

The effect of formoterol on the late asthmatic phenomena in guinea pigs

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We investigated the effects of formoterol, a new, long-acting, selective β_2 -adrenoceptor agonist, on the antigen-induced late asthmatic response (LAR) and airway inflammation in guinea pigs. Animals were sensitized by exposure to aerosolized ovalbumin (2% in saline). After antigen challenge, preceded by administration of an H_1 -receptor antagonist, specific airway conductance was measured with a two-chambered whole-body plethysmograph. An aerosolized solution of formoterol, isoproterenol, or saline was inhaled 15 minutes before challenge. Bronchoalveolar lavage (BAL) was performed 24 hours after challenge. The provocative concentrations of histamine required to decrease specific airway conductance by 50% were obtained before challenge, at 24 hours, and at 72 hours after challenge. The LAR ($52.7\% \pm 7.7\%$ of the baseline; $p < 0.02$) was observed 6 to 8 hours after antigen challenge. An increased cellular influx in BAL (mainly eosinophils and macrophages) and an increased bronchial responsiveness to histamine occurred 24 hours after antigen challenge. Formoterol completely inhibited the LAR and the cellular increase in BAL; however, isoproterenol failed to prevent either the cellular infiltration or the LAR. Formoterol also decreased the antigen-induced increase in bronchial reactivity. These findings suggest that formoterol has inhibitory effects on the underlying inflammatory processes in antigen-induced asthma in addition to prolonged bronchodilation. (J ALLERGY CLIN IMMUNOL 1992;89:858-66.)

Key words: Formoterol, long-acting β_2 -agonist, late asthmatic response, airway inflammation, increased bronchial responsiveness, guinea pigs, bronchoalveolar lavage

In sensitized individuals, a variety of pulmonary asthmatic response patterns can develop after appropriate antigen challenge. After an IAR, an LAR can occur in some allergic subjects with asthma 4 to 6 hours after antigen challenge.¹ Although the mechanisms underlying the LAR are not clear, the LAR is known to be accompanied by an increase in bronchial responsiveness to nonspecific stimulation and by a cellular influx into the bronchial lumen.^{2,3} Many studies have therefore focused on the LAR phenomena. Various animal models suitable for the study of the LAR have been developed to understand the mechanisms involved and to establish new methods of diagnosis, prevention, and treatment.⁴⁻⁷

Abbreviations used

β_2 :	β_2 -Adrenoceptor
IAR:	Immediate asthmatic response
LAR:	Late asthmatic response
SG _{aw} :	Specific airway conductance
BAL:	Bronchoalveolar lavage
BALF:	Bronchoalveolar lavage fluid
PC ₅₀ :	Provocative concentration of histamine causing a 50% reduction in SG _{aw}
OA:	Ovalbumin
HBSS:	Hanks' balanced salt solution

β_2 -Agonists, administered by inhalation, are in routine use for the therapy of bronchial asthma. β_2 -Agonists are reported to have an in vitro inhibitory effect on mast cell mediator release⁸ in addition to their direct bronchodilating effect. These activities can contribute to an inhibitory effect on the antigen-induced IAR. Conventional short-acting β_2 -agonists have not, however, been demonstrated to inhibit the LAR after antigen challenge or the accompanying rise in nonspecific bronchial responsiveness.^{9,10} In previous studies,

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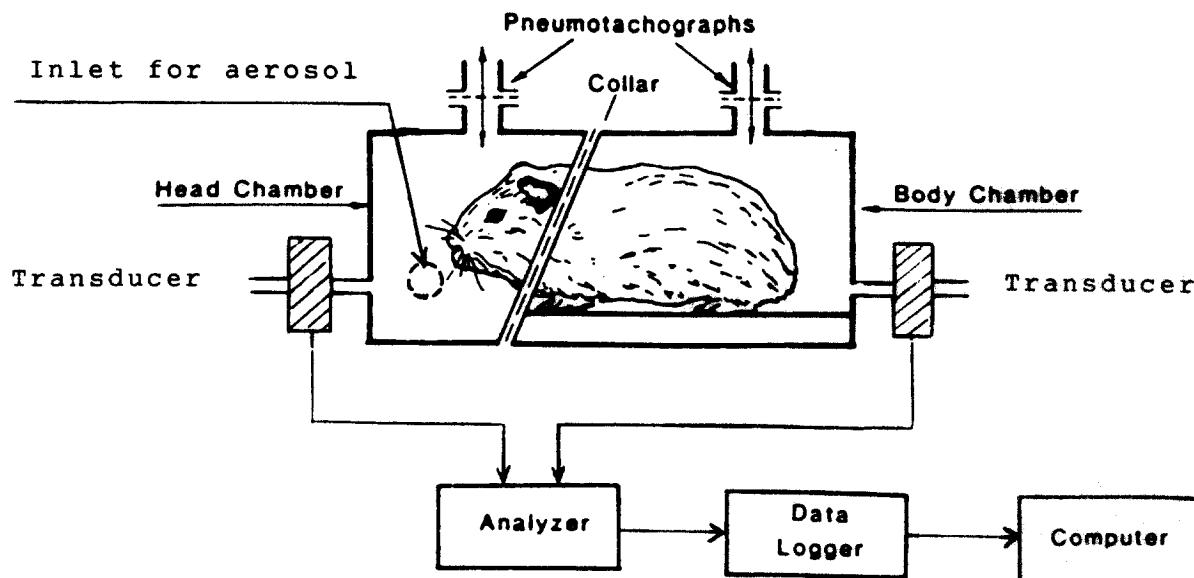


FIG. 1. Two-chambered plethysmograph system used for measuring SG_{aw} .

β_2 -agonists were administered before antigen challenge, and their effect of bronchodilation persisted from 4 to 6 hours. The protective effect of β_2 -agonists disappeared before the LAR or was essentially non-protective more than 6 hours after antigen challenge. If a sufficiently long-acting β_2 -agonist is administered before antigen challenge, it may be possible to prevent the occurrence of the LAR and the antigen-induced rise in nonspecific bronchial responsiveness.

Formoterol is a new long-acting, selective β_2 -agonist. Inhaled formoterol is five to 15 times more potent than salbutamol in patients with asthma, and the duration of action is usually at least 8 to 12 hours.¹¹⁻¹³ Formoterol is reported to have a longer duration of action than salbutamol against bronchoconstriction induced by exercise or hyperventilation of cold air in subjects with asthma.^{14, 15}

We planned this study to examine the effect of inhaled formoterol on the LAR in guinea pigs, a model of asthma that we have developed. We also investigated the effects of formoterol on the increase in nonspecific bronchial responsiveness and the cellular influx into the airway that accompanied the LAR phenomena in our guinea pig model.

MATERIAL AND METHODS

Animal and sensitization

Male Hartley guinea pigs, weighing 350 to 450 gm (Sasco, Omaha, Neb.) were sensitized by exposure to aerosolized OA (2% in saline) for 5 minutes on two occasions, separated by 7 days. The animals were positioned in a two-chambered, whole-body plethysmograph (Fig. 1) while they

inhaled aerosolized antigen or drug. The aerosol was generated by an ultrasonic nebulizer (model 65, DeVilbiss Co., Somerset, Pa.) with an airflow of 10 ml/sec. Seven days after the second sensitization, the animals were used for the antigen challenge. These protocols were approved by the Creighton University School of Medicine Animal Research Committee.

Drug treatment and antigen challenge

The animals were pretreated with pyrilamine maleate (10 mg/kg, intraperitoneally), an H_1 -receptor antagonist, 30 minutes before antigen challenge to prevent lethality from endogenously released histamine. An aerosolized solution of formoterol (10 μ g/ml in saline), isoproterenol (1 mg/ml in saline), or saline was inhaled for 2 minutes, 15 minutes before challenge. The animal was then placed in a two-chambered, whole-body plethysmograph. Five minutes after measuring the baseline pulmonary function, the animal was challenged with 1% OA or saline aerosol for 5 minutes.

Measurement of pulmonary function

Pulmonary function in unanesthetized guinea pigs was measured with a two-chambered, whole-body plethysmograph, previously described by Gerlach et al.¹⁶ The conscious animal was placed with its neck extending through the partition of a two-chambered, rectangular plastic box. The box was separated at the partition for insertion of the animal, the two chambers were joined by spring clamps, and a foam-latex collar between chambers formed a seal around the neck. Each chamber was fitted with identical wire screen pneumotachographs (2.5 cm in diameter with six mesh stainless steel cloth screens) and identical differential pressure transducers (model DP-103, Validyne Corp., Northridge, Calif.). These transducers were connected to a

respiratory analyzer (model 6, Buxco Electronics, Inc., Sharon, Conn.). SG_{aw} was measured with a data-logging analyzer (model LS-14, Buxco Electronics, Inc.) according to the method described by Pennock et al.¹⁷ with the formula below:

$$\tan \theta = W \times R \times C$$

where θ equals phase difference between the thoracic and head chamber flow signals, W equals $2\pi \times$ respiratory frequency, R equals pulmonary resistance, and C equals compressibility of lung volume;

$$= V / (P_{atm} - 47) \times 1.36$$

where V equals thoracic gas volume, P_{atm} equals barometric pressure in millimeters of mercury, and 1.36 equals factor to convert millimeters of mercury to centimeters of H_2O ; therefore:

$$SG_{aw} = 1/R \times V = W / (P_{atm} - 47) \times 1.36 \times \tan \theta$$

The parameters of pulmonary function were averaged during 5-second periods and monitored for 5 minutes. The mean value of SG_{aw} in a steady state was used as a representative of SG_{aw} at the time of measurement. SG_{aw} was expressed as the percentage of the prechallenge baseline value. The animals were removed from the plethysmograph between measurements at each time point.

Bronchoalveolar lavage

BAL was performed 24 hours after antigen challenge because, in a preliminary study, the increase of total cells in BAL reached a peak at 24 hours after antigen challenge. Animals were anesthetized intraperitoneally with sodium pentobarbital (Nembutal, 50 mg/kg; Abbott Laboratories, Abbott Park, Ill.), and killed by exsanguination from the heart. The trachea was exposed and cannulated with a polyethylene tube connected to a syringe. The lung was lavaged by flushing with five 10 ml aliquots of HBSS at 37°C instilled through the tracheal cannula. The recovered fluid collections (usually 85% to 90% of the instilled volume) were combined and filtered through surgical gauze. The lavage fluid was centrifuged (200 g for 10 minutes at 4°C) to remove the supernatant. The cell pellet was washed once and resuspended in 1 ml of HBSS. Total cell counts were done with a hemocytometer with Turk's stain. Differential cell counts were undertaken on cytocentrifuged preparations (Cytospin 2; Shandon, Pittsburgh, Pa.) stained with Wright's Giemsa stain. A minimum of 500 cells were counted and classified as macrophages, eosinophils, neutrophils, and lymphocytes based on normal morphologic criteria.

Histamine challenge

The histamine challenge was performed before challenge, at 24 hours, and at 72 hours after antigen challenge in the control (saline-pretreated), the isoproterenol-pretreated, and the formoterol-pretreated guinea pigs. Histamine solutions were prepared at the concentrations of 6.25, 12.5, 25, 50, and 100 $\mu\text{g/ml}$. The aerosol was generated with an ultrasonic nebulizer. After the animal breathed spontaneously in

the aerosol mist for 1 minute, the aerosol was pulled by vacuum ($-0.01 \text{ cm } H_2O$) through the chamber, and the pulmonary function was measured for 3 minutes. The dose of histamine was increased until there was at least a 50% reduction in SG_{aw} . The concentration of histamine which produced PC_{50} value was then interpolated from each dose-response curve.

Drugs and chemicals

Pyrilamine, OA (grