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Sustained protection against distilled water provocation by a single dose of salmeterol in patients with asthma

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ABSTRACT: The long-acting β_2-agonist salmeterol inhibits *in vitro* the release of inflammatory mediators up to 20 h. These mediators are involved in ultrasonically nebulized distilled water (UNDW)-induced bronchoconstriction. We investigated whether salmeterol provides prolonged protection against UNDW provocation and whether this effect was paralleled by its bronchodilator effects.

Nineteen asthmatic patients (mean forced expiratory volume in one second (FEV_1) 84.8% predicted, mean provocative concentration of histamine producing a 20% decrease in FEV_1 0.65 $mg \cdot mL^{-1}$) participated in this randomized, double-blind, placebo-controlled crossover trial. After measuring baseline FEV_1, patients inhaled 50 μg salmeterol or placebo by metered-dose inhaler. FEV_1 was measured after 20 and 40 min, and UNDW provocations and FEV_1 measurements were performed after 10, 20 and 34 h.

Compared to placebo, salmeterol caused marked bronchodilatation from 20 min up to 20 h after inhalation. Salmeterol also provided more than 20 h of protection against UNDW provocation (still more than one doubling dose). Protection beyond the period of bronchodilatation did not occur. Eleven subjects had a significant reduction in provocative dose of UNDW causing a 20% fall in FEV_1 ($PD_{20,UNDW}$) values between 10 and 20 h, at a time when there was still persistent bronchodilation. No correlation existed between changes in FEV_1 and changes in $PD_{20,UNDW}$. From the equations of regression lines between FEV_1 and corresponding $PD_{20,UNDW}$ values, it was calculated that only ~25% of the afforded protection was explained by bronchodilatation.

In conclusion, a single dose of salmeterol induces both bronchodilatation and protection independently of this bronchodilation against a physiological bronchoconstrictor stimulus for more than 20 h.

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The long-acting β_2-agonist salmeterol xinafoate has a higher potency and much longer duration of action than the short-acting β_2-agonists such as salbutamol [1]. Unlike the short-acting β_2-agonists, it has been suggested that salmeterol has some anti-inflammatory properties. *In vitro* data showed that salmeterol blocked mast cell mediator release 10–35 times more potently than salbutamol, with effects persisting for more than 20 h [2]. Salmeterol, but not salbutamol, also had inhibitory effects on other inflammatory cells such as eosinophils and alveolar macrophages [3], and afforded long-lasting inhibition of increases in vascular permeability [4]. Despite these cellular and vascular effects, evidence that they are of clinical relevance is still lacking. No change in bronchial hyperresponsiveness (BHR) was reported after 6 weeks of treatment with salmeterol [5] and analysis of bronchoalveolar lavage (BAL) cell profile has not shown convincing evidence of an anti-inflammatory effect [6].

On the other hand, TWENTYMAN *et al.* [7] suggested that salmeterol has some additional effects, *i.e.* preventing the increase in BHR after allergen provocation, beyond the time of bronchodilation. PEDERSEN *et al.* [8] also reported that salmeterol blocked the late asthmatic response and increase in BHR after allergen provocation.

In contrast to pharmacological stimuli such as histamine and methacholine, ultrasonically nebulized distilled water (UNDW) induces airway narrowing indirectly, by causing the release of endogenous mediators and possibly by initiating vagal reflex mechanisms [9, 10]. Challenge with UNDW may increase BHR and induce a late asthmatic response, in the same manner as allergen exposure [10]. Thus, the mechanism by which UNDW provocation induces bronchoconstriction is likely to be similar to those involved in asthma provoked by naturally occurring stimuli [9]. If the above-mentioned long-lasting cell-stabilizing effect of salmeterol were present *in vivo*, this drug might be expected to afford prolonged protection against UNDW provocation.

The present study was, therefore, designed to assess whether a single dose of salmeterol provided long-lasting protection against UNDW provocation and whether or not this was caused by its bronchodilating properties.

