

Bronchial hyperresponsiveness induced by chronic treatment with albuterol: Role of sensory nerves

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Background: It has recently been suggested that regular treatment with racemic β_2 -adrenoceptor agonists might result in bronchial hyperresponsiveness (BHR) to a range of spasmogens, and this might be due to adverse effects of the distomer. **Objective:** We sought to determine whether BHR induced by means of continuous exposure to racemic and S-albuterol was mediated by sensory nerves.

Methods: Naive or ovalbumin-sensitized guinea pigs were treated for 10 days with RS-, R-, or S-albuterol (1 mg·kg⁻¹·d⁻¹) through subcutaneously implanted minipumps. Lung function was then determined in response to a number of spasmogens and assessed on the basis of an increase in total airway resistance. A separate group of animals were chronically treated with capsaicin (80 mg/kg) before the albuterol treatment.

Results: Treatment with RS- or S-albuterol increased airway responsiveness to bradykinin, leukotriene C₄, and capsaicin in naive guinea pigs ($P < .05$) and to histamine and ovalbumin in immunized guinea pigs ($P < .05$). Chronic treatment with capsaicin prevented the development of RS- and S-albuterol-induced BHR in these models. The bronchodilator efficacy of acute intravenously administered RS-albuterol was unaffected in RS-, R-, or S-albuterol-treated guinea pigs compared with in vehicle-treated animals.

Conclusion: We have provided evidence demonstrating that continuous exposure to RS- and S-albuterol increases bronchial responsiveness to a range of stimuli, an effect not attributed to β -adrenoceptor occupancy or desensitization. Furthermore, capsaicin-sensitive sensory nerves mediate the development of BHR, at least in part. (*J Allergy Clin Immunol* 2002;110:388-94.)

Key words: Albuterol, enantiomers, bronchial hyperresponsiveness, heterogeneity, capsaicin

β_2 -Adrenoceptor agonists are used extensively for the relief of the symptoms of asthma and remain the first choice in bronchodilator therapy.¹ They afford protection against a range of both specific and nonspecific bronchoconstrictor stimuli, including antigen, exercise, and various chemical irritants. Rapid bronchodilation results from their action on airway smooth muscle, antagonizing

Abbreviations used

BHR: Bronchial hyperresponsiveness
LTC₄: Leukotriene C₄
R_L: Lung resistance

muscle contraction induced by a wide range of stimuli. Furthermore, β_2 -adrenoceptor agonists have other actions in the airway, including inhibition of mast cell degranulation and reducing microvascular leakage by inhibiting endothelial cell contraction.² However, it is now recognized that regular treatment with short-acting β_2 -adrenoceptor agonists, such as albuterol,³⁻⁵ fenoterol,^{6,7} and terbutaline,^{8,9} appears to result in a loss of protection against both specific and nonspecific bronchoconstrictor stimuli. Similarly, other consequences of regular treatment with β -agonists include an increase in bronchial hyperresponsiveness (BHR) to a range of spasmogens,^{3,10} exacerbation of the late asthmatic response,¹¹ and a worsening of asthma control.¹² Such changes do not appear to be related to any loss of bronchodilator effect, thereby ruling out β_2 -adrenoceptor desensitization as a mechanism that accounts for these clinical observations.⁵ However, the precise mechanisms whereby β_2 -agonists induce BHR remains to be established.

Few studies have attempted to address the mechanism of this phenomenon directly, although a number of hypotheses have been raised. For example, it has been suggested that regular treatment with β_2 -agonists might allow subjects to tolerate exposure to greater antigen loads¹³ or remain exposed for longer periods of time in the presence of antigen, thereby leading to enhanced penetration of allergen to the lung.¹⁴ This could explain the exacerbation of the underlying inflammatory response that has been observed experimentally and clinically after regular treatment with β -agonists.^{5,15} Most β_2 -agonists administered to patients are available as the racemate, and the increase in BHR observed after regular racemic β_2 -agonist treatment has been suggested to be due to an untoward effect of the S enantiomer of the β_2 -agonist.¹⁶ However, the mechanism by which S-albuterol, which has little or no effect on β -adrenoceptors at relevant concentrations, mediates BHR remains unclear. Many studies have now shown that capsaicin pretreatment reduces BHR induced by a wide range of stimuli, including antigen,¹⁷ platelet-activating factor,¹⁸ 15-hydroperoxyicosatetraenoic acid,¹⁹ toluene diisocyanate,²⁰ LPS,²¹ ozone,²² and propranolol.²³ In the guinea pig, chronic treatment with capsaicin is associated with a greater than

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90% loss in neuropeptide content and leads to the complete abrogation of capsaicin-induced bronchoconstriction,²⁴ suggesting chemical inactivation of sensory nerve function. In the present study we have therefore examined whether BHR induced by means of continuous exposure with racemic albuterol and its individual enantiomers (R and S) is mediated through capsaicin-sensitive nerves.

