

Mast cell tryptase release and asthmatic responses to allergen increase with regular use of salbutamol

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Background: Increased asthmatic responses to allergen, both early and late, have been demonstrated after regular use of β_2 -agonists in as few as 7 days. Desensitization of β_2 -adrenergic receptors on airway mast cells may contribute to this effect by allowing greater release of mast cell mediator on allergen-induced degranulation. Tryptase released from lung mast cells can be measured in serum 1 hour after allergen challenge and serves as a marker of mast cell degranulation.

Objective: To examine the effect of regular treatment with salbutamol, a β_2 -agonist, on mast cell mediator release after allergen challenge and its influence on the early asthmatic response (EAR) and the late allergic response, we measured the EAR, serum tryptase levels, the 7-hour FEV₁, and sputum tryptase levels and cell profiles.

Methods: We conducted a placebo-controlled, double-blind, randomized cross-over comparison of treatments for 10 days with either a salbutamol metered-dose inhaler (100 μ g, 2 puffs 4 times daily) or a matched placebo inhaler with at least a 7-day washout between treatments. Atopic subjects ($n = 14$) with mild-to-moderate asthma performed same-dose allergen inhalation tests after both treatments 12 to 15 hours after the last dose of study inhaler. Baseline and 7-hour FEV₁ and the EAR to allergen were measured by using spirometry; venous blood was drawn at 1 hour for analysis of serum tryptase; and sputum was induced and collected at 1 and 7 hours.

Results: Salbutamol treatment resulted in a significantly greater EAR ($20\% \pm 1.6\%$ [SEM] vs $15\% \pm 2.1\%$; $P = .047$); increased 1-hour serum tryptase levels (9.09 ± 1.57 vs 7.52 ± 1.12 μ g/L; $P = .011$); increased proportions of eosinophils in the 7-hour sputum sample ($39.1\% \pm 5.1\%$ vs $28.4\% \pm 4.4\%$; $P < .05$); increased proportion of metachromatic cells in the 7-hour sputum sample ($4.4\% \pm 1.1\%$ vs $2.2\% \pm 0.6\%$; $P = .032$); and lower 7-hour FEV₁ (2.77 ± 0.18 vs 2.97 ± 0.20 L; $P = .014$). Baseline FEV₁ was not significantly different after salbutamol treatment compared with values after placebo treatment (2.90 ± 0.20 vs 3.00 ± 0.19 L; $P = .11$).

Conclusion: Regular 10-day treatment with salbutamol increases the allergen-induced release of mediator from airway mast cells, and this is reflected in an increased EAR to allergen. Late-phase responses to allergen were also enhanced, as demonstrated by decreased 7-hour FEV₁ and increased eosinophilia and percentage of metachromatic cells in the 7-hour sputum sample. Increased allergen-induced mast cell degranulation could, in part, explain the increased asthmatic responses to allergen after β_2 -agonist treatment and could contribute to the deterioration of asthma control that is associated with regular use of β_2 -agonist by potentiating allergic inflammation. (*J Allergy Clin Immunol* 2000;106:57-64.)

Key words: β -Agonists, allergen, asthma, mast cell, tryptase, eosinophils

The β_2 -adrenergic agonists remain the most widely used and effective bronchodilating drugs in asthma management. The appropriate frequency of use in asthma patients has been questioned in recent years because of the demonstration of poorer asthma control in patients using regular daily β_2 -agonist in comparison with as-needed use.¹ Subsequent research has shown losses in the ability of the β_2 -agonist to protect against bronchoconstriction after regular β_2 -agonist use.^{2,3} Several investigations have shown increased responsiveness to histamine and methacholine^{1,4-7} or losses in the bronchodilating effect^{8,9} of these drugs after regular use, although others have not.^{10,11} Increased bronchospasm after exercise¹² and loss of the protection provided by β_2 -agonists against exercise-induced^{13,14} and allergen-induced bronchospasm¹⁵ have also been demonstrated after regular β_2 -agonist treatment.

We^{10,16,17} and others¹⁸ have previously reported increased airway responses to bronchial allergen challenge after regular treatment with β_2 -agonist. β_2 -Agonist treatment induced increases in both the early asthmatic response (EAR)^{10,16} and late asthmatic response (LAR),^{17,18} and this effect on the EAR is not eliminated with concomitant high-dose inhaled corticosteroid.¹⁶

The increased early response to allergen after regular β_2 -agonist treatment could be due, in part, to desensitization of β_2 -adrenergic receptors on the airway mast cells. Mast cells can degranulate by the cross-linking of receptor-bound IgE with allergen. β_2 -Adrenergic receptor stimulation confers stability to mast cells, thereby preventing degranulation and release of mediator.¹⁹ Because

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Abbreviations used

EAR: Early asthmatic response
 LAR: Late asthmatic response
 PC₁₅: Provocation concentration causing a 15% fall in FEV₁

tryptase is a major component of the human pulmonary mast cell secretory granule, its presence in serum can serve as a marker of mast cell activation. Thus the levels of serum tryptase measured 1 to 2 hours after allergen inhalation challenge indicate the extent of allergen-induced mast cell activation.²⁰ We undertook a study of subjects with atopic asthma to measure the effect of 10-day treatments of salbutamol (200 µg administered 4 times daily) and matched placebo on the asthmatic responses to allergen. We assessed the EAR and LAR and measured serum tryptase levels 1 hour after bronchial allergen challenge performed after each treatment. Sputum was examined at 1 and 7 hours after allergen challenge for tryptase content and eosinophil and metachromatic cell numbers.

METHODS**Design**

Sixteen subjects with asthma were enrolled in a randomized, double-blind, placebo-controlled, cross-over study to compare the effects of 10-day treatment periods each of salbutamol and placebo on the allergen-induced asthmatic responses. Treatments consisted of salbutamol (100 µg/puff, Ventolin; Glaxo Wellcome Inc, Mississauga, Ontario, Canada) or identical placebo metered-dose inhaler (2 puffs administered 4 times daily). All evaluated subjects completed both treatments. The second treatment period began after at least 7 days washout from the first. The last dose of study inhaler was taken 12 to 15 hours before allergen inhalation challenge. Allergen challenges were performed the morning after each treatment period and were followed by two sputum inductions at 1 and 7 hours. Venous blood was drawn at 1 hour for analysis of serum tryptase. The same dose of allergen was administered for each subject after both treatments. Parameters assessed were the baseline and 7-hour FEV₁, the EAR measured as a maximum percentage fall in FEV₁ from baseline during the first hour, sputum and serum tryptase levels measured at 1 hour after allergen challenge, and the eosinophil and metachromatic cell differential counts in the 7-hour sputum samples. All subjects provided informed signed consent, and the protocol was approved by the University of Saskatchewan Ethics Committee.

Baseline FEV₁ was taken as the highest of 3 forced expiratory maneuvers performed just before allergen challenge after 10 to 15 minutes of rest in the laboratory. The greater of two FEV₁ values measured just before the 7-hour sputum induction was considered the 7-hour FEV₁.

Subjects

Anthropometric data and relevant asthma information are given in Table I. Sixteen subjects, 20 to 51 years of age, had an FEV₁ of 70% or greater predicted values. All subjects demonstrated airway responsiveness to methacholine inhalation challenge with a PC₂₀ of 8 mg/mL or less, except subject 5. All showed atopy for one or more inhalant allergens by skin prick test and had demonstrated an EAR

to allergen of at least a 15% fall in FEV₁ on inhalation challenge in the laboratory. Five subjects were using regular inhaled corticosteroid, one subject used sodium cromoglycate before exercise 1 to 2 times per week, and all but two subjects had used inhaled salbutamol as needed. Those taking inhaled corticosteroid were instructed to continue this treatment as prescribed in a stable dose for the entire study. All subjects withheld β₂-agonist use for at least 2 weeks before and for the duration of the study but were allowed to use ipratropium bromide (Atrovent; Boehringer Ingelheim Ltd, Burlington, Ontario, Canada) for symptom relief. All subjects were free of respiratory tract infection and known allergen exposure for the duration of and at least 4 weeks before participation in the study.

Allergen inhalation test

Subjects completed two 7-hour allergen screening days before enrollment into the study, as previously described.¹⁷ The first day, the diluent used in the allergen dilutions was aerosolized and inhaled as a control. The second day was the initial allergen inhalation day. Diluent was 0.9% saline solution with 0.4% phenol and 0.03% human serum albumin (Bayer Corp, Spokane, Wash). Aerosols were generated with a Wright nebulizer (Aerosol Medical Ltd, Colchester, Essex, UK) calibrated to give an output of 0.13 mL/min and passed into a one-way Hans-Rudolph valve (Hans Rudolph Inc, Kansas City, Mo) and then to the patient through a mouthpiece. Two ventilator filters were placed in series on the expiratory side of the valve to prevent aerosol from escaping into the room. The subjects performed three 2-minute inhalations 10 minutes apart using tidal breathing. FEV₁ was initially measured in triplicate 60 seconds apart before inhalations and in duplicate 10 minutes after each inhalation. After the final inhalation, FEV₁ was measured at 10, 20, 30, 40, 50, 60, 90, 120, 180, 240, 300, 360, and 420 minutes. The highest value at each time was used for analysis.

