Effect of a single dose of inhaled salmeterol on baseline airway caliber and methacholine-induced airway obstruction in asthmatic children

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Background: *Salmeterol is a new inhaled selelctive β_2-adrenergic receptor agonist with a long duration of action. We studied the duration of the bronchodilation and the protective effect against methacholine-induced airway obstruction of a single dose of salmeterol in a double-blind, randomized, placebo-controlled, crossover design.*
Methods: *Seventeen boys and three girls with mild-to-moderate asthma participated in the study. On two separate days either 50 μg salmeterol or placebo was inhaled. FEV_1 and PD_{20} methacholine were determined before and 1, 4, 8, 12, and 24 hours after inhalation.*
Results: *Salmeterol resulted in a significant bronchodilation compared with placebo, up to 12 hours ($p = 0.0001$). At 24 hours there was a residual effect that approached significance; mean FEV_1 being 8.3% ± 2.4% above baseline ($p = 0.06$). Significant protection against airway sensitivity to methacholine after salmeterol inhalation was found at all time points ($p < 0.005$). Twenty-four hours after administration mean PD_{20} was still 1.22 ± 0.29 doubling dose above baseline. No important adverse effects were noted.*
Conclusion: *We conclude that a single dose of 50 μg salmeterol in children with asthma gives a long-lasting bronchodilation, exceeding 12 hours, which is comparable to the results in adult studies. The duration of the protection against airway sensitivity to methacholine exceeds 24 hours. (J ALLERGY CLIN IMMUNOL 1993;91:127-34.)*

Key words: *Salmeterol, bronchodilation, airway responsiveness, asthma, children*

Asthma is characterized by a variable degree of airway obstruction and airway hyperresponsiveness to several stimuli.[1] Persistent airway obstruction and airway hyperresponsiveness predispose to chronic symptoms and constitute a risk factor for the continuation of childhood asthma into adulthood.[2, 3] Airway hyperresponsiveness can be characterized by an increase in airway sensitivity and by an increase in maximal response.[4] Currently available short-acting inhaled β_2-agonists, like salbutamol, are strong bronchodilators. Their bronchodilating effect lasts for approximately 4 to 6 hours.[5] After inhalation of therapeutic doses, protection against histamine-induced airway responsiveness and other acute challenges, such as allergen, exercise, and cold air, lasts less than 4 hours.[5, 6] They do not influence the maximal degree of airway narrowing; moreover, a steepening of the dose-response curve for methacholine and histamine has been reported.[7]

Salmeterol xinafoate (Glaxo, England) is a new selective β_2-agonist with a long duration of action on

Abbreviations used
PD_{20}: Provocative dose of methacholine causing a 20% decrease in FEV_1
FEV_1: Forced expiratory volume in 1 second
FVC: Forced vital capacity

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TABLE I. Patient characteristics at entry of the study

Subject	Age (yr)	Sex	FEV_1 (%pred)	FEV_1/FVC (%)	PD_{20} (μg)	Maintenance treatment
1	9	M	94	77	19	B, C, S
2	10	M	103	86	80	B, C, S
3	10	M	86	76	83	B, S
4	14	M	65	64	25	B, C, S
5	13	M	76	90	109	B
6	9	M	82	78	94	B, S
7	13	M	57	60	3	B, S
8	16	M	88	79	24	B, S
9	12	M	103	77	14	B, S
10	16	M	61	52	26	B, S
11	11	M	81	63	19	B, S
12	12	M	90	86	35	B, C
13	10	M	81	69	16	B, S
14	11	M	88	83	47	B, S
15	16	M	99	78	123	B
16	12	F	85	77	41	B, C
17	8	M	90	74	42	B, S
18	7	F	89	69	16	B, S
19	10	F	71	70	7	B, C
20	9	M	81	66	22	B, S
Mean	11.8		84	74	30*	
SD	2.7		12.7	9.6		

M, Male; *F*, female; *B*, inhaled β_2-agonist on demand; *C*, inhaled disodium cromoglycate; *S*, inhaled corticosteroid.
*Geometric mean

smooth muscle contractility in vitro.[8] Its long-lasting bronchodilating effect in vivo has been established in several studies in adult asthmatic patients.[9-11] More than one half of the maximum bronchodilation remains 12 hours after administration.[9] In healthy volunteers, protection against histamine-induced airway obstruction lasted 12 hours after inhalation of 200 μg salmeterol.[12] In adult asthmatic patients protection against methacholine- and histamine-induced airway obstruction remained for at least 12 hours after salmeterol doses of 50 and 100 μg and appeared to be dose-related.[10, 11]

The aim of our study was to establish the duration of the bronchodilating effect and the degree and duration of the protection against airway sensitivity to methacholine of a single dose of 50 μg salmeterol in children with mild-to-moderate asthma.

