

A comparative study in atopic subjects with asthma of the effects of salmeterol and salbutamol on allergen-induced bronchoconstriction, increase in airway reactivity, and increase in urinary leukotriene E₄ excretion

Ian K. Taylor, BSc, MRCP, Kevin M. O'Shaughnessy, DPhil, MRCP,
Nozhat B. Choudry, PhD, Mitsuru Adachi, MD,
James B. D. Palmer, MRCP,* and Richard W. Fuller, MD, MRCP
London and Greenford, England

Salmeterol (SM) is a novel β_2 -adrenoceptor agonist with a duration of action in excess of 12 hours. Evidence from in vitro studies has also demonstrated that, unlike the short-acting β_2 -agonists, such as salbutamol (SB), it may have some anti-inflammatory properties. With a randomized, double-blind, crossover design, we have compared the inhibitory effects of SM (50 μ g) and SB (200 μ g) delivered by metered-dose inhaler on allergen-induced bronchoconstriction, changes in airway reactivity, and urinary leukotriene (LT) E₄ excretion in 12 atopic subjects with mild asthma. The immediate bronchoconstriction to allergen was significantly reduced by both β_2 -agonists ($p < 0.005$), when reduction was expressed either in terms of maximum fall in FEV₁ at 15 minutes after allergen (percent fall in FEV₁, mean $\pm$ SEM: 6.2 $\pm$ 4.9, SM; 5.7 $\pm$ 2.5, SB; 40.4 $\pm$ 6.3, placebo) or the area under the FEV₁ time curve (AUC) for the first 120 minutes after allergen. Four hours after challenge, results in the SB-treated and placebo-treated groups were not significantly different and demonstrated a small persistent bronchoconstriction compared to bronchodilatation in the SM-treated group (percent fall in FEV₁, respectively, 9.3 $\pm$ 3.7, 14.3 $\pm$ 7.1, and -6.3 $\pm$ 2.7; $p < 0.005$, SM versus SB; $p < 0.02$, SM versus placebo). Expressed in terms of AUC, only SM significantly reduced bronchoconstriction in the period 120 to 240 minutes after allergen ($p < 0.01$). The changes in airway reactivity 4 hours after allergen were (doubling doses of histamine, median, and range) -1.34 (-4.06 to 0.56) placebo; -0.20 (-3.29 to 1.14) SB; and 1.39 (-1.22 to 5.60) SM ($p < 0.005$, SB and SM versus placebo; $p < 0.005$, SM versus SB). There were no differences between groups in urinary LTE₄ excretion for either the 0 to 120-, 120 to 240-, or total 0 to 240-minute collection periods after allergen inhalation ($p > 0.1$). Therefore, although both drugs have inhibitory effects on allergen-induced bronchoconstriction, neither drug significantly reduced allergen-induced cysteinyl leukotriene production. This finding suggests that the major effect of inhaled β_2 -agonists in allergen challenge is relaxation of airway smooth muscle and not the inhibition of mediator release from pulmonary inflammatory cells. (J ALLERGY CLIN IMMUNOL 1992;89:575-83.)

Key words: Allergen challenge, asthma, cysteinyl leukotrienes, leukotriene E₄, β_2 -agonists

From the Department of Clinical Pharmacology, Royal Postgraduate Medical School, London, and *Department of Respiratory Medicine, Glaxo Group Research, Ltd., Greenford, Middlesex, England.

Supported by National Asthma Campaign, Glaxo Pharmaceuticals, and a grant from the Medical Research Council.

Received for publication May 29, 1991.

Revised Aug. 28, 1991.

Accepted for publication Oct. 7, 1991.

Reprint requests: I. K. Taylor, MD, Department of Clinical Pharmacology, Royal Postgrad. Med. School, Du Cane Rd., London W12 0NN, England.

1/1/34259

The present therapeutic strategies in asthma are directed toward both reducing airway inflammation and promoting bronchodilatation.¹ Of the currently available bronchodilators in clinical use, β_2 -selective adrenergic agonists are by far the most effective, although all are limited by their relatively short duration of action. Considerable emphasis has been placed on studies demonstrating that inhaled β_2 -agonists,² in contrast to glucocorticosteroids,² nedocromil sodium,³ and sodium cromoglycate,² do not inhibit either late bronchoconstrictor responses to allergen or the subsequent increase in bronchial responsiveness. Moreover, chronic administration of β -adrenergic agonists,⁴ unlike glucocorticosteroids,⁴ does not reduce bronchial responsiveness and may even cause a small rebound increase in airway reactivity when therapy is stopped.⁵ This evidence has therefore highlighted the potential inadequacy of β_2 -agonists as disease-modifying agents in asthma and leads to the concept that they are of value merely in the symptomatic relief of airflow obstruction.

The molecular mechanisms by which β_2 -agonists mediate bronchodilatation are well understood.⁶ Although reversal of airway smooth muscle contraction is believed to represent the principal mode of action, considerable in vitro evidence exists suggesting additional properties, including attenuation of mast cell-mediator release,^{7,8} reduction in airway microvascular leakage,⁹ and inhibition of cholinergic neurotransmission within the airway.¹⁰ However, direct evidence in vivo for any anti-inflammatory effects of β_2 -agonists is limited.¹¹

SM is a new sympathomimetic bronchodilator agent (Fig. 1) with a prolonged duration of action and, as such, may represent an important therapeutic advance in the treatment of asthma. In vitro evidence of its prolonged effects on airway smooth muscle has been confirmed in animal models in vivo,^{12,13} and more recent clinical evaluation¹⁴⁻¹⁶ suggests a duration of action of 7 to 12 hours. In addition to producing sustained bronchodilation, SM attenuates release of inflammatory mediators from antigen-stimulated human lung fragments in vitro for up to 20 hours¹⁷ and microvascular leakage and inflammatory cell infiltration in guinea pig lung for 6 to 8 hours.¹³ Furthermore, SM, over concentration ranges at which short-acting β_2 -agonists are ineffective, has marked inhibitory activity on mediator release in vitro from neutrophils¹³ and alveolar macrophages,¹⁸ the latter putatively via a mechanism independent of β_2 -receptor activation.

