Respiratory pathophysiologic responses

Airway responsiveness after a single dose of salmeterol and during four months of treatment in children with asthma

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Background: *Inhalation of a single dose of the long-acting β_2-adrenoceptor agonist salmeterol protects against methacholine-induced airway obstruction and other bronchoconstricting stimuli for at least 12 hours. Hypothetically, twice daily dosing of salmeterol may result in continuous protection.*
Objective: *This study was designed to investigate the protective effect of a single dose of salmeterol and of continuous twice daily treatment on airway responsiveness to methacholine.*
Methods: *In a double-blind, parallel study, salmeterol 50 μg twice daily was compared with salbutamol 200 μg twice daily. Thirty children with mild asthma, who had little or no bronchial obstruction and were hyperresponsive to methacholine ($PD_{20} \leq 150$ μg) were allocated to receive either salmeterol or salbutamol. Airway responsiveness was measured before study entry, 12 hours after a single dose of drug was given, and monthly during 4 months of daily treatment. Measurements were always performed at the same time of the day, 12 hours after the last dose of medication was administered.*
Results: *No significant differences in FEV_1 were found between treatments at any time point. PD_{20} significantly increased after the first dose of salmeterol was given (geometric mean, 100 μg). Geometric mean PD_{20} values were significantly better during salmeterol treatment than during salbutamol treatment, 52 and 25 μg, respectively ($p = 0.005$).*
Conclusion: *The protection provided by salmeterol during maintenance treatment was less than that provided after the first dose ($p < 0.001$). However, protection did not diminish during the 4-month treatment period and remained significant compared with baseline ($p = 0.003$). (J ALLERGY CLIN IMMUNOL 1996;97:938-46.)*

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

Salmeterol xinafoate has a bronchodilating effect that lasts for at least 12 hours when administered as a single dose of 50 μg in adults and children with asthma.[1-4] Protection against methacholine-induced[2, 3] and histamine-induced airway obstruction lasts for 12 up to 24 hours. Single-dose studies also show a prolonged protection against other bronchoconstricting stimuli, such as exercise,[5] hyperventilation with dry cold air,[6] and allergen.[7] Theoretically, twice daily dosing of salmeterol can provide 24-hour protection against

Abbreviations used
DD: Doubling dose
FVC: Forced vital capacity
PEFR: Peak expiratory flow rate

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various bronchoconstricting stimuli and therefore decrease symptoms in patients with asthma. Studies comparing salmeterol 50 μg twice daily with salbutamol 200 μg four times daily during 12 weeks have indeed shown better symptom control in the group treated with salmeterol.[8-10] In patients with mild asthma a reduction of the acute protective effect of salmeterol against methacholine-induced bronchoconstriction from a 3.3 doubling dose (DD) after the first dose to a 1.0 DD after stopping maintenance treatment at 4 and 8 weeks was found.[11] The bronchodilating effect did not change during the study period. Another study in patients with mild to moderate asthma, of whom the majority were treated with inhaled corticosteroids, did not show this reduction in protection against methacholine-induced airway obstruction.[12] The two studies differ in the time point at which methacholine challenges were performed, 1 and 12 hours after salmeterol administration, respectively; whereas in the study by Cheung et al.[11] maintenance treatment was also stopped for 36 hours. A recent study in adult patients with symptomatic asthma, who were already being treated with a low dose of inhaled corticosteroids, showed a response more favorable in symptoms and peak flow values when salmeterol was added than when the inhaled corticosteroid dose was increased.[13] This study, however, does not include data on airway responsiveness. We investigated the protective effect of salmeterol against methacholine-induced bronchoconstriction after a single dose and during 4 months of maintenance treatment and compared this with the effect of salbutamol.

METHODS

Subjects

Between July 1992 and January 1993, 30 children, aged 7 to 16 years, with mild asthma according to the American Thoracic Society's criteria,[14] were recruited from the outpatient department for Pediatric Respiratory Medicine, Sophia Children's Hospital, Rotterdam. The patients had to be capable of performing lung function tests reproducibly (i.e., a coefficient of variation in three consecutive measurements of FEV_1 less than 5%). Because airway responsiveness is partly determined by the degree of bronchial smooth muscle constriction,[15] we selected children who had a consistent increase in airway responsiveness but little or no bronchoconstriction. They had to meet the following criteria: (1) a dose of methacholine that produced a 20% fall in FEV_1 (PD_{20} methacholine) equal to or less than 150 μg (this being more than 2 standard deviations below the mean value in healthy children),[16] (2) a baseline FEV_1 and forced vital capacity (FVC) greater than 70% of predicted value (reference values according to Zapletal et al.[17]), and (3) an FEV_1/FVC greater than 70%. The inclusion criteria had to be fulfilled at a prestudy visit. All patients were atopic to one or more inhaled allergens, as determined by measurement of specific IgE in serum and/or positive skin test results. Their households were adapted to reduce house dust mite exposure, and keeping of domestic animals was discouraged. Asthma treatment before the study consisted of an inhaled β_2-agonist on demand only or in combination with maintenance treatment with disodium cromoglycate. Inhaled corticosteroids and maintenance treatment with oral corticosteroids were not allowed in the year preceding the study. Disodium cromoglycate was stopped 2 weeks before the start of the run-in period. If during this period the symptoms of asthma increased significantly, the patient was excluded from the study. None of the children had acute episodes of asthma or respiratory tract infections for at least 1 month before entry into the study.

Thirty children, 20 boys and 10 girls, were allocated randomly to treatment groups (15 in each group). The baseline characteristics were the same for each treatment group (Table I). Nine children in each treatment group had had disodium cromoglycate medication discontinued. The median duration of asthma was 5 and 6 years, respectively, for the salmeterol and salbutamol groups. The exacerbation rate was low in both groups, reflected by the mean number of prednisolone courses per patient in the preceding year, respectively, 0.13 and 0.20. None of the children had been hospitalized for treatment of asthma in the year before entering the study.

Study design

The study had a double-blind, parallel-group design and consisted of a 2-week run-in period, a 4-month treatment period, and a 2-week follow-up period. The study was based on an intention-to-treat principle. At the first visit to the lung function laboratory, before the start of the run-in period, children were randomly allocated to receive either salmeterol 50 μg twice daily or salbutamol 200 μg twice daily. During the run-in period no medication was given, except for salbutamol in case of symptoms. The first dose of the study drug was taken at the end of the run-in period, 12 hours before the second visit. Thereafter, the 4-month treatment period started, and children took their study medication two times a day with an interval of approximately 12 hours. For relief of acute asthma symptoms, salbutamol was allowed at a maximum dose of 200 μg six times daily. Exacerbations of asthma were treated with a standard short course of prednisolone (starting with 30 mg on the first day and tapering off to 0 in 1 week according to a scheme that depended on body weight). Salbutamol and the study medication were administered as Rotadisks in combination with a Diskhaler (Glaxo, Greenford, U.K.). All children were instructed in use of this inhalation

TABLE I. Patient characteristics at time of entry into the study

Subject No.*	Sex	Age (yr)	FEV_1 (% pred)	FVC (% pred)	FEV_1/FVC (%)	PD_{20} (μg)	Medication	Atopy
Salmeterol group								
1	M	9	93	97	80	34	B	HD, C, D
2	F	13	90	112	68	6	B	HD, Gr, D
3	M	7	106	107	84	23	BC	HD
4	M	11	99	90	92	75	B	HD, Gr
5	M	15	96	115	68	17	BC	HD, Gr, C, D
6	F	12	104	107	82	4	BC	HD, Gr, C, D
7	M	6	87	94	78	48	B	HD, Gr, C
8	M	11	74	82	75	16	B	Gr
9	M	7	84	79	90	92	BC	HD, Gr
10	M	7	102	97	89	49	BC	HD, Gr, C, D
11	F	10	94	95	85	66	BC	HD, Gr, C, D
12	M	8	95	87	92	39	B	HD, C, D
13	F	12	99	99	85	136	BC	Gr
14	M	10	97	96	85	54	BC	HD, Gr, C, D
15	M	10	76	77	83	25	BC	C
Mean		10.3	93.0	95.5	82.5	32.4†		
SD		2.5	9.5	11.6	7.6			
Salbutamol group								
16	F	11	103	106	82	101	B	HD
17	M	7	111	121	80	28	BC	HD, Gr, C
18	M	11	103	113	76	123	B	HD, Gr
19	M	11	81	103	66	27	BC	HD, Gr, C, D
20	F	7	96	111	75	14	BC	HD, Gr, C
21	F	11	84	89	81	24	BC	HD, Gr, C
22	M	12	91	84	90	83	BC	HD, D
23	F	11	92	94	83	107	BC	HD
24	F	7	103	103	86	25	B	HD, Gr, C
25	F	7	87	81	93	20	BC	HD, Gr, C, D
26	M	12	82	86	80	27	BC	HD
27	M	12	77	82	78	26	B	HD
28	M	10	73	73	83	45	B	HD
29	M	8	96	88	91	41	B	HD, C, D
30	M	10	89	97	78	21	BC	HD
Mean		10.3	91.2	95.3	81.5	37.2†		
SD		2.0	10.9	13.6	7.0			

