Single-isomer R-salbutamol is not superior to racemate regarding protection for bronchial hyperresponsiveness

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Summary Bronchial hyper-reactivity (BHR) has been suggested to follow cessation of regular medication with racemic salbutamol. This study aimed at investigating the effects from medication with R,S- and R-salbutamol on bronchial response to provocation with isocapnic hyperventilation of cold air (IHCA).

Twenty-six patients with mild to moderate asthma were enrolled in a double-blind, randomised, cross-over study. Bronchial response to provocation was measured before and after 1 week's medication. Doses of 0.63 mg R-salbutamol or 1.25 mg R,S-salbutamol were inhaled three times daily during medication-weeks and a wash-out week intervened. Tests were performed 6 h after the last dose of test drug. Impulse oscillometry and forced expiratory volume during one second were methods used to identify bronchial response to provocation. Two patients withdrew from the investigation due to side-effects, one from R- the other from R,S-salbutamol.

Comparable resting bronchial conditions were indicated by differences in baseline lung function values of <2% between study days. No statistically significant medication-dependent differences in BHR could be demonstrated between treatment groups. However, 15 patients exhibited higher \( P = 0.03 \) post-treatment BHR after pure R-salbutamol than after R,S-salbutamol. Furthermore, plasma concentrations of R-salbutamol tended to be lower \( P = 0.08 \) after medication with R- than after R,S-salbutamol despite equal doses of R-salbutamol given during the two separate treatment periods. We also found that considerable amounts of S-salbutamol were retrieved in plasma after medication with pure R-salbutamol.

We conclude that we were unable to demonstrate favourable effects of R-salbutamol over R,S-salbutamol regarding response to provocation with IHCA after regular medication of 1 week's duration.

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Introduction

Asthma is characterised by airway inflammation, variable airway constriction and abnormal
bronchial responses to various stimuli, i.e. bronchial hyperresponsiveness (BHR). It is assumed that bronchodilatation and BHR are effected via different mechanisms. Bronchodilatation may be the result of beta-2-receptor stimulation as induced by a beta-2-receptor agonist.1 The bronchodilating properties of Salbutamol, a racemic beta-2-receptor agonist, follow the R-enantiomer. The racemate undergoes stereoselective inactivation in man through sulphation by sulphotransferases, mainly in the gut and liver. Bronchodilating R-salbutamol undergoes faster and up to 10-fold more effective metabolism than the S-enantiomer, leading to S/R-salbutamol ratios in serum exceeding one. Repeated administrations of racemate may lead to accumulation of S-salbutamol2 and after cessation of bronchodilatation, accomplished by R,S-salbutamol, a distinguishable amount of S-salbutamol can still be found in plasma.

BHR may be the result of converging factors or cascades3,4 and might also be mediated via unknown mechanisms and also suggested to correlate with genetic variability of the beta-2-receptor gene.5 A pro-inflammatory effect has been suggested to occur following cessation of medication with racemic salbutamol, reflected by accumulation of eosinophils as well as enhanced BHR.7,8 This has been proposed to be connected with S-salbutamol,9,10 suggesting a stereoselective pro-inflammatory effect. In contrast, both R- and S-enantiomers of another beta-receptor-agonist, isoprenaline, has been suggested to increase airway hyperreactivity in guinea pigs.11,12 The connection between BHR and presence of S-salbutamol in man is under debate, and a relation between S-salbutamol levels in serum and lung-physiology has until now not been demonstrated in humans.

The aim of this study was to evaluate whether bronchial responsiveness elicited by isocapnic hyperventilation of cold air (IHCA) would increase after repeated dosing during 1 week with R,S-salbutamol relative to corresponding treatment with pure R-salbutamol. Provided that adequate plasma levels of S-enantiomer are still present and that masking of BHR by remaining bronchodilation exerted by R-salbutamol is not at hand, enhanced BHR would indirectly suggest the S-enantiomer to be associated with post-treatment increases of BHR. Based on previous findings,2 such prerequisites were presumed to occur not earlier than 6 h after a final dose of R,S-salbutamol.

