Eur Respir J 2005; 25: 864–872
DOI: 10.1183/09031936.05.00102203

Effects of salbutamol and enantiomers on allergen-induced asthmatic reactions and airway hyperreactivity

F.J. Westerhof, A.B. Zuidhof, L. Kok, H. Meurs and J. Zaagsma

ABSTRACT: Salbutamol consists of a racemic mixture of *R*- and *S*-salbutamol. *R*-salbutamol (levalbuterol) is the active bronchodilating enantiomer, whereas *S*-salbutamol is thought to be pharmacologically inactive or to exert adverse effects.

This study evaluated the bronchoprotective effects of inhalation of therapeutically relevant doses of the racemate and individual enantiomers in guinea pigs.

It was found that basal airway reactivity to histamine was similarly reduced 30 min after inhalation of equivalent doses of *RS*- and *R*-salbutamol; this protective effect disappeared within 3 h. Inhalation of *RS*- and *R*-salbutamol 30 min before and 5.5 h after allergen challenge suppressed allergen-induced airway hyperreactivity to histamine after the early and late asthmatic reaction, completely inhibiting the early asthmatic reaction and tending to reduce the development of the late asthmatic reaction. At 5 h after allergen challenge, the inhibition of airway hyperreactivity was more pronounced in animals treated with *R*-salbutamol compared to racemate-treated animals. Both basal airway reactivity and allergen–induced hyperreactivity were not affected by *S*-salbutamol. Inflammatory cell infiltration was not affected by the racemate or the individual enantiomers.

In conclusion, inhalation of therapeutically relevant doses of *R*- and *RS*-salbutamol effectively suppress allergen-induced airway reactivity after the early and late asthmatic reactions, the *R*-enantiomer being slightly more potent with respect to early airway reactivity than the racemate. No adverse effects were observed for the *S*-enantiomer.

KEYWORDS: Airway hyperreactivity, allergic asthma, enantiomers, inflammation, salbutamol

AFFILIATIONS
Dept of Molecular Pharmacology, University Centre for Pharmacy, Groningen, The Netherlands.

CORRESPONDENCE
J. Zaagsma
Dept of Molecular Pharmacology
University Centre for Pharmacy
A. Deusinglaan 1
9713 AV Groningen
The Netherlands
Fax: 31 503636908
E-mail: j.zaagsma@rug.nl

Received:
September 10 2003
Accepted after revision:
December 27 2004

SUPPORT STATEMENT
This study was supported by Sepracor, Inc. (Marlborough, MA, USA).

Although β_2-agonists, including salbutamol, are the most effective bronchodilators available for the treatment of asthma, long-term use of these drugs has been associated with adverse effects, including the development of airway hyperreactivity (AHR) and tolerance to β_2-agonist-induced bronchoprotection against allergic and pharmacological stimuli [1–4]. These adverse effects are thought to be caused by desensitisation of β_2-adrenoceptors on airway smooth muscle as well as on inflammatory cells [5–7].

Salbutamol is a mixture of *R*- and *S*-salbutamol. Potent agonist-induced bronchodilator properties have been ascribed to *R*-salbutamol, whereas the *S*-enantiomer was, for a long time, thought to be pharmacologically inactive [8]. However, recent studies have suggested that *S*-salbutamol may exhibit the potential to exert adverse effects, counteracting the bronchoprotective properties of the *R*-enantiomer. Thus, in mild asthmatics, a single inhalation of *S*-salbutamol was found to increase airway responsiveness to methacholine 3 h after administration [9], although this was not invariably found by others [10, 11]. *In vitro*, *S*-salbutamol was shown to increase the contractile response to carbachol in guinea pig tracheal smooth muscle preparations [12], intracellular calcium levels in bovine airway smooth muscle cells [13], and histamine and interleukin-4 production in immunoglobulin (Ig)E-stimulated murine mast cells [14]. If *S*-salbutamol has biological activities, counteracting the bronchodilator properties of the *R*-enantiomer, enantiomerically pure formulations might be recommended in asthma therapy.

To date, the effects of the individual salbutamol enantiomers on allergen-induced early (EAR) and late (LAR) asthmatic reactions, AHR and airway inflammation have not been established. Therefore, in this study, a guinea pig model of allergic asthma was used to compare

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

the protective effects of inhaled racemic salbutamol and the equivalent doses of the *R*- and *S*-enantiomers on the magnitudes of the EAR and LAR, AHR to histamine after both the EAR and LAR, and airway inflammation after the LAR. In addition, the acute protective effects against histamine-induced bronchoconstriction were assessed in both normoreactive and hyperreactive airways, after both the EAR and the LAR.

MATERIALS AND METHODS

Animals

Male specific-pathogen-free guinea pigs (Harlan, Gannat, France), weighing 500–700 g, were used in the present study. All animals were sensitised to ovalbumin (grade III ovalbumin; Sigma Chemical Co., St Louis, MO, USA) at 3 weeks of age as described previously [15]. In order to obtain a shift to IgE-class antibodies, an allergen solution containing 100 $\mu g \cdot mL^{-1}$ ovalbumin and 100 $mg \cdot mL^{-1}$ $Al(OH)_3$ (J.T. Baker Chemical Co., Phillipsburg, NJ, USA) in saline was used. The allergen solution was gently rotated for 60 min to obtain an alu-gel; 0.5 mL was injected intraperitoneally and another 0.5 mL was divided over seven intracutaneous injection sites in the proximity of lymph nodes in the paws, lumbar regions and neck. Animals were operated on 3 weeks after sensitisation and used experimentally 4–8 weeks after sensitisation. The animals were housed in individual cages in climate-controlled animal quarters and given water and food *ad libitum*. All protocols described were approved by the University of Groningen Committee for Animal Experimentation (University of Groningen, Groningen, The Netherlands).

Measurement of airway function

Airway function was assessed by on-line measurement of pleural pressure (P_{pl}) under unrestrained conditions as described previously [15]. In brief, a small fluid-filled latex balloon connected to a saline-filled cannula was surgically implanted inside the thoracic cavity. The free end of the cannula was driven subcutaneously to and permanently attached in the neck of the animal. The pleural balloon was connected to a pressure transducer (Ohmeda DTX™; Spectramed, Bilthoven, The Netherlands) *via* an external fluid-filled cannula. P_{pl} was measured continuously using an on-line computer system. Using a combination of flow measurement with a pneumotachograph, implanted inside the trachea, and pressure measurement with the pleural balloon, it has previously been shown that changes in P_{pl} are linearly correlated with changes in airway resistance [15]. In this way, airway function can be monitored repeatedly and continuously for prolonged periods of time, with the animals unaware of the measurements being taken. However, since guinea pigs are nose breathers, changes in P_{pl} may possibly also reflect upper airway resistance. In a carefully designed study, FINNEY and FORSBERG [16] quantified the involvement of the nose, using a technique that allowed the separate provocation and measurement of respiratory function in the nasal and lower airways of anaesthetised guinea pigs. It was found that aerosols of histamine, carbachol and ovalbumin delivered to the nose of sensitised animals did not affect nasal conductance, even at doses 100-times higher than that required to reduce pulmonary conductance. Thus, nasal resistance is unaffected by the aerosol drugs studied. The nasal contribution to the changes in P_{pl} was, therefore, considered negligible.