MATERIAL AND METHODS

Patients

Twenty children with mild-to-moderate asthma, 17 boys and 3 girls, were studied. The patient characteristics are summarized in Table I. The children were selected from the outpatient department of Pediatric Respiratory Medicine, Sophia Children's Hospital, University of Rotterdam. The following inclusion criteria were fulfilled at a prestudy visit: (1) mild-to-moderate asthma according to American Thoracic Society criteria,[1] that is, reversible airway obstruction with an increase of at least 15% in forced expiratory volume in 1 second (FEV_1) after inhalation of a bronchodilator, (2) age between 7 and 16 years, (3) baseline forced FEV_1 greater than 60% predicted, (4) airway hyperresponsiveness to methacholine, that is, the dose of methacholine to produce a 20% fall in FEV_1 (PD_{20}) equal to or less than 150 μg,[13] (5) ability to produce reproducible lung function tests, that is, coefficient of variation in three consecutive measurements of FEV_1 less than 5%. All children were atopic to one or more inhaled allergens. Their asthma had been stable for at least 1 month, with no respiratory tract infections. Maintenance treatment, which had not been changed during the previous 6 months, consisted of inhaled corticosteroid (12 children), disodium cromoglycate (3 children), or both (3 children). All patients used a β_2-agonist on demand.

Study design

The study was double-blind, randomized, placebo-controlled, and crossover. The children visited the lung function laboratory on two separate occasions within 4 weeks; the interval between visits being no shorter than 3 days, preferably approximately 1 week. They arrived at 8 AM, having abstained from inhaled bronchodilators for at least 12 hours. After a short rest (15 minutes), baseline heart rate, blood

pressure, and FEV_1 were measured. Then a methacholine provocation test was performed. At least 1 hour after the last concentration of methacholine, when FEV_1 had returned to within 10% of baseline, the study medication was inhaled. This consisted of either placebo or 50 μg salmeterol administered as two puffs from a metered dose inhaler in conjunction with a Volumatic (Glaxo) spacer device. The two puffs were given one by one. After each puff the child had to take five breaths of sufficient magnitude to move the valve of the Volumatic.[14] During the study days no other bronchodilators were allowed except the study medication. Inhaled corticosteroid or disodium cromoglycate or both were continued as before entry into the study. On both study days they were inhaled at the same time of the day for all patients.

Repeated measurements of heart rate, blood pressure, and FEV_1 as well as repeated methacholine provocation tests were performed 1, 4, 8, 12, and 24 hours after administration of the study medication. During the day the children stayed in the laboratory of the outpatient department of pediatric respiratory medicine, during the night they slept in the hotel accomodation of the hospital together with one of their parents.

The study was approved by the medical ethics committee of the University Hospital/Sophia Children's Hospital Rotterdam. Informed consent was obtained from all patients and their parents

Lung function measurements

All FEV_1 measurements were performed on a spirometer with a digital volume transducer (Vicatest-P2; Mijnhardt, Zeist, The Netherlands). Reference values used were those of the European Community for Coal and Steel.[15] Before each methacholine provocation, FEV_1 was measured in triplicate; the best value was taken. Methacholine provocation was performed according to standardization recommendations.[16, 17] Aerosol-dispersed methacholine bromide in unbuffered saline solution was given in doubling concentrations (0.125 to 32 mg/ml). The aerosol was generated by a DeVilbiss 646 (De Vilbiss Co., Somerset, Pa.) nebulizer, which was operated with 3 ml solution in the nebulizer cup. The nebulizer was attached to a Rosenthal-French dosimeter (Laboratory for Applied Immunology, Fairfax, Va.) driven by air at 137.8 kPa (20 psi). The aerosol was delivered directly into the mouth through a mouthpiece. The patient inspired slowly from functional residual capacity to total lung capacity. During inspiration the dosimeter was triggered for 0.6 seconds. After the inspiration had been completed, the child was asked to hold his or her breath for approximately 2 seconds. A total of 20 μl of aerosolized solution was delivered to the mouth in four consecutive breaths. Mouth doses were 2.5 to 640 μg methacholine. To exclude reactions to the diluent, saline solution was inhaled before methacholine in a similar way. FEV_1 was measured in triplicate 3 minutes after saline solution or methacholine inhalation. The interval between consecutive doses was 5 minutes. The next methacholine dose was not given if FEV_1 had fallen below 80% of baseline. PD_{20} was calculated from a log dose-response plot with linear interpolation of data points.