Apart from tests on BHR to IHCA, values of eosinophil cationic protein (ECP) in serum were taken as an estimate of eosinophil related inflammation and used as a secondary endpoint. Genetic variability at amino acid 16 and 27 of the beta-2-receptor gene has been shown to be linked with characteristics of asthma.13,14 Possible associations between drug induced BHR and polymorphisms of the beta-2-receptor gene (Gly16 and Glu 27) or salbutamol metabolising enzyme gene (SULT 1A3) were studied.

Materials and methods

Patients

Twenty-six patients with mild to moderate asthma were selected, as a consequence of distinctly identifiable bronchial reactivity to IHCA, from a pool of 36 patients with a history of asthma. Fourteen out of 26 were female and 20 of the patients were allergic, mostly to common aeroallergens or animal dander. Demographic data are presented in Table 1.

Criteria for inclusion were asthma according to GINA criteria15 and a significant response to provocation, i.e. \( \geq 30\% \) increase of total airway resistance as measured by impulse oscillometry (IOS). Patients with more than 800 micrograms (\( \mu \)g) daily of inhaled budesonide or corresponding amounts of other inhaled steroids or any oral steroids were not included in this study. Patients should not have been suffering from any other serious condition beside asthma. Prior to any test, short acting beta-agonists other than study-medication were not allowed or if used on an “as required basis” it was withheld for at least 8 h, long acting beta-agonists for 24 h, leucotriene-receptor antagonists for 3 days and antihistamines for 1 week. Fourteen of our patients were on regular inhaled budesonide in doses ranging between 200 and 800 \( \mu \)g daily. This medication was allowed unchanged throughout the study. Two patients withdrew from the study after inclusion due to side effects, one (No. 1, Table 1) with worsening of asthma with nightly wheezes during medication with R,S-salbutamol, and the other (No. 10) with troublesome palpitations during medication with R-salbutamol.

All patients gave written informed consent before entering the study that was approved by the Medical Products Agency, Sweden and the Ethics Research Committee, Faculty of Health, University of Linköping, Sweden.

Study design

Patients were enrolled in a double blind, randomised, crossover study including medication with
week with either R- or R,S-salbutamol was done at all patients. Randomisation to medication during 1 stable conditions with PEF variability below 10% in peak expiratory flow (PEF) measurements, revealed entering the study. A run-in week with twice daily examination was performed for each person before and allergy was obtained and a brief medical intervals. At the first visit, a history of asthma GlaxoSmithKline, M

- reported for testing five times with 6 in Fig. 1. During the study-period each patient BHR before and after study treatments as outlined

s

R-salbutamol (Xopenex

992 K.N. Sjöswärd, MA, USA) and R,S-salbutamol (Ventoline

s

GlaxoSmithKlineKline, Mölnlad, Sweden), and test for BHR before and after study treatments as outlined in Fig. 1. During the study-period each patient reported for testing five times with 6-8 day intervals. At the first visit, a history of asthma and allergy was obtained and a brief medical examination was performed for each person before entering the study. A run-in week with twice daily peak expiratory flow (PEF) measurements, revealed stable conditions with PEF variability below 10% in all patients. Randomisation to medication during 1 week with either R- or R,S-salbutamol was done at the second visit, using a pre-designed randomisation list. During study-weeks two and four, only the test drugs (i.e., R-salbutamol or R,S-salbutamol, respectively) was allowed as rescue medication. To avoid masking of BHR by remaining bronchodilation, tests and provocation at the end of medication-weeks were performed 6 h after the last dose of either medication. A wash-out period of more than 6 days, corresponding to more than 20 times the half-life of S-salbutamol[16] was carried out during the third study week. Each visit started with blood-sampling for analysis of ECP in serum and salbutamol-isomers in plasma as well as control of bronchial status. After challenge by means of IHCA, IOS was measured at every second minute for a period of 8 min and FEV1 was assessed 10 min after the challenge. All medication used during the four study-weeks was documented by the patients in a medication-diary that was scrutinised by one researcher (KNS) to confirm adherence to protocol. Evaluation of all results was performed blinded to medication.