B, Inhaled β_2-agonist on demand; *HD,* house dust; *C,* cat; *D,* dog; *Gr,* grass; *BC,* inhaled β_2-agonist and disodium cromoglycate.
*Subject numbers do not indicate the sequence of entry into the study.
†Geometric mean.

device before entry into the study, and technique was checked at every visit. During the follow-up period after the study medication was stopped, salbutamol was used as needed. Children visited the lung function laboratory at the start and at the end of the run-in period, monthly during the treatment period, and at the end of the follow-up period. At each visit heart rate, blood pressure, FEV_1, peak expiratory flow rate (PEFR), and airway responsiveness to methacholine were measured. All lung function measurements were performed between 8:30 and 9:30 AM, 12 hours after the last dose of the study drug was given. To verify compliance with the last dose, patients were asked for the exact time of drug inhalation. If this was not 12 hours earlier, lung function measurements were rescheduled. Rescue salbutamol was allowed up to 8 hours before the measurements were taken. No FEV_1 measurements or methacholine provocation tests were performed within the first 4 weeks after prednisolone was taken. During the run-in and follow-up periods and during the first 2 weeks of every month of treatment, a record card was completed daily. Separate daytime and nighttime scores from 0 to 3 were given for the presence and severity of cough, wheezing, and dyspnea. PEFR was recorded in triplicate twice daily before inhalation of the study drug with the use of a mini-Wright peak flow meter (Clemente Clarke International Ltd., Harlow, Essex, U.K.). The use of rescue salbutamol was also recorded. At the start and

end of the treatment period, blood samples were taken and analyzed for hematologic and biochemical parameters, and urine was analyzed for protein, glucose, and blood.

The study was approved by the Medical Ethics Committee of the University Hospital/Sophia Children's Hospital Rotterdam. Written informed consent was obtained from all patients and their parents.

Lung function measurements

FEV_1 was measured according to the European Community for Steel and Coal recommendations[18] with a water-sealed spirometer (Mijnhardt, Zeist, The Netherlands). The largest value from an envelope curve consisting of three to five attempts was recorded. Reference values of Zapletal et al.[17] were used. PEFR was measured in triplicate, and the best value was recorded with the use of the patient's own mini-Wright peak flow meter. Methacholine provocation tests were performed with a modification of the dosimeter method of Chai, as described previously.[19] Nebulized 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 nebulizer (DeVilbiss Co., Somerset, Pa.), attached to a Rosenthal-French dosimeter (Laboratory for Applied Immunology, Fairfax, Va.) and driven by air at 137.8 kPa (20 psi) with a timing adjustment of 0.6 second. 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 of methacholine. Saline solution was inhaled before methacholine to exclude a nonspecific response. The effect of each dose was determined by measuring FEV_1 in triplicate 3 minutes after each administration. The PD_{20} methacholine was calculated from a log dose-response plot by linear interpolation of data points.

Statistical analysis

FEV_1 results were expressed as percent predicted value according to reference values.[17] All PD_{20} values were logarithmically transformed before analysis. For patients in whom a 20% fall in FEV_1 was not reached after the maximum dose of 640 μg methacholine, PD_{20} was considered to be 640 μg. Because this only occurred in two patients, both in the salmeterol group, this has resulted in a slight underestimation of the effect of salmeterol. PD_{20} values were analyzed as geometric mean, as well as changes from baseline, expressed in DDs. Comparisons of PD_{20} and of FEV_1 between and within treatment groups were done by using repeated-measures analysis of variance.[20] Comparisons of PD_{20} and of FEV_1 at and between specific time points were done by using the *t* test and the paired *t* test, respectively. The percentage of days with symptoms and mean morning and evening PEFR for individual patients were calculated from the daily record card for each study period. If the number of days scored on the daily record card was less than 7 (of the required 14 days), the percentage of days with symptoms or peak flow rates were considered inestimable for that item in that period and were not included in the analysis. Comparisons of the percentages of days with symptoms in various study periods were done by using the Mann-Whitney U test. Comparisons of mean morning and mean evening PEFRs at and between specific study periods were done by using the *t* test and the paired *t* test, respectively. For all analyses, a *p* value of 0.05 (two-sided) was considered the limit of significance.

RESULTS

During the study, six prednisolone courses were given: three during salmeterol treatment (subject 2, one course; and subject 7, two courses) and three during salbutamol treatment (subjects 18, 19, and 29). Two children (subjects 7 and 18), one in each treatment group, withdrew during the treatment period because of an increase in symptoms; it was considered unethical to continue administration of blinded medication. After withdrawal, both children began receiving inhaled corticosteroids. Measurements obtained from these subjects were included up to the last visit before withdrawal. Compliance with treatment schedules were checked by counting the used blister packs at each visit. The compliance gradually improved during the treatment period in both treatment groups: from 1.11 to 1.76 blister packs/day for the group treated with salmeterol and from 1.10 to 1.90 blister packs/day for the group treated with salbutamol.

Airway caliber

Results of FEV_1, expressed in liters and percent predicted value, are listed in Table II. At the beginning of the run-in period, FEV_1 was similar in both groups. Twelve hours after the first dose of either of the study drugs was administered, we found no significant change in FEV_1 compared with baseline values. At no time, either after the first dose was given or during the treatment period, were there any significant changes in FEV_1 within or between the two treatment groups. Two weeks after the discontinuation of salmeterol treatment, a small but significant ($p = 0.005$) decrease in FEV_1 occurred. However, the fall in FEV_1 after continuous treatment was stopped did not differ between the salmeterol and salbutamol groups ($p = 0.10$).

Airway responsiveness

Baseline PD_{20} methacholine values were similar in the salmeterol and salbutamol groups (geometric mean, 32 and 37 μg methacholine, respective-

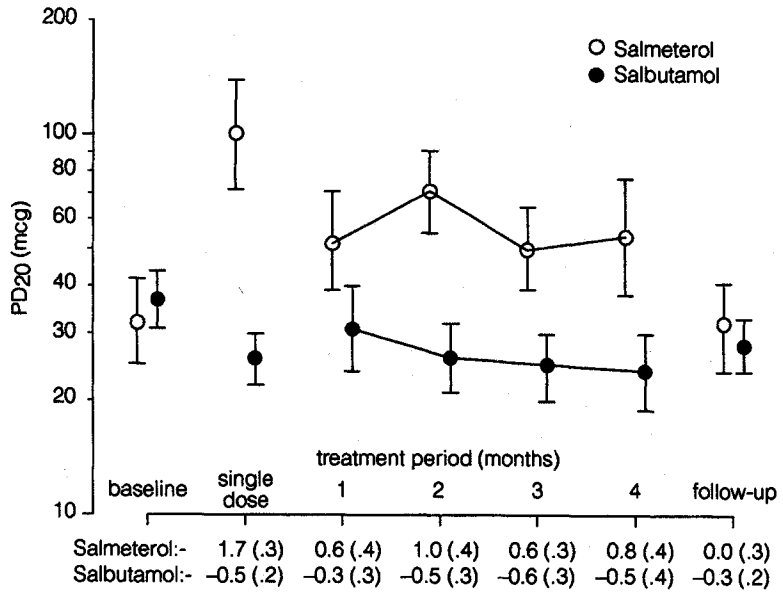

FIG. 1. PD_{20} methacholine (geometric mean ± SEM) at all time points. Mean PD_{20} changes from baseline in DDs (±SEM) for salmeterol and salbutamol treatment are listed below the time points.