Statistical analysis

Wilcoxon's signed-rank test was used to compare active treatment with placebo after verifying that no significant treatment-order and period effects were present.[18] To allow for the multiplicity of testing at five time points, p values less than 0.01 (0.05/5) were considered to be statistically significant according to Bonferroni's procedure. The effect of study treatment on FEV_1 was expressed as percent change from baseline FEV_1 on the same day. Logarithmic transformation was performed in all analyses of PD_{20}. The magnitude of the protection against methacholine-induced airway sensitivity was expressed in doubling dose change from baseline PD_{20} on the same day. In patients who did not reach a 20% fall in FEV_1 after the maximum dose of 640 μg methacholine, PD_{20} was considered to be 640 μg for statistical calculations. As in these cases, PD_{20} is known to exceed 640 μg, and all but one of these occurred during active treatment; this will underestimate the treatment effect of salmeterol. Therefore an additional analysis was performed, with use of survival analysis techniques, allowing for such censored data. With these methods full use is made of the information that some PD_{20} values are known to exceed 640 μg, instead of assuming them to be equal to 640 μg. Because PD_{20} values appeared log normally distributed, it was possible to make adjusted estimates (i.e., which take account of the censored values) of the mean difference in doubling doses at the different time points.[19]

RESULTS

Twenty children entered the study. Mean baseline values of FEV_1 and baseline geometric means of PD_{20} at the two different treatment days did not differ significantly; 2.20 and 2.13 L (83% and 82% predicted), respectively, 34.7 and 30.9 μg, respectively, methacholine during placebo and active treatment. There were no effects of treatment order or period effects on FEV_1 and PD_{20} at all time points.

During placebo administration two children had an exacerbation of their asthma at 8 and 12 hours, respectively, after administration. Both children received salbutamol for relief of their symptoms. Measurements of FEV_1 and methacholine responsiveness within 12 hours after the salbutamol dose were not performed.

Effect on airway caliber

Salmeterol gave a long-lasting bronchodilation (Fig. 1). Compared with placebo the effect on FEV_1 was highly significant at all time points up to 12 hours ($p = 0.0001$). The maximum mean effect on FEV_1 was reached after 4 hours; the change from baseline being 18.6% ± 2.5% (mean ± SEM). However, after 1 hour, bronchodilation was near its maximum

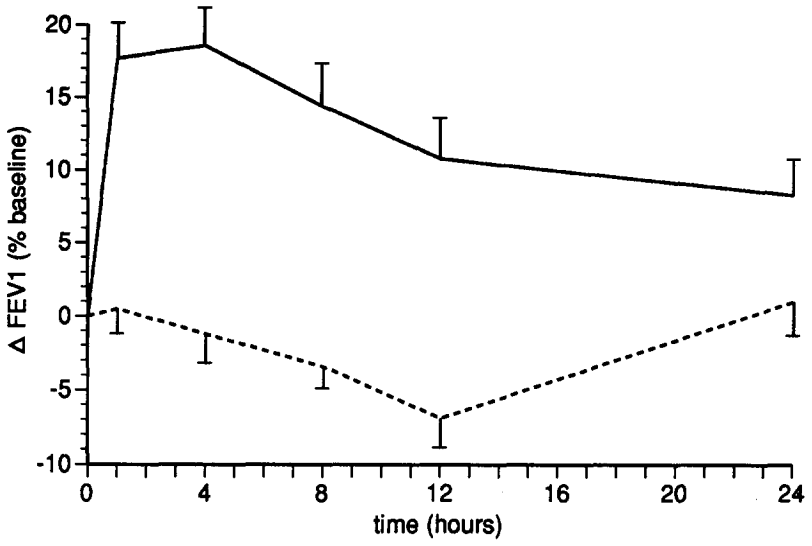

FIG. 1. Change in FEV_1 in percent change from baseline (SEM). *Solid line,* salmeterol; *broken line,* placebo.