On this first screening day, subjects also underwent duplicate skin prick tests with serial doubling dilutions (1:8-≥1:1024) of the aqueous allergen extract (Table I) to be used for allergen inhalation. Allergen extracts of cat hair, horse hair, and grass and weed pollens were diluted from stock concentrations ranging from 10,000 to 83,000 protein nitrogen units per milliliter. Each wheal produced by the extract was measured in two perpendicular directions (one maximal) at 10 to 15 minutes, and the mean wheal diameter for each concentration was determined. The skin test endpoint was defined as the lowest concentration of allergen producing a mean wheal diameter of at least 2 mm larger than that produced by the diluent. A PC₁₅ for the allergen inhalation test could then be predicted from the skin test endpoint and the methacholine PC₂₀.¹⁷

On the second screening day, an allergen inhalation was performed in a fashion similar to that for the diluent inhalation, starting 3 to 4 concentrations below the predicted PC₁₅. Allergen inhalations were continued in increasing concentrations until the FEV₁ had fallen by 15% or greater at 10 minutes, and then the FEV₁ was monitored for 7 hours, as on the diluent day. The candidate subjects who showed a positive response to allergen challenge (≥15% fall in FEV₁) were enrolled in the study.

The allergen challenges performed after the treatment periods 12 to 15 hours after the last dose of study inhaler were started at 2 to 3 concentrations lower than the final concentration used during the screening allergen challenge. After treatment 1, allergen was administered until a 15% or greater fall in FEV₁ occurred, and this same dose of allergen was administered after treatment 2. FEV₁ was then measured every 10 minutes for the first hour and then again at 7 hours. Those subjects who had previously exhibited LARs to allergen inhalation were monitored hourly until 7 hours; those who had exhibited only an EAR were allowed to leave the laboratory and return at 6 hours but were disallowed from sleeping, exercising, or leaving the building.

TABLE I. Anthropometric and asthma data

Patient No.	Age (y)	Sex	Height (cm)	Methacholine PC ₂₀ (mg/mL)	FEV ₁ (L)		Medications (μg/d)	Allergen
					Baseline	% Predicted		
1	20	F	160	0.50	3.21	103	FP 250, salbutamol prn	Horse*
2	51	M	170	0.45	2.38	72	Nil	Grass*
3	21	M	191	4.2	4.83	94	Salbutamol prn	Horse*
4	22	F	160	0.56	3.09	100	Salbutamol prn	Horse*
5	36	M	171	NA	3.58	94	Salbutamol prn	Grass*
6	21	F	161	0.71	2.65	85	SCG prn, salbutamol prn	Grass†
7	29	F	154	0.31	2.36	86	Salbutamol prn	Cat‡
8	23	F	177	0.80	3.52	97	BDP 50	Cat*
9	21	M	188	0.39	4.24	85	BUD 800, salbutamol prn	Weed†
10	22	M	170	2.8	3.48	85	BDP 1000	Horse*
11	33	F	178	0.72	2.60	75	Nil	Cat†
12	23	F	154	1.2	2.53	89	BDP 200	Grass*
13	25	M	182	0.35	4.21	86	Salbutamol prn	Weed†
14	22	F	173	1.0	3.30	94	Salbutamol prn	Horse§
15	28	F	160	0.81	2.44	82	Salbutamol prn	Cat*
16	29	F	168	0.45	3.33	104	Salbutamol prn	Cat§

FP, Fluticasone propionate; prn, as needed; SCG, sodium cromoglycate; BDP, beclomethasone dipropionate; BUD, budesonide.

*Greer Laboratories Inc, Lenoir, NC.

†Bencard Laboratories, Mississauga, Ontario, Canada.

‡Bayer, Inc, Etobicoke, Ontario, Canada.

§Western Allergy Services, Richmond, British Columbia, Canada.

As in previous studies,¹⁷ if the dose of allergen used after the first treatment could not be safely administered after the second treatment because a significant fall in FEV₁ occurred at a weaker concentration, then the treatments were crossed over once again so that the first 10-day treatment was repeated, but the following allergen challenge was stopped at the concentration achieved after the second treatment. The data used for analysis were from the same-dose allergen challenge tests.