Materials and methods

Study design

This randomized, double-blind, placebo controlled, crossover trial consisted of two identical 3 day study periods, with a minimal interval of 1 week between the start of the two periods, in order to prevent any carry-over effect. Subjects withheld rescue medication (salbutamol 100 μg by metered dose inhaler (MDI)) at least 6 h before each visit and rested for at least 15 min before starting measurements.

On the first day, at 22.00 h, baseline forced expiratory volume in one second (FEV_1) was measured. Subsequently, study medication was administered, consisting of two inhalations of 25 μg of salmeterol or placebo by MDI in random order. Flow-volume curves were recorded 20 and 40 min afterwards. On the second and third day, *i.e*. 10 h (at 08.00 h), 20 h (at 18.00 h), and 34 h (08.00 h the next day) after inhalation of the study medication, FEV_1 measurements and a UNDW provocation were performed. Baseline FEV_1 on the starting evening of both periods had to be within 10%, otherwise the second period was postponed to a later day.

Subjects

Nineteen nonsmoking asthmatic patients (6 males, 13 females) according to the criteria of the American Thoracic Society [11], aged 16–54 (mean 28) yrs, entered the study. Sixteen persons were atopic, defined by an elevated specific immunoglobulin E or positive intracutaneous tests against house dust mite or two of seven other tested common aero-allergens [12]. At study entry, FEV_1 had to be ≥50% predicted, and reversibility had to be ≥15% from prebronchodilator values in response to 200 μg salbutamol by MDI. The provocative concentration of histamine causing a 20% fall in FEV_1 ($PC_{20,H}$) [13] had to be below 4 $mg \cdot mL^{-1}$ for all subjects. None had any significant medical condition or an upper or lower respiratory tract infection within 6 weeks before the study. Seasonally allergic persons were not measured during the time when exposure to such allergen was likely. During the study, the subjects used only salbutamol by MDI (100 μg) as needed to control symptoms. Anti-inflammatory treatment (inhaled corticosteroids, nedocromil sodium, and cromolyn sodium) were withheld for at least 6 weeks preceding the study and systemic steroids for at least 6 months. Methylxanthines were stopped at least 48 h, anticholinergics and antihistamines at least 24 h, before the start of the trial. The study was approved by the local hospital Ethics Committee; written informed consent was obtained from all participants.

Methods

The bronchodilator response and reactions to UNDW provocation were assessed by FEV_1, obtained from flow-volume curves recorded on a heated pneumotachograph (Spiro analyser ST 250®; Fukuda Sangyo Co., Tokyo). Baseline FEV_1 was recorded from the best of three reproducible values (within 5%).

The UNDW provocation test was performed according to the method described by GROOT *et al.* [14]. An ultrasonic nebulizer (Ultraneb 99, DeVilbiss, Somerset, PA, USA) was used at a fixed output of 2.00±0.05 $mL \cdot min^{-1}$. The patient inhaled UNDW during tidal breathing through a mouthpiece with tightened lips and the nose clipped. A Wright respirometer (British Oxygen Co., London, UK) was connected to a two-way valve (Laerdal IV, Stavanger, Norway), placed in-between the aerosol hose and the mouthpiece, to measure the total volume of inhaled air. After inhalation of 20 L of ambient air through the system, doubling volumes of air with UNDW (3, 5, 10, up to 160 L) were successively inhaled at 5 min intervals. The response to inhaled UNDW was assessed by FEV_1 after 90 and 180 s of each dose. The test was stopped if FEV_1 dropped by at least 20% or if 160 L of air with UNDW was inhaled. Before and after each test, the nebulizer chamber and aerosol hose were weighted. The cumulative dose of inhaled distilled water in mL H_2O causing a 20% fall in FEV_1 from post-air values ($PD_{20,UNDW}$), was calculated by linear interpolation on a semilogarithmic curve.

Pretrial $PC_{20,H}$ was measured according to the method of COCKROFT *et al.* [13]. In short, the patient inhaled doubling doses of histamine phosphate from 0.03 to 16 $mg \cdot mL^{-1}$. The test was stopped if FEV_1 fell 20% from baseline, and a log dose-response curve was constructed. The $PC_{20,H}$ was calculated in $mg \cdot mL^{-1}$ by linear interpolation.