Provocation procedures

Ovalbumin and histamine provocations were performed by inhalation of aerosolised solutions produced by a DeVilbiss nebuliser (DeVilbiss 646; DeVilbiss, Somerset, PA, USA), driven by an airflow of 8 $L \cdot min^{-1}$, resulting in an output of 0.33 $mL \cdot min^{-1}$. The provocations were performed in a specially designed perspex cage with a volume of 9 L, in which the animals could move freely [15]. The animals were habituated to the experimental conditions on 2 sequential days $\geq$1 week after the surgery, when preoperative weight was restored. On the 1st day, the animals were placed into the provocation cage unconnected to the pressure transducer. After an adaptation period of $\geq$30 min, three consecutive provocations with saline were performed, each provocation lasting 3 min, and separated by 7-min intervals. The next day, this procedure was repeated with the animals attached to the measurement system.

On the experimental days following the habituational procedure, allergen and histamine provocations were performed as described below. All provocations were preceded by an adaptation period of $\geq$30 min, followed by two consecutive provocations with saline as described above. Baseline P_{pl} was calculated by averaging the P_{pl} of the last 20 min of the adaptation period. In order to assess airway reactivity to histamine, histamine provocations were performed with an initial concentration of 25 $\mu g \cdot mL^{-1}$ in saline, followed by increasing dosage steps of 25 $\mu g \cdot mL^{-1}$. Each provocation lasted 3 min and provocations were separated by 7-min intervals. Animals were challenged until P_{pl} was increased by >100% for $\geq$3 min consecutively. P_{pl} returned to baseline within 15 min after the last histamine provocation. Airway reactivity to histamine was expressed as the provocative concentration of histamine causing a 100% increase in P_{pl} (PC_{100}), which was calculated by linear intrapolation of the concentration–P_{pl} response curve.

Allergen provocations were performed by increasing aerosol concentrations of 0.1, 0.3 and 0.5 $mg \cdot mL^{-1}$ ovalbumin in saline for 3 min, each separated by 7-min intervals. Allergen inhalations were discontinued when an increase in P_{pl} of >100% was observed. Using these conditions, none of the animals developed anaphylactic shock after allergen provocation.

Bronchoalveolar lavage

At 25 h after the ovalbumin provocation, bronchoalveaolar lavage (BAL) was performed in all animals as described previously [17]. In short, the animals were anaesthetised with 20 $mg \cdot kg$ body $weight^{-1}$ Brietal® (methohexital sodium; Eli Lilly, Nieuwegein, The Netherlands), 35 $mg \cdot kg$ body $weight^{-1}$ Ketalar® (ketamine hydrochloride; Parke-Davis, Hoofddorp, The Netherlands) and 6 $mg \cdot kg$ body $weight^{-1}$ Rompun® (2-(2,6-xylidino)-5,6-dihydro-4*H*-1,3-thiazine-hydrochloride, methylparaben; Bayer, Leverkusen, Germany), administered intraperitoneally. The trachea was exposed and cannulated, and the lungs were lavaged gently using 5 mL of sterile saline at 37℃, followed by three subsequent 8-mL aliquots of saline.

The recovered lavage samples were cooled on ice and centrifuged at $200 \times g$ for 10 min at 4℃. The pellets were combined and resuspended in phosphate-buffered saline at a final volume of 1 mL and total cell numbers were counted using a Coulter counter. For cytological examination, cytospin preparations were stained with May–Grünwald and Giemsa stains (Sigma). Cell differentials were determined by counting $\geqslant$400 cells in duplicate.

Experimental protocols

Effects of *RS*-, *R*- and *S*-salbutamol on basal airway reactivity

In the first group of animals, the effect and duration of action of inhaled racemic salbutamol, *R*-salbutamol and *S*-salbutamol (gifts from Sepracor, Inc., Marlborough, MA, USA) on basal airway reactivity were assessed. At 30 min after the assessment of basal PC_{100}, aerosols of salbutamol enantiomers (1.25 mM for 3 min) or the equivalent concentration of racemate (2.5 mM for 3 min) were inhaled, and PC_{100} was measured again after 30 min and 1.5, 3, 6, 12 and 24 h. The nebuliser concentrations of the drugs were selected on the basis of preliminary experiments with *RS*-salbutamol in order to provide a clinically relevant three-to-four-fold shift in histamine reactivity.

Bronchoprotective effects of *RS*-, *R*- and *S*-salbutamol

In the second series of animals, the effects of racemic salbutamol and the individual enantiomers on allergen-induced EAR and LAR, AHR after the EAR and LAR, and airway inflammation were determined. In the 1st week, all guinea pigs inhaled vehicle (saline) for 3 min, 30 min before and 5.5 h (between the EAR and LAR) and 23.5 h (after the LAR) after ovalbumin provocation.

In the 2nd week, the animals were subdivided into four groups, inhaling either saline (control group), or *RS*-salbutamol (2.5 mM), *R*-salbutamol (1.25 mM) or *S*-salbutamol (1.25 mM) for 3 min at the time points indicated above. In each animal, allergen provocations performed in weeks 1 and 2 were identical with respect to the ovalbumin dose used.

Basal PC_{100} was determined 24 h before each allergen provocation, and 30 min later either saline or (in the 2nd week) *RS*-, *R*- or *S*-salbutamol were inhaled, and a second PC_{100} determined after another 30 min in order to determine the effects on basal airway reactivity. On the next day, PC_{100} were assessed again 5 h (after the EAR) and 23 h (after the LAR) after ovalbumin provocation in all animals in order to determine allergen-induced AHR at these time points. In order to investigate acute bronchoprotective effects after the EAR and LAR, respectively, aerosols of saline or the drug were again inhaled 5.5 and 23.5 h, and PC_{100} were redetermined 6 and 24 h, after ovalbumin provocation, respectively.

A schematic illustration of the protocol is given in figure 1.

For quantitative assessment of the EAR (0–5 h after allergen provocation) and LAR (8–23 h after allergen provocation), airway function was continuously monitored over the whole time-span. Between measurements of PC_{100} at 6 and 23 h, the animals were placed in their home cage (0.16 m^2), in which water and food were available *ad libitum*, and where they could move around freely. During this transfer, the animals remained connected to the measurement system.