Serum tryptase

Approximately 5 mL of blood was drawn by means of venipuncture from each subject 1 hour after the last inhalation of allergen before sputum induction. Blood samples were allowed to clot at room temperature, and then the serum was removed after centrifugation, placed in aliquots, and stored at -70°C to -80°C. Serum tryptase levels were then assayed by using the UniCAP fluoroenzyme immunoassay (Pharmacia and Upjohn Diagnostics, Uppsala, Sweden) according to the manufacturer's instructions. This assay uses a human mast cell tryptase-specific murine monoclonal antibody. All samples were analyzed in the same run with use of the same calibrator and reagent. The assay will detect tryptase levels greater than 1 μg/L.

Sputum induction and processing

Sputum inductions were performed at 1 and 7 hours after allergen challenge by using a modified version of a previously established method.²¹ Contrary to standard protocol and for technical reasons, subjects were not premedicated with salbutamol before saline inhalation. Sterile saline solutions (3%, 4%, and 5%) were nebulized in 12-mL quantities by using an ultrasonic nebulizer (Ultra-Neb 99; Devilbiss Co, Somerset, Pa) at maximum setting for an average output of 3 mL/min. Subjects inhaled the saline aerosol through a mouthpiece with an open valve by means of tidal breathing. Inhalations began with 3% saline and continued until the 12 mL

was completely nebulized. Subjects thoroughly rinsed their mouths and pharynx with water to eliminate saliva before each attempt to bring up sputum into a sterile specimen cup. Saline inhalation was continued, at higher concentrations if necessary and tolerated by the subject, until 1 to 2 mL of good quality sputum was produced. Within 2 hours, sputum (whole expectorate) was processed by the addition of 2 mL of 0.1% dithiothreitol in saline and brief low-speed vortexing to disperse sputum clumps. Some of this sputum mixture was reserved and frozen at -70°C for tryptase analysis. The cells were separated from the sputum/dithiothreitol mixture by centrifugation for 10 minutes at 400g at room temperature, washed twice with an excess of Dulbecco's modified Eagle's medium (Gibco/BRL, Grand Island, NY), fixed for 30 minutes on ice in 85% ethanol/5% glacial acetic acid/10% formalin, and finally transferred to and stored at -20°C in 70% ethanol. Differential cell counts (metachromatic cells, eosinophils, lymphocytes, and monocytes/macrophages) were established from Giemsa-stained cytocentrifuge slides by counting 200 cells per sample.

Analysis

Treatment effects between salbutamol and placebo for mean values of baseline FEV₁ and 7-hour FEV₁ and the EAR (expressed as percentage fall from baseline FEV₁) were compared by using the paired *t* test method on the Statistix 4.1 software program (Analytical Software, Tallahassee, Fla). Serum tryptase levels (in micrograms per liter) were compared by using the Wilcoxon signed rank test. Eosinophils and metachromatic cells were reported as the mean percentage of total sputum cells and compared by using the Wilcoxon signed rank test. All data are reported as mean values ± SEM.

RESULTS

Two subjects were withdrawn from the study. Subject 3 was excluded because of an inability to withhold rescue

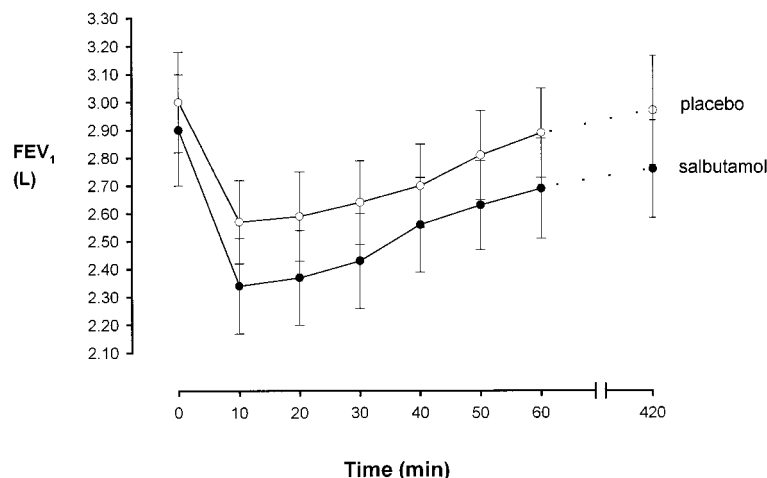


FIG 1. FEV₁ versus time in minutes after allergen inhalation challenge in 14 subjects comparing regular use of salbutamol and placebo. Results are expressed as means for all data at each time point.

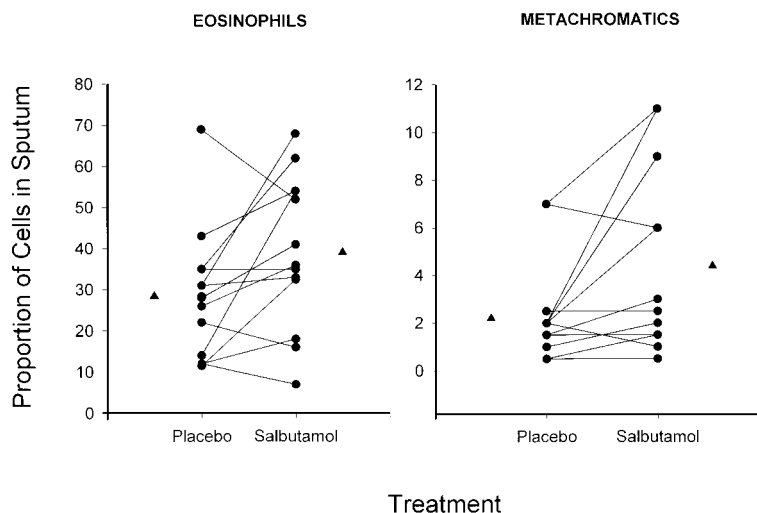


FIG 2. Proportion of eosinophils ($n = 13$) and basophils ($n = 13$) as a percentage of the total sputum cells in the 7-hour sputum samples. The percentage of eosinophils was greater after salbutamol treatment in comparison with placebo treatment ($39.1\% \pm 5.1\%$ vs $28.4\% \pm 4.4\%$; $P < .05$) as was the percentage of metachromatic cells ($4.4\% \pm 1.1\%$ vs $2.2\% \pm 0.6\%$; $P = .032$). Triangles represent means of the data.

inhaler use during the night before allergen challenges. Subject 16 was unable to complete the second treatment period because of a deterioration of asthma control that was coincident with the onset of spring mold sporing season. Subject 12 had no measurable serum tryptase. Thus there were evaluable data on 14 subjects for baseline FEV₁, 7-hour FEV₁, and EAR, and evaluable data on 13 subjects for serum tryptase and sputum cell differentials. Of the subjects to have completed the study, 8 had taken placebo and 6 had taken salbutamol in the first treatment period. It was necessary to repeat the first treatment period in one subject (subject 14) because the same dose of allergen could not be safely administered after the second treatment.

The EAR after the salbutamol treatment was significantly larger than that after placebo treatment (Table II and Fig 1). Serum tryptase measured at 1 hour was significantly higher after salbutamol treatment in comparison with placebo treatment (Table II). Tryptase was not detected in the sputum collected at 1 hour. Cell differentials in the 1-hour sputum sample were not significantly different between treatments: eosinophils comprised $29.4\% \pm 4.7\%$ and $27.3\% \pm 6.8\%$ ($P = .64$) of sputum cells after salbutamol and placebo treatments, respectively, and metachromatic cells comprised $0.96\% \pm 0.2\%$ after salbutamol and $1.9\% \pm 0.7\%$ after placebo ($P = .16$). The paired 7-hour sputum differential counts for salbutamol and placebo

TABLE II. Results

Patient No.	Baseline FEV ₁ (L)		7-h FEV ₁ (L)		EAR (% fall in FEV ₁)		Tryptase (μg/L)	
	Salbutamol	Placebo	Salbutamol	Placebo	Salbutamol	Placebo	Salbutamol	Placebo
1	2.84	3.16	3.02	3.14	31	19*	7.6	7.2
2	2.25	2.26	1.94	2.34	28	17*	10.7	7.1
4	3.34	3.18	3.09	3.17	18*	20	6.8	7.5
5	3.51	3.50	3.20	2.90	22*	31	9.8	9.2
6	2.60	2.44	2.45	2.64	14*	10	26.8	19.6
7	1.67	2.12	1.84	1.92	20*	0	5.7	4.0
8	2.65	2.85	2.49	2.84	18	20*	10.9	9.9
9	4.18	4.36	3.31	4.22	15	14*	8.2	4.0
10	3.12	3.48	3.24	3.48	12	13*	6.7	6.4
11	2.55	2.69	2.12	2.38	22*	9	7.1	6.6
12	2.62	2.49	2.50	2.48	13	20*	Nil	Nil
13	4.28	4.11	4.19	4.57	27	15*	6.5	5.0
14	2.10	2.25	2.16	2.41	20†	4	7.0	6.6
15	2.86	3.06	3.17	3.15	25	20*	4.4	4.7
Mean ± SEM	2.90 ± 0.20	3.00 ± 0.19	2.77 ± 0.18	2.97 ± 0.20	20 ± 1.6	15 ± 2.1	9.09 ± 1.57	7.52 ± 1.12
	<i>P</i> = .112		<i>P</i> = .014		<i>P</i> = .047		<i>P</i> = .011	

*First treatment period.

