjects with allergic asthma but also increased the incidence and degree of activation of eosinophils in the lumen of asthmatic airways.[29] This finding implies that, like antigen, racemic albuterol may activate eosinophils. Such conclusion is no longer surprising, given that (S)-albuterol has been shown to intensify superoxide production by human eosinophils, in marked contrast to (R)-albuterol, whose actions are inhibitory.[59] As noted for anomalous hyperresponsiveness, the effect of (S)-albuterol may become predominant during regular administration of racemic albuterol because of stereoselective elimination of (R)-albuterol.

The guinea pig was selected for asthma research because allergic reactions in this species were manifest primarily as changed pulmonary function and showed close similarity with allergic reactions of patients with asthma.[60] Reaffirmation of this opinion after an interval

of almost 50 years is grounds for considering that effects of racemic albuterol in guinea pig airways might prove predictive for asthmatic airways.[61] Of particular note has been the finding that regular administration of racemic albuterol induces changes in the airways that are also elicited by allergic reactions. Thus continuous infusion of racemic albuterol produced pronounced hyperresponsiveness of the airways. The seven spasmogens that were used to define changed airway responsiveness had different rank orders from those observed after an allergic reaction. Even so, in both circumstances, the most pronounced increased responsiveness was detected when LTC_4 was the test spasmogen. Moreover, when increased responsiveness to LTC_4 was produced by infusion of racemic albuterol, hyperresponsiveness was intensified further after an allergic reaction. In these studies, a threshold dose of antigen was infused over 60 minutes to minimize the bronchoconstrictor reaction to antigen so that lethal allergic bronchospasm was rare (<1%). This may be contrasted with the frequent (>30%) deaths that occurred in those animals that had previously been exposed to racemic albuterol and then exposure to this low dose of antigen or to allergic mediators.[28,62]

Interaction between sympathomimetics and antigens were recognized by early investigators who studied allergic bronchospasm to detect anomalous effects of sympathomimetics.[48] These early observations have been confirmed repeatedly, but no basis has been proposed that would explain how racemic albuterol might interact with allergic reactions to produce such severely increased responsiveness. In the guinea pig, it is noticeable that whereas responsiveness of the airways increases after regular exposure to racemic albuterol, there is no corresponding change of threshold sensitivity. One possibility that merits consideration is that decreased airway elastance may determine this outcome by increasing the maximal degree of shortening. Such changed behavior of airway smooth muscle follows either regular inhalation of racemic albuterol or regular inhalation of antigen, so that additive or even synergistic interaction should not be considered exceptional.[63-65] Detailed morphometric studies of airway smooth muscle after exposure to regular fenoterol revealed changes of the extracellular matrix, which could limit the extent of shortening.[63] Such a change might be initiated by infiltrating inflammatory cells and, once established, this structural modification may be difficult to reverse or even irreversible. In view of the recent evidence that (S)-albuterol may promote activation of an inflammatory leukocyte and thereby nullify the anti-inflammatory action of (R)-albuterol, it becomes apparent that decreased elastance may be a feature of asthmatic airways whose origin could be iatrogenic, in whole or in part. Whether racemic albuterol is capable of initiating or amplifying the decreased elastance of asthmatic airways has not been contemplated, and experimental investigation of the capacity of stereoisomers of albuterol to induce decreased elastance merits urgent investigation.

The acute hyperresponsiveness that was produced by infusion of (S)-albuterol was abolished when vagal section preceded the infusion.[38] From this observation, it may be inferred that (S)-albuterol can modulate neural activities and thereby modify sensitivity of the airways to contractile stimuli. Such interpretation is supported directly by the recent demonstration that (S)-albuterol preferentially enhances responsiveness to bradykinin[66] and indirectly by the finding that increased efflux of ^{3}H-norepinephrine from rat brain slices caused by racemic clenbuterol is determined solely by an action of the distomer.[67] Some insight into this response pattern is provided by other agencies of hyperresponsiveness in this species. Thus both ozone and toluene di-isocyanate (TDI) have been shown to produce hyperresponsiveness that could be preempted but not reversed by vagal section. Use of antagonists has revealed that development of hyperresponsiveness in reaction to these agents may be secondary to release of sensory neuropeptides. Thus responses to ozone were preempted but not reversed by CP99994 and responses to TDI were preempted but not reversed by spantidine.[68,69] If release of neuropeptides determines the changed airway responsiveness produced by ozone or TDI, this would accommodate the capacity of vagal section to preempt (but not reverse) the changed responsiveness. Persistence may be conferred on this reaction if it is determined by expression of the transcription factor and immediate early gene, c-*fos*, as has been shown for capsaicin.[70] By analogy, similar changes may be induced by β_2-sympathomimetics, although whether this reflects activation of β_2-adrenoceptors has yet to be investigated.[71]

Involvement of neural processes in the development of airway hyperresponsiveness caused by racemic albuterol could contribute to the selectivity of increased responsiveness that characterizes this reaction. For instance, in guinea pigs that have received a continuous infusion of racemic albuterol over 6 days, constrictor responses to some spasmogens (acetylcholine, histamine, serotonin, prostaglandin $F_{2\alpha}$) were diminished by comparison with control animals, whereas responses to other spasmogens (bradykinin and peptidoleukotrienes C_4 and E_4) were exaggerated.[28,62] These differential effects have a counterpart in the airways of asthmatic subjects, whose sensitivity to inhaled spasmogens is markedly heterogeneous.[47] Thus the increased airway responsiveness that results from regular use of racemic albuterol was selective, with significant increased sensitivity to bradykinin, even though responsiveness to methacholine or histamine showed no increase overall.[72,73] Whether individual enantiomers determine these differential effects is currently under investigation, and preliminary results are consistent with such expectation.[74]

CONCLUSIONS

Racemic albuterol is established as the most widely used bronchodilator in asthma therapy. The predominance of racemic albuterol is testament to its bronchodilator efficacy. Even so, it is well established that loss of protective efficacy is a deficiency that leads to increased use of this drug, a practice that is in conflict

with the recommendations of expert committees who have proposed strategies for treatment of asthma. Comparison of the pharmacologic actions of enantiomers of albuterol has proved enlightening by providing a rationale for many of the anomalous actions of racemic albuterol, which can now be attributed to unsuspected properties of the distomer (S)-albuterol (Table I).[75] This extensive body of preclinical pharmacology anticipates that formulation of albuterol as homochiral (R)-albuterol will offer the prospect of a major improvement in maintenance therapy for asthma. When albuterol has been used as a racemate, actions of (S)-albuterol may have limited the anti-inflammatory and anti-allergic potential of (R)-albuterol. The possibility of using (R)-albuterol both to treat symptoms (bronchodilation, bronchoprotection) and to preempt tissue modification (anti-allergic, anti-inflammatory) may be an unexpected bonus of the process of drug purification.

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