In this comparative study, we have evaluated the effect of a single inhaled dose of SM (50 μ g) and SB (200 μ g) on the airflow obstruction and change in bronchial reactivity that follows allergen challenge in

Abbreviations used

AUC:	Area under the curve
LT:	Leukotriene
SB:	Salbutamol
SM:	Salmeterol
PC ₂₀ :	Provocative concentration causing a 20% fall in FEV ₁
HPLC:	High-performance liquid chromatography
Δ DDPC ₂₀ :	Doubling-dose change in PC ₂₀ histamine values

atopic subjects with asthma. We have also measured urinary LTE₄ excretion as an integrated measure of mediator release from lung inflammatory cells after inhalation of allergen.¹⁹

METHODS

Twelve atopic subjects with mild asthma participated after giving written and informed consent for the study that was approved by the Hammersmith and Queen Charlotte's Special Area Health Authority. All subjects gave a history of periodic wheeze and demonstrated a reversibility of at least 20% in peak expiratory flow rate either spontaneously or with bronchodilator therapy. None of the subjects smoked, seven subjects were male, and their ages ranged between 20 to 40 years. All subjects were clinically stable at the time of the study and had no evidence of systemic or respiratory disease. Their serum biochemical profile and full blood counts were all within normal limits. FEV₁ was >60% predicted, and histamine PC₂₀ <32 mg/ml at inclusion into the study. At the time of study, subjects were either taking no therapy or occasional inhaled SB alone and were skin test positive to either mixed grass pollen or house dust-mite (*Dermatophagoides pteronyssinus*) extracts. Subjects' characteristics are summarized in Table I. Bronchodilator therapy, in which it was applicable, was withheld for at least 8 hours and caffeine-containing drinks for 12 hours before bronchial challenges.

Bronchial challenges

Airway caliber was measured before and during the inhalation challenges as FEV₁ by use of a dry-wedge spirometer (Vitalograph, Buckingham, U.K.). Serial dilutions of histamine acid phosphate were prepared freshly in 0.9% wt/vol of saline from a stock solution containing 32 mg/ml. Extracts of allergen (*D. pteronyssinus*/Aquagen SQ or mixed grass pollens/Spectralgen, Pharmacia, Milton Keynes, U.K.) were prepared in 0.9% saline to produce a range of concentrations from 500 to 25000 U/ml. Bronchial challenges were administered from a compressed air-driven nebulizer attached to a breath-activated dosimeter (P. K. Morgan, Gillingham, Kent, U.K.) with a delivery time of 1 second. Subjects inhaled the aerosol from a mouthpiece by taking five consecutive breaths from functional

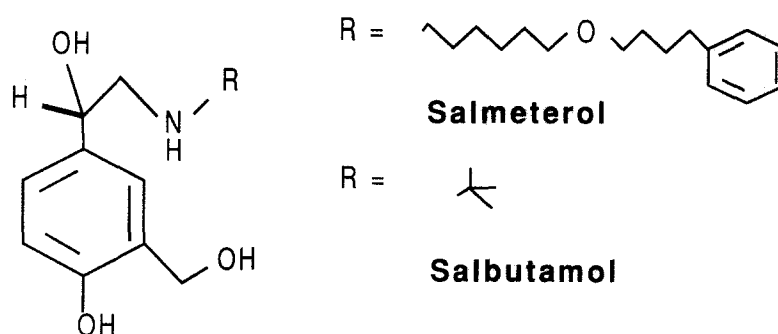


FIG. 1. Structures of SB and SM.

TABLE I. Subjects' characteristics

Subject	Age (yr)	Sex	Height (cm)	Smoker Y/N	FEV ₁ * (L)	% Predicted FEV ₁ *	PC ₂₀ histamine (mg/ml*)	Treatment
1	25	F	168	N	2.98	86.4	1.52	None
2	32	M	175	N	4.10	102.5	11.65	SB
3	28	F	151	N	2.80	100.4	3.43	SB
4	22	F	160	N	3.30	105.8	3.01	SB
5	22	F	163	N	1.90	64.2	0.15	SB
6	38	M	181	N	4.28	106.5	28.3	SB
7	23	M	173	N	3.23	80.8	0.99	SB
8	39	M	180	N	4.20	108.5	6.67	SB
9	20	M	166	N	3.53	92.9	1.66	SB
10	29	M	183	N	3.82	87.6	7.87	SB
11	40	M	180	N	2.48	64.1	0.31	SB
12	25	F	162	N	2.78	89.1	19.50	None

*Values at inclusion into the study (visit 1).

residual capacity to total lung capacity; under these conditions, 8 μ l was delivered per breath.

Assessment of PC₂₀ histamine. One FEV₁ measurement was made before and 1 minute after the inhalation of five breaths of 0.9% saline; the postsaline value was designated the basal value. Postsaline FEV₁ measurements had to be within 10% of measurements recorded before saline inhalation. Doubling-dose concentrations of histamine acid phosphate were then inhaled, and FEV₁ was measured 1 minute later until at least PC₂₀ had been achieved or the subject had inhaled until at least a 20% fall from baseline had been achieved or the highest concentration of histamine used (32 mg/ml). PC₂₀ histamine was obtained by linear interpolation of the last two points of the dose response. Five subjects who received SM had a fall in FEV₁ of <20% after 32 mg/ml of histamine and were assigned a PC₂₀ histamine of 32 mg/ml.

Allergen challenge. *D. pteronyssinus* or grass-pollen extract was chosen for allergen inhalation, depending on the results of skin prick testing. After determination of the postsaline baseline FEV₁ (always within 10% of the presaline values), subjects inhaled from an initial solution of allergen

chosen as that which caused at least a 3 mm diameter wheal on intradermal injection. FEV₁ was then measured at 5 and 10 minutes. If FEV₁ recorded at 10 minutes had fallen <10% of the baseline, a fivefold increase in allergen concentration was inhaled. If the fall was >10% but <15%, a 2.5 increase in allergen concentration was administered. If the fall was >15%, no further allergen was administered. No subject received more than three doses of allergen. Cumulative allergen administered ranged from 6000 to 31,000 U/ml. FEV₁ measurements were made at 5, 10, 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, and 240 minutes after the last dose of allergen. At 4 hours, postchallenge PC₂₀ histamine was again determined.

Study design

Subjects attended the clinical laboratory at 8 AM on four separate occasions at least 1 week apart. On visit 1, subjects were evaluated for inclusion into the double-blind part of the study. Provided baseline spirometry was >60% predicted and histamine PC₂₀ was <32 mg/ml, an allergen challenge was performed 1 hour after the completion of the histamine challenge. On subsequent study days, subjects'

PC₂₀ histamine was determined on arrival in the laboratory and had to be within 1 doubling dose of the value recorded at their initial visit. One hour later, the trial medication was administered as two actuations from identical metered-dose inhalers containing SM (25 µg per puff), SB (100 µg per puff), or placebo. Dosing was randomized and double blinded; 15 minutes later, FEV₁ was measured (posttreatment FEV₁) followed immediately by determination of the postsaline baseline (prechallenge) FEV₁ and the start of the allergen challenge. Subjects inhaled exactly the same doses of allergen during the same time as visit 1. Administration of the final dose of allergen was designated time 0, and postchallenge FEV₁ measurements were started. At 4 hours, PC₂₀ histamine was again determined. The study was then concluded by administering inhaled SB (200 µg) and beclomethasone dipropionate (2 mg).

Urine collections

On all study days, subjects voided their bladder immediately before allergen challenge and then drank 500 ml of water. Urine was voided at 120 minutes after inhalation of the last dose of allergen. The duration of this urine collection was minimally 120 minutes or maximally 140 minutes, depending on the number of doses of allergen administered. The subjects then drank an additional 500 ml of water, and at 240 minutes after completion of the allergen challenge, urine was again collected. The duration of this collection was always 120 minutes. Possible diurnal variation in urinary cysteinyl leukotriene excretion was minimized by performing allergen challenges at the same time on each of the study limbs.

Mediator analysis

Urinary LTE₄ was measured with single-step extraction HPLC coupled with RIA.²⁰ Urine (30 ml) was spiked with ~5000 disintegrations per minute of [³H]LTE₄ (Du Pont [U.K.] Ltd., Stevenage, Herts, U.K.), 38.4 Ci/mmol, immediately after collection and then stored at -80° C. Samples were allowed to thaw at room temperature just before analysis. The single-step extraction and HPLC were performed as previously described in which handling is reduced to a minimum, and consequently, yields increased. The presence of the [³H]LTE₄ allows the elution position of endogenous LTE₄ to be defined accurately for each given sample. The methanol/water solvent of eight 2 ml fractions across the elution position of LTE₄ was removed under vacuum before immunoassay with a standard kit (Amersham International, Amersham, Buckinghamshire, U.K.). The HPLC fractions were resuspended in 600 µl of immunoassay buffer; 200 µl was used to determine recovery (of the order of 40%) and 2 × 100 µl aliquots to assay for LTE₄ immunoreactivity in duplicate. LTC₄ was used as the radioligand, and samples were quantified against a LTC₄ standard curve (12.5 to 1600 pg). The antibody used was raised against LTD₄; the cross-reactivity of this antiserum toward LTE₄ was 51%. The presence of LTE₄ was accepted only when there was concomitant rise and fall of both the immunoreactivity and radioactivity across the HPLC profile; this also allows the background cross-reacting impurities to be defined and quantified.

Data analysis

Changes in histamine reactivity after allergen challenge were expressed as ΔDDPC_{20} , calculated as the $\Delta\log \text{PC}_{20}/\log_{10}2$. Because of the truncation of a number of histamine challenges with a PC₂₀ of 32, ΔDDPC_{20} histamine is presented as a median, and range and differences between groups were analyzed nonparametrically with a Wilcoxon's signed-rank test. The response to allergen was also computed by trapezoid integration of the FEV₁ AUC for the first (AUC₀₋₁₂₀) and second (AUC₁₂₀₋₂₄₀) 2-hour periods to coincide with the urine collections after allergen challenge. The first 2-hour period actually varied, depending on the number of doses of allergen administered to individual subjects; this was minimally 120 minutes or maximally 140 minutes. The value AUC₀₋₂₄₀ is the sum of these two values. Differences in AUC and falls in FEV₁ on both placebo-treated and active-treated days were assessed by Wilcoxon's signed-rank analysis. Urinary leukotriene excretion was quantified as nanograms per millimole of creatinine. The distribution of LTE₄ is log normal¹⁹ and hence was log transformed before use of a two-way analysis of variance with collection time and treatment as independent variables. Significance was assigned at $p < 0.05$ throughout.

RESULTS

No adverse clinical or biochemical sequelae were noted in relation to either treatment during the study.

The FEV₁ values both before and after histamine challenge, trial medication, and immediately before allergen challenge are presented in Table II. Values either before and after histamine challenge were not significantly different among the three treatment limbs. Inhalation of both β_2 -agonists caused a small bronchodilator effect (Table II), but at the time of allergen-challenge, it did not reach statistical significance.