†Repeat cross-over.

treatments for eosinophils and metachromatic cells are depicted in Fig 2. The proportion of eosinophils was greater after salbutamol treatment ($39.3\% \pm 4.8\%$ vs $29.3\% \pm 4.2\%$; $P = .049$) as was the proportion of metachromatic cells ($4.4\% \pm 1.1\%$ vs $2.2\% \pm 0.6\%$; $P = .032$).

Macrophages and monocytes were the predominant cells present in the sputum, and the proportion of these cells in the 7-hour sputum samples was significantly decreased after salbutamol treatment ($42.3\% \pm 4.5\%$ after salbutamol vs $57.5\% \pm 4.3\%$ after placebo; $P = .0081$).

Baseline FEV₁ measured just before allergen challenge (Table II) was slightly but not significantly ($P = .11$) lower after salbutamol treatment. The FEV₁ measured at 7 hours (Table II) just before sputum induction was significantly lower after salbutamol treatment compared with that after placebo.

Subjects were monitored by means of spirometry during sputum induction particularly because β_2 -agonist pretreatment was not used to prevent saline-induced bronchoconstriction. During the 1-hour sputum induction, only 2 subjects (7 and 10) required rescue bronchodilator because of falls in FEV₁ that were 20% or greater after saline inhalation. The other subjects experienced falls that were 15% or less and of short duration. Five subjects showed no decrease in FEV₁ after saline inhalation. Any fall in FEV₁ that occurred during the 7-hour sputum induction was treated with salbutamol as required.

DISCUSSION

We have demonstrated a significant increase in the serum tryptase 1 hour after a same-dose allergen challenge, along with a significant increase in the EAR to allergen after treatment with salbutamol in comparison with placebo. Salbutamol treatment was also associated with a lower FEV₁ and increased sputum eosinophil and metachromatic cell counts at 7 hours after bronchial allergen challenge.

Previous investigations have demonstrated the increased EAR to allergen after regular (4 times daily) administration of salbutamol,^{10,16,22} although this has not been a consistent finding.¹⁸ This effect was also seen after regular use of the long-acting β_2 -agonist salmeterol²³ and was not eliminated by concomitant treatment with high-dose inhaled corticosteroid.¹⁶ Regular treatment with salbutamol is also associated with a significantly greater LAR to allergen challenge^{17,18} and significantly increased eosinophil infiltration and eosinophil cationic protein (ECP) levels measured in sputum at 7 hours after allergen challenge.¹⁸

We chose to examine tryptase because it is a prominent constituent of, and specific to, the mast cell and is relatively more stable than histamine in serum. It is considered to be a reliable indicator of mast cell degranulation.²⁰ The EAR to allergen is initiated by allergen binding to and cross-linking specific IgE molecules that are bound to mast cells by the high-affinity IgE receptor Fc ϵ RI. This causes the mast cell to degranulate and release numerous preformed mediators, including histamine, tryptase, and heparin, as well as produce prostaglandins and leukotrienes from membrane-derived arachidonic acid by the action of cyclooxygenase and lipoxygenase.²⁴ An array of inflammatory and immunoregulatory cytokines are also released from the mast cell over the next few minutes to couple of hours. IL-4 and IL-13 are involved in switching B lymphocytes to IgE production, and IL-5, TNF- α , and GM-CSF are important in increasing the traffic of eosinophils in the lung.²⁵ Lung eosinophilia is the most distinguishing pathologic feature of inflammation in allergic asthma and has been shown to correlate positively with clinical asthma severity²⁶ and to increase with allergen exposure.²⁷

We propose that β_2 -agonist treatment potentiates the inflammatory response to allergen in atopic patients with asthma and that the combination of β_2 -agonist and aller-