TABLE II. No. of patients in which PD_{20} exceeded 640 μg methacholine

Time	No. of patients in which PD_{20} exceeded 640 μg	Unadjusted mean differences in PD_{20} (doubling doses)	Adjusted mean differences in PD_{20} (doubling doses)
1 hr	10	3.7 (3.1-4.2)	4.5 (3.8-5.2)
4 hr	5	3.7 (3.0-4.4)	4.1 (3.3-4.9)
8 hr	3	3.2 (2.5-3.9)	3.5 (2.9-4.1)
12 hr	2	3.0 (2.3-3.7)	3.2 (2.5-3.9)
24 hr	1*	1.3 (0.5-2.1)	1.2 (0.4-2.0)

Except the one marked (*), this occurred after salmeterol treatment. Unadjusted and adjusted mean differences (95% confidence intervals) in PD_{20}, expressed as doubling doses at different time points between salmeterol and placebo treatment are given. Unadjusted values calculated, assuming PD_{20} in these patients was 640 μg. Adjusted mean differences calculated, with survival analysis techniques, taking account for censored PD_{20} values, which actually exceed 640 μg.

(17.7% ± 2.3%). Twelve hours after inhalation of salmeterol mean FEV_1 was 10.8% ± 2.7% (p = 0.0001) above baseline, and after 24 hours it was still 8.3% ± 2.4% above baseline. Compared with placebo this value was approaching significance (p = 0.06). After placebo administration mean FEV_1 decreased with a maximum of 6.9% ± 2.0% after 12 hours. Results of FEV_1/forced vital capacity (FVC)% were comparable to those of FEV_1. Salmeterol inhalation resulted in a significant increase in FEV_1/FVC% at 1, 4, 8, and 12 hours compared with placebo ($p < 0.002$). After 24 hours the mean FEV_1/FVC% was respectively 0.77 and 0.75 after salmeterol and placebo (p = 0.19).

After salmeterol, individual results showed an increase in FEV_1 from baseline of at least 10% in 17 of 20 children. The maximum bronchodilating effect in these responders varied between 12% and 46%. Seven patients had their peak effect after 1 hour, seven after 4 hours, and three after 8 hours. Also the time period for which FEV_1 was more than 10% above baseline varied between patients. Eight of the 17 responders had FEV_1 values of more than 10% above baseline during 24 hours. In the remainder of responders FEV_1 values of more than 10% above baseline were maintained for up to 12 hours in three patients, up to 8 hours in one, and up to 4 hours in five.

Effect on airway sensitivity to methacholine

The protection against methacholine-induced airway obstruction lasted for 24 hours. Fig. 2 shows the geometric means of PD_{20} after salmeterol and placebo. The changes from baseline, expressed in doubling doses, are shown in Fig. 3. Salmeterol resulted in a decrease in airway sensitivity to methacholine, with a maximum mean effect of nearly four doubling doses

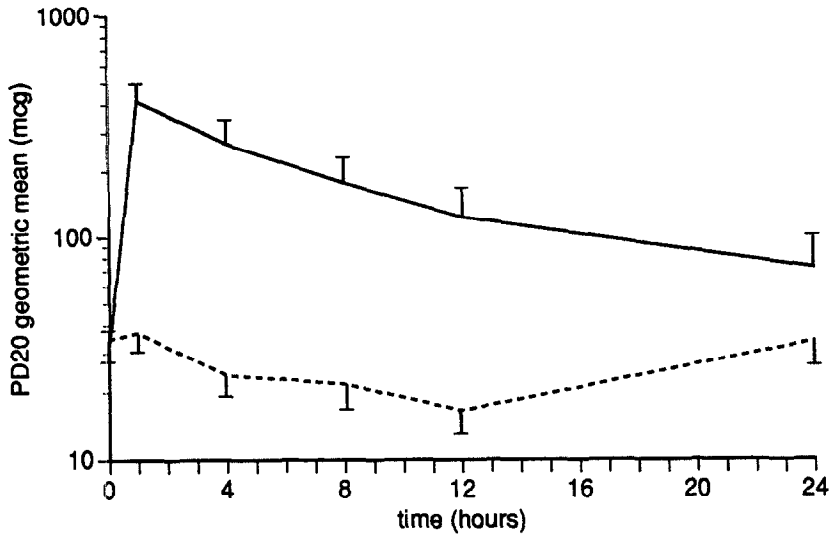

FIG. 2. PD_{20} methacholine (geometric mean ± SEM). *Solid line,* salmeterol; *broken line,* placebo.