Statistical analysis

All $PD_{20,UNDW}$ data were log_{10} transformed before analysis. FEV_1 data were expressed as % pred [15]. To calculate the treatment effect of salmeterol, differences between values (FEV_1 and $PD_{20,UNDW}$) on salmeterol and on placebo were calculated and tested at each time-point with the Wilcoxon signed rank test. The change in UNDW responsiveness ($\Delta PD_{20,UNDW}$) was expressed in doubling doses (DD), calculated as:

$$((\log PD_{20,UNDW}\text{-salmeterol}) - (\log PD_{20,UNDW}\text{-placebo}))/\log 2$$

Period and carry-over effects were analysed according to KOCH [16]. The coefficient of repeatability for $PD_{20,UNDW}$ was calculated for each subject using the two UNDW provocations in the placebo period (baseline), at the same time of the day (08:00 h) according to the method of BLAND and ALTMAN [17]. Correlations between variables were performed with the Spearman correlation test. Regression lines were compared with analysis of variance (ANOVA) of repeated measurements. For multiple comparisons, a Bonferroni correction was used. A p-value of 0.05 or less was considered significant for one test. For multiple comparisons, this boundary was set at 0.01. Data are reported as mean values (SEM).

Results

Patient characteristics are listed in table 1. Seventeen patients completed the study. Two persons (subjects No. 2 and 8) failed to return to the laboratory for lung function and provocation tests for the second treatment period (both after placebo in period one) and were withdrawn from the study. There were no period or carry-over effects between the two study periods at any time-point with regard to FEV_1 and $PD_{20,UNDW}$ data.

Baseline FEV_1 and changes during study medication

Baseline FEV_1 at 22.00 h on the starting day of both periods was similar with a variation of 1.8% (range 0.3–9.7% pred).

Salmeterol caused a significant and substantial degree of bronchodilatation *versus* placebo from 20 min up to 20 h after inhalation (per cent increase from baseline after salmeterol 14.8 (2.1), 17.7 (2.3), 13.9 (3.2) and 12.7 (1.7) 1% after 20 and 40 min and 10 and 20 h, respectively; all time-points significantly different from placebo ($p<0.001$), except for 34 h ($p=0.55$)) (table 2).

Table 1. – Characteristics of the study subjects

Subject No.	Sex	Age yrs	Atopic	FEV_1 % pred	Reversibility* %	$PC_{20,H}$ $mg \cdot mL^{-1}$	Medication+
1	M	33	Yes	85.2	15.1	0.33	B
2	F	23	Yes	95.0	15.6	0.09	B
3	M	26	Yes	99.6	17.4	0.36	B
4	M	25	Yes	64.0	24.5	1.09	B
5	F	18	Yes	56.2	31.7	0.08	B, T
6	M	39	Yes	85.6	18.5	0.71	B, C
7	F	42	No	59.0	26.9	0.07	B
8	F	26	Yes	99.7	15.6	0.15	B
9	F	16	Yes	106.3	15.6	0.10	B
10	M	22	Yes	77.6	26.1	0.62	B
11	F	27	Yes	96.3	19.2	0.15	B
12	F	22	No	99.7	15.1	1.05	B
13	F	26	Yes	100.3	27.9	0.25	B
14	F	23	Yes	90.3	19.7	0.90	B
15	M	18	Yes	60.2	46.4	0.07	A,B
16	F	54	No	103.3	30.9	1.98	B
17	F	31	Yes	76.4	16.7	3.62	B
18	F	27	Yes	84.9	18.7	0.25	B, IC
19	F	26	Yes	77.3	22.5	0.54	B, C
Mean		27.6		85.1	22.3	0.65	
SEM		2.1		3.7	1.8	0.20	

*: reversibility to salbutamol 200 μg by metered-dose inhaler (% change from prebronchodilator value). +: therapy until 6 weeks before participation in the study. $PC_{20,H}$: provocative concentration of histamine causing a 20% fall in forced expiratory volume in one second; A: anticholinergic; B: β_2-agonist; C: cromolyn sodium; IC: inhaled corticosteroids; T: oral theophylline; M: male; F: female.