METHODS

Animals

Male Dunkin-Hartley guinea pigs (250-450 g, Charles River) were used throughout this study. Guinea pigs were housed in the animal unit on site for at least 24 hours before experimentation and given free access to food and water.

Immunization protocol

In some experiments guinea pigs were actively immunized to ovalbumin (10 μ g) in saline (0.1 mL) added to aluminum hydroxide (0.9 mL). This was injected intraperitoneally into recipients, and after 14 days, this procedure was repeated. After a further 7 days, blood was collected by means of cardiac puncture after achievement of anesthesia with urethane (1.75 g/kg administered intraperitoneally) and added to heparin (0.2 mL; 100 U/mL). Blood was centrifuged (1500g for 15 minutes), and the plasma was removed and stored (-20°C). For passive sensitization, the anti-ovalbumin guinea pig plasma (1 mL per animal administered intravenously) was injected 1 day before the implantation of minipumps.

Capsaicin treatment

Naive or passively sensitized guinea pigs were anesthetized with an intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg). An intraperitoneal injection of mepyramine (10 mg/kg) and R-albuterol (1 mg/kg) was then administered. Fifteen minutes later, animals were subcutaneously injected with capsaicin or vehicle (ethanol/Tween 80/0.9% saline, 1:1:8) in the dorsal region of the neck. Animals were treated twice a day (6 hours apart) on 3 consecutive days, receiving a total of 80 mg/kg capsaicin, as previously described.²⁵

Implantation of minipumps

Animals were shaved in the dorsal region of the neck. After achievement of light anesthesia with halothane, an approximately 2-cm-long incision was made. Blunt scissors were inserted laterally and opened, separating the epidermal layer and connective tissue. Osmotic minipumps (Alzet model 2002) were then implanted, and the wound was sutured. The minipumps contained RS-albuterol (1 mg $\cdot$ kg $^{-1}\cdot$ d $^{-1}$), R-albuterol (1 mg $\cdot$ kg $^{-1}\cdot$ d $^{-1}$), S-albuterol (1 mg $\cdot$ kg $^{-1}\cdot$ d $^{-1}$), or vehicle (sterile 0.9% physiologic saline). In the case of vehicle- and capsaicin-treated animals, minipumps were implanted 1 hour after the last injection of capsaicin.

Measurement of pulmonary function

On the day of the experiment to perform lung function, guinea pigs were anesthetized by means of an intraperitoneal injection of urethane (1.75 g/kg), and the minipumps were removed. Lung function was assessed 1 hour later. Guinea pigs were ventilated through a tracheal cannula connected to a pneumotachograph and a pressure transducer (± 2 cm H₂O, model MP-45-14-871, Validyne Engineering). Changes in airflow were measured by using a lung function recording system (Pulmonary Monitoring System, version 3.0, Mumed) and displayed in real time on a personal computer. The flow signal was integrated to give a measure of tidal volume. An intrathoracic cannula was inserted between the third and fifth intercostal space and connected to the negative side of the pressure transducer

(± 20 cm H₂O, Validyne). The positive side of the transducer was connected to the side of the pneumotachograph proximal to the animal. The difference between mouth and thoracic pressure was used as a measure of transpulmonary pressure. Total lung resistance (R_L ; in square centimeters of water per liter per second) was calculated from flow, tidal volume, and transpulmonary pressure by means of integration. The jugular vein was cannulated for the administration of spasmogens, with blood pressure and heart rate being recorded with a pressure transducer attached to the carotid artery.

Bronchoconstriction was measured in response to histamine (0.5, 1, 2, and 5 μ g/kg) administered at 5-minute intervals. Bradykinin (2 and 5 μ g/kg) and leukotriene C₄ (LTC₄; 0.1, 0.2, and 0.4 μ g/kg) were administered in different groups of animals at 15-minute intervals.