### Table 1 Demographic data for patients in this study.

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<th>Sex</th>
<th>BMI (kg/cm²)</th>
<th>Allergy</th>
<th>Reg. med.</th>
<th>FEV1 med. (% pred)</th>
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<td>a,b</td>
<td>116</td>
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95% CI 32-43 — 26-31 — 90-102

Females (F) and males (M), body mass index (BMI). Allergy according to case history: non-pollen allergy = + and pollen allergy = + p. Regular medication (Reg. med.): a = inhaled glucocorticosteroids, b = long-acting beta agonists, c = short-acting beta agonists, d = anti-histamine, e = leukotrien-receptor antagonist. Forced expiratory volume over 1 s (FEV1) expressed as percentage of the predicted normal value.

**Lung function tests**

Bronchial status was studied with IOS via an MS-IOS Digital instrument (Erich Jaeger AG, Würzburg, Germany) attached to a computer. Resistance at 5 Hz (R5) was defined to represent total airway resistance, and resistance at 20 Hz (R20) to reflect status in the central airways. Baseline values of these parameters were determined as the mean of two values measured during tidal breathing before provocation. Reactance at 5 Hz (X5) and resonant frequency (Fres) — the frequency where reactance is zero — were also documented. IOS-values were obtained before and at 2, 4, 6 and 8 min after bronchial provocation. Artefacts from the upper airways were minimised by manual control, a firm pressure of cheek and chin, and reproducibility of bronchial status ascertained by two consecutive
measurements with a week’s interval prior to randomisation, with a difference in baseline-values of R5 not exceeding 0.1 kPa/l/s. Forced expiratory volume over 1 s (FEV1) was performed before and at 10 min after provocation. Previously published reference values were used and the better of two volumes, recorded before provocation, defined as the baseline value.

Bronchial challenge

For a period of 4 min, dry cold air, admixed with 5% CO2, was delivered to the patients via a respiratory heat exchange system (RHES, Erich Jaeger AG, Würzburg, Germany) and a one-way valve. Temperature was maintained at −15°C and minute-volume set by a rotameter (Platon, Platon Parks Viables, Hants, UK), corresponding to approximately 70% of maximal voluntary ventilation, calculated by 25 × FEV1. To attain comparable dosing of cold air, a balloon on the inspiratory side was to be emptied at every inspiration and a breathing rate around 25/min. encouraged. Bronchial responsiveness to IHCA was calculated as the maximal percentage rise from baseline in R5 and BHR was defined by a rise in R5 of at least 30% of the baseline value. A rise in R5 exceeding a rise in R20 was taken as being an indirect indicator of events in the peripheral airways.

Medication

During medication-weeks 3 ml of equipotent doses of either R-salbutamol (Xopenex®, Sepracor Inc., Marlborough, MA, USA) 0.63 mg/3 ml or racemic R,S-salbutamol (Ventoline®, GlaxoSmithKline, Möln达尔, Sweden) 1.25 mg/3 ml were inhaled three times daily via a jet nebuliser (PARI Turbo BOY attached to PARI LC, PARI GmbH, Starnberg, Germany). Rescue doses of the same medication were provided and allowed. Doses were delivered in individual ampoules, prepared by the research-laboratory at the Hospital Pharmacy (J.J. Berzelius, University Hospital, Linköping, Sweden). Xopenex was kept in its original ampoules with the name safely covered and Ventoline was diluted to the desired strength at the pharmacy during strictly sterile conditions. Left over doses were returned to the pharmacy and were counted there. Study subjects and experimenters were blinded to type of medication throughout the study period.

Pollen

Pollen profiles were continuously obtained for the region and time of interest (The Palynological Laboratory, Swedish Museum of Natural History, Stockholm, Sweden).

Assay of ECP

Venous blood was drawn and kept at room temperature for 60 ± 15 min, and then centrifuged at 3000 rpm for 10 min. The supernatant was stored at −70°C until analysed as previously described. Assay of isomers

Samples of venous blood were collected in 7 ml heparinised glass tubes (Vacutainer, Becton Dickinson and Co, Rutherford, NJ, USA) and centrifuged (3000 rpm for 10 min) within 45 min after collection. The supernatant plasma was separated and stored at −70°C until analysis. Analysis of R- and S-salbutamol was carried out by means of a stereoselective high performance liquid chromatography assay (HPLC) with fluorescence detection, as previously described.