Data analysis

The magnitudes of the allergen-induced EAR and LAR were expressed as the area under the P_{pl} time–response curve (AUC) between 0 and 5 h after allergen provocation for the EAR, and between 8 and 23 h after provocation for the LAR. P_{pl} was expressed as percentage change from baseline, and the AUC was calculated by trapezoid integration over discrete (5 min) time periods. Based on saline control provocations, threshold values of AUC (mean+2SD (95% confidence interval)) were defined as 1,185%·5 min^{-1} for a positive EAR and 2,790%·5 min^{-1} for a positive LAR, respectively [17]. Using these criteria, animals were characterised as single early responders and dual responders (*i.e.* animals expressing both an EAR and a LAR). Inherent to the research question, only dual-responding animals (72% of the animals) were included in the present study.

Changes in airway reactivity towards histamine after allergen or saline challenge were expressed as the pre-/post-treatment PC_{100} ratio. PC_{100} before and after inhalation of allergen, salbutamol or saline, as well as the changes in EAR and LAR, were compared using the Wilcoxon signed-rank test.

Statistical analysis of the changes in basal airway reactivity at several time points after salbutamol inhalation was performed using the Kruskal–Wallis ANOVA on ranks. When significance was observed ($p<0.05$), a complementary Newman–Keuls test was performed. For comparison between groups, an unpaired Mann–Whitney rank-sum test was used.

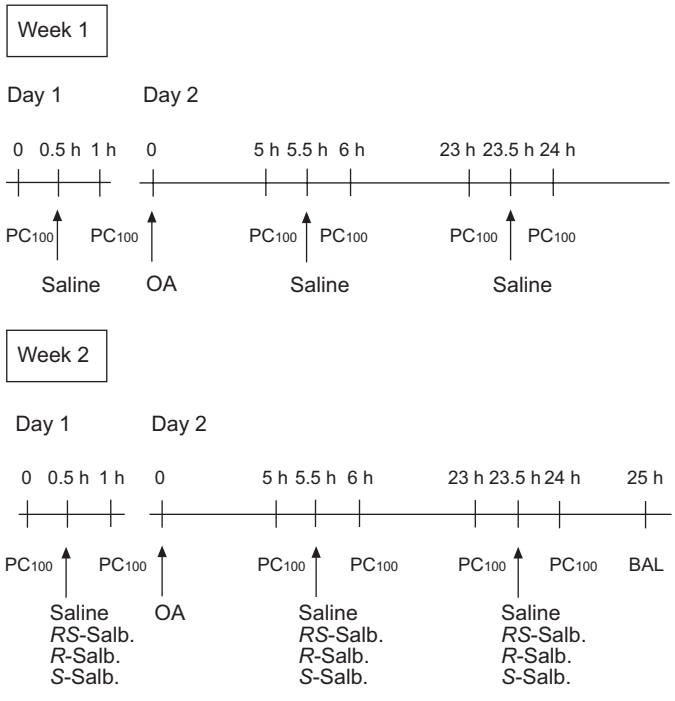

FIGURE 1. Schematic illustration of the study protocol. In week 1, all four groups of animals were subjected to vehicle (saline) inhalation, and, in week 2, to saline, *RS*-, *R*- or *S*-salbutamol (Salb.) inhalation, respectively. Vertical arrows indicate inhalation. OA: ovalbumin; PC_{100}: provocative concentration of histamine causing a 100% increase in pleural pressure; BAL: bronchoalveolar lavage.

RESULTS

Effects of R-, S- *and* RS-*salbutamol on basal airway reactivity to histamine*

Inhalation of *RS*- and *R*-salbutamol caused similar decreases in basal airway reactivity to histamine 30 min after inhalation, with a mean±SEM 3.73±0.30- and 3.56±0.84-fold increase in PC_{100}, respectively (both $p<0.05$) (fig. 2a and b). Both protective effects disappeared within 3 h, the effect of *R*-salbutamol, but not that of the racemate, still being significant 1.5 h after inhalation ($p<0.05$). In contrast to *R*- and *RS*-salbutamol, the *S*-enantiomer exhibited no effect on PC_{100} (fig. 2c). Figure 2 also shows that, during the repeated histamine challenges, no tachyphylaxis developed.

Effects of R-, S- *and* RS-*salbutamol on early and late asthmatic reactions*

The effects of salbutamol and its enantiomers on the EAR and LAR are shown in figure 3. In the saline-treated group of animals, the magnitudes of the EAR and LAR were similar in weeks 1 and 2. The magnitudes of the EAR in animals that inhaled *RS*-salbutamol or *R*-salbutamol 30 min prior to ovalbumin were 1,781.7±566.3%·5 min^{-1} and 1,225.5±523.7%·5 min^{-1}, respectively, which do not differ significantly from the threshold value for a positive EAR (1,185%·5 min^{-1}), indicating that the EAR is suppressed (fig. 3a). This suppression of the EAR in week 2 by *RS*- and *R*-salbutamol is significant when compared to the control EAR (saline-treated) in week 1 ($p<0.01$ and $p<0.05$, respectively). In contrast, the *S*-enantiomer did not affect the magnitude of the EAR.

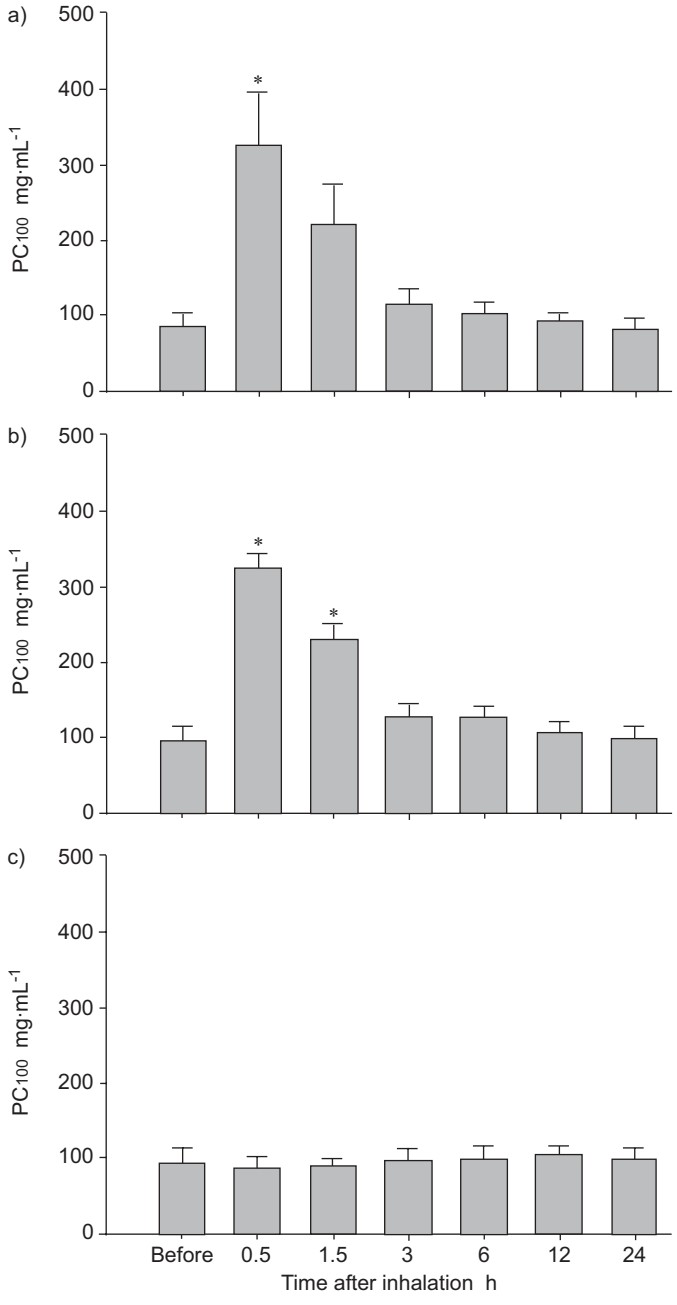

FIGURE 2. Time course of the acute bronchoprotective effects of single inhalations of: a) *RS*-, b) *R*- and c) *S*-salbutamol on histamine-induced bronchoconstriction in sensitised guinea pigs. Data are presented as mean±SEM (n=3 animals). PC_{100}: provocative concentration of histamine causing a 100% increase in pleural pressure. *: $p<0.05$ *versus* before inhalation.

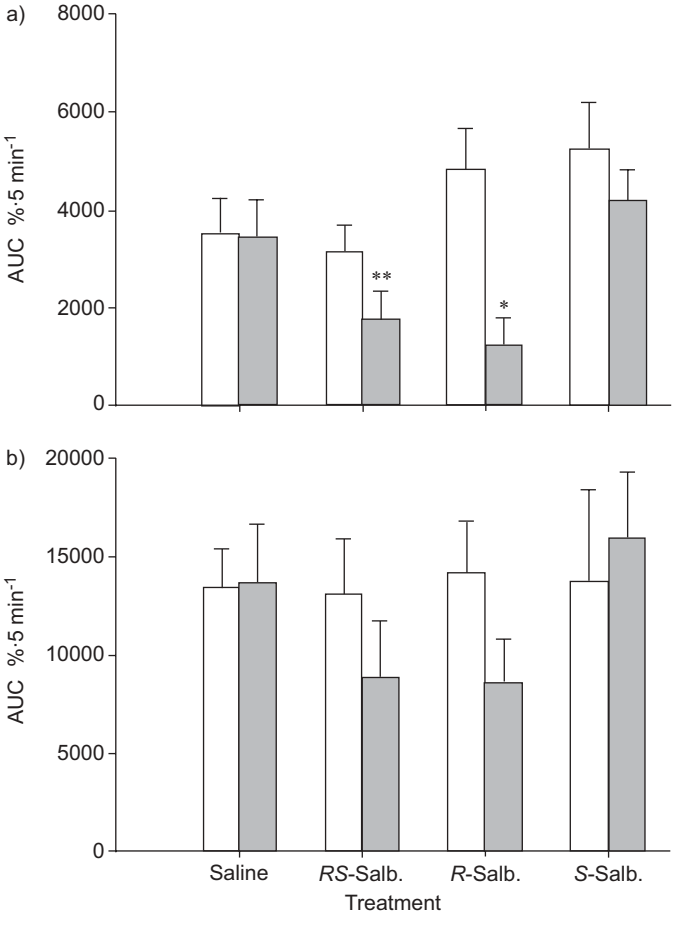

FIGURE 3. Effects of *RS*-, *R*- and *S*-salbutamol (Salb.) inhalation on the magnitude of the: a) early, and b) late asthmatic reaction (□: week 1 (saline); ■: week 2 (treatment)). Data are presented as mean±SEM (n=5–7 animals). AUC: area under the pleural pressure time–response curve. *: $p<0.05$; **: $p<0.01$ *versus* saline (Mann–Whitney rank-sum test).

Similar results were obtained when the immediate (maximal) increases in P_{pl} after allergic provocation, which is predominantly determined by mediators acutely released after mast cell degranulation, were compared (fig. 4). Both *RS*- and *R*-salbutamol, but not *S*-salbutamol, strongly suppressed the peak increase in P_{pl} during the EAR (both $p<0.01$; fig. 4).

The LAR tended to be slightly suppressed in *RS*- and *R*-salbutamol-treated animals compared to the saline group; however, this difference did not reach significance (fig. 3b).

Effects of R-, S- *and* RS-*salbutamol on allergen-induced airway hyperreactivity*

In week 1, all groups of animals received saline and developed comparable AHR to histamine, at both 5 h (after the EAR) and 23 h (after the LAR) after allergen challenge (fig. 5). In week 2, inhalation of *RS*- and *R*- salbutamol, given 30 min before and 5.5 h after ovalbumin challenge, caused significant inhibition of allergen-induced AHR at 5 h (fig. 6a) and 23 h (fig. 6b) after the challenge compared to the saline control inhalation ($p<0.05$ and $p<0.01$, respectively). Inhibition of AHR was more pronounced in animals treated with *R*-salbutamol compared to racemate-treated animals (from 3.51 ± 0.82-fold in controls to 1.29 ± 0.11-fold in *RS*- *versus* 0.81 ± 0.03-fold in *R*-salbutamol-treated animals; $p<0.01$) 5 h after challenge. Both 5 and 23 h after allergen challenge, *S*-salbutamol exhibited no significant effect on allergen-induced AHR (fig. 6).

Figure 7 shows the acute effects of *RS*-, *R*- and *S*-salbutamol or saline on PC_{100} 24 h before and 5 and 23 h after allergen challenge, demonstrating similar bronchoprotective effects of *RS*- and *R*-salbutamol at all time points, whereas *S*-salbutamol was ineffective.

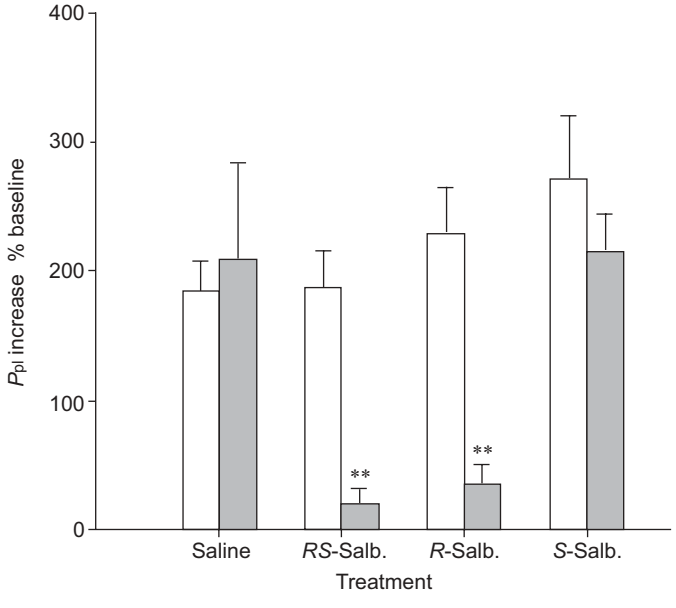

FIGURE 4. Effects of *RS*-, *R*- and *S*-salbutamol (Salb.) inhalation on the immediate (maximal) increase in pleural pressure (P_{pl}) after allergen provocation (□: week 1 (saline); ■: week 2 (treatment)). Data are presented as mean±SEM (n=5–7 animals). **: $p<0.01$ *versus* saline (Mann–Whitney rank-sum test).

Effects of R-, S- *and* RS-*salbutamol on allergen-induced airway inflammation*

Treatment with *R*-, *S*- and *RS*-salbutamol before and 5.5 h after allergen challenge did not cause a significant reduction in the numbers of total cells, or of eosinophils, neutrophils, lymphocytes, macrophages and ciliated epithelial cells in BAL fluid, as determined 25 h after allergen challenge (fig. 8).

DISCUSSION

Inhalation of *RS*- and *R*-salbutamol reduced the basal airway reactivity of guinea pigs to histamine effectively and to a similar extent when using identical doses of the *R*-enantiomer (2.5 mM *RS*- and 1.25 mM *R*-salbutamol). The duration of action of the *R*-enantiomer may be somewhat longer, given the observation that its effect at 1.5 h after inhalation was still significant, whereas no significance was observed with the racemate at this time point (fig. 1). No protection by *S*-salbutamol (1.25 mM) was observed, nor was there an increase in basal histamine reactivity at the dose used.

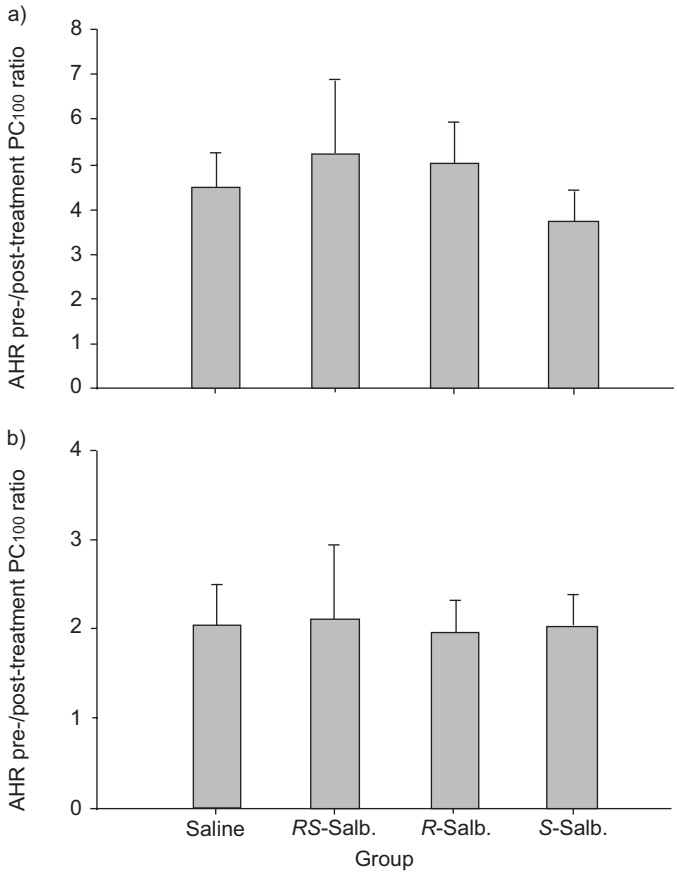

FIGURE 5. Airway hyperreactivity (AHR) to histamine in week 1: a) 5 h (after early asthmatic reaction), and b) 23 h (after late asthmatic reaction) after ovalbumin challenge in the four groups of guinea pigs. All animals underwent saline inhalation 30 min prior to ovalbumin. Data are presented as mean±SEM (n=5–7 animals). There were no significant differences (Mann–Whitney rank-sum test). PC_{100}: provocative concentration of histamine causing a 100% increase in pleural pressure; Salb.: salbutamol.

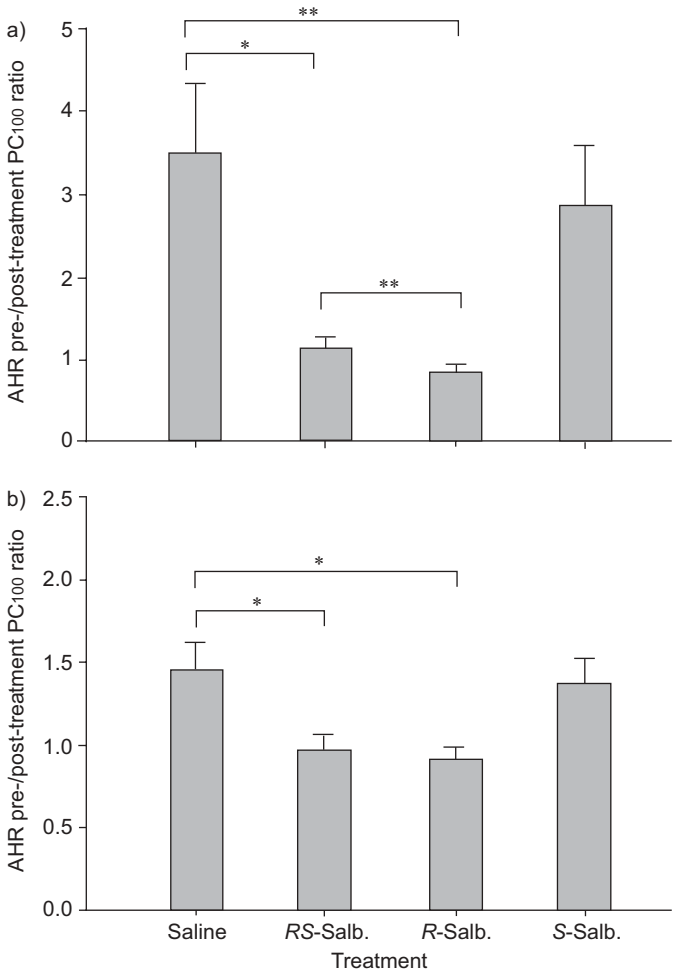

FIGURE 6. Effects of *RS*-, *R*- and *S*-salbutamol (Salb.) inhalation on airway hyperreactivity (AHR) to histamine: a) 5 h (after early asthmatic reaction), and b) 23 h (after late asthmatic reaction) after ovalbumin challenge in the four groups of guinea pigs. Animals underwent saline, *RS*-, *R*- or *S*-Salb. inhalation 30 min prior to and 5.5 h after ovalbumin (week 2). Data are presented as mean±SEM (n=5–7 animals). PC_{100}: provocative concentration of histamine causing a 100% increase in pleural pressure. *: $p<0.05$; **: $p<0.01$ (Mann–Whitney rank-sum test).