In bronchodilator experiments bronchoconstriction was induced by means of continuous intravenous administration of bombesin (0.1-0.2 μ g $\cdot$ kg $^{-1}\cdot$ min $^{-1}$). After reaching a submaximal level of bronchoconstriction (approximately 200% above baseline R_L), RS-albuterol was intravenously administered through a separate cannula to reverse the bombesin-induced bronchoconstriction.

Plasma albuterol concentration

Guinea pigs were implanted with minipumps containing RS-salbutamol (1 mg $\cdot$ kg $^{-1}\cdot$ d $^{-1}$) for a period of 10 days, after which time samples of plasma were obtained (12 mL per animal, $n = 4$) for subsequent analysis of plasma salbutamol levels. Guinea pig plasma was spiked with an internal standard ([²H₃]-salbutamol) and loaded onto a preconditioned Bond Elute Si column, and the sample (0.5 mL) was allowed to pass through the column. The column was then washed and dried by using the Vac Elute system that was connected to a vacuum pump. The salbutamol that was retained in the column was then eluted, collected, and dried under a stream of nitrogen gas at 60°C for 15 minutes. The dried samples were derivatized, and a 2- μ L aliquot of the derivatized sample was analyzed by using a gas chromatography-mass spectrometry system, with helium gas at 0.6 mL/min as a carrier. Analysis was accomplished by means of selected monitoring of ions at m/e 369 for the trimethylsilyl ether derivative of salbutamol and m/e 372 for the trimethylsilyl ether derivative of [²H₃]-salbutamol.

Drugs

The following reagents were used: chicken egg ovalbumin, urethane, histamine, bradykinin, bombesin, LTC₄, Tween 80, mepyramine, and capsaicin (Sigma); aluminum hydroxide solution (Sanofi); RS-albuterol, R-albuterol, and S-albuterol (Sepracor); and halothane (Rhone-Merieux).

Data analysis

Bronchoconstriction was calculated as a percentage increase in total R_L . Bronchodilation was calculated as the percentage reversal of the increase in resistance by bombesin. The bronchodilator potency was calculated as the effective dose of albuterol that reversed bombesin-induced bronchoconstriction by 50%. Data are expressed as arithmetic means $\pm$ SEM. The effect of drug treatment on the position of the dose-response curve was analyzed by means of 1-way ANOVA with repeated measures, in which appropriate differences in mean values were compared with the Dunnett t test for multiple comparisons and considered statistically significant at a P value of less than .05.

RESULTS

Effect of continuous albuterol treatment on airway responsiveness

Naive study. The plasma concentration after chronic treatment with RS-salbutamol (1 mg $\cdot$ kg $^{-1}\cdot$ d $^{-1}$ for 10 days)