Genotyping

Allele frequencies for the normal population was determined from DNA isolated from white blood cells, from 206 individuals randomly collected in the south-eastern region of Sweden, representing the same study base as the asthma patients. The two polymorphisms in the β2-adrenergic receptor, GGA16 > AGA and CAA27 > GAA (Gly > Arg and Gln > Glu, respectively) were determined by direct DNA sequencing in both sense and anti-sense direction, using fluorescently labelled dideoxy terminators in a Megabase (Amersham-Pharmacia) capillary sequencer. Polymorphic sites in the two different isoforms of sulphotransferases (SULT), 1A1 and 1A2 were analysed according to published methods based on PCR-restriction fragment length polymorphism (RFLP). SULT1A3 displays two polymorphic sites in close proximity to each other in exon 7; CAC143 > TAC (His > Tyr) and GAA146 > GCA. We therefore choose the method of pyrosequencing to analyse both these genetic variants simultaneously.

Statistics

Mean and 95% confidence intervals (95%CI) were used to describe data, unless otherwise stated. T-test for dependent or independent samples and Mann-Whitney U-test were used in the statistical evaluations, performed by a commercially available program (Statistica 6.0, Statsoft Inc, Tulsa, OK, USA).

Sample size was chosen to give 90% power to detect a 50% difference in mean responses (as delta
R5) assuming a common standard deviation of 0.2 kPa/l/s. These data were based on earlier studies. The power calculation was made by means of a commercially available computer program (nQuery Adviser 3.0, Statistical Solutions, Saugus, MA, USA).

**Results**

Stable pre-challenge bronchial conditions were recorded in all patients on the five different study days, and there were no statistically significant differences over time in baseline values obtained before any challenge, whether seen as IOS-measurements or FEV1 (P > 0.05 and 0.05, respectively, Table 2).

IHCA elicited approximately four times larger increases of values of R5 than of R20 (mean increases of R5 ranged from 0.21 to 0.24 kPa/l/s as compared to mean increases of R20 ranging from 0.05 to 0.06 kPa/l/s, Fig. 2) in all test-days. Bronchial responses to provocation through hyperventilation of cold air, were similar on all 3 days not preceded by study medication, and did not differ significantly from the days after 1 week of medication with either R- or R,S-salbutamol (Table 3). The increases of R5 remained for at least 8 min after completion of the hyperventilation challenge, and there were no significant differences between values of R5 recorded at 8 min after challenge after either treatment or non-treatment weeks (P > 0.05, all comparisons, data not shown). Nor were there any differences in FEV1 recorded 10 min after IHCA.

The spread of data on post-challenge increases of R5 however tended to be higher after treatment with pure R-salbutamol than after R,S-salbutamol or after non-treatment weeks. After each treatment-session (R- followed by R,S-salbutamol or reverse) nine of 24 patients displayed a lessened response to challenge whereas 15 of 24 patients of each treatment group showed an increased response relative to the day before commencement of medication. There was no significant difference between treatment groups when all values were included for statistical evaluation. However, the increase in bronchial reactivity after treatment with R-salbutamol reached higher levels than corresponding values in connection with R,S-salbutamol (P = 0.03, Fig. 3). Patients with attenuated reactivity showed no such treatment-related difference (P = 0.45). These observations were not reflected in FEV1 readings. BHR recorded after each treatment week was also compared with the

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**Table 2**

Baseline values i.e., before provocation, of bronchial status, lung function and serum ECP.

<table>
<thead>
<tr>
<th></th>
<th>R5 (kPa/l/s)</th>
<th>R20 (kPa/l/s)</th>
<th>X5 (kPa/l/s)</th>
<th>Fres (Hz)</th>
<th>FEV1 (L)</th>
<th>s-ECP (mcg/l)</th>
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</thead>
<tbody>
<tr>
<td>Without preceding treatment</td>
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<td></td>
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<tr>
<td>Visit 1, screening</td>
<td>0.32 (0.28-0.36)</td>
<td>0.32 (0.28-0.35)</td>
<td>0.32 (0.28-0.36)</td>
<td>0.12 (-0.16 to 0.09)</td>
<td>14.3 (12.2-16.4)</td>
<td>3.6 (3.1-4.1)</td>
</tr>
<tr>
<td>Visit 2</td>
<td>0.32 (0.28-0.36)</td>
<td>0.32 (0.28-0.35)</td>
<td>0.32 (0.28-0.36)</td>
<td>0.12 (-0.15 to 0.09)</td>
<td>15.3 (13.2-17.4)</td>
<td>3.5 (3.0-4.0)</td>
</tr>
<tr>
<td>Visit 3 and 5</td>
<td>0.32 (0.28-0.36)</td>
<td>0.32 (0.28-0.35)</td>
<td>0.32 (0.28-0.36)</td>
<td>0.12 (-0.15 to 0.10)</td>
<td>15.7 (13.4-18.1)</td>
<td>3.5 (3.0-3.9)</td>
</tr>
<tr>
<td>After treatment</td>
<td>0.41 (0.34-0.48)</td>
<td>0.32 (0.28-0.36)</td>
<td>0.32 (0.28-0.36)</td>
<td>0.13 (-0.16 to 0.09)</td>
<td>15.3 (12.9-17.6)</td>
<td>3.5 (3.0-3.9)</td>
</tr>
<tr>
<td>R,S-salbutamol</td>
<td>0.31 (0.28-0.34)</td>
<td>0.31 (0.28-0.34)</td>
<td>0.31 (0.28-0.34)</td>
<td>0.13 (-0.17 to 0.09)</td>
<td>15.4 (13.0-17.8)</td>
<td>3.5 (3.0-3.9)</td>
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<tr>
<td>R-salbutamol</td>
<td>0.41 (0.34-0.47)</td>
<td>0.32 (0.28-0.36)</td>
<td>0.32 (0.28-0.36)</td>
<td>0.13 (-0.17 to 0.09)</td>
<td>15.3 (12.9-17.6)</td>
<td>3.5 (3.0-3.9)</td>
</tr>
</tbody>
</table>

Mean (95% CI) are given. Resistance at 5 Hz (R5), and resonant frequency (fR5) are values from impulse Oscillometry (FEV1 = forced expiratory volume in 1 s. s-ECP = eosinophil cationic protein in serum.)
individual values formed by the mean value of BHR recorded after the three separate non-treatment weeks ("reference response"). Airway responses after R-salbutamol treatment were significantly more pronounced than the "reference response" ($P = 0.04$, paired analysis). In contrast airway responses after R,S-salbutamol treatment were similar to the reference values ($P > 0.05$).

Plasma-levels of isomers were measured at the end of each medication week, 6 h after the latest inhaled dose. Plasma concentrations of R-salbutamol tended to be lower after regular use of pure R-salbutamol than after R,S-salbutamol (0.13 ng/ml (0.09–0.16) ng/ml vs. 0.10 (0.07–0.14) ng/ml, $P = 0.08$, paired analyses). When analysing data on an individual basis, 18 of 24 samples showed lower plasma levels of R-salbutamol after regular use of R-salbutamol than after R,S-salbutamol. No significant difference was found between treatment sorts in values below zero ($n = 9$ in each group; $P = 0.45$).

$P$-values are given in Table 3.
treatment week, significant amounts of S-salbutamol were found in 15 of the 24 plasma samples collected after 1 week’s treatment with R-salbutamol (range 0.05–0.51 ng/ml).

Lower levels of R-salbutamol in plasma after treatment with pure R-salbutamol, tended to be associated with higher bronchial reactivity. Of 12 patients whose bronchial response to provocation was more intense following R-salbutamol than following R,S-salbutamol we found 10 to have lower plasma levels of R-salbutamol after treatment with pure R-salbutamol during 1 week than after a R,S-salbutamol week.