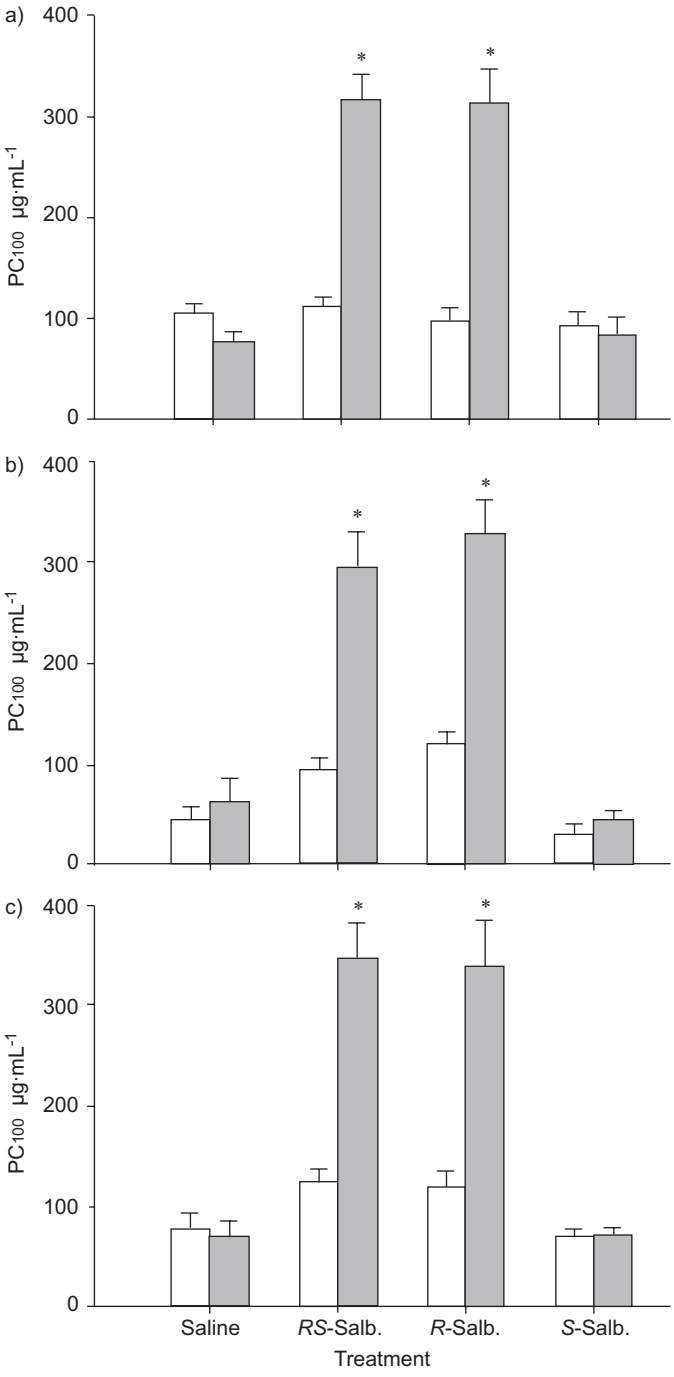

FIGURE 7. Acute bronchoprotective effects of *RS*-, *R*- and *S*-salbutamol (Salb.) inhalation on histamine-induced bronchoconstriction: a) 24 h before, and b) 5 h and c) 23 h after ovalbumin challenge (□: 30 min before inhalation; ■: 30 min after inhalation). Data are presented as mean±SEM (n=5–7 animals). PC_{100}: provocative concentration of histamine causing a 100% increase in pleural pressure. *: $p<0.05$ *versus* 30 min before inhalation (Wilcoxon signed-rank test).

Similar levels of bronchoprotection against methacholine [11] and against methacholine and adenosine monophosphate [10] by racemic salbutamol and the equivalent dose of the *R*-enantiomer have also been reported in asthmatic patients following single-dose inhalations of *RS*-, *R*- and *S*-salbutamol; notably, in the former study, 10-fold higher doses of the racemate and the individual enantiomers were applied. These studies also found no adverse effects of the distomer, and no difference in the duration of action between the racemate and the eutomer, with respect to both bronchoprotection [11] and bronchodilatation [10, 11]. A recent study in asthmatic patients, in which a wide range of cumulatively administered doses were compared, also showed identical bronchodilatory effects at all equivalent doses of *RS*- and *R*-salbutamol [18].

The above-described clinical findings were all obtained in mildly to moderately severe asthmatics. The aim of the present study was, therefore, to investigate the acute bronchoprotective effects of salbutamol and its enantiomers not only in normoreactive but also in hyperreactive guinea pigs, after both the EAR and the LAR. Surprisingly, however, when administered 30 min before and 5.5 h after the allergen