Percent change in baseline FEV₁ values after allergen challenge are illustrated in Fig. 2. After placebo treatment, there was a fall in FEV₁ with a maximum fall of $40.4\% \pm 6.3\%$ at 15 minutes after the administration of the last dose of allergen. In contrast, after inhalation of SM and SB, respective maximal falls in FEV₁ were $6.2\% \pm 4.9\%$ (at 15 minutes) and $6.5\% \pm 2.7\%$ (at 10 minutes) after the last dose of allergen.

Data relating to the FEV₁ AUCs are illustrated in Fig. 3. Both SB and SM, compared to placebo, significantly attenuated the AUC₀₋₁₂₀ ($p < 0.005$ versus placebo), but there was no significant difference between the β_2 -agonists.

At 240 minutes after allergen challenge, the airflow obstruction on the placebo and SB limbs was not significantly different (Fig. 2). In contrast, after SM, bronchodilatation was observed at this time point ($p < 0.02$, SM versus placebo; $p < 0.005$, SM versus SB). Data relating to the AUC₁₂₀₋₂₄₀ and the

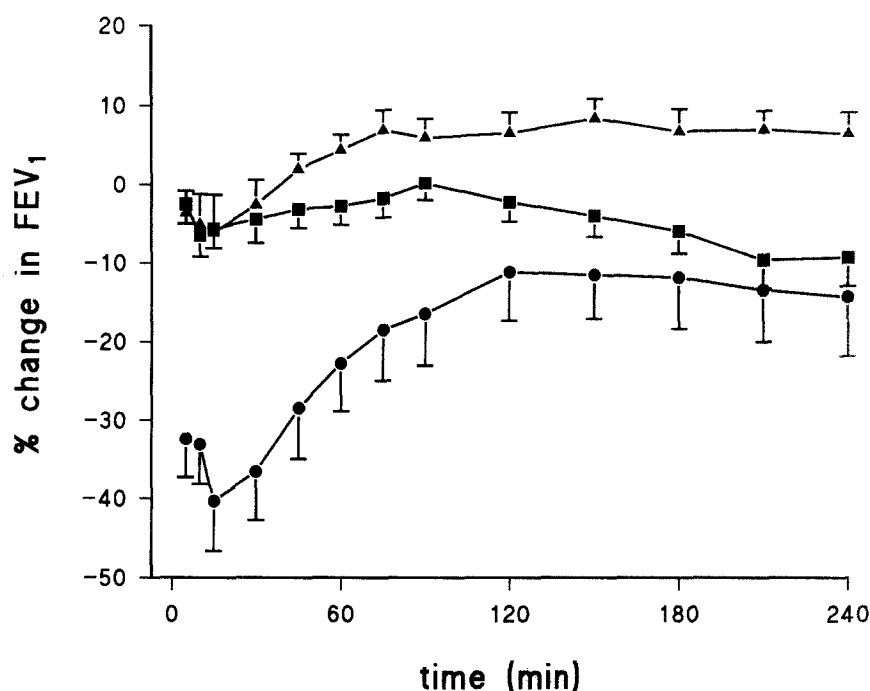


FIG. 2. Effect of SB (■), SM (▲), and placebo (●) on allergen-induced bronchoconstriction. Each point represents the mean ($\pm$ SEM) FEV₁, expressed as a percentage of the preallergen challenge baseline in 12 atopic subjects with asthma. Time zero represents the administration of the last dose of allergen.

TABLE II. Baseline FEV₁ (L) data

Treatment	Before histamine*	After histamine†	After medication‡	Before challenge§	After challenge
Placebo	3.02 $\pm$ 0.23	3.11 $\pm$ 0.23	3.05 $\pm$ 0.22	3.05 $\pm$ 0.24	2.68 $\pm$ 0.32
SB	2.96 $\pm$ 0.23	3.01 $\pm$ 0.21	3.40 $\pm$ 0.18	3.39 $\pm$ 0.18	3.07 $\pm$ 0.20
SM	3.03 $\pm$ 0.22	3.03 $\pm$ 0.22	3.26 $\pm$ 0.19	3.27 $\pm$ 0.17	3.44 $\pm$ 0.17

*All values recorded after saline.

†Recorded 60 minutes after initial histamine challenge.

‡Recorded 15 minutes after study medication.

§The preallergen-challenge value was the basal value for postallergen-challenge measurements.

||Recorded 4 hours after allergen challenge and before the second histamine challenge.

AUC₀₋₂₄₀ are illustrated in Fig. 3. There was significant reduction in the AUC₁₂₀₋₂₄₀ by SM ($p < 0.01$, SM versus placebo; $p < 0.005$, SM versus SB) but not by SB. Both SM and SB significantly attenuated the AUC₀₋₂₄₀ ($p < 0.01$, SB versus placebo; $p < 0.005$, SM versus placebo) as did SM compared to SB ($p < 0.005$).

The increase in airway reactivity after allergen exposure expressed in terms of Δ DDPC₂₀ histamine was significantly attenuated by both SB (median, -0.20 [range, -3.29 to 1.14]) and SM (median, 1.39 [range, -1.22 to 5.60]) compared to placebo (median, -1.34 [range, -4.06 to 0.56]) ($p < 0.005$, versus placebo; Fig. 4). The Δ DDPC₂₀ after treatment

with SM was also significantly different to that after SB treatment ($p < 0.005$). Thus, at 4 hours after the last dose of allergen was administered, the change in airway responsiveness was, respectively, 1.14 and 2.73 Δ DDPC₂₀ after SB and SM compared to placebo.