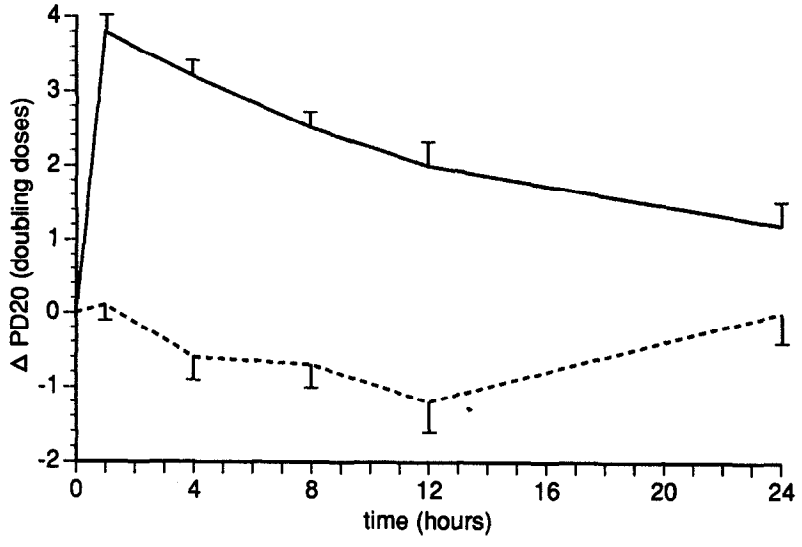

FIG. 3. PD_{20} changes from baseline (doubling doses ± SEM). *Solid line,* salmeterol; *broken line,* placebo.

1 hour after inhalation, which gradually diminished to slightly more than one doubling dose after 24 hours. Compared with placebo the protection of salmeterol was highly significant at all time points ($p < 0.0002$ up to 12 hours, $p = 0.005$ at 24 hours). After placebo administration no changes of more than one doubling dose occurred.

No PD_{20} was reached after the maximum methacholine dose, that is, 640 μg, in a substantial number of the children after salmeterol administration and in one patient 24 hours after placebo. Because in these subjects PD_{20} was considered to be 640 μg, the above-mentioned results underestimate the treatment effect. Taking into account that PD_{20} actually exceeded 640 μg in these patients, adjusted mean differences between salmeterol and placebo treatment were calculated (Table II).

One hour after administration of salmeterol 18 patients had PD_{20} values in the normal range, that is, PD_{20} equal to or greater than 150 μg methacholine.[13] For the other subsequent time points after 4, 8, 12, and 24 hours, respectively, 17, 11, 8, and 5 children had their PD_{20} in the normal range.

The peak response of the protective effect was mea-

sured at 1 hour after salmeterol administration in all subjects and varied between 2.2 and 6.1 doubling doses. In only five patients the protective effect became less than one doubling dose within 24 hours.

No correlation was found between the degree of the protective effect and the degree of bronchodilation at different time points ($r = -0.14$, -0.15, -0.12, -0.14, and 0.11, respectively, $p > 0.5$).

Adverse events

No adverse effects, like tremor or palpitations, were noted during the study. Two children complained of headache; one during placebo and one during salmeterol treatment. Heart rate and systolic and diastolic blood pressure during active treatment did not significantly differ between salmeterol and placebo.

DISCUSSION

In adult studies 50 μg salmeterol had been shown to be efficacious.[9-11] In a first study in children this dose was well tolerated.[20]

In this study the bronchodilating effect of 50 μg salmeterol exceeded in most patients 12 hours, although in a few patients it was less. Twenty-four hours after administration there was still a small but significant reduction of airway sensitivity to methacholine. Although at this time point the bronchodilating effect of salmeterol was not significantly different from that after placebo, a residual effect on FEV_1 seemed to exist in approximately one half of the patients. The changes of FEV_1 and PD_{20} after placebo administration were small and likely to be caused by diurnal variation.