There was no obvious association of bronchial reactivity and plasma levels of S-salbutamol, and although we did not design this study to assess bronchodilatation as a result to treatment with salbutamol enantiomers, we found no trace of association between plasma levels of R-salbutamol and bronchial tone as judged from R5 values at baseline 6 h after the last inhaled dose. Nor did we find that the order of treatments had any impact of the results of either baseline lung function, BHR or plasma concentration of R- or S-salbutamol, suggesting no carry-over effect.

There were no statistically significant differences in serum-ECP values from the five different days (Table 2). Furthermore changes in pollen density had no impact on serum values of ECP or lung function whether lung function was expressed as baseline lung function or as increases of resistance after IHCA. Furthermore, plasma-levels of ECP obtained at the start of every test-day did not differ significantly between allergic and non-allergic patients or between allergic patients who reported for testing on days with low-versus on days with high pollen-density in the air (Table 4). Nor were there any differences in consumption of rescue medication during wash out- or treatment weeks (data not shown). Treatment with moderate or low doses of inhaled corticosteroids had no detectable impact on the results (data not shown).

The frequency of polymorphism of the Gly 16- and Glu 27-alleles were 52% and 48%, respectively, in the asthmatics as compared to 54% and 40%, respectively, in control subjects. There were no polymorphisms detected in SULT 1A-, 2 or 3- genes. There were no obvious associations between any detected polymorphism and/or clinical symptoms that may have affected the drug response.

**Discussion**

We were unable to demonstrate any favourable protecting effect of R-salbutamol over racemic salbutamol following a week’s regular medication, when effects were studied 6 h after cessation of medication. This contradicts statements about the superiority of medication with the single R-enantiomer and indirectly about a negative effect from presence of S-salbutamol.23 On the contrary, when only those medication-weeks that resulted in enhanced reactivity were studied, or when results were related to an individual reference response formed by the average bronchial responses to IHCA after the three separate non-treatment weeks, medication over 1 week with R-salbutamol resulted in more pronounced reactivity to provocation than did 1 week’s medication with R,S-salbutamol.

We could not relate our findings of increased susceptibility to IHCA to any confounding factor.
such as worsening in the asthmatic status or to increased density of airborne pollen. Steroid-users in this study did not differ from the cortisone-naive individuals regarding our results, although steroid-medication in previously ordered doses (not exceeding 800 µg/day) was allowed throughout the study period. These findings may agree with previous observations on hyperventilation challenge in patients treated with inhaled steroids.4,24 Our finding of minimal intra-individual variability of IOS readings and serum ECP values as well as the fact that almost no rescue medication was needed during active treatment- or non-treatment weeks, also suggests the asthmatic inflammation to be mild in our patients. Since baseline lung function values were almost identical when comparing data from before treatment and at 6 h after completed medication with R- or R,S-salbutamol, bronchial dilatation was judged to have tapered off in all cases and initial conditions to be comparable. We therefore conclude that masking of BHR by bronchodilatation was highly unlikely in our patients.

Interestingly, we could trace a connection between enhanced bronchial reactivity to IHCA and signs of increased inactivation of R-salbutamol after medication with the pure R-enantiomer. Competition for salbutamol degrading enzymes might possibly be one conceivable explanation for abnormal metabolism of R-salbutamol when pure R-salbutamol is used. Salbutamol is inactivated by sulphotransferase 1A3,25 and any functional polymorphism of the SULT1A3 gene might result in abnormal metabolism. Genotyping of the SULT1A3 gene did however not disclose any abnormality in our patients. It is therefore implicated that additional, and up to now unknown, metabolic routes may operate in metabolism of R-salbutamol. Since both R- and S-salbutamol are metabolised by the same sulphotransferases, theoretically, in the absence of S-salbutamol, more R-salbutamol would be inactivated by sulphation. Animal experiments have pointed to the inhibition of contractile responses in bronchi in connection with beta-receptor-agonists, possibly as a result of modulated release of tachykinins from airway sensory nerves.26 The association to our results would then theoretically be less beta-agonist (R-salbutamol) available to influence bronchial contractions via tachykinins. Less available R-salbutamol might also have an impact on mediator-release from mastcells and basophils and on the function of eosinophils and basophils, thus leading to increased inflammatory actions.26

