

Differential effects of (S)- and (R)-enantiomers of albuterol in a mouse asthma model

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Background: (R)- and (S)-Enantiomers of albuterol likely exert differential effects in patients with asthma. The (R)-enantiomer binds to the β_2 -adrenergic receptor with greater affinity than the (S)-enantiomer and is responsible for albuterol's bronchodilating activity. (S)-Albuterol augments bronchospasm and has proinflammatory actions.

Objective: The study aim was to determine whether the (S)-enantiomer, in contrast to the (R)-enantiomer, has adverse effects on allergic airway inflammation and hyperresponsiveness in a mouse asthma model.

Methods: Mice sensitized to ovalbumin (OVA) intraperitoneally on days 0 and 14 were challenged with OVA intranasally on days 14, 25, and 35. On day 36, 24 hours after the final allergen challenge, the effect of the (R)- and (S)-enantiomers of albuterol ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ administered by means of a miniosmotic pump from days 13-36) on airway inflammation and hyperreactivity was determined.

Results: In OVA-sensitized/OVA-challenged mice, (R)-albuterol significantly reduced the influx of eosinophils into the bronchoalveolar lavage fluid and airway tissue. (R)-Albuterol also significantly decreased airway goblet cell hyperplasia and mucus occlusion and levels of IL-4 in bronchoalveolar lavage fluid and OVA-specific IgE in plasma. Although (S)-albuterol significantly reduced airway eosinophil infiltration, goblet cell hyperplasia, and mucus occlusion, it increased airway edema and responsiveness to methacholine in OVA-sensitized/OVA-challenged mice. Allergen-induced airway edema and pulmonary mechanics were unaffected by (R)-albuterol.

Conclusion: Both (R)- and (S)-enantiomers of albuterol reduce airway eosinophil trafficking and mucus hypersecretion in a mouse model of asthma. However, (S)-albuterol increases allergen-induced airway edema and hyperresponsiveness. These adverse effects of the (S)-enantiomer on lung function might limit the clinical efficacy of racemic albuterol. (*J Allergy Clin Immunol* 2005;116:332-40.)

Key words: β_2 -adrenergic agonist enantiomers, airways, mucus, edema, inflammation, hyperresponsiveness

Adrenergic receptors are composed of α - and β -receptors that bind endogenous catecholamines, such as epinephrine. Although 3 subtypes of β -adrenergic receptors exist, smooth muscle relaxation producing vasodilation and bronchodilation is mediated by the β_2 -receptor. Short-acting β_2 -adrenergic receptor agonists rapidly induce bronchodilation in patients with asthma and are used for relief of acute symptoms, prevention of exercise-induced asthma, and management of acute severe asthma.

Racemic albuterol contains equal concentrations (50:50) of the (R)- and (S)-enantiomers (ie, enantiomers that are nonsuperimposable mirror images).¹ The (R)-enantiomer of albuterol binds to β_2 -adrenergic receptors with nearly 100-fold greater affinity than the (S)-enantiomer, suggesting that the (S)-enantiomer does not act through β -adrenergic receptor activation.¹ Whereas the (R)-enantiomer of albuterol (levalbuterol) exerts the bronchodilating properties of albuterol, the (S)-enantiomer has adverse effects, including augmentation of bronchospasm and proinflammatory activities.²⁻⁵ In murine mast cells (S)-albuterol increases IgE-induced histamine and IL-4 production, whereas the (R)-enantiomer lacks these effects.² Anti-inflammatory effects of (R)-albuterol, such as inhibition of T-cell proliferation, might be negated by the presence of the (S)-enantiomer.³

Differential effects of the enantiomers might result from differences in pharmacokinetics.¹ The initial step in the metabolism of the (S)- and (R)-enantiomers is sulfate conjugation, a stereospecific process in human airway epithelial cells and other cells and tissues.⁶ The greater rate of sulfate conjugation of (R)-albuterol might lead to lower plasma levels of (R)- than (S)-albuterol in human subjects.⁷ Potential adverse effects of (S)-albuterol on asthma control might also be augmented by increased binding to lung tissue.

In this study we characterized the effects of the (R)- and (S)-enantiomers of albuterol on allergic airway inflammation and hyperresponsiveness in a mouse asthma model that mimics key features of human asthma.⁸ Although prior studies in guinea pigs and human subjects have demonstrated that the (S)-enantiomer of albuterol can induce airway hyperreactivity, there are no prior studies examining the effect of (S)-albuterol versus (R)-albuterol on both airway hyperresponsiveness and the T_H2 phenotype (ie, allergen-induced airway eosinophil trafficking, mucus metaplasia, edema, and T_H2 cytokine release) in an *in vivo* asthma model. We report that both

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Abbreviations used

AP-1: Activator protein 1
BAL: Bronchoalveolar lavage
OVA: Ovalbumin
 P_{enh} : Enhanced pause

(R)- and (S)-enantiomers reduce allergen-induced airway eosinophil and mucus gland hyperplasia. However, only (S)-albuterol increases airway edema and responsiveness to methacholine, effects that would decrease the therapeutic efficacy of racemic albuterol.

METHODS

Study protocol

All animal use procedures were approved by the University of Washington Animal Care Committee. Female BALB/c mice (6–8 weeks of age; The Jackson Laboratory, Bar Harbor, Me) received an intraperitoneal injection of 100 μ g of ovalbumin (OVA; 0.2 mL of 500 μ g/mL) complexed with alum on days 0 and 14 (Fig 1). Mice were anesthetized with 0.2 mL of ketamine (6.5 mg/mL)/xylazine (0.44 mg/mL) in normal saline administered intraperitoneally before receiving an intranasal dose of 50 μ g of OVA (50 μ L of 1 mg/mL) on days 14, 25, and 35 (Fig 1). The control group received 0.2 mL of normal saline with alum administered intraperitoneally on days 0 and 14 and 0.4 mL of saline without alum administered intranasally on days 14, 25, and 35. In both the saline- and OVA-treated groups, miniosmotic pumps (200 μ L, Alzet Model 2004; Durect Corp, Cupertino, Calif) containing either (R)- or (S)-albuterol (1 mg $\cdot$ kg⁻¹ $\cdot$ d⁻¹, 6 μ L/d delivery administration) were inserted subcutaneously on the back slightly posterior to the scapulae on day 13 and remained in place until study conclusion on day 36 (Fig 1). Absorption of the compounds by local capillaries results in systemic administration. Each study group consisted of 4 to 6 animals. The 1 mg $\cdot$ kg⁻¹ $\cdot$ d⁻¹ dose of albuterol enantiomer infusion was selected on the basis of prior work by Sartori et al,⁹ demonstrating that continuous release of racemic albuterol (2 mg $\cdot$ kg⁻¹ $\cdot$ d⁻¹) subcutaneously by means of miniosmotic pump produced steady-state, high-plasma levels of albuterol (10⁻⁵ M) in mice.

Pulmonary function testing

In vivo airway responsiveness to methacholine was determined on day 36 in conscious, freely moving, spontaneously breathing mice by using whole-body plethysmography (Model PLY 3211; Buxco Electronics Inc, Sharon, Conn), as described by Hamelmann et al.¹⁰ Mice were challenged with aerosolized saline or increasing doses (2 and 10 mg/mL) of methacholine generated by an ultrasonic nebulizer (DeVilbiss Health Care, Inc, Somerset, Pa) for 2 minutes. The degree of bronchoconstriction was expressed as enhanced pause (P_{enh}), a calculated dimensionless value that correlates with measurement of airway resistance, impedance, and intrapleural pressure.^{9–11} P_{enh} readings were taken and averaged for 4 minutes after each nebulization challenge. P_{enh} is calculated as follows: $P_{enh} = [(T_e/T_r - 1) \times (PEF/PIF)]$, where T_e is expiration time, T_r is relaxation time, PEF is peak expiratory flow, and PIF is peak inspiratory flow $\times$ 0.67 coefficient. The time for the box pressure to change from a maximum to a user-defined percentage of the maximum represents the relaxation time. The T_r measurement begins at the maximum box pressure and ends at 40%. Because P_{enh} is the ratio of measurements obtained during the same breath, it is mainly

independent of functional residual capacity, tidal volume, and respiratory rate.

Light microscopy-morphometry

After pulmonary function testing, bronchoalveolar lavage (BAL) was performed on the right lung, with total BAL fluid cells counted and eosinophils identified by means of eosin staining.¹² Left lung tissue was obtained for histopathology, and plasma was obtained for OVA-specific IgE levels. Ten lung sections per animal were randomly selected and examined in a blinded manner. Sections were stained with hematoxylin and eosin, the total inflammatory cell infiltrate was assessed on a semiquantitative scale (0–4+), the number of eosinophils per unit of airway area (2200 μ m²) was determined by using a point-counting system (Image-Pro Plus point-counting system software, Version 1.2 for Windows; Media Cybernetics, Silver Spring, Md),¹² and interstitial and perivascular airway edema were assessed.^{13,14} Airway goblet cells (as a percentage of total airway cells) were identified by means of Alcian blue staining,¹² and the degree of mucus plugging of the airways (0.5 mm to 0.8 mm in diameter) with the percentage occlusion of the airway diameter was classified on a 0 to 4+ scale on the basis of the following criteria: 0, no mucus; 1, approximately 10% occlusion; 2, approximately 30% occlusion; 3, approximately 50% occlusion; 4, greater than approximately 80% occlusion.¹²

Cytokine assays

IL-4, IL-5, IL-10, GM-CSF, TNF- α , IL-2, and IFN- γ were assayed in BAL fluid with Bio-Plex Mouse Cytokine assays (Bio-Rad Laboratories, Hercules, Calif) that are bead-based multiplex sandwich immunoassays with a limit of detection of less than 10 pg/mL. IL-13 was assayed in BAL fluid with a mouse IL-13 immunoassay (Quantikine M; R&D Systems, Minneapolis, Minn), with a limit of detection of less than 1.5 pg/mL.

OVA-specific IgE assay

OVA-specific IgE was assayed by modification of the method of Iio et al.¹⁵ Nunc 96-well flat-bottom plates (Nalge Nunc International, Rochester, NY) were coated with 50 μ g/mL OVA in 1 $\times$ PBS overnight at room temperature, washed 3 times with 1 $\times$ PBS plus 0.05% Tween-20 (wash buffer), blocked with 3% BSA in 1 $\times$ PBS for 1 hour at room temperature, and washed 4 times with wash buffer. Fifty-microliter plasma samples (1:1 in 1 $\times$ PBS) were added per well and incubated for 90 minutes at 37°C, then washed 4 times with wash buffer, and blotted dry by inverting over paper towels. One hundred microliters (1:100 in 1 $\times$ PBS) of biotin-conjugated rat anti-mouse IgE mAb (clone R35-72; BD Biosciences, San Diego, Calif) was added to each well and incubated overnight at 4°C and then washed 4 times with wash buffer and blotted dry. Then 100 μ L per well (1:1000 in 1 $\times$ PBS) streptavidin-horseradish peroxidase-conjugated secondary antibody (BD Biosciences) was added, and samples were incubated at 37°C for 90 minutes, then washed 4 times with wash buffer, and blotted dry. One hundred microliters of substrate solution (ie, 1 tablet of 2,2'-azinobis [3-ethylbenzthiazoline-sulfonic acid, ABTS; Sigma Chemical Co, St Louis, Mo] dissolved in 100 mL of 0.05 M phosphate-citrate buffer, pH 5.0, and 25 μ L of 30% H₂O₂) was added per well, and color was developed for 30 minutes at room temperature. OD 405 nm was measured by using an AD 340C absorbance detector (Beckman Coulter Inc, Fullerton, Calif). For the IgE standard curve, a sandwich ELISA was used in which in separate assay plates biotin-conjugated rat anti-mouse IgE mAb (clone R35-72, BD Biosciences) was used to coat the wells, and instead of plasma samples, known concentrations of purified anti-mouse IgE (clone C38-2, BD Biosciences)

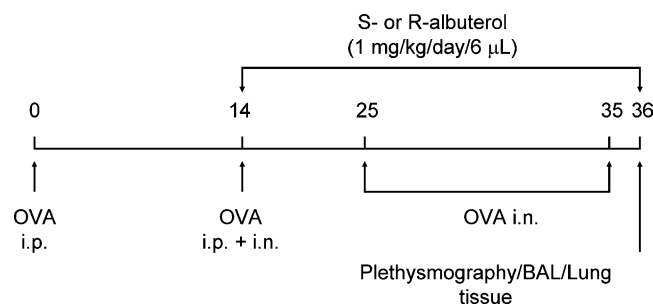


FIG 1. Study protocol. *i.p.*, Intraperitoneal; *i.n.*, intranasal

were incubated; assays were run as described above. The standard curve was constructed by using a linear regression analysis of the absorbances against serial dilutions of known concentrations of mouse IgE. Pooled mouse plasma from OVA-sensitized/OVA-challenged mice was used as a positive control.

Statistical analysis

The data are reported as the means $\pm$ SE of the combined experiments. Differences were analyzed for significance ($P < .05$) by means of ANOVA with the protected least-significant-difference method (Statview II; Abacus Concepts, Berkeley, Calif).

RESULTS

Effect of (R)- and (S)-enantiomers of albuterol on allergen-induced airway inflammation

Airway infiltration by eosinophils. A marked infiltration of inflammatory cells that were predominantly eosinophils around the airways and pulmonary blood vessels was observed in the lung interstitium of OVA-treated mice (Fig 2, B) compared with that seen in saline-treated control mice (Fig 2, A) on day 36, 24 hours after the last intranasal OVA or saline challenge. By means of morphometric analysis (Fig 3, A and B) of the histologic sections (Fig 2, C and D vs B), administration of (R)- and (S)-albuterol by means of miniosmotic pumps ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ dose from days 13-36) significantly decreased the influx of total inflammatory cells ($P = .0035$, R-albuterol/OVA vs OVA; $P = .0226$, S-albuterol/OVA vs OVA; Fig 3, A) and eosinophils ($P = .021$, R-albuterol/OVA vs OVA; $P = .008$, S-albuterol/OVA vs OVA; Fig 3, B) into the lung interstitium. Compared with the saline group (Fig 3, C), OVA-sensitized/OVA-challenged mice exhibited a marked increase in BAL fluid eosinophils to $2.5 \pm 0.5 \times 10^5$ eosinophils/mL ($P < .0001$, OVA vs saline; Fig 3, C), which represented 41.8% of total BAL fluid cells. In OVA-sensitized/OVA-challenged mice, treatment with (R)-albuterol significantly inhibited the influx of eosinophils into BAL fluid by 40.6% ($P = .0043$, R-albuterol/OVA vs OVA; Fig 3, C). In contrast, (S)-albuterol had no significant effect on eosinophil influx into the BAL fluid of OVA-treated mice (Fig 3, C).

Airway mucus hypersecretion. Hyperplasia of airway goblet cells and hypersecretion of mucus were observed in OVA-treated mice (Fig 2, B) compared with in control

mice (Fig 2, A). Airway goblet cells increased to 38.0% of total airway cells in OVA-treated mice compared with 0.4% in saline control mice ($P = .0001$, OVA vs saline; Fig 4, A). The mucus occlusion of the airway diameter morphometric score increased 15-fold in the OVA-treated mice compared with control mice ($P < .0001$, OVA vs saline; Fig 4, B). Allergen-induced goblet cell hyperplasia and mucus occlusion of airway diameter were inhibited by both the (R)-enantiomer (Fig 2, C) and (S)-enantiomer (Fig 2, D) of albuterol. By means of morphometric analysis, (R)-albuterol decreased goblet cell hyperplasia by 48.9% ($P = .0066$, R-albuterol/OVA vs OVA; Fig 4, A) and airway mucus occlusion by 41.4% ($P = .0042$, R-albuterol/OVA vs OVA; Fig 4, B). (S)-Albuterol reduced goblet cell hyperplasia by 44.8% ($P = .0095$, S-albuterol/OVA vs OVA; Fig 4, A) and mucus occlusion of the airways by 35.7% ($P = .0088$, S-albuterol/OVA vs OVA; Fig 4, B).

Airway edema. Airway edema was observed in the lungs of OVA-treated mice (Fig 2, B) compared with that seen in saline-treated control mice (Fig 2, A). (S)-Albuterol markedly increased airway edema in OVA-treated mice (Fig 2, D vs B). In contrast, (R)-albuterol had no effect on allergen-induced edema in the airways of OVA-treated mice (Fig 2, C).

Cytokine release. Significant levels ($P < .05$) of IL-4, IL-5, IL-13, and GM-CSF were found in the BAL fluid of OVA-treated mice compared with levels in the saline control group (Fig 5). The increased levels of IL-4 in OVA-treated mice were reduced 70.5% and 52.2% by (R)-albuterol and (S)-albuterol, respectively; the reduction was statistically significant only for the (R)-enantiomer ($P = .043$, R-albuterol/OVA vs OVA; Fig 5). There was no significant effect of either the (R)- or (S)-enantiomer of albuterol on the increased BAL fluid levels of IL-5, IL-13, and GM-CSF in the OVA-treated animals. The levels of IL-10, TNF- α , IL-2, and IFN- γ were not significantly increased in the BAL fluid of OVA-treated mice compared with levels seen in saline-treated control mice; neither enantiomer affected the levels of these cytokines in OVA-treated animals (Fig 5).

OVA-specific IgE. Plasma OVA-specific IgE was absent in saline-treated control mice and present in OVA-treated control mice (Fig 6). (R)-Albuterol and (S)-albuterol reduced OVA-specific IgE levels in OVA-treated mice; the reduction was statistically significant

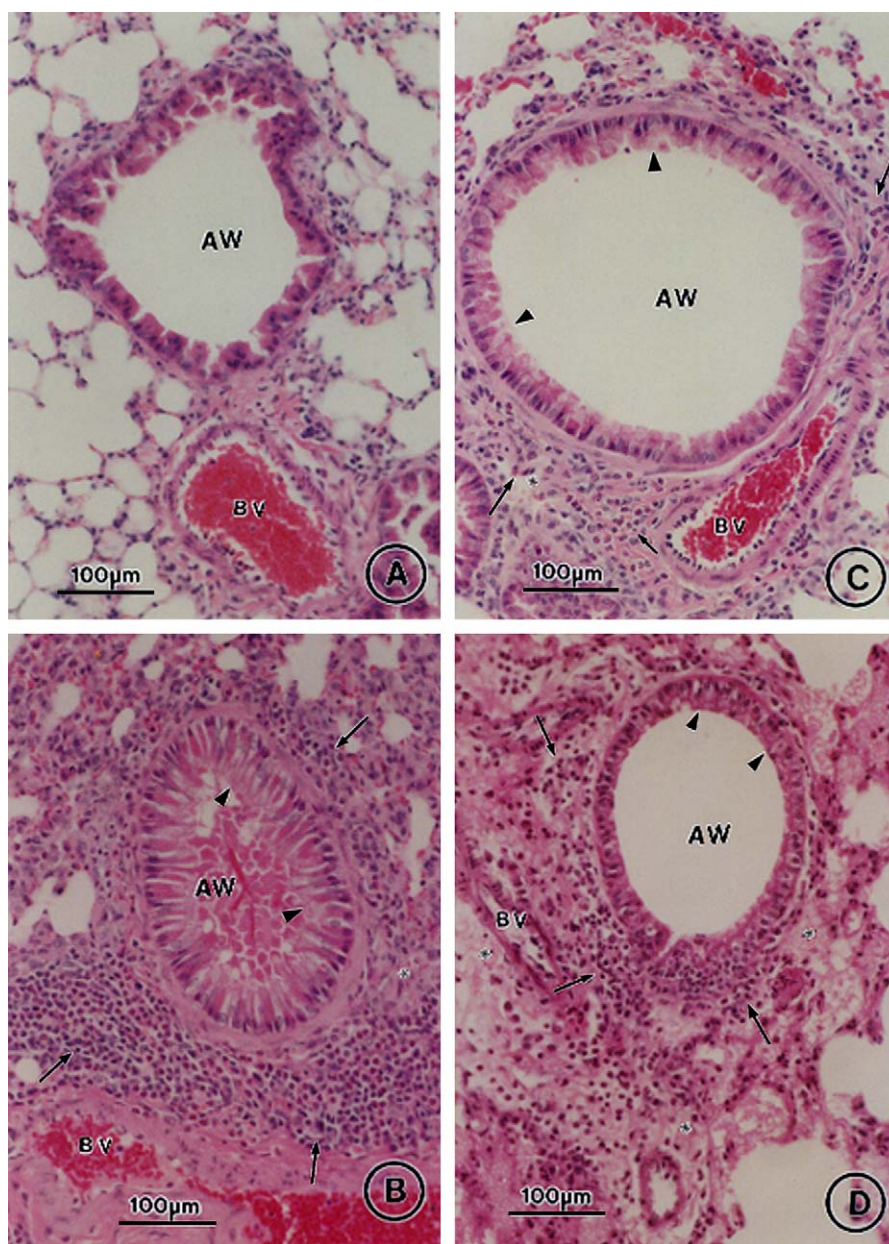


FIG 2. Effect of (R)- and (S)-albuterol enantiomers on airway histopathology in a mouse asthma model. Lung tissue was obtained on day 36 from saline-treated control animals (**A**), OVA-treated control animals (**B**), OVA-treated mice administered (R)-albuterol (**C**), and OVA-treated mice administered (S)-albuterol (**D**), and sections were stained with hematoxylin and eosin. Arrows indicate eosinophils and other inflammatory cells, arrowheads indicate mucus, and asterisks indicate edema. AW, Airway; BV, blood vessel. Bars = 100 μ m.

only for the (R)-enantiomer ($P = .0120$, R-albuterol/OVA vs OVA; Fig 6).

Effect of (S)- and (R)-enantiomers of albuterol on allergen-induced airway hyperresponsiveness

Pulmonary mechanics were assessed in response to aerosolized methacholine by means of noninvasive *in vivo* plethysmography on day 36, 24 hours after the last intranasal OVA challenge. The OVA-sensitized mice

had been challenged with 3 intranasal doses of OVA, a protocol that we have previously shown to induce airway inflammation and mucus hypersecretion but that is suboptimal for inducing airway hyperresponsiveness.^{16,17} This protocol was used because an augmenting effect of (S)-albuterol on airway hyperresponsiveness could have been masked in our mouse asthma model protocol, in which bronchial hyperresponsiveness is achieved through administration of 4 intranasal doses of OVA in mice sensitized by 2 intraperitoneal OVA doses.^{16,17}

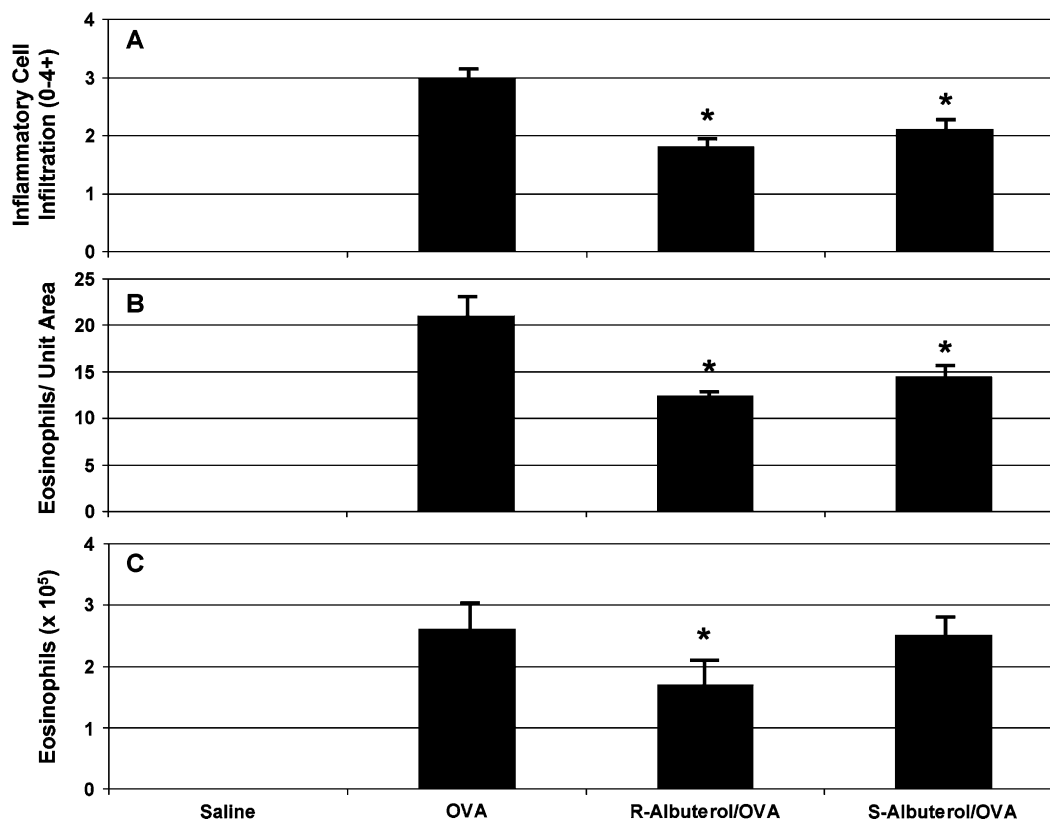


FIG 3. Effect of (R)- and (S)-albuterol enantiomers on allergen-induced airway inflammatory cell infiltration. The total inflammatory cell infiltration of the airways (**A**), the number of eosinophils per unit area (2200 μm^2) of lung tissue (**B**), and eosinophils per milliliter of BAL fluid (**C**) were determined. * $P < .05$ versus OVA.

In OVA-treated mice (S)-albuterol significantly increased bronchial responsiveness to methacholine (Fig 7). In contrast, (R)-albuterol did not alter airway responsiveness to methacholine in OVA-sensitized/OVA-challenged mice (Fig 7).

Effect of (R)- and (S)-enantiomers of albuterol in non-OVA-sensitized/OVA-challenged mice

Miniosmotic pumps containing either (R)- or (S)-albuterol were placed subcutaneously in saline control animals for a 24-day treatment period before pulmonary function testing and assessment of lung histopathology (Fig 8) to examine the effect of the albuterol enantiomers independently of a modification of the allergic inflammatory response.

Lung morphology. The (S)-enantiomer of albuterol did not induce airway edema independently of an allergic response. No airway edema or inflammation was seen in saline-treated mice administered either (S)-albuterol (Fig 8, B) or (R)-albuterol (Fig 8, C).

Pulmonary mechanics. The (S)-enantiomer of albuterol did not affect airway reactivity in non-OVA-sensitized/OVA-challenged mice. At 0, 2, and 10 mg/mL methacholine challenge doses, P_{enh} (percentage of air) values were, respectively, 105.1%, 92.3%, and 100.0% of the values of saline control animals. Similarly, (R)-albuterol

had no effect on P_{enh} (percentage of air) values of the saline control group. At 0, 2, and 10 mg/mL methacholine doses, non-OVA-sensitized/OVA-challenged mice administered (R)-albuterol had 106.4%, 95.7%, and 101.1% P_{enh} (percentage of air) values of the saline control animals.

DISCUSSION

In this mouse model of asthma, we found both overlapping and distinct actions of the (S)- and (R)-enantiomers of albuterol on key features of allergen-induced airway inflammation and responsiveness to methacholine. (R)-Albuterol significantly reduced the following features of allergen-induced airway inflammation: BAL fluid levels of IL-4 and eosinophils, airway eosinophil infiltration, goblet cell hyperplasia, and mucus occlusion and circulating levels of OVA-specific IgE. Although (S)-albuterol also decreased airway tissue eosinophilia, goblet cell hyperplasia, and mucus plugging of the airways, no significant effect on the influx of eosinophils into the BAL fluid was observed. (S)-Albuterol, but not (R)-albuterol, had adverse effects on airway function by increasing airway edema and hyperresponsiveness in OVA-treated mice.

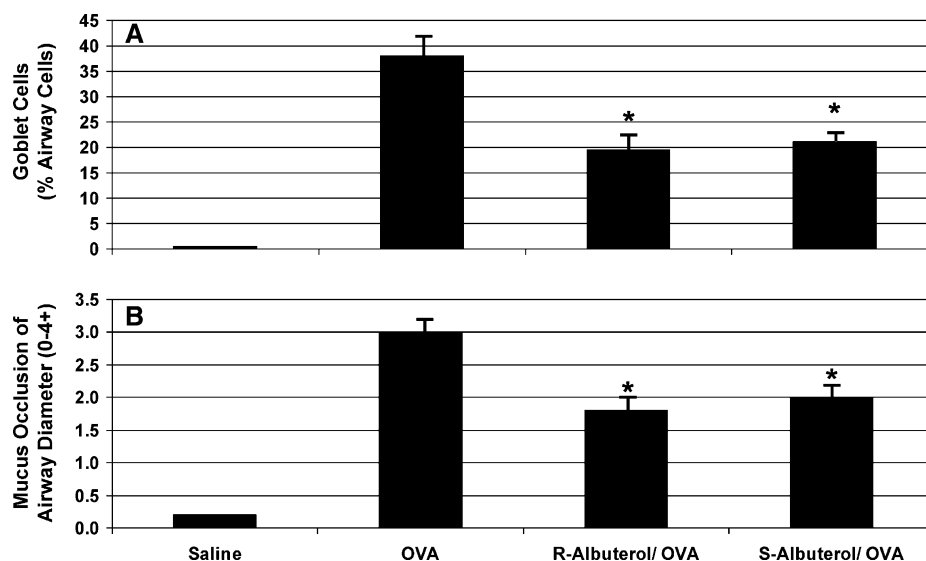


FIG 4. Effect of (R)- and (S)-albuterol enantiomers on allergen-induced airway mucus hypersecretion. The number of goblet cells (**A**) and mucus occlusion of airway diameter (**B**) were determined. * $P < .05$ versus OVA.

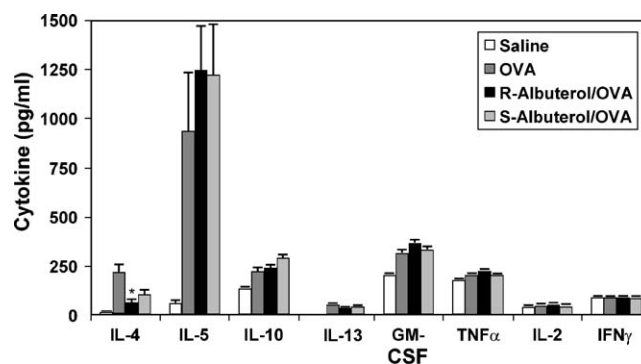


FIG 5. Effect of (R)- and (S)-albuterol enantiomers on BAL fluid cytokine levels in OVA-treated mice. BAL fluid was assayed for T_H1 and T_H2 cytokines. * $P < .05$ versus OVA.

We found in this mouse asthma model that both (S)- and (R)-albuterol inhibited infiltration of eosinophils into airway tissue, but only (R)-albuterol significantly reduced eosinophil influx into BAL fluid. Although a correlation between BAL fluid levels of IL-5 and eosinophilia is typically seen, there was no reduction in the increased IL-5 levels of OVA-treated mice administered either albuterol enantiomer. Short- and long-acting β_2 -adrenergic agonists might facilitate eosinophil apoptosis, thereby reducing airway eosinophilia.¹⁸ Albuterol (0.1-10 μ M) *in vitro* dose dependently decreases colony numbers and increases apoptosis of eosinophil progenitor cells from the blood of patients with asthma.¹⁹ In contrast, racemic albuterol, (R)-albuterol, and (S)-albuterol do not affect apoptosis of antigen-specific human T-cell lines.³

An unexpected finding of this study was the reduction in airway goblet cell hyperplasia and mucus hypersecretion by both the (R)- and (S)-enantiomers of albuterol. Limited data exist regarding the effect of (R)- and (S)-enantiomers of albuterol on airway mucus gland

function. Sympathetic (ie, adrenergic), parasympathetic (ie, cholinergic), and sensory-efferent (ie, tachykinin-mediated) pathways regulate mucus secretion from airway epithelial goblet cells and submucosal glands.²⁰ In human airways the cholinergic response is predominant and is mediated by muscarinic M_3 -receptors on the mucus secretory cells.²⁰ In ovine tracheal epithelial cells (R)-albuterol increased ciliary beat frequency, whereas (S)-albuterol had no significant effect.²¹ Increased mucociliary clearance rates by β_2 -adrenergic agonists have been reported in some patients with asthma.²² The T_H2 cytokines IL-4 and IL-13 have potent effects on mucus secretion.^{23,24} Administration of each cytokine independently stimulates airway mucus accumulation in mice.²⁴ MUC5AC gene expression and BAL fluid mucus protein release are increased in IL-4 transgenic mice.²³ In addition, inhibition of IL-4 by administration of soluble IL-4 receptor reduces airway mucus hypersecretion and inflammatory cell trafficking to the lungs in OVA-treated mice.²⁵ We found that the increased BAL fluid levels of IL-4, but not IL-13,

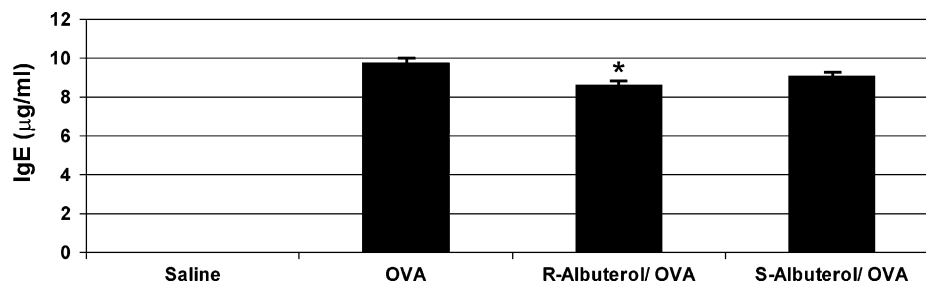


FIG 6. (R)-Albuterol decreases OVA-specific IgE levels in OVA-treated mice. Plasma OVA-specific IgE levels were determined. * $P < .05$ versus OVA.

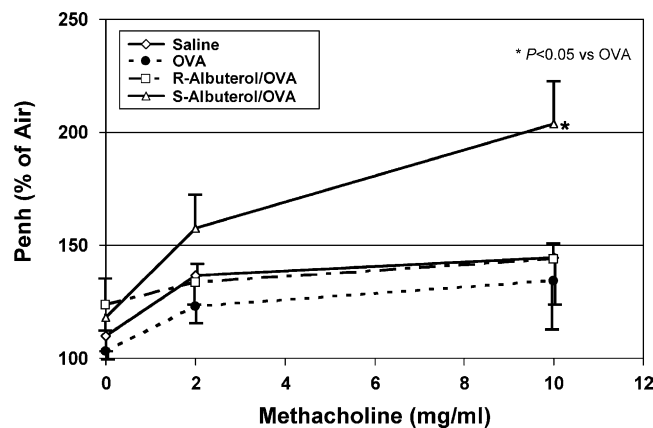


FIG 7. (S)-Albuterol increases allergen-induced airway hyperresponsiveness. The degree of bronchoconstriction to aerosolized methacholine (0, 2, and 10 mg/mL) was expressed as P_{enh} (percentage of air as control). * $P < .05$ versus OVA.

in OVA-treated mice were significantly reduced by (R)-albuterol, with a trend toward IL-4 reduction by (S)-albuterol. Thus the albuterol enantiomers might modulate allergen-induced airway inflammation and mucus hypersecretion through IL-4, rather than IL-5 or IL-13, signaling. We have recently demonstrated a similar discordance between IL-4 and IL-5/IL-13 in mediation of allergen-induced airway eosinophilia and mucus hypersecretion.¹⁴ In a mouse asthma model the selective redox effector factor 1 inhibitor PNRI-299, which inhibits the transcription factor activator protein-1 (AP-1), significantly decreased airway eosinophil infiltration and mucus occlusion and lung gene expression of IL-4 but not IL-5 or IL-13.¹⁴ During T-cell activation, a complex interaction between nuclear factor of activated T cells and AP-1 is necessary for inducible expression of IL-4.²⁶ In contrast, transcriptional regulation of IL-5 and IL-13 might be independent of AP-1 binding.²⁷ The induction of goblet cell hyperplasia and eosinophilia by IL-4 in triple IL-5/IL-9/IL-13-knockout mice further demonstrates the key role IL-4 exerts in the development of the T_H2 phenotype.²⁸ In our studies the reduction in IL-4 in OVA-treated mice administered (R)-albuterol correlated with a decrease in circulating OVA-specific IgE.

We found that (S)-albuterol, but not (R)-albuterol, augmented the interstitial edema observed in OVA-treated

mice, suggesting a proinflammatory effect unique to (S)-albuterol. This effect of (S)-albuterol was not observed in nonsensitized/nonchallenged mice. In 2 sheep models of altered lung fluid balance, the effect of aerosolized racemic albuterol and its (R)- and (S)-enantiomers on lung epithelial permeability has been examined.²⁹ Pretreatment with (S)-albuterol increased the level of albumin in the epithelial lining fluid in sheep receiving histamine to increase lung permeability. This effect was not observed after pretreatment with either (R)-albuterol or its racemate. In sheep receiving an increase in left atrial pressure to increase hydrostatic forces, only (S)-albuterol increased lung water volume. Thus in the presence of altered lung fluid balance, (S)-albuterol, but not (R)-albuterol, might increase lung epithelial permeability. Our finding that (S)-albuterol increases allergen-induced interstitial edema indicates a potential adverse effect of this enantiomer in patients with asthma.