gen exposure results in greater inflammation than allergen alone. Studies of regular β_2 -agonist use from other centers have demonstrated significant decreases in baseline FEV₁ and increases in airway response to methacholine (a test of airway responsiveness),^{4-7,12} whereas studies conducted at our center have not shown these effects. This difference may be due to the ability of our subjects to almost completely avoid environmental allergen exposure during studies. Our studies are conducted during the winter months when snow cover and consistently subfreezing temperatures prevent dispersal of pollens and outdoor fungal spores, and low indoor humidity inhibits survival of house dust mite. This is evidenced by a low (almost nonexistent) incidence of house dust mite sensitivity in the local population and the fact that many subjects with seasonal allergic asthma remain well controlled with little or no medications during winter months. At most other sites, some allergen exposure, especially that of house dust mites, is unavoidable. We believe it is this combination of environmental allergen exposure and β_2 -agonist, and not β_2 -agonist alone, that results in the deterioration of airway caliber and increased airway responsiveness at other study sites to a greater degree than chronic allergen exposure alone (as would be seen during placebo treatment). It is possible that this near-complete lack of allergen exposure among our subjects is responsible for the low levels of serum tryptase that were measured in our study. However, even at our site, some allergen exposure, such as cat allergen transported on clothing and fungal spores from indoor plant soil, is unavoidable. This could explain the lower baseline FEV₁ in some of our subjects after salbutamol treatment in the present study. The implication of not finding increased airway responsiveness to methacholine after salbutamol treatment during our previous studies is that the increased response to allergen is a result of alteration to the mechanism of allergy and not a function of general increase in airway responsiveness.

The results of the current investigation may indicate one mechanism by which increased inflammation could occur. After salbutamol treatment, the same dose of allergen elicited increased levels of serum tryptase, indicating an increase of mast cell mediator release. Concomitant increases in the release of inflammatory mediators, such as IL-5, TNF- α , and GM-CSF, could lead to increased eosinophil infiltration and survival in the lung. It has been shown experimentally that IL-5 administered by inhalation to asthmatic patients induces lung eosinophilia and elevated sputum ECP levels and causes increased airway responsiveness to methacholine at 24 and 48 hours.²⁸ These results reflect the ability of IL-5 to chemoattract and enhance the adhesion, viability, and degranulation of eosinophils.²⁸ Indeed, in our study increased proportions of eosinophils and metachromatic cells were observed at 7 hours after allergen challenge in the sputum of salbutamol-treated subjects. Hence if subjects are chronically exposed to allergic stimuli from the natural environment concurrent with regular use of β_2 -agonist, it is possible that the allergy-induced inflamma-

tion is enhanced, as indicated by decreased FEV₁ and increased methacholine responsiveness. Further evidence for this effect is given by Manolitsas et al²⁹ in a study in which increases in total and activated eosinophils and ECP were found in bronchoscopy and bronchoalveolar lavage specimens from human subjects after 16 weeks of albuterol (salbutamol) treatment in comparison with baseline values.

Increased degranulation of mast cells could be the result of decreased cell membrane stability caused by β_2 -adrenergic receptor desensitization on these cells. Most mammalian cells have β_2 -adrenoceptors, and the desensitization of this receptor is a well-established phenomenon. Desensitization occurs in stages of phosphorylation activated by stimulation of the receptor. This phosphorylation can result in an uncoupling of the receptor from the effector system, causing functional impairment. Prolonged stimulation increases phosphorylation of the receptor and leads to binding by β -arrestin, which completely disables the system. The receptor is then internalized or sequestered intracellularly. With continued exposure to agonist, the receptor may be lysed, resulting in a net loss of receptors on the cell surface (downregulation). Furthermore, Nishikawa et al³⁰ have shown that sustained exposure to agonist leads to decreased β_2 -adrenoceptor mRNA expression in guinea pigs. Functional desensitization has been demonstrated in human pulmonary cells both in vitro^{31,32} and in vivo.³³ Functional desensitization of β_2 -adrenoceptors on mast cells would decrease the stabilizing effect of endogenous or exogenous β_2 -agonist and render the cell more apt to degranulate on immunologic activation. In this way the same dose of allergen could cause degranulation of a greater number of mast cells, thereby causing an increased airway response to allergen.

Previous research has demonstrated relatively good preservation of the bronchodilator smooth muscle-mediated response after chronic β_2 -agonist use and yet significant and sometimes complete loss of the bronchoprotective effect against mast cell-mediated stimuli, such as allergen or adenosine monophosphate.^{2,15} It is possible that desensitization of the β_2 -adrenoceptor on smooth muscle occurs to a lesser degree than that which occurs on mast cells. McGraw and Liggett³⁴ compared airway smooth muscle, epithelial, and mast cell lines for β_2 -adrenergic receptor kinase expression and activity. This kinase is the primary phosphorylating agent responsible for β_2 -adrenergic receptor desensitization. They found the expression and activity of the kinase in epithelial and smooth muscle cells to be about 50% and 10% of that in mast cells. This indicates a 10 times greater propensity for desensitization in mast cells.