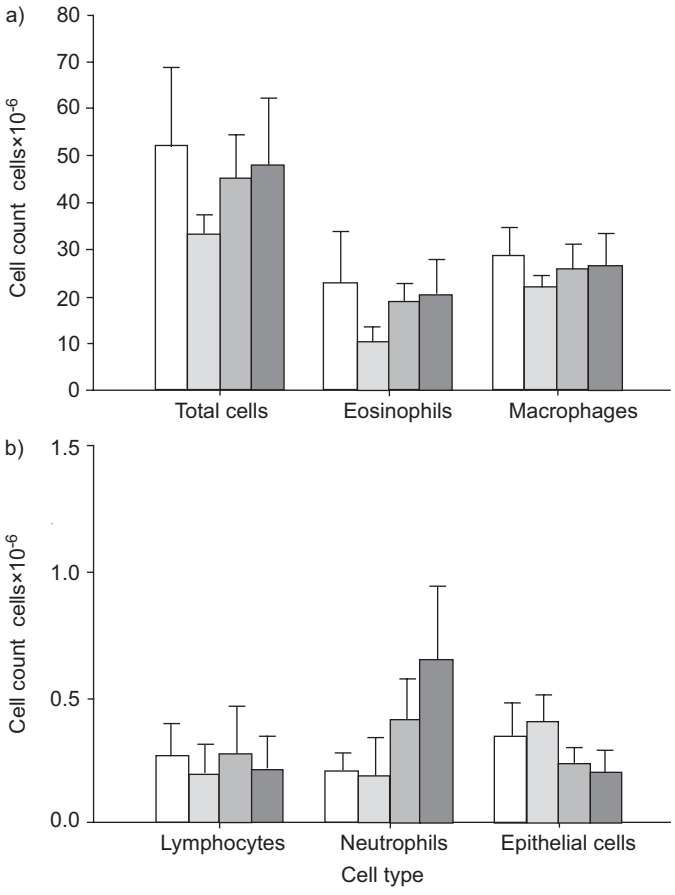

FIGURE 8. Effects of *RS*- (▫), *R*- (■) and *S*-salbutamol (■) inhalation on bronchoalveolar lavage fluid cell counts 25 h after ovalbumin challenge (□: saline): a) total cells, eosinophils and macrophages; and b) lymphocytes, neutrophils and epithelial cells. Data are presented as mean±SEM (n=5–6 animals).

challenge, *RS*- and *R*-salbutamol effectively inhibited the allergen-induced AHR towards histamine at both 5 h, after the EAR, and 23 h, after the LAR. With respect to AHR after the EAR, the protective effect of the *R*-enantiomer was slightly but significantly greater than that of the racemate. This inhibition of the allergen-induced AHR observed 5 and 23 h after challenge was unexpected, since the PC_{100} measurements were performed 5.5 and 17.5 h after the latest inhalation of the β-agonist, respectively, whereas the duration of action of the acute (bronchoprotective) effects of *R*- and *RS*-salbutamol on basal histamine reactivity was <3 h (fig. 2). This strongly points to the involvement of indirect effects of *R*- and *RS*-salbutamol, unrelated to smooth muscle relaxation or acute inhibition of mediator release from mast cells, which suppresses the EAR (fig. 3), in particular the immediate rise in P_{pl} following antigen challenge (fig. 4).

It could be argued, however, that the reduction in AHR after the EAR and LAR might yet be related to inhibition of histamine release during the EAR, since, in the same animal model, histamine H_1 receptor blockade using mepyramine was found to suppress both early and late AHR and to dampen the severity not only of the EAR but also of the LAR [19]. However, although mepyramine inhalation had also reduced inflammatory cell infiltration 24 h after allergen challenge [19], both salbutamol and its eutomer were without any effect on BAL fluid cell content and composition (fig. 8) as monitored at the time at which they had effectively suppressed late AHR. This would imply that inhibition of activation, rather than of influx of inflammatory cells, is involved. Indeed, *RS*- and *R*-salbutamol were found to suppress superoxide anion production and eosinophil peroxidase release from activated human eosinophils [20, 21]. It was recently observed that the generation of superoxide anions is associated with the AHR of guinea pigs after the LAR [22], whereas eosinophil-derived polycations, such as major basic protein, are importantly involved in the AHR after the EAR [23]. Hence inhibition of inflammatory cell activation by *RS*- and *R*-salbutamol may well be associated with the observed suppression of early and late AHR. Presumably, this may also underlie the tendency of inhibition, by both the racemate and the eutomer, to reduce the magnitude of the LAR, starting in guinea pigs 7–8 h after allergen challenge [17]; however, this reduction did not reach significance (fig. 3).

It should be mentioned that, contrary to the general belief that short-acting β-agonists do not suppress the LAR, occasional reports have indicated that nebulised salbutamol is able to reduce the development of the LAR in asthmatic patients, not only at high [24] but also at normal doses [25].

The acute bronchoprotective effects of salbutamol and its enantiomers were measured before and 5 and 23 h after allergen provocation. Two consecutive PC_{100} determinations were performed for this purpose at 1-h intervals, the second one preceded, 30 min before, by inhalation of the β-agonist. As illustrated in figure 7, the equivalent doses of *RS*- and *R*-salbutamol provided very similar levels of bronchoprotection at all time points, whereas the *S*-enantiomer was without effect, irrespective of the time point. This latter result is at variance with the enhanced responsiveness to histamine observed in anaesthetised guinea pigs after intravenous administration of *S*-salbutamol [26]. As discussed elsewhere, acute administration of the *S*-enantiomers of β-agonists has been associated with enhanced airway reactivity of guinea pigs towards spasmogens, especially if the animals are sensitised [10]. Beside direct pro-inflammatory effects [14], *S*-salbutamol has been reported to increase the cytosolic calcium concentration in airway smooth muscle cells through a phospholipase C-dependent mechanism [13]. Obviously, such properties might enhance airway reactivity.

However, in unanaesthetised freely moving sensitised guinea pigs, no changes in airway responsiveness to histamine were observed as a result of *S*-salbutamol inhalation in either normoreactive (before allergen challenge) or hyperreactive airways, after the EAR and the LAR (fig. 7).

Interestingly, KEIR *et al*. [27] have recently shown that long-term continuous treatment of guinea pigs, using osmotic minipumps, with *S*- and also with *RS*-salbutamol for 10 days evoked AHR to several bronchoconstrictive agonists that was not observed after chronic treatment with the *R*-enantiomer. Evidence was presented that, in the development of this AHR, which was obviously unrelated to β_2-adrenenoceptor

desensitisation, sensory nerves played an important role, since capsaicin-induced neurokinin depletion, performed prior to *RS*- and *S*-salbutamol treatment, prevented the AHR to an important extent.

A most interesting finding of the present study was that salbutamol (both the racemate and the eutomer), inhaled at a low therapeutically relevant concentration before allergen provocation, suppresses the development of AHR after the EAR as well as after the LAR (fig. 6). Although, in animal models, such effects have not been reported before with short-acting β-agonists, only one study has addressed the question as to whether salbutamol is able to influence the allergen-induced AHR in asthmatic patients. Using a high dose (2.5 mg) of nebulised salbutamol, TWENTYMAN *et al*. [24] found not only complete abolition of the EAR, but also inhibition of the LAR, as well as suppression of the attendant AHR to histamine by mechanisms that could not be accounted for by bronchodilatation or functional antagonism of bronchoconstriction.

In conclusion, the present study has shown that inhalation of equivalent and clinically relevant doses of *RS*- and *R*-salbutamol exhibit similar bronchoprotective properties towards histamine in unchallenged guinea pigs, and effectively suppress allergen-induced airway hyperreactivity, after both the early and the late asthmatic reaction, through mechanisms not related to direct smooth muscle relaxation; *R*-salbutamol was slightly more efficacious towards the early airway hyperreactivity than the racemate. No adverse effects were observed for the individual *S*-enantiomer.

REFERENCES

1 Vathenen AS, Knox AJ, Higgins BG, Britton JT, Tattersfield AE. Rebound increase in bronchial hyperresponsiveness after treatment with inhaled terbutaline. *Lancet* 1988; 1: 554–558.