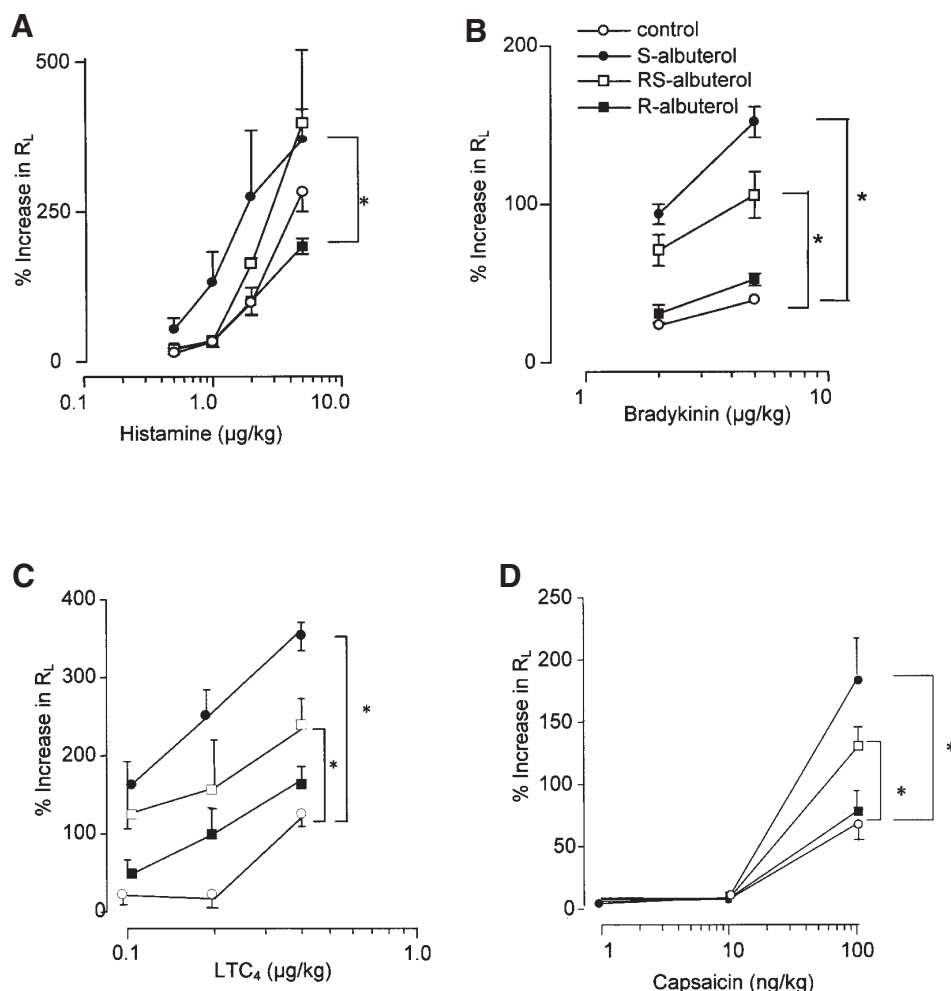


FIG 1. Bronchoconstriction (percentage increase in baseline R_L) to histamine (**A**), bradykinin (**B**), LTC_4 (**C**), and capsaicin (**D**) in animals treated for 10 days with RS-, R-, or S-albuterol ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) or vehicle (0.9% saline). * $P < .05$ versus saline ($n = 6-8$ for each treatment group).

was $3.65 \pm 0.7 \text{ ng/mL}$ ($n = 4$, measured in duplicate). There was no difference in baseline lung function (R_L , $n = 6-8$ per group) between saline-treated animals ($183 \pm 12 \text{ cm H}_2\text{O} \cdot \text{L}^{-1} \cdot \text{s}^{-1}$) and animals treated with RS- ($176 \pm 11 \text{ cm H}_2\text{O} \cdot \text{L}^{-1} \cdot \text{s}^{-1}$), R- ($170 \pm 8 \text{ cm H}_2\text{O} \cdot \text{L}^{-1} \cdot \text{s}^{-1}$), or S-albuterol ($174 \pm 9 \text{ cm H}_2\text{O} \cdot \text{L}^{-1} \cdot \text{s}^{-1}$).

The intravenous administration of histamine, bradykinin, LTC_4 , and capsaicin increased total airway resistance in a dose-dependent manner. Animals treated for 10 days with albuterol were significantly more responsive to bradykinin, LTC_4 , and capsaicin compared with saline-treated animals ($P < .001$, ANOVA in each case). This was reflected by a significantly greater bronchoconstrictor response to the different spasmogens in animals treated with RS-albuterol compared with those treated with saline ($P < .05$, Dunnett t test; $n = 6$; Fig 1). Furthermore, animals treated with S-albuterol were also significantly more responsive to each of the spasmogens tested compared with saline-treated animals ($P < .05$, $n = 6$, Fig 1). However, animals treated with R-albuterol showed no significant increase in airway responsiveness to any of

the spasmogens tested ($P > .05$, $n = 6$, Fig 1). In further studies bronchodilation to intravenously administered RS-albuterol was performed in saline- and albuterol-treated animals. The bronchodilator potency (effective dose of albuterol that reversed bombesin-induced bronchoconstriction by 50%, $n = 5$) was not significantly different between saline-treated ($0.88 \pm 0.16 \mu\text{g/kg}$) and RS- ($0.96 \pm 0.18 \mu\text{g/kg}$), R- ($1.01 \pm 0.2 \mu\text{g/kg}$), and S-albuterol-treated ($1.02 \pm 0.22 \mu\text{g/kg}$) animals.