(S)-Albuterol significantly increased bronchial responsiveness to methacholine challenge in OVA-sensitized/OVA-challenged mice, an adverse effect on airway function not shared by (R)-albuterol. In nonsensitized/nonchallenged mice (S)-albuterol did not independently affect the airway response to methacholine. We used whole-body plethysmography to assess airway hyperactivity to methacholine in the study groups. Although

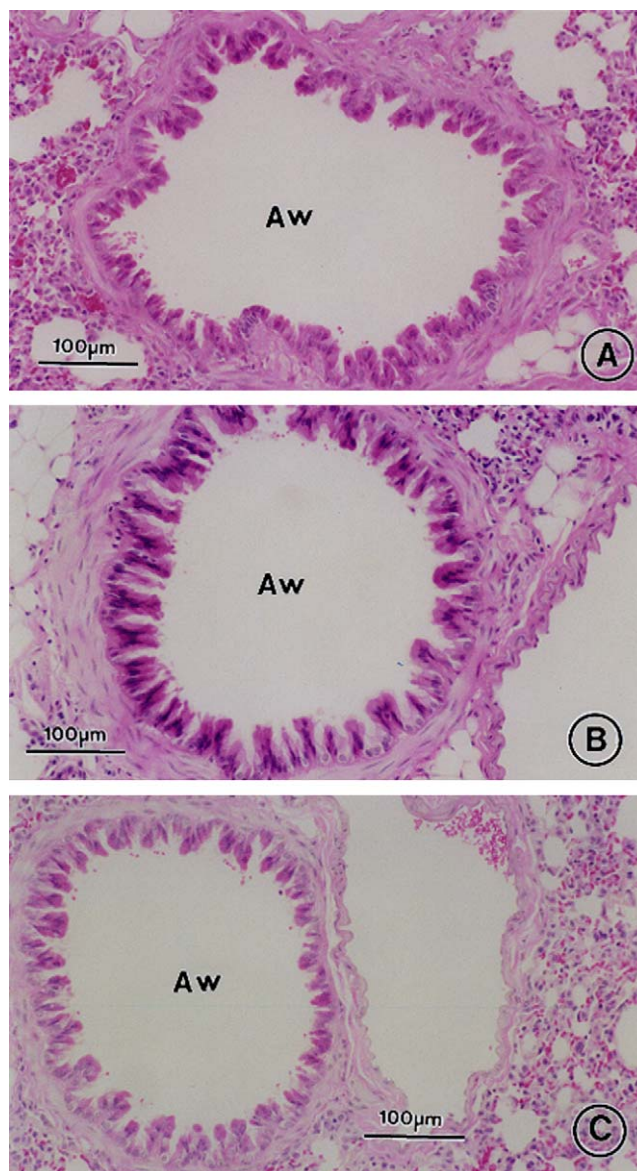


FIG 8. Effect of (R)- and (S)-albuterol enantiomers on airway histology in non-OVA-sensitized/non-OVA-challenged mice. Lung tissue was obtained on day 36 from saline control animals (A), saline-treated mice administered (S)-albuterol (B), and saline-treated mice administered (R)-albuterol (C), and sections were stained with hematoxylin and eosin. AW, Airway. Bars = 100 µm.

there has been recent controversy regarding the use of P_{enh} as an indirect measure of pulmonary mechanics,³⁰⁻³² P_{enh} values correlate well with airway resistance measured directly in anesthetized, tracheotomized, and mechanically ventilated mice.^{10,11} A strong correlation also exists between P_{enh} values and the intensity of the allergen-induced airway eosinophil infiltration in the mouse asthma model.³³

(S)-Albuterol might mediate its augmenting effect on bronchial hyperresponsiveness through several modes of action, including parasympathetic and sympathetic pathways. In OVA-sensitized guinea pigs continuous exposure to (R,S)- and (S)-albuterol, but not (R)-albuterol,

for a 10-day period increased bronchial hyperresponsiveness to both histamine and OVA.⁵ Chronic capsaicin treatment prevented the (R,S)- and (S)-albuterol-induced bronchial hyperresponsiveness in this model to indicate the importance of capsaicin-sensitive sensory nerves in (S)-albuterol-mediated development of airway hyperresponsiveness.⁵ Recent studies by Agrawal et al⁴ indicate that (S)-albuterol activates proconstrictor and proinflammatory pathways in human bronchial smooth muscle cells. (S)-Albuterol significantly increased the expression and activity of $G_{i\alpha-1}$ protein and reduced G_s protein in these cells.⁴ (S)-Albuterol also increased the intracellular free calcium concentration in the bronchial smooth

muscle cells after methacholine stimulation.⁴ These proconstrictor effects of (S)-albuterol were accompanied by stimulation of phosphatidylinositol 3'-OH-kinase and nuclear factor κ B proinflammatory pathways in the smooth muscle cells. (R)-Albuterol induced the opposite effects on $G_{i\alpha-1}$, G_s , and intracellular free calcium concentration in the bronchial smooth muscle cells, which indicates separate mechanisms of action of the enantiomers.⁴

In summary, the actions of the (R)- and (S)-enantiomers of albuterol in the lungs of allergen-sensitized/allergen-challenged mice are complex. Although both enantiomers reduce mucus hypersecretion and trafficking of eosinophils to the lungs after allergen challenge, only the (S)-enantiomer induces airway edema and hyperresponsiveness to methacholine. Additional studies are needed to delineate the specific effects of the (R)- and (S)-enantiomers of albuterol on airway inflammation and hyperresponsiveness in patients with asthma.

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