Table 2. – Individual data of forced expiratory volume in one second (percentage of predicted value)

Patient No.		Time after inhalation									
		20 min		40 min		10 h		20 h		34 h	
	Baseline	SLM	Placebo	SLM	Placebo	SLM	Placebo	SLM	Placebo	SLM	Placebo
1	84.5	103.1	84.3	105.6	87.2	93.1	81.1	92.8	82.8	82.8	80.6
3	106.0	115.8	104.9	119.0	108.0	113.6	106.0	113.4	116.0	98.4	103.0
4	55.1	63.2	56.6	67.3	60.9	63.8	44.1	60.7	56.4	54.3	44.6
5	54.2	64.3	56.4	67.9	53.4	46.0	38.9	64.6	55.3	57.8	60.5
6	85.0	91.0	86.4	96.2	85.8	95.1	73.1	88.4	89.9	75.2	81.2
7	49.0	53.5	50.9	53.5	51.2	59.7	50.9	66.4	50.9	52.7	50.5
9	109.4	119.4	108.0	120.2	109.1	121.7	112.8	117.1	115.7	117.1	113.4
10	76.7	95.0	73.8	98.6	77.3	95.8	71.2	99.0	80.9	88.8	66.6
11	95.6	114.5	99.6	112.3	101.8	124.3	100.9	108.0	100.6	99.0	100.3
12	94.6	100.5	94.0	107.0	82.1	98.3	70.8	108.2	79.8	63.4	73.6
13	68.8	105.5	60.0	104.3	58.3	106.1	62.9	90.9	72.3	68.8	83.0
14	94.9	111.1	94.4	111.7	100.0	111.1	90.3	111.9	102.5	100.2	92.8
15	61.2	75.4	65.1	82.5	60.6	86.0	56.5	75.2	49.8	46.2	46.4
16	87.7	118.7	80.2	123.9	81.6	102.3	89.6	104.2	89.6	84.0	86.3
17	81.2	92.6	81.8	94.2	83.4	92.9	80.8	92.3	85.0	81.5	78.2
18	90.6	98.1	83.7	97.2	86.1	100.6	78.6	94.8	83.1	82.5	54.2
19	93.8	97.3	90.2	95.8	91.4	97.0	90.2	92.9	93.5	86.1	81.4
Mean	81.7	95.2	80.6	97.5	81.1	94.5	76.4	93.0	82.6	78.7	76.3
SEM	4.4	4.9	4.3	4.8	4.5	5.1	5.1	4.2	5.0	4.7	4.9

SLM: salmeterol.

The early morning dip seen after treatment with placebo (at 08.00 h) (mean change in FEV_1 -7.2%, range -28 to +5, compared to 22.00 h) was completely abolished in all but one patient ($p<0.001$). The next morning (34 h after inhalation), salmeterol no longer provided protection against a morning dip.

UNDW provocation tests

Two of the 17 persons differed in their response to UNDW provocation from the others. Subject No. 1 appeared to be unresponsive to UNDW provocation. He recovered very fast from the constrictor effects of UNDW and showed a plateau in reaction of FEV_1 at 80% of the post-air values. Subject No. 10 turned out to be refractory to subsequent UNDW provocation tests. $PD_{20,UNDW}$ increased at subsequent tests, and he ended totally unresponsive at the third test (table 3). For these two patients, no real treatment effect of salmeterol could be calculated, but exclusion of their data did not alter the levels of significance for the major outcome variables. The other 15 subjects demonstrated a good short-term reproducibility of $PD_{20,UNDW}$. The standard deviation of the differences for baseline UNDW provocations was 0.67 DD.

In the whole group (n=17), treatment with salmeterol resulted in protection against UNDW-induced bronchoconstriction for at least 20 h (table 3). Ten hours after the inhalation of salmeterol, a significant increase was observed in the $PD_{20,UNDW}$ of 16.7 (2.3) mL H_2O as compared with 3.3 (1.4) mL H_2O after placebo (treatment effect of 2.82 (0.35) DD, $p<0.0001$). In nine of the 17 subjects, the maximum dose of UNDW was reached. In these patients, the total amount of mL H_2O inhaled at that time was taken for analysis, since no $PD_{20,UNDW}$ could be reached after salmeterol. Twenty hours after inhalation (at 18.00 h) there was still a significant protection for UNDW, with a $PD_{20,UNDW}$ of 7.0 (1.5) mL H_2O after salmeterol as compared with 3.3 (0.7) mL H_2O after placebo (treatment effect of 1.09 (0.23) DD, $p=0.0008$). After 34 h (at 08.00 h) $PD_{20,UNDW}$ values returned to placebo level (4.8 (1.6) mLH_2O after salmeterol as compared with 5.0 (2.0) mL H_2O after placebo (treatment effect of 0.1 (0.2) DD, $p=0.55$)).

Table 3. – Individual data of PD_{20}

Patient No.	Time after inhalation					
	10 h		20 h		34 h	
	SLM	Placebo#	SLM	Placebo	SLM	Placebo#
1	19.3	25.0	6.1	10.9	11.2	21.2
3	15.4	2.6	2.7	4.8	1.3	1.9
4	1.7	0.9	1.3	1.2	1.7	1.3
5	1.0	0.3	1.9	0.4	0.2	0.5
6	18.5	6.3	10.6	7.6	10.0	6.5
7	21.7	0.7	6.7	1.8	2.2	2.8
9	19.9	0.9	4.7	1.0	2.7	1.3
10	30.1	1.7	25.6	6.1	27.3	31.2
11	12.2	3.4	7.7	3.0	2.1	3.8
12	23.1	1.9	3.2	2.2	2.7	1.9
13	12.9	0.9	1.9	0.9	0.8	1.3
14	17.5	1.7	17.3	5.7	5.3	2.1
15	8.9	1.0	1.7	1.0	2.0	1.0
16	11.9	2.4	4.0	1.5	3.74	2.9
17	8.3	1.8	10.7	3.2	1.2	2.0
18	37.2	1.6	6.8	1.4	1.9	0.8
19	23.2	1.6	5.6	2.8	5.4	2.7
Mean	16.7	3.3	7.0	3.3	4.8	5.0
SEM	2.3	1.4	1.5	0.7	1.6	2.0

SLM: salmeterol; PD_{20}: provocative dose causing a 20% fall in forced expiratory volume in one second; #: data used for the coefficient of repeatability (excluded patients Nos. 1 and 10; see text).

Relationship between UNDW provocation and airway calibre

For each time-point, $\Delta PD_{20,UNDW}$ was not correlated with the corresponding change in FEV_1 (ΔFEV_1) from placebo to salmeterol (all $r<0.11$, $p>0.65$).

Figure 1 shows the FEV_1 and corresponding $PD_{20,UNDW}$ values of the individual patients 10 and 20 h (fig. 1a), and 20 and 34 h (fig. 1b) after inhalation of salmeterol. From 10 to 20 h, in most individual patients and as a group, FEV_1 did not change ($p=0.38$), while $PD_{20,UNDW}$ dropped significantly ($p=0.002$, fig. 1b). From 20 to 34 h, both mean FEV_1 and mean $PD_{20,UNDW}$ decreased significantly ($p<0.001$ and $p=0.02$, respectively), but again some individual patients showed (almost) no decrease in FEV_1, while $PD_{20,UNDW}$ dropped (lines appear roughly vertical), while other patients exhibited a decrease in FEV_1 with no change in PD_{20}, (lines are more or less horizontal). Both time courses indicate that the protection afforded by salmeterol was independent from bronchodilation.