Passive sensitization study. There was no significant difference in baseline R_L ($n = 5$) in passively sensitized animals treated with saline ($195 \pm 10 \text{ cm H}_2\text{O} \cdot \text{L}^{-1} \cdot \text{s}^{-1}$) and RS- ($185 \pm 10 \text{ cm H}_2\text{O} \cdot \text{L}^{-1} \cdot \text{s}^{-1}$), R- ($179 \pm 10 \text{ cm H}_2\text{O} \cdot \text{L}^{-1} \cdot \text{s}^{-1}$), and S-albuterol ($180 \pm 9 \text{ cm H}_2\text{O} \cdot \text{L}^{-1} \cdot \text{s}^{-1}$). The intravenous administration of histamine and ovalbumin increased total airway resistance (Fig 2). Animals treated for 10 days with RS- and S-albuterol were significantly more responsive to both histamine and ovalbumin compared with saline-treated animals ($P < .05$, ANOVA in each case). This was reflected by a significantly greater bronchoconstrictor response to histamine and

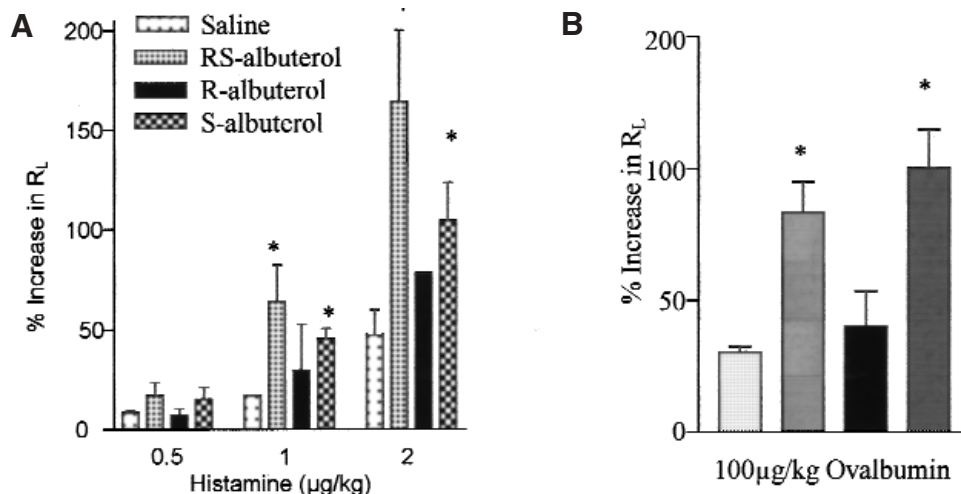


FIG 2. Bronchoconstriction (percentage increase in baseline R_L) to histamine (A) and ovalbumin (B) in ovalbumin-sensitized guinea pigs after continuous exposure to RS-, R-, or S-albuterol ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) or vehicle (0.9% saline). * $P < .05$ versus saline ($n = 6-8$ for each group of animals).

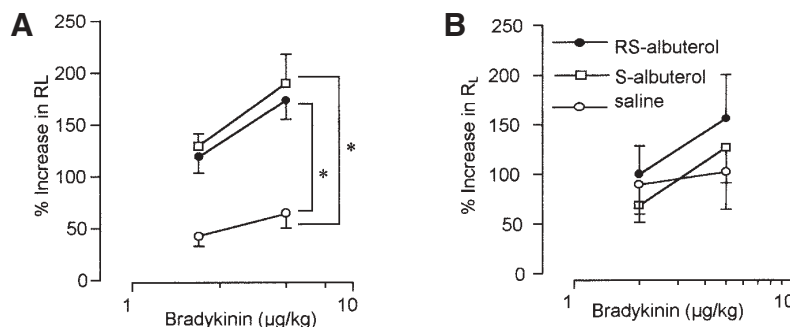


FIG 3. Effect of treatment with capsaicin and RS-albuterol on airway responsiveness to bradykinin (2 and 5 $\mu\text{g/kg}$) in naive guinea pigs. Animals were pretreated for 3 days with vehicle of capsaicin (ethanol/Tween 80/saline, 1:1:8; A) or capsaicin (80 mg/kg; B). This was followed by 10-day subcutaneous infusion of RS-albuterol ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) or its vehicle (0.9% saline). * $P < .05$ versus saline ($n = 6-8$ for each group of animals).

ovalbumin in passively sensitized animals chronically treated with RS-albuterol ($P < .05$ vs saline in each case, Dunnett t test) and S-albuterol ($P < .05$ vs saline), but not R-albuterol ($P > .05$ vs saline).