TABLE II. Results of FEV_1 expressed in liters and as percent predicted for both treatment groups at different time points

	Salmeterol		Salbutamol	
	L	% pred	L	% pred
Baseline	1.98 ± 0.17	93.0 ± 2.5	2.00 ± 0.13	91.2 ± 2.8
Visit 2 (12 hr after first dose)	2.08 ± 0.22	95.5 ± 4.2	1.99 ± 0.12	90.3 ± 2.8
Treatment period				
1 mo	2.04 ± 0.19	94.3 ± 3.1	2.03 ± 0.14	90.3 ± 3.4
2 mo	1.97 ± 0.17	92.8 ± 2.9	2.06 ± 0.12	92.0 ± 2.4
3 mo	2.07 ± 0.19	93.2 ± 3.0	1.98 ± 0.12	88.0 ± 2.1
4 mo	2.02 ± 0.19	90.5 ± 4.1	1.96 ± 0.15	87.3 ± 3.1
Follow-up	1.95 ± 0.18	85.7 ± 3.1	1.97 ± 0.14	86.2 ± 2.1

Values are expressed as means ± SEM.

ly). There was a strong correlation between PD_{20} values at the different time points and baseline values of PD_{20} within treatment groups. Therefore to reduce the variation caused by interindividual differences in baseline PD_{20}, not only geometric mean PD_{20} values were analyzed but also changes in PD_{20} from baseline. The results of these analyses were similar. Fig. 1 shows both geometric mean PD_{20} values and PD_{20} changes in DDs. Table III shows individual data at each time point.

At the end of the run-in period, 12 hours after the first 50 μg dose of salmeterol was given, PD_{20} methacholine increased by 1.66 DD compared with baseline. After administration of 200 μg of salbutamol, PD_{20} fell with a 0.54 DD ($p < 0.001$ salmeterol vs salbutamol). Geometric mean PD_{20} values at this time point were 100 and 26 μg methacholine, respectively, for salmeterol and salbutamol ($p = 0.001$). The individual results are plotted in Fig. 2.

During the treatment period no significant changes in geometric mean PD_{20} were found within both groups from 1 to 4 months. The geometric mean PD_{20} during the treatment period was 52 μg methacholine for the salmeterol group, compared with 25 μg methacholine for the salbu-

TABLE III. Individual data for PD_{20} (in micrograms of methacholine) at different time points

Subject No.	Baseline	Single dose	Treatment period (mo) 1	2	3	4	Follow up
Salmeterol group							
1	34	89	127	73	24	21	11
2	6	14	*	*	13	12	11
3	23	13	20	26	41	35	13
4	75	>640	428	504	103	221	199
5	17	410	169	179	56	216	51
6	4	32	18	28	21	11	8
7	48	55	*	201	*	*	*
8	16	20	19	19	*	21	32
9	92	338	142	118	50	101	105
10	49	208	59	51	63	68	48
11	66	167	35	31	28	136	20
12	39	108	10	101	65	13	50
13	136	>640	101	117	>640	>640	69
14	54	99	51	50	54	44	20
15	25	97	23	46	44	*	*
Geometric mean	32	100	52	71	50	54	32
Salbutamol group							
16	101	36	77	31	29	52	90
17	28	13	35	11	34	60	58
18	123	28	30	31	23	*	*
19	27	28	123	62	45	*	43
20	14	9	10	9	10	10	16
21	24	29	7	55	33	19	21
22	83	80	174	183	140	37	75
23	107	52	56	31	72	26	33
24	25	37	29	17	16	13	13
25	20	24	7	11	13	10	12
26	27	28	20	12	16	24	16
27	26	20	20	15	21	51	22
28	45	32	47	67	7	11	24
29	41	9	*	11	8	9	20
30	21	25	30	36	50	104	44
Geometric mean	37	26	31	26	25	24	28

*No measurements.

tamol group ($p = 0.005$). The geometric mean PD_{20} was less during maintenance treatment with salmeterol than after the first dose was given ($p < 0.001$) but still significant compared with baseline ($p = 0.003$). Two weeks after maintenance treatment was stopped, geometric mean PD_{20} values were not significantly different between groups. For both treatment groups these values did not differ significantly from the values at the time of entry into the study.

Daily record cards

Symptom scores were generally low. Mean percentages of days with at least one symptom were 46% and 40%, respectively, for the salbutamol and salmeterol groups during the run-in period. For the group treated with salmeterol, these percentages were 32%, 25%, 22%, and 23%, respectively during the four consecutive treatment periods. These percentages did not significantly differ from the percentages for the group treated with salbutamol, which were 35%, 34%, 30%, and 25%, respectively (all $p > 0.59$). No significant differences were found when the various symptoms—cough, wheezing, and shortness of breath—were analyzed separately. This also applied to the separate morning and evening symptom scores. Morning and evening PEFRs did not differ significantly within or between groups, although both tended to increase during salmeterol treatment.