Urinary LTE₄ excretion was greater during the first 0 to 120-minute collection compared to the second collection (120 to 240-minute collection) on placebo geometric mean, 30.0 [95% confidence interval, 17.4 to 51.6] versus 20.5 [11.7 to 36.0] ng/mmol of creatinine, SB (25.0 [14.9 to 41.7] versus 16.1 [9.2 to 28.2]) and SM (25.1 [16.0 to 39.4] versus 16.9 [10.1 to 28.3]) treatment days (Fig. 5). During either the 0 to 120- or 120 to 240-minute period, there was no

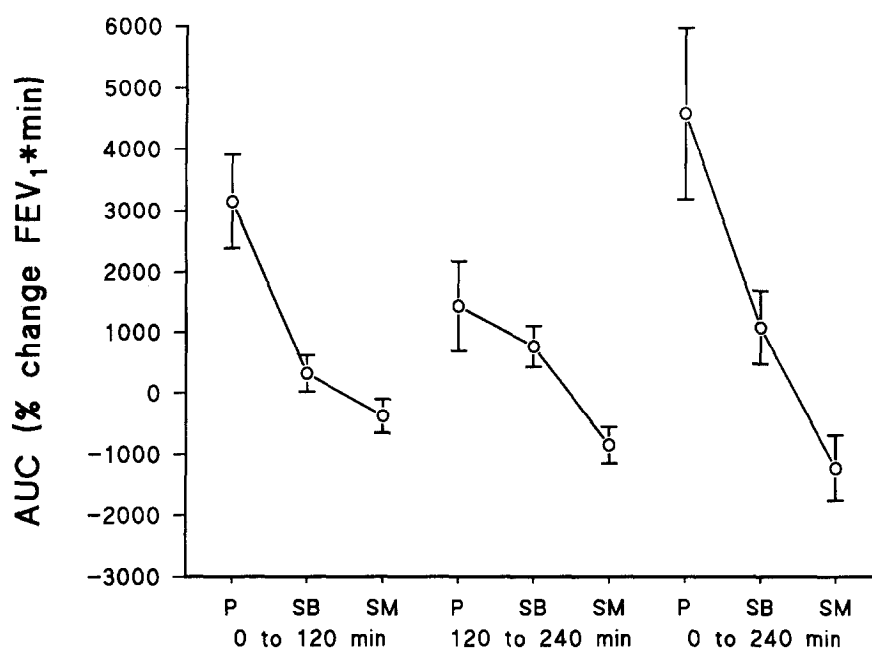


FIG. 3. FEV₁ AUCs after allergen challenge and treatment with placebo (P), SB, and SM. Data (mean $\pm$ SEM) on the three time periods, 0 to 120, 120 to 240, and 0 to 240 minutes, are illustrated. A positive value represents bronchoconstriction.

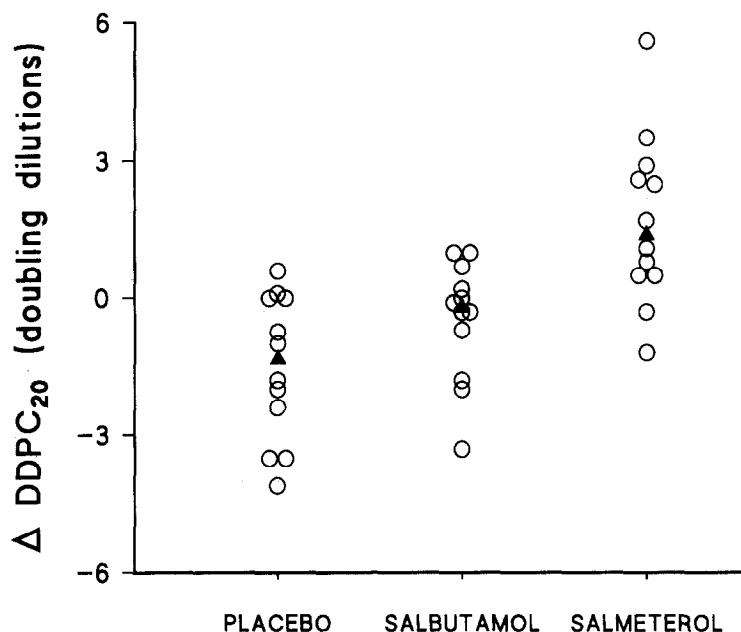


FIG. 4. Effect of SM, SB, and placebo on allergen-induced changes in bronchial reactivity expressed as Δ DDPC₂₀; median values (▲).

significant difference in urinary LTE₄ excretion between treatments ($p > 0.1$; Fig. 5). Expressed as the total LTE₄ excreted after allergen challenge (0 to 240 minutes) (placebo, 25.9 [15.3 to 43.8]; SB, 20.0 [12.8 to 31.3]; and SM, 20.4 [13.8 to 30.3] ng/mmol of creatinine), there was still no significant treatment effect ($p > 0.1$).

DISCUSSION

We undertook this study to compare the effects of single therapeutic doses of inhaled SM (50 μ g) and SB (200 μ g) on allergen-induced bronchoconstriction, airway reactivity, and endogenous cysteinyl leukotriene release in atopic subjects with asthma. This study confirms the inhibitory effect of single-dose in-