Genetic polymorphism in the beta-2-receptor gene leading to structural and functional variance of the receptor has been suggested, including differences in expression and down regulation of the receptor. Conflicting results has been presented and while polymorphisms in codons 16 and 27 have been suggested to be associated with enhanced bronchial responsiveness, others found no individual polymorphism of codons 27 to be associated to increased bronchial responsiveness.5,27,28 In contrast a protective effect was suggested to be associated with the Gly16 or Gln27 haplotype.29 Receptor down regulation may occur after prolonged exposure of beta-2 agonists and the total mass of available receptors is thereby decreased. This has been associated with polymorphism of β2-adrenoceptor with Glu at the 27 position.30 Polymorphism in codon 16 of the beta-2-receptor has been shown to be associated to airway function in asthmatics after regular treatment with an inhaled beta-adrenergic agonist.14 We however found no association between polymorphism in codon 16 or 27 of the beta-2-receptor gene and any clinical feature in our patients with asthma.

Bronchial tone may be brought about by many different means which is supposed to involve various pathways of action. We chose an indirect or non-specific test, IHCA, resembling a common-day encounter. This test has high sensitivity and specificity to identify asthmatics and does not give noticeable bronchial constriction in a non-asthmatic subject.4,31 An in-flow of cold air corresponding to 70% of maximal ventilatory capacity was arranged in our patients, by thorough instruction and by supportive supervision during a 4 min challenge. Similarity in results from test days following non-medication weeks gave us the confidence to rely on results from the method as performed.

It is recognized that results of bronchial provocation may depend on the method used for assessment of effects. We chose IOS as a stable and sensitive indicator for IHCA-induced bronchial reactions.31 The main advantage lies in not having to perform any extreme breathing efforts and enabling serial evaluation of bronchial conditions during tidal breathing. Any deep inspiration, such as the one preceding a FEV1 manoeuvre, may have an impact on the status within the respiratory system.12,31 This may be the reason why we did not find parallel readings in IOS and FEV1 after challenge. In agreement with earlier publications, our measurements after IHCA showed changes in R5 that were considerably larger than changes in R20, indicating that the rise in R5 reflects changes in the peripheral airways.34

Both Fres and R5 are suggested to have high sensitivity and specificity in identifying bronchial obstruction by IHCA. Since we had the impression,
in this study, that Frs-values swayed more than R5-values we chose the latter as a stable and sensitive indicator for bronchial reactions.

In conclusion we did not find support for the proposed superiority in R-salbutamol over R,S-salbutamol and neither, indirectly, for the suspicion that presence of S-salbutamol would give rise to diminished protection against bronchial hyperreactivity or, indeed, increase in BHR. On the contrary, a number of our patients had a more pronounced response to IHCA after regular use of the pure enantiomer R-salbutamol and the majority of these patients had lower than expected plasma levels of R-salbutamol after treatment with pure R-salbutamol, suggesting enhanced metabolism of R-salbutamol when given as a single isomer. These data agree with previously published pharmacokinetic data. Furthermore, we found no genetic polymorphism of the SULT 1A3 gene that could explain altered metabolism. This suggests either a lack of competition for metabolising enzymes or perhaps that additional metabolic pathways for degradation of salbutamol may exist in our patients. We also found significant levels of S-salbutamol in plasma after the treatment period with pure R-salbutamol in some of our patients, suggesting significant in vivo inter-conversion of enantiomers. The fact that S-salbutamol is metabolised much slower than R-salbutamol may have augmented the increases of plasma levels of S-salbutamol in a number of patients.

Theoretically results from a study population consisting of a subgroup of asthma patients with mild disease and reproducible bronchial responses to IHCA might not be valid for a larger group of asthmatics with severe disease. Further studies on the effect of R-salbutamol in larger populations of asthma patients with various phenotypic and genotypic characteristics, therefore seems warranted.

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