The slopes of the regression lines through these points after 10 and 20, but not after 34 h on salmeterol and placebo differed significantly from zero ($p=0.001$ and $p=0.03$, respectively), indicating a (linear) relationship between starting airway calibre and BHR. The slopes between the regression lines of salmeterol in comparison with placebo were not different at any time-point (all $p>0.49$), but again, both after 10 and 20 h, the treatment effect of salmeterol was highly significant, placing the lines after salmeterol parallel at a higher level compared to placebo ($p=0.000$ and $p=0.002$, respectively). Previously, it has been shown that there is a linear relationship between FEV_1 and the provocative concentration of methacholine causing a 20% fall in FEV_1 ($PC_{20,M}$) [18]. Under the assumption of a similar relationship between FEV_1 and $PD_{20,UNDW}$ and because the measurements on salmeterol and on placebo are paired, the relationship between $PD_{20,UNDW}$, and FEV_1 can be describe statistically with one equation for the regression lines at each time-point:

$$\text{after 10 h: } PD_{20,UNDW} = -0.747 + 1.075 \text{ treatment} + 0.035 \times FEV_1$$

$$\text{after 20 h: } PD_{20,UNDW} = -0.636 + 0.408 \text{ treatment} + 0.028 \times FEV_1$$

where treatment is assigned a value of +1 for salmeterol and -1 for placebo.

$\Delta PD_{20,UNDW}$ is 2.8 and 1.1 DD, and ΔFEV_1 is 18.4% and 10.4%, after 10 and 20 h, respectively. It follows from the equation that 10 h after inhaling salmeterol,

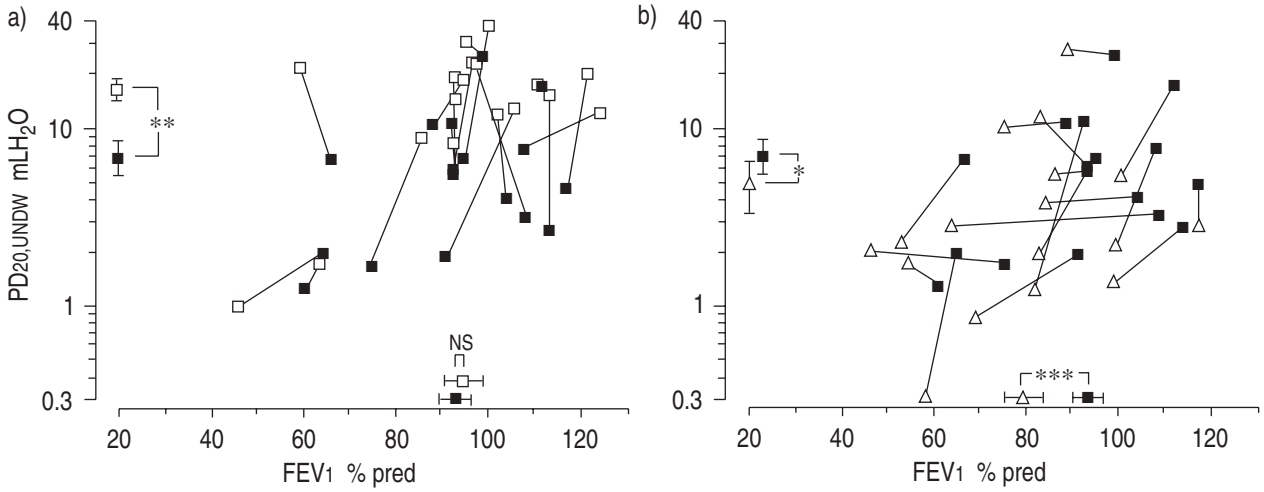

Fig. 1. – The change in FEV1 and corresponding PD20,UNDW points in each patient, from: a) 10–20 h; and b) 20–34 h after inhalation of salmeterol. ❑: 10 h; ■: 20 h; Δ: 34 h after inhalation of salmeterol. Mean (SEM) values of FEV1 and PD20,UNDW are shown at the corresponding axes: *: p<0.05; **: p<0.01; ***: p<0.001; NS: nonsignificant. FEV1: forced expiratory volume in one second; PD20,UNDW: provocative dose of ultrasonically nebulized distilled water causing a 20% fall of FEV1; % pred: percentage of predicted value.