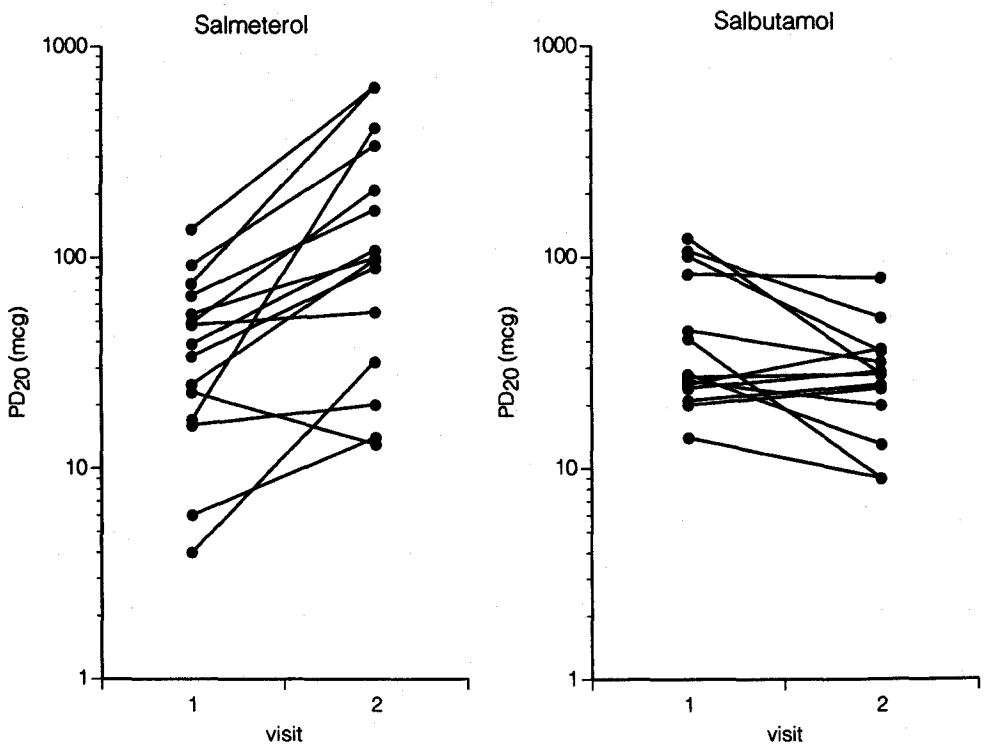

FIG. 2. Individual results of PD_{20} methacholine for both treatment groups at baseline period (visit 1) and 12 hours after the first dose of the study drug was given (visit 2).

Adverse events

During salmeterol treatment 17 adverse events were reported in 10 patients; during salbutamol treatment 36 adverse events were reported in 12 patients. Most adverse events were upper respiratory tract symptoms. Headache occurred slightly more often during salbutamol treatment (eight periods of headache in four patients) than during salmeterol treatment (one headache). There were no significant changes in systolic and diastolic blood pressure or heart rate in any group during treatment.

DISCUSSION

The results of this study show that twice daily treatment with salmeterol in children with mild asthma results in continuous, stable protection against methacholine-induced bronchoconstriction. This protection, however, is less than the protection provided after a first single dose. After maintenance treatment was stopped for 2 weeks, no residual protection remained, indicating that there was no sustained reduction of airway responsiveness.

Salbutamol instead of placebo was used in the control group; otherwise, because of its bronchodilatory effect salmeterol could be recognized as the effective treatment. Twice daily treatment with salbutamol resulted in a slight but not significant decrease ($p = 0.06$) in PD_{20} during the 4-month period compared with baseline measurements; this was probably the result of withdrawal of disodium cromoglycate in more than half of the children before they entered the study. Some authors have suggested an increase in airway responsiveness and a deterioration of asthma as a result of regular β_2-agonist treatment.[21, 22] It is unlikely, however, that regular use of salbutamol is the explanation for the decrease of PD_{20} in the salbutamol group in our study, because the decrease in PD_{20} was already present at the end of the run-in period in which children used salbutamol "as needed."