We have assumed that lung mast cell numbers and IgE levels did not change during the 10-day treatments to affect the outcome of allergen challenges. Manolitsas et al²⁹ found no change in bronchial mast cell numbers, as assessed by biopsy, after regular 16-week treatment with albuterol. Although Coqueret et al³⁵ have shown that the presence of β_2 -agonist can potentiate IL-4-induced pro-

duction of IgE in vitro, this has not yet been demonstrated in vivo. It has also been shown that baseline preallergen numbers of eosinophils are not substantially increased in sputum after 7 days of regular β_2 -agonist treatment.¹⁸

Increased mast cell degranulation may be one of many factors contributing to the increased responses to allergen. There are several cell types, other than the mast cell, involved in the asthmatic responses to allergen, and they may be affected by regular β_2 -agonist treatment. Several mechanisms have been described whereby β_2 -agonists could be proinflammatory and thus detrimental in asthmatic patients. Regular exposure may interfere with the action of glucocorticosteroid (endogenous or exogenous),^{36,37} desensitize cells to the beneficial effects of PGE₂ by upregulating phosphodiesterase 4,³⁸ or increase IgE production in lymphocytes.³⁵ It has also been shown in vitro that prolonged β -agonist exposure can potentiate eosinophil generation of toxic oxygen metabolite, a component of eosinophilic inflammatory action.³⁷

β_2 -Agonist drugs, such as salbutamol and the longer acting salmeterol, have been given anti-inflammatory status by investigators who have found inhibition of mediator release from inflammatory cells by the drug.^{39,40} However, the demonstration of acute inhibition does not adequately address the desensitization and downregulation of the β_2 -adrenoceptor that may occur with chronic, sustained, in vivo β_2 -agonist exposure. Ultimately, no improvements in the inflammatory indices of asthma have ever been demonstrated in asthmatic patients after regular use of either drug.⁴¹

In this study we analyzed the 7-hour FEV₁ rather than measure the LAR for several reasons. Only 5 subjects had shown a late response at previous allergen challenges; the balance were subjects demonstrating only an isolated EAR. Also, 5 participants were taking regular inhaled corticosteroids, which are known to inhibit or eliminate the LAR. The study was not designed to properly measure a late response because it was uncertain how the 1-hour sputum induction would affect the LAR, with a possibility of interference from the saline inhalation. Finally, several subjects had lower baseline FEV₁ values after the salbutamol treatment arm of the study. Because the LAR is measured as a percentage fall from baseline, the LAR would have underestimated the deterioration of asthma in these subjects. This lowered baseline airway caliber after salbutamol treatment has been noted in other studies.¹²

Thorough investigations have demonstrated a certain loss of bronchodilation after regular use of both short- and long-acting β_2 -agonists.^{8,9} This loss has been regarded as clinically less important because most of the bronchodilation is preserved. Loss in the ability of β_2 -agonists to protect against bronchoconstriction after regular use has drawn greater concern. It has been shown that there is almost complete loss in the ability of the same dose of β_2 -agonist to protect against bronchoconstriction induced by the same dose of allergen or exercise.¹³⁻¹⁵ Although these losses in bronchodilation and bronchoprotection could possibly be compensated for by increasing the amount of drug acutely administered, this

is not an appropriate solution if the drug is also potentiating the inflammatory response. In the clinical setting it would be difficult to distinguish the contribution of the β_2 -agonist to the inflammation that is being brought on by exposure to environmental allergen. Because the patient is being exposed to the allergen, inflammatory mechanisms will be induced and asthma symptoms will increase, probably necessitating use of a relief medication (β_2 -agonist). Although the β_2 -agonist would continue to issue most of its bronchodilating effect after regular long-term use, it would be difficult for the patient or the clinician to perceive that the drug is increasing the inflammation because any worsening of asthma control would simply be attributed to the allergen exposure. It is clinically relevant to patients if their anti-inflammatory therapy needs are increased during allergen exposure because of the use of a symptom-relieving medication.

We have demonstrated an increase in the tryptase levels measured in the serum of atopic asthmatic patients 1 hour after allergen inhalation challenge following regular treatment with salbutamol in comparison with placebo. This was in conjunction with a greater EAR to allergen and increased late-phase responses as indicated by the lower FEV₁ measured at 7 hours and increased numbers of eosinophils and metachromatic cells in the sputum taken 7 hours after allergen challenge. These findings indicate a potentiating effect of regular salbutamol on allergic inflammation. This is further evidence that β_2 -agonists in regular sustained use may worsen asthma control and increase the need for anti-inflammatory therapy, reinforcing the recommendations of international guidelines to limit the use of these drugs in the proper management of asthma.

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