2 Kerrebijn KF, Essen-Zandvliet EE, Neijens HJ. Effect of long-term treatment with inhaled corticosteroids and β-agonists on the bronchial responsiveness in children with asthma. *J Allergy Clin Immunol* 1987; 79: 653–659.

3 Kraan J, Koeter GH, van der Mark TW, Sluiter HJ, de Vries K. Changes in bronchial hyperreactivity induced by 4 weeks of treatment with antiasthmatic drugs in patients with allergic asthma: a comparison between budesonide and terbutaline. *J Allergy Clin Immunol* 1985; 76: 628–636.

4 Cockcroft DW, Swystun VA. Functional antagonism: tolerance produced by inhaled β_2 agonists. *Thorax* 1996; 51: 1051–1056.

5 Cockcroft DW, Davis BE, Swystun VA, Marciniuk DD. Tolerance to the bronchoprotective effect of β_2-agonists: comparison of the enantiomers of salbutamol with racemic salbutamol and placebo. *J Allergy Clin Immunol* 1999; 103: 1049–1053.

6 Jokic R, Swystun VA, Davis BE, Cockcroft DW. Regular inhaled salbutamol: effect on airway responsiveness to methacholine and adenosine 5′-monophosphate and tolerance to bronchoprotection. *Chest* 2001; 119: 370–375.

7 Mio M, Kirino Y, Kamei C. Desensitization of β_2-adrenoceptor and hypersensitization to phosphodiesterase inhibitors elicited by β_2-agonists in guinea pig eosinophils. *J Allergy Clin Immunol* 2000; 106: 896–903.

8 Penn RB, Frielle T, McCullough JR, Aberg G, Benovic JL. Comparison of *R*-, *S*-, and *RS*-albuterol interaction with human β_1- and β_2-adrenergic receptors. *Clin Rev Allergy Immunol* 1996; 14: 37–45.

9 Perrin FM, Blum PS, Morley J, Grosclaude M, Chambe MT. Differential responses of asthmatic airways to enantiomers of albuterol. Implications for clinical treatment of asthma. *Clin Rev Allergy Immunol* 1996; 14: 139–147.

10 Ramsay CM, Cowan J, Flannery E, McLachlan C, Taylor DR. Bronchoprotective and bronchodilator effects of single doses of *S*-salbutamol, *R*-salbutamol and racemic salbutamol in patients with bronchial asthma. *Eur J Clin Pharmacol* 1999; 55: 353–359.

11 Cockcroft DW, Swystun VA. Effect of single doses of (*S*)-salbutamol, (*R*)-salbutamol, racemic salbutamol, and placebo on the airway response to methacholine. *Thorax* 1997; 52: 845–848.

12 Johansson F, Rydberg I, Aberg G, Andersson RG. Effects of albuterol enantiomers on *in vitro* bronchial reactivity. *Clin Rev Allergy Immunol* 1996; 14: 57–64.

13 Mitra S, Ugur M, Ugur O, Goodman HM, McCullough JR, Yamaguchi H. (*S*)-Albuterol increases intracellular free calcium by muscarinic receptor activation and a phospholipase C-dependent mechanism in airway smooth muscle. *Mol Pharmacol* 1998; 53: 347–354.

14 Cho SH, Hartleroad JY, Oh CK. (*S*)-albuterol increases the production of histamine and IL-4 in mast cells. *Int Arch Allergy Immunol* 2001; 124: 478–484.

15 Santing RE, Meurs H, van der Mark TW, *et al.* A novel method to assess airway function parameters in chronically instrumented, unrestrained guinea-pigs. *Pulm Pharmacol* 1992; 5: 265–272.

16 Finney MJ, Forsberg KI. Quantification of nasal involvement in a guinea pig plethysmograph. *J Appl Physiol* 1994; 76: 1432–1438.

17 Santing RE, Olymulder CG, Zaagsma J, Meurs H. Relationships among allergen-induced early and late phase airway obstructions, bronchial hyperreactivity, and inflammation in conscious, unrestrained guinea pigs. *J Allergy Clin Immunol* 1994; 93: 1021–1030.

18 Lötvall J, Palmqvist M, Arvidsson P, Maloney A, Ventresca GP, Ward J. The therapeutic ratio of *R*-albuterol is comparable with that of *RS*-albuterol in asthmatic patients. *J Allergy Clin Immunol* 2001; 108: 726–731.

19 Santing RE, Schraa EO, Wachters A, Olymulder CG, Zaagsma J, Meurs H. Role of histamine in allergen-induced asthmatic reactions, bronchial hyperreactivity and inflammation in unrestrained guinea pigs. *Eur J Pharmacol* 1994; 254: 49–57.

20 Leff AR, Herrnreiter A, Naclerio RM, Baroody FM, Handley DA, Munoz NM. Effect of enantiomeric forms of albuterol on stimulated secretion of granular protein from human eosinophils. *Pulm Pharmacol Ther* 1997; 10: 97–104.

21 Volcheck GW, Gleich GJ, Kita H. Pro- and anti-inflammatory effects of β-adrenergic agonists on eosinophil response to IL-5. *J Allergy Clin Immunol* 1998; 101: Suppl. 2, S35.

22 De Boer J, Meurs H, Flendrig L, Koopal M, Zaagsma J. Role of nitric oxide and superoxide in allergen-induced airway hyperreactivity after the late asthmatic reaction in guinea-pigs. *Br J Pharmacol* 2001; 133: 1235–1242.

23 Meurs H, Schuurman FE, Duyvendak M, Zaagsma J. Deficiency of nitric oxide in polycation-induced airway hyperreactivity. *Br J Pharmacol* 1999; 126: 559–562.

24 Twentyman OP, Finnerty JP, Holgate ST. The inhibitory effect of nebulized albuterol on the early and late asthmatic reactions and increase in airway responsiveness provoked by inhaled allergen in asthma. *Am Rev Respir Dis* 1991; 144: 782–787.

25 Palmqvist M, Balder B, Lowhagen O, Melander B, Svedmyr N, Wählander L. Late asthmatic reaction decreased after pretreatment with salbutamol and formoterol, a new long-acting β_2-agonist. *J Allergy Clin Immunol* 1992; 89: 844–849.

26 Mazzoni L, Naef R, Chapman ID, Morley J. Hyperresponsiveness of the airways following exposure of guinea-pigs to racemic mixtures and distomers of β_2-selective sympathomimetics. *Pulm Pharmacol* 1994; 7: 367–376.

27 Keir S, Page C, Spina D. Bronchial hyperresponsiveness induced by chronic treatment with albuterol: role of sensory nerves. *J Allergy Clin Immunol* 2002; 110: 388–394.