In adult studies the peak level of FEV_1 after a single dose of 50 μg salmeterol was comparable with that after 200 μg salbutamol.[10, 11, 21] Mean peak increases were 15%, 12%, and 18%, respectively, which is comparable with the results in our study (18.6% ± 2.5%). The relatively small increase in FEV_1 may be due to the mild degree of airway obstruction at entry. Studies in adults report a significant bronchodilation after single doses of 50 and 100 μg salmeterol up to 12 hours after inhalation.[9-11] Our results are in keeping with those of Ullman and Svedmyr,[9] who found that one half of the bronchodilator effect remained after 12 hours. Another study showed almost identical mean FEV_1 values 1 and 12 hours after 50 μg salmeterol.[10] No data are available on the bronchodilating effect 24 hours after inhalation. The protective effect against methacholine-induced airway obstruction was studied in adult asthmatic patients by Derom et al.[11] Salmeterol (50 and 100 μg) gave a significant dose-related protection up to 12 hours after inhalation, whereas the effect of 200 μg salbutamol was no longer significantly different from that of placebo at 4 hours. Twelve hours after salmeterol, mean PC_{20} was 1.5 doubling doses after 50 μg and 2.4 doubling doses after 100 μg salmeterol, which is comparable with 2.0 doubling doses in our study after 50 μg. The peak effect was smaller, 2.4 and 3.3 doubling doses, respectively, after 50 and 100 μg salmeterol. In a study on histamine-induced airway obstruction, the peak effect was 2.7 doubling doses, which is also less than we obtained, but the protection 12 hours after inhalation of 50 μg salmeterol was again similar (1.79 doubling doses).[10] Whether these differences result from differences in patient characteristics or differences in pharmacokinetics between children and adults is unknown. Campos Gongora et al.[10] found 50 μg salmeterol four times as potent as 200 μg salbutamol in regard to the protective effect against histamine-induced airway obstruction, whereas 50 μg salmeterol equaled the protective effect against methacholine of the same dose of salbutamol in another study.[11] No data are available on the effect of methacholine- and histamine-induced airway obstruction 24 hours after inhalation. In a recent saline challenge study 32 hours after 50 μg salmeterol, there was no significant effect on either FEV_1 or airway responsiveness to histamine.[22] Malo et al.[21] studied the effect of a single dose of 50 μg salmeterol on hyperventilation with cold dry air up to 24 hours. The mean duration of the protective effect of salmeterol was 15.9 hours, compared with 3.5 hours for salbutamol. However, 24 hours after inhalation only one patient showed a significant protection. In our study 24 hours after inhalation 15 of the 20 children showed a clinically relevant protection against methacholine-induced airway obstruction, that is, more than one doubling dose above baseline. So the duration and degree of the protective effect of salmeterol may be different for different stimuli, which is also known for short-acting β_2-agonists.[5]

Nonbronchodilating properties of salmeterol have been suggested from in vitro studies on human lung fragments[23] as well as from the study by Twentyman et al.[22] who revealed a complete inhibition of the rise in nonspecific airway responsiveness over a 34-hour period after allergen challenge. Mechanisms other than bronchodilation and functional antagonism were held responsible for the protection of a short-acting β_2-agonist against several constrictor stimuli in guinea pig treachea preparations.[24] The data in our study do not allow any conclusion on whether the long-lasting effect of salmeterol on airway sensitivity is the result of inhibition of smooth muscle contractility or of other

nonbronchodilating properties. The fact that no correlation was found between the degree of the protective effect and the bronchodilating effect, which is also known for short-acting β_2-agonists,[5] supports the view that mechanisms in addition to smooth muscle inhibition play a role.

It is unlikely that our results have been influenced by the concomitant use of inhaled corticosteroids or disodium cromoglycate or both. No significant direct protective effect on methacholine-induced airway responsiveness of these drugs exists.[6] During both study days these drugs were administered at the same time point for all patients, so they only could have influenced the placebo and the active treatment in the same way. Because all patients were using their maintenance treatment for at least 6 months, it is unlikely that the chronic dosing effect has changed within the study period. It is in agreement with this that no period effect was found.

In this study a dose of 50 μg salmeterol was shown to have a long-lasting brochodilating effect and a prolonged duration of action against airway sensitivity to methacholine in children with mild-to-moderate asthma. So, twice daily dosing may result in a 24-hour protection. This is of considerable clinical importance because in general the tolerance to various external stimuli increases together with a decrease in airway sensitivity.[25] A direct correlation has been found between the degree of airway sensitivity to a nonspecific stimulus and the amount of allergen that can be tolerated.[26, 27] However, for short-acting β_2-agonists a steepening of the dose-response curve is known, and maximal airway narrowing is not influenced.[7] Because we studied only airway sensitivity and not the effect on maximal airway narrowing after methacholine, we can not speculate on the potential hazardous effects of higher doses of allergen or other strong stimuli reaching the airways because of long-lasting better patency. Further studies will be necessary to determine this effect as well as the degree and duration of the protective effect of salmeterol against other stimuli in asthmatic children and to determine the effect on the nonspecific airway responsiveness during long-term treatment.

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