Effect of capsaicin on albuterol-induced BHR

Naive and passively sensitized guinea pigs treated with capsaicin failed to respond to intravenous administration of capsaicin (100 $\mu\text{g/kg}$) compared with vehicle-treated animals (data not shown).

Capsaicin treatment significantly impaired the ability of albuterol to induce BHR to bradykinin in naive animals ($P < .05$, ANOVA; Fig 3). Vehicle-treated animals continuously exposed for 10 days to RS- or S-albuterol were significantly more responsive to bradykinin compared with saline-treated animals ($P < .05$, Dunnett t test; Fig 3, A). In contrast, animals chronically treated with capsaicin failed to exhibit BHR after exposure to RS- or S-albuterol ($P > .05$; Fig 3, B).

Capsaicin treatment of passively immunized guinea pigs significantly inhibited the albuterol-induced BHR to

histamine ($P < .05$, ANOVA; Fig 4, A and B) and ovalbumin ($P < .05$, ANOVA; Fig 4, C and D). Passively sensitized guinea pigs continuously exposed for 10 days to RS- or S-albuterol were significantly more responsive to histamine ($P < .05$ in each case; $n = 6$; Fig 4, A) and ovalbumin ($P < .05$ in each case; $n = 6$; Fig 4, B) compared with saline-treated animals. In contrast, chronic treatment with capsaicin prevented the development of BHR to RS- and S-albuterol in response to histamine (Fig 4, C) and ovalbumin (Fig 4, D).

DISCUSSION

We have extended previously published findings that regular treatment with S-albuterol induces BHR to a number of different stimuli. However, we have also demonstrated the novel finding that this phenomenon appears to be dependent on a capsaicin-sensitive pathway, highlighting a role for sensory nerves in this response.

Sympathomimetic bronchodilators exert their bronchodilator action primarily through the activation of β_2 -