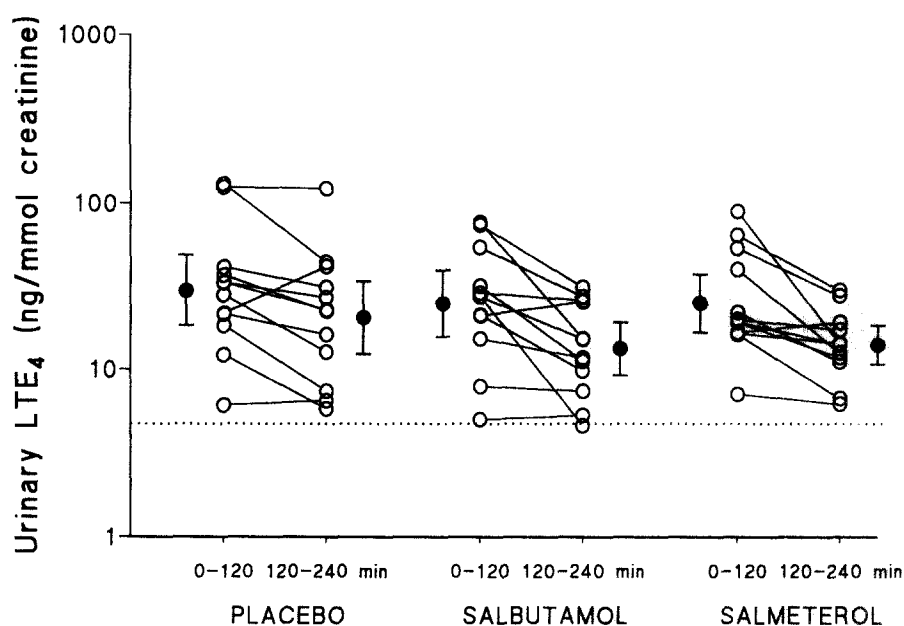


FIG. 5. Effect of SB, SM, and placebo on urinary LTE₄ excretion. Data are expressed as nanograms per millimole of creatinine for the collection periods 0 to 120 and 120 to 240 minutes after allergen challenge; geometric means for each group with 95% confidence interval (●); upper limit of the normal laboratory range for urinary LTE₄ excretion (· · ·), that is, <5 ng/mmol of creatinine.

haled SB on immediate bronchoconstriction after allergen challenge. At later time points (2 to 4 hours), SB and placebo had similar FEV₁ time profiles, and the AUCs were not significantly different. In contrast, SM significantly attenuated the bronchoconstrictor response to allergen during the entire 4-hour observation period after allergen challenge. Neither SB or the longer-acting SM significantly attenuated the allergen-induced increase in urinary LTE₄ excretion.

Human airway smooth muscle possess β_2 -adrenergic receptors,²¹ and activation of these receptors causes bronchodilatation. The long duration of action of SM is believed to reflect exoreceptor binding.²² The long lipophilic chain of the molecule (Fig. 1) facilitates high-affinity binding to a site adjacent to the β_2 -receptor in the cell membrane (the "exoreceptor" site). The findings of this study are therefore in accordance with previous data on the duration of action of SM on airway smooth muscle both in vitro,^{12, 13} in animal models in vivo,¹³ and in clinical evaluation in asthma.¹⁴⁻¹⁶ Four hours after allergen challenge, the airflow obstruction after placebo and SB treatment was indistinguishable. In contrast, after SM, bronchodilatation was observed at this point. The airway effects of SM presumably reflect sustained β_2 -adrenergic-receptor stimulation of the airway smooth muscle that provides functional antagonism of the effects of allergen.

The immediate bronchoconstriction that occurs af-

ter allergen challenge in atopic subjects with asthma has been conventionally associated with the IgE-dependent activation of lung mast cells,²³ although immunologic activation of other cells, such as alveolar macrophages, are likely to be important.²⁴ Activation of mast cells is associated with the release of histamine and spasmogenic products of arachidonic acid metabolism, including prostaglandin D₂, thromboxane A₂, and the cysteinyl leukotrienes (LTC₄, LTD₄, and LTE₄).²³ Furthermore, the generation of cysteinyl leukotrienes in vitro has been described from a variety of tissues, including allergen-challenged lungs of subjects with asthma²⁵ and purified inflammatory cells, of which mast cells²⁶ and eosinophils²⁷ are particularly relevant.

In addition to their effects on airway smooth muscle, β_2 -adrenergic agonists attenuate the release in vitro of histamine and cysteinyl leukotrienes from lung mast cells and sensitized human lung fragments after immunologic challenge.^{7, 28, 29} The duration and magnitude of this inhibition is considerably greater with SM at equipotent concentrations than shorter-acting β_2 -agonists.¹⁷ Such effects in vivo have been suggested by the observations that SB in therapeutic doses suppresses the rise in plasma histamine after allergen challenge.¹¹ These increments are, however, transient (levels peak in 5 minutes and return to baseline within 30 minutes³⁰), often precede the maximum bronchoconstrictor response, and do not provide an integrated

assessment of endogenous mediator release.³¹ Urinary measurements circumvent these problems. After allergen challenge, small increases in the urinary excretion of histamine and thromboxane A₂ metabolites have been reported,^{32, 33} although other studies have failed to verify these findings.^{31, 34} In contrast, there is a large increment in urinary LTE₄ excretion coincident with allergen-induced early phase bronchoconstriction *in vivo*.^{19, 35, 36} We have therefore used urinary LTE₄ measurements as an index of inflammatory-cell mediator release during allergen challenge to assess the effect of inhaled β_2 -agonists on inflammatory-cell activation.

The kinetics of LTE₄ excretion into the urine have been addressed in several studies.³⁵⁻³⁸ After inhalation of nebulized LTD₄,³⁷ approximately 50% of total urinary LTE₄ is excreted in the first 2 hours. Similarly, most LTE₄ enters the urine within 4 hours after intravenous administration of LTC₄.³⁸ Allergen-provoked release of urinary LTE₄ is greatest within 2 to 3 hours of challenge and declines rapidly thereafter.^{19, 35, 36} Hence, any attenuating effect of β_2 -agonists on urinary LTE₄ excretion is most likely to occur within the first few hours of an allergen challenge.