the treatment effect is +2.15 DD and the effect through FEV1 is 0.035×18.4=0.65, hence 2.8 DD as found in the study. After 20 h, the treatment effect is +0.82 and the effect through FEV1 is 0.028×10.4=0.29 (hence the real found protection of 1.1 DD). This means that only 23% (0.65/2.80×100%) of the afforded protection can be explained by bronchodilation, and 77% by a direct effect of salmeterol. After 20 h these values are 26 and 74%, respectively.

Discussion

This study shows that a single dose of salmeterol affords both bronchodilation and protection against UNDW provocation up to 20 h in asthmatic patients who did not use anti-inflammatory medication. Protection against UNDW provocation beyond the period of bronchodilation did not occur. In 11 of 17 patients, the inhibition of bronchoconstriction to UNDW decreased significantly between 10 and 20 h, at which time bronchodilation persisted. However, protection was still more than 1 DD after 20 h, and up to this time, only a maximum of 26% of the protection could be explained by the bronchodilating effect of salmeterol.

The duration of protection against UNDW challenge was in line with the *in vitro* activity of salmeterol. UNDW provocation is thought to be mediated by the release of mast cell mediators [9, 10]. Salmeterol inhibits the release of these mediators from sensitized human lung fragments for more than 20 h [2]. In the present study, a single dose of salmeterol afforded protection at 10 h of almost 3 DD, and, although the magnitude of the protection weaned, protection was still more than 1 DD after 20 h. In this way, salmeterol showed, *in vivo,* a relevant protection during the period of blocking mediators *in vitro* [2].

In accordance with BOOTH *et al.* [19], no increase in BHR after withdrawal of salmeterol was found in our study. Thirty four hours after inhalation of salmeterol (more than three times the half-life), no rebound BHR to UNDW occurred, the PD20,UNDW being 0.12 DD above placebo.

Salmeterol also induced bronchodilation for more than 20 h, and protected against the early morning dip 10 h after inhalation. TWENTYMAN *et al.* [7] tested bronchodilation of a single dose of salmeterol up to 34 h, but regular measurements were discontinued after 9.5 h. When starting measurements again after 32 h, salmeterol no longer afforded bronchodilation. In a group of asthmatic patients with similar characteristics as in the present study, RABE *et al.* [20] showed that salmeterol decreased airway tone significantly over a whole 24 h period, compared with placebo. Because of multiple comparisons, however, the bronchodilating effect was not significant beyond 12 h at the individual time-points. Our study clearly shows a bronchodilation up to 20 h, which disappeared after 34 h.

Besides bronchodilation, an important property of salmeterol could be the ability to afford protection of airways smooth muscle against bronchoconstrictor mediators with time-course characteristics different from those observed for bronchodilation [7]. Since baseline airway function correlates somewhat with airway reactivity [18], the inhibitory effect of a bronchodilator could be due to a change in airway calibre. In this study, both bronchodilation and protection lasted more than 20 h but less than 34 h. More measurements of UNDW provocation during this period would be needed to determine exactly the duration of action and to distinguish between protection and bronchodilation. However, we made measurements at 10 h intervals to avoid confounding problems such as a temporary (small) increase in BHR after UNDW provocation [10, 21], and to avoid refractoriness after repeated UNDW measurements [14, 21], which may persist up to 4 h after the last challenge [22, 23]. Despite this, two patients (subjects No. 2 (dropped out) and 10) became refractory to successive UNDW provocations.