Asthma, rhinitis,
other respiratory
diseases

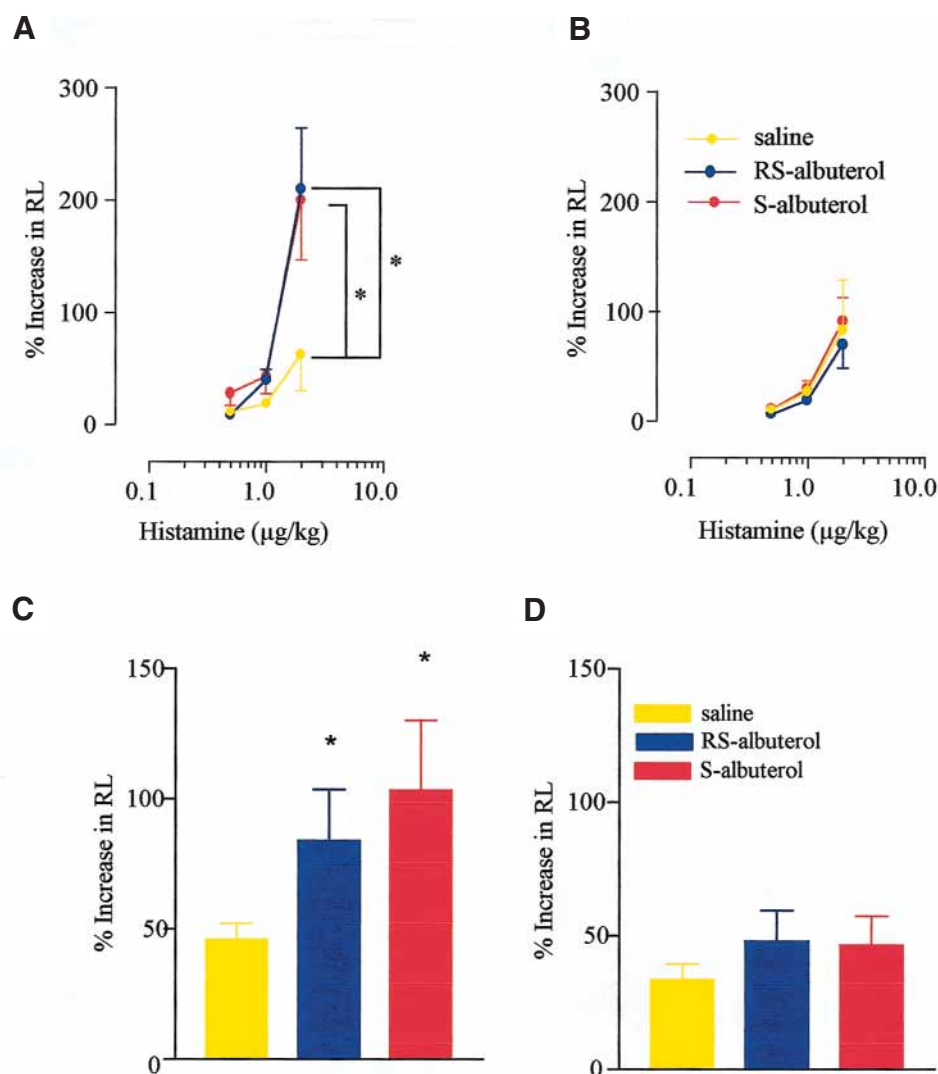


FIG 4. Effect of treatment with capsaicin and RS- and S-albuterol on airway responsiveness to histamine (0.5, 1, and 2 $\mu\text{g/kg}$) and ovalbumin (100 $\mu\text{g/kg}$) in immunized guinea pigs. Animals were pretreated for 3 days with vehicle (ethanol/Tween 80/saline, 1:1:8; **A** and **C**) or capsaicin (80 mg/kg; **B** and **D**). This was followed by 10-day subcutaneous infusion of RS-albuterol (1 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$) or its vehicle (0.9% saline). * $P < .05$ versus saline ($n = 6-8$ for each group of animals).

adrenoceptors, and this is caused, almost exclusively, by the actions of the eutomer, which in the case of albuterol (and other β_2 -adrenoceptor agonists) is the R enantiomer, and the distomer (S enantiomer) makes little or no contribution to bronchodilator efficacy.²⁶ We have confirmed previous studies showing that chronic treatment with RS-albuterol is not associated with loss of bronchodilator activity.²⁷ After chronic administration of albuterol in the guinea pig, the plasma levels were found to be 3.89 ± 0.66 ng/mL. In treating asthma the usual inhaled dose of albuterol is 0.8 to 1.6 mg/d (for adults), and although plasma levels of this agonist are not known after chronic treatment, it has been shown that peak plasma levels between 10 and 15 ng/mL were observed 30 minutes after inhalation of 0.54 mg of albuterol administered by means of metered-dose inhaler.²⁸ The concentration decreased to 6

ng/mL after 60 minutes and was sustained for 4 hours. Therefore the plasma levels achieved in our experiments after 10-day subcutaneous exposure with RS-albuterol (3-4 ng/mL) are in the order of that observed during regular treatment in asthmatic subjects. Furthermore, ingestion of racemic albuterol (4 mg administered 4 times daily) produces plasma levels ranging from 10 to 15 ng/mL.²⁹ In severe asthma very high doses are administered by means of nebulization, increasing plasma levels to greater than 340 ng/mL,³⁰ although these are likely to be peak plasma levels, whereas in our study we had continuously moderate plasma levels. Thus the adverse effects of chronic administration of β_2 -adrenoceptor agonists occur within the dose range that occurs in subjects with asthma, and we believe the results are unlikely to be due to β_2 -adrenoceptor desensitization because the bronchodilator effect was

not compromised. It is now well established that BHR is defined by an increase in the reactivity and sensitivity of the airways after exposure to a range of chemical and physical stimuli,³¹ a phenomenon that is heterogeneous and dependent on the agonist that is used. This is consistent with the view that various pharmacologic stimuli are divided into direct- or indirect-acting spasmogens, with asthmatic subjects showing a preference for these indirect-acting stimuli.³² A number of studies have shown that intravenous infusion of (+)-isoprenaline and its enantiomers induced BHR to histamine and bombesin in the guinea pig,^{16,33} suggesting that BHR to histamine was independent of β -adrenoceptor activation. Similarly, infusion of albuterol and adrenaline also induced BHR to histamine.^{27,33,34} However, we have investigated the effect of the racemate and individual enantiomers of albuterol to a range of different stimuli in the same study. Chronic subcutaneous infusion of RS-albuterol for 10 days induced BHR to bradykinin, LTC₄, and capsaicin. Similarly, treatment with S-albuterol promoted BHR to bradykinin, LTC₄, capsaicin, and histamine. In contrast, R-albuterol had no effect on airway responsiveness to any of the spasmogens tested. The lack of effect of R-albuterol on BHR suggests that the adverse effects observed after treatment with the racemate primarily lies with the S-enantiomer and is therefore unrelated to β -adrenoceptor desensitization.

Clinical studies in asthma have found that chronic treatment with β -adrenoceptor agonists induces BHR to a number of bronchoconstrictor stimuli, including methacholine,⁶ histamine,³⁵ exercise,³⁶ and allergen.³ It was of potential interest that bronchoconstriction to allergen after chronic albuterol treatment of asthmatic subjects was far greater than the response to methacholine,^{3,37} suggesting that allergic status might amplify pathways stimulated by allergen challenge. However, in passively sensitized guinea pigs, chronic treatment with R-albuterol once again failed to elicit BHR to any of the spasmogens tested, although guinea pigs chronically treated with both RS- or S-albuterol had significantly increased responsiveness to both histamine and ovalbumin. The results obtained in passively sensitized animals support previous studies investigating RS-albuterol-induced BHR in passively sensitized guinea pigs³⁸ and clinical studies showing augmented bronchoconstriction to allergen challenge in subjects receiving regular β_2 -agonists.^{3,37} It is of interest that BHR to histamine after chronic treatment with β_2 -agonist is consistently observed in passively sensitized animals. This might explain a number of the conflicting reports in the literature concerning the effect of regular treatment with β_2 -agonist on BHR to various stimuli, including histamine, in animal models. It is clear that allergic status plays an important role in this regard, and different sensitization procedures might contribute to the variability reported in the literature surrounding this phenomenon.³⁹

A considerable body of evidence supports the notion that BHR might be linked to alterations in afferent activity. Thus exacerbation of underlying inflammation promotes selective augmentation of responsiveness to bradykinin compared with methacholine. Asthmatic sub-

jects are considerably more responsive to indirect-acting stimuli, including exercise and bradykinin, compared with normal subjects,³² and repeated exposure to β_2 -adrenoceptor agonists promotes greater changes in responsiveness to allergen compared with methacholine.³⁷ Therefore it is of interest that in experimental models chronic treatment with capsaicin, which leads to an impairment of sensory nerve activity, abolishes BHR to a variety of stimuli, including antigen,¹⁷ platelet-activating factor,¹⁸ 15-hydroperoxyeicosatetraenoic acid,¹⁹ toluene diisocyanate,²⁰ LPS,²¹ ozone,²² and propranolol.²³ In the present study we have shown that 3-day chronic treatment with capsaicin resulted in the complete abrogation of capsaicin-induced bronchoconstriction, suggesting chemical inactivation of sensory nerve function. This is consistent with previous studies showing that chronic treatment with capsaicin in the guinea pig is associated with a greater than 90% loss in neuropeptide content.²⁴ However, in capsaicin-treated naive guinea pigs, the BHR to bradykinin in RS- and S-albuterol-treated animals was also abolished. Furthermore, in sensitized animals after treatment with RS- and S-albuterol, BHR to ovalbumin and histamine was suppressed. These results support the view that capsaicin-sensitive pathways mediate BHR to regular racemic and S-albuterol treatment in the guinea pig. The hypothesis is consistent with previous reports demonstrating that intravenous infusion of isoprenaline induced BHR to histamine and bombesin in the guinea pig that was pre-empted by bilateral vagotomy.¹⁶ Further evidence to support a role of sensory nerves in BHR to S-albuterol comes from additional experiments in naive guinea pigs chronically treated with RS- or S-albuterol. These animals had BHR to capsaicin, whereas R-albuterol-treated animals were unaffected, indicating a possible involvement of sensory nerves in the development of RS- and S-albuterol-induced BHR. The exact molecular events that occur to explain this observation remain unclear at present. However, previous studies have shown that the catechol moiety of β_2 -adrenoceptor agonists increases Ca²⁺ channel activity and promotes phospholipase C activation, thereby possibly augmenting neuronal activity irrespective of stereoselectivity.⁴⁰

In conclusion, chronic treatment with RS- and S-albuterol induces BHR to a range of spasmogens, including histamine, bradykinin, LTC₄, and ovalbumin. Furthermore, chronic treatment with capsaicin prevented the development of RS- and S-albuterol-induced BHR, suggesting that capsaicin-sensitive sensory nerves are involved in the development of S-albuterol-induced BHR.

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