The failure of both β_2 -agonists to suppress urinary excretion of LTE₄ in the 4 hours after allergen challenge was unexpected and stands in marked contrast to their clearly separable effects on airway caliber during the same period. There are several possible explanations of these conflicting findings. First, the tacit assumption has been made that the allergen-evoked rise in urinary LTE₄ represents cysteinyl leukotriene generation within the lung. In fact, their precise origin has not been established, and they could equally arise from an extrapulmonary site(s), such as the blood. A recent study with nebulized sodium cromoglycate suggests, however, that in some subjects the allergen-evoked rise in urinary LTE₄ does indeed come from mast cells in the lung.³⁹ Second, although β_2 -agonists are potent inhibitors of cysteinyl leukotriene generation by the lung *in vitro*,^{7, 17, 28} this inhibition may not occur to a significant extent after inhalation *in vivo*. Inhaled β_2 -agonists do, nevertheless, block the allergen-evoked rise in plasma histamine that suggests they are able to inhibit lung mast cells *in vivo*.¹¹ A more likely explanation, and the one favored in this study, is that, functionally, the most important action of inhaled β_2 -agonists in humans is not the inhibition of lung mast cells but the relaxation of airway smooth muscle.

In accordance with other studies, SB treatment only partially attenuated the increase in bronchial reactivity after allergen challenge.² Conversely, treatment with SM resulted in a substantial decrease in histamine

reactivity after allergen challenge, equivalent to a reduction of 2.73 and 1.59 doubling dilutions of histamine compared to that of placebo and SB. This shift in the PC₂₀ histamine is probably not a simple consequence of bronchodilatation, since inhaled ipratropium bromide causes similar levels of bronchodilatation in subjects with asthma without any effect on their histamine reactivity.⁴⁰ The observation most likely reflects a prolonged functional antagonism of the bronchoconstrictor effect of histamine, although an additional anti-inflammatory effect cannot be excluded. This issue has been addressed further in a recent study by Twentyman et al.⁴¹ that evaluated the effect of SM on allergen challenge during a 34-hour period, although this study was unblinded in the sense that a second β_2 -agonist was not used as an active control. These authors found that SM protected against histamine-induced bronchoconstriction at least up to 9½ hours. In addition, it prevented allergen-induced early and late-phase responses for up to 7½ hours, and the accompanying increases in bronchial reactivity up to 34 hours. The authors concluded that the attenuation of airway responsiveness by SM after allergen challenge could not be accounted purely on the basis of bronchodilation and functional antagonism and that it may be the result of some additional anti-inflammatory properties of the drug. However, our data suggest that these effects are independent of inhibition of cysteinyl leukotriene release, at least during the early phase, and that the mechanism of these putative effects require further investigation.

We thank Dr. O. Twentyman and Professor S. Holgate of the University of Southampton, and Drs. R. Coleman and M. Johnson of the Peripheral Pharmacology Unit, Glaxo Pharmaceuticals, for preprints of their recent manuscripts (Ball et al.,¹² Butchers et al.,¹⁷ and Twentyman et al.⁴¹).

REFERENCES

1. Barnes PJ. A new approach to the treatment of asthma. *N Engl J Med* 1989;321:1517-27.
2. Cockcroft DW, Murdock KY. Comparative effects of inhaled salbutamol, sodium cromoglycate, and beclomethasone dipropionate on allergen-induced early asthmatic responses, late asthmatic responses, and increased bronchial responsiveness to histamine. *J ALLERGY CLIN IMMUNOL* 1987;79:734-40.
3. Dahl R, Pedersen B. Influence of nedocromil sodium on the dual asthmatic reaction after allergen challenge: a double-blind, placebo-controlled study. *Eur J Respir Dis* 1986;69:263-5.
4. Kraan J, Koeter GH, van der Mark TW, Sluiter HJ, de Vries K. Changes in bronchial hyperreactivity induced by 4 weeks of treatment with antiasthmatic drugs in patients with allergic asthma: a comparison between budesonide and terbutaline. *J ALLERGY CLIN IMMUNOL* 1985;76:628-36.
5. Vathenen AS, Knox AJ, Higgins BG, Britton JR, Tattersfield AE. Rebound increase in bronchial responsiveness after treatment with inhaled terbutaline. *Lancet* 1988;1:554-8.
6. Stiles GL, Caron MG, Lefkowitz RJ. β -Adrenergic receptors:

- biochemical mechanisms of physiological regulation. *Physiol Rev* 1984;64:661-743.
7. Church MK, Hiroi J. Inhibition of IgE-dependent histamine release from human-dispersed lung mast cells by anti-allergic drugs and salbutamol. *Br J Pharmacol* 1987;90:421-9.
8. Butchers PR, Skidmore IF, Vardey CJ, Wheeldon A. Characterization of the receptor mediating the anti-anaphylactic effects of β -adrenoceptor agonists in human lung tissue in vitro. *Br J Pharmacol* 1980;71:663-7.
9. Erjefalt I, Persson CG. Anti-asthma drugs attenuate inflammatory leakage of plasma into airway lumen. *Acta Physiol Scand* 1986;128:653-4.
10. Rhoden KJ, Meldrum LA, Barnes PJ. Inhibition of cholinergic neurotransmission in human airways by β_2 -adrenoceptors. *J Appl Physiol* 1988;65:700-5.
11. Howarth PH, Durham SR, Lee TH, Kay AB, Church MK, Holgate ST. Influence of albuterol, cromolyn sodium, and ipratropium bromide on the airway and circulating mediator responses to allergen bronchial provocation in asthma. *Am Rev Respir Dis* 1985;132:986-92.
12. Ball DI, Brittain RT, Coleman RA, et al. Salmeterol, a novel, long-acting β_2 -adrenoceptor agonist: characterization of pharmacological activity in vitro. *Br J Pharmacol* 1991;104:665-71.
13. Johnson M. The pharmacology of salmeterol. *Lung* 1990;168(suppl):115-9.
14. Ullman A, Svedmyr N. Salmeterol, a new long-acting inhaled β_2 -adrenoceptor agonist: comparison with salbutamol in adult asthmatic patients. *Thorax* 1988;43:674-8.
15. Boyd G, Anderson K, Carter R. A placebo-controlled comparison of the bronchodilator performance of salmeterol and salbutamol over 12 hours. *Thorax* 1990;45:340P.
16. Palmer JBD. The early clinical development of salmeterol. *Eur Respir J* 1990;3(suppl 10):225S.
17. Butchers PR, Vardey CJ, Johnson M. Salmeterol: a potent and long-acting inhibitor of inflammatory mediator release from human lung. *Br J Pharmacol* 1991;104:672-6.
18. Baker AJ, Fuller RW. Anti-inflammatory effect of salmeterol on human alveolar macrophages. *Am Rev Respir Dis* 1990;141:A394.
19. Taylor GW, Taylor IK, Black P, et al. Urinary leukotriene E_4 after antigen challenge and in acute asthma and allergic rhinitis. *Lancet* 1989;1:584-8.
20. Richmond R, Turner NC, Maltby NH, et al. Single step procedure for the extraction and purification of leukotrienes B_4 , C_4 , and D_4 . *J Chromatog* 1987;417:241-51.
21. Carstairs JR, Nimmo AJ, Barnes PJ. Autoradiographic visualization of beta-adrenoceptor subtypes in human lung. *Am Rev Respir Dis* 1985;132:541-7.
22. Brittain RT. Approaches to a long-acting, selective β_2 -adrenoceptor stimulant. *Lung* 1990;168(suppl):111-4.
23. Holgate ST, Finnerty JP. Recent advances in understanding the pathogenesis of asthma and its clinical implications. *Q J Med* 1988;66:5-19.
24. Fuller RW, Morris PK, Richmond RR, et al. Immunoglobulin E-dependent stimulation of human alveolar macrophages: significance in type I hypersensitivity. *Clin Exp Immunol* 1986;65:416-26.
25. Dahlen SE, Hansson G, Hedqvist P, Bjork T, Granstrom E, Dahlen B. Allergen challenge of lung tissue from asthmatics elicits bronchial contraction that correlates with the release of leukotrienes C_4 , D_4 , and E_4 . *Proc Natl Sci USA* 1983;80:1712-6.
26. MacGlashan DW Jr, Schleimer RP, Peters SP. Generation of leukotrienes by purified human lung mast cells. *J Clin Invest* 1982;70:747-51.
27. Weller PF, Lee CW, Foster DW, Corey EJ, Austen KF, Lewis RA. Generation and metabolism of 5-lipoxygenase pathway leukotrienes by human eosinophils: predominant production of leukotriene C_4 . *Proc Natl Sci USA* 1983;80:7626-30.
28. Hughes JM, Seale JP, Temple DM. Effect of fenoterol of immunologically release of leukotrienes and histamine from human lung in vitro: selective antagonism by β -adrenoceptor antagonists. *Eur J Pharmacol* 1983;95:239-45.
29. Church MK, Young KD. The characteristics of inhibition of histamine release from human lung fragments by sodium cromoglycate, salbutamol, and chlorpromazine. *Br J Pharmacol* 1983;78:671-9.
30. Busse WW, Swenson CA. The relationship between plasma histamine concentrations and bronchial obstruction to antigen challenge in allergic rhinitis. *J ALLERGY CLIN IMMUNOL* 1989;84:658-66.
31. Taylor IK, O'Malley G, Murray S, et al. Urinary N^6 -methylimidazole acetic acid excretion in respiratory disease. *J Appl Physiol* 1990;69:591-6.
32. Keyzer JJ, Kauffman HF, de Monchy JGR, Keyzer-Udding JJ, de Vries K. Urinary N^6 -methylhistamine during early and late allergen-induced bronchial-obstructive reactions. *J ALLERGY CLIN IMMUNOL* 1984;74:240-5.
33. Lupinetti MD, Sheller JR, Catella F, FitzGerald GA. Thromboxane biosynthesis in allergen-induced bronchospasm: evidence for platelet activation. *Am Rev Respir Dis* 1989;140:932-5.
34. Taylor IK, Ward PS, O'Shaughnessy KM, et al. Thromboxane A_2 biosynthesis in acute asthma and after antigen challenge. *Am Rev Respir Dis* 1991;143:119-25.
35. Sladek K, Dworski R, Fitzgerald GA, et al. Allergen-stimulated release of thromboxane A_2 and leukotriene E_4 in humans. *Am Rev Respir Dis* 1990;141:1441-5.
36. Manning PJ, Rokach J, Malo J-L, et al. Urinary leukotriene E_4 levels during early and late asthmatic responses. *J ALLERGY CLIN IMMUNOL* 1990;86:211-20.
37. Verhagen J, Bel EH, Kijne GM, et al. The excretion of leukotriene E_4 into the urine following inhalation of leukotriene D_4 by human individuals. *Biochem Biophys Res Commun* 1987;148:864-8.
38. Maltby NH, Taylor GW, Ritter JM, Moore K, Fuller RW, Dollery CT. Leukotriene C_4 elimination and metabolism in man. *J ALLERGY CLIN IMMUNOL* 1990;85:3-9.
39. Westcott JY, Smith HR, Wenzel SE, et al. Urinary leukotriene E_4 in patients with asthma: effects of airway reactivity and sodium cromoglycate. *Am Rev Respir Dis* 1991;143:1322-8.
40. Britton J, Hanley SP, Garrett HV, Hadfield JW, Tattersfield AE. Dose-related effects of salbutamol and ipratropium bromide on airway calibre and reactivity in subjects with asthma. *Thorax* 1988;43:300-5.
41. Twentyman OP, Finnerty JP, Harris A, Palmer J, Holgate ST. The long-acting β_2 -agonist salmeterol protects against allergen-induced inflammatory events in asthma. *Lancet* 1990;2:1338-42.