No correlation was found between bronchodilation (ΔFEV1) and protection (ΔPD20,UNDW) provided by salmeterol at any time-point, indicating that protection was not caused by bronchodilation. However, the number of patients in our study is probably too small to state that there might not be a correlation with a much larger

population. On the other hand, figure 1 shows that in individual patients the protection afforded by salmeterol is independent of airway calibre, and there were moderate-to-severe responses to inhalation to water at a time when airway calibre was optimal. The regression lines through these points again show a highly significant treatment effect of salmeterol, by shifting the lines at 10 and 20 h parallel to higher levels than after placebo. From the equations of the regression lines on salmeterol and on placebo at the various time-points, it can be calculated that up to 20 h, only a maximum of 26% of the protection can be explained by the bronchodilating effect of salmeterol. Therefore, there seems to be a differential effect of salmeterol on lung function and the response to UNDW. A similar dissociation has been shown with sodium cromoglycate, which had no effect on lung function, but did block UNDW provocation [24]. Conversely, ipratropium bromide in doses up to 160 μg caused bronchodilation, but did not change the response to challenge with UNDW [25].

Other mechanisms are thus likely to be involved in the protective effects of salmeterol against UNDW-induced bronchoconstriction. The term functional antagonism is often used to describe the protective effects of β_2-agonists during provocation tests. β_2-agonists may prevent smooth muscle contraction, irrespective of the constrictor mediator, by acting on a different receptor on the same cell, which opposes this constriction [26]. In this way, pharmacological effects of β_2-agonists are different between smooth muscle relaxation and protection against bronchoconstriction [27]. It has previously been shown that β_2-agonists provide true functional antagonistic protection at the level of the smooth muscle against direct pharmacological stimuli as histamine and methacholine [28]. UNDW, however, is thought not to act directly at the level of the smooth muscle, but to induce airway narrowing indirectly [9, 10]. Therefore, mechanisms other than bronchodilation and functional antagonism should be considered to explain this apparent dissociation.

O'CONNOR *et al.* [29] showed that β_2-agonists have an additional inhibitory nonsmooth muscle effect on bronchoconstrictor stimuli that involve mast cell activation, in affording a greater protection against adenosine monophosphate- than methacholine-induced bronchoconstriction. Salmeterol has several acute anti-inflammatory effects *in vitro* that may contribute, *e.g.* the strong inhibition of the release of mast cell mediators [2], involved in the mechanism of action of UNDW. Thus, although the evidence is only indirect, this protection may indicate long-lasting cell-stabilizing effects of salmeterol *in vivo* up to 20 h rather than functional antagonism.

Finally, in a number of patients, the protection of salmeterol against UNDW decreased, while bronchodilation persisted. This dichotomy between duration of bronchodilation and protection against a bronchoconstrictor stimulus has already been described by AHRENS *et al.* [30]. These differences in time course could reflect differences in the mechanism for these two β_2-agonistic actions. However, an alternative explanation could be the differences in potency of the bronchoconstrictor stimulus. A larger concentration of a β_2-agonist may be required to prevent contraction to a potent stimulus as a provocation test, as compared with the concentration of the drug to produce relaxation of the relatively modest level of bronchospasm at baseline [31]. Several studies showed a relationship between bronchodilator dose and the degree of inhibition of provocation [32]. A greater concentration of salmeterol may be required to prevent contraction to UNDW provocation than is required to produce relaxation. However, the concentration of salmeterol required to prevent mast cell mediator release may similarly be higher than the concentration required to prevent contraction of the muscle by the mediators released.

Whether this nonbronchodilator effect of salmeterol also provides clinically relevant effects or persists after prolonged therapy, is at present unclear. No change in BHR was reported after 6 weeks of treatment with salmeterol [7]. On the other hand, salmeterol significantly improved the treatment of (chronic) bronchial asthma and resulted in a clinical significant improvement in quality of life *versus* placebo and salbutamol [33]. GREENING *et al.* [34] showed that adding salmeterol to inhaled corticosteroid therapy was more appropriate for patients with inadequately controlled asthma on low-dose inhaled corticosteroids than doubling this dose. Finally, in this study, salmeterol afforded a significant protection of more than 20 h against a naturally occurring stimulus, which may be very relevant for asthma management.

In conclusion, our study shows that a single dose of salmeterol in mild-to-moderate asthma causes bronchodilation and protection independently of this bronchodilation against a physiological bronchoconstrictor stimulus for more than 20 h.

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