We selected children with mild asthma, who were hyperresponsive but had little or no bronchoconstriction, to avoid interference with airway caliber and PD_{20}. We chose to measure airway responsiveness 12 hours after the last dose of the study drug was administered, which is the normal dose interval during maintenance treatment with salmeterol and therefore clinically relevant. Furthermore, a longer interval might introduce a rebound increase in airway responsiveness, as has been shown for up to 59 hours after stopping regular treatment with the short-acting β_2-agonists

terbutaline[23] and salbutamol.[24] Until now, a rebound increase in PD_{20} after stopping regular treatment with salmeterol has not been demonstrated.[11, 12, 25, 26]

In our study the protective effect of salmeterol was probably caused by the prolonged effect of the drug on airway smooth muscle. This is functional antagonism, a well-known phenomenon associated with other β_2-adrenoceptor agonists.[27] In vitro experiments show evidence of an interaction of β_2-agonists and methacholine at the level of intracellular signal transduction through phosphoinositide metabolism.[28]

After the first dose of salmeterol was given, we found an improvement in PD_{20} of 1.7 DD, which is comparable with the results of previous studies.[2, 3, 29] During the treatment period from 1 to 4 months, this protection was constant but reduced to 0.7 DD.

Two studies in adult patients with asthma investigated the immediate protective effect of salmeterol and the effect during regular twice daily treatment.[11, 12] Booth et al.[12] examined 26 patients with mild to moderate asthma in a parallel-group, placebo controlled study. The majority of their patients were also receiving inhaled corticosteroid treatment. As in our study, the interval between salmeterol administration and measurement of airway responsiveness to methacholine was 12 hours. They found a small but significant protection during 8 weeks of salmeterol treatment, which did not differ from the single dose effect.[12] A reduction in protection during regular twice daily treatment with salmeterol was found by Cheung et al.[11] They reported a reduction in protection from 3.3 DD after the single dose of salmeterol was given to 1.0 DD after 4 and 8 weeks of treatment. Airway responsiveness was measured 1 hour after salmeterol administration, and maintenance treatment was stopped for 36 hours. Although unlikely from the data at the end of their study, a possible rebound increase in airway responsiveness could not be excluded. As in our study, Cheung et al.[11] selected subjects with mild asthma who were not treated with inhaled corticosteroids. The reduction in protection occurred within 4 weeks after the start of maintenance treatment and remained at the same level after 8 weeks of treatment. The explanation for this tolerance remains unclear but may be the result of receptor downregulation. Tachyphylaxis to nonpulmonary effects (e.g., tremor, increased QTc interval, and elevated blood glucose levels) has been found after 2 weeks of treatment with salmeterol in healthy subjects.[30] The use of inhaled corticosteroids may protect against the development of tachyphylaxis to pulmonary effects of β_2-agonists and may explain the different results obtained by Booth et al.[12] Reversal of tachyphylaxis by systemic corticosteroids has been shown in vitro and in vivo.[31]

In our study significant, stable protection remained throughout the 4 months of treatment with salmeterol. So, if any downregulation of the β_2-receptors occurs, this seems incomplete. Because we selected children with little or no bronchoconstriction, the effect could not be explained by an improvement in airway caliber.[14] This is supported by the fact that no significant changes occurred in FEV_1 and that no correlation was found between the changes in PD_{20} and in FEV_1. Although the protection after 4 weeks of treatment was less than that after the first dose was given, our data do not indicate an ongoing increase in airway responsiveness. A significant degree of protection remained during treatment, and this may be of clinical relevance, because a decrease in airway responsiveness will improve the tolerance to other exogenous stimuli. A direct correlation has been found between the degree of airway responsiveness to a nonspecific stimulus and the amount of allergen that can be tolerated.[32, 33] In our study the changes in airway responsiveness were not reflected by changes in symptom scores. However, this may be the result of selecting patients with mild asthma who already have very low symptom scores before the start of the study.

We conclude that the protective effect of salmeterol against methacholine-induced airway obstruction during 4 months of treatment is lower than the protection offered by a single dose. However, twice daily administration of salmeterol provides significant, stable protection compared with baseline and salbutamol treatment.

According to international consensus reports, asthma therapy should be directed against airway inflammation, and inhaled corticosteroids are now the mainstay of asthma treatment.[34] It is unlikely from the data now available that salmeterol in itself influences chronic airway wall inflammation.[35] Addition of salmeterol to inhaled corticosteroid treatment may have beneficial effects on symptom scores and airway responsiveness.[13] Studies are now being performed in children with asthma to evaluate the effect of addition of salmeterol to treatment with a conventional dose of an inhaled corticosteroid, as compared with increasing the dose of an inhaled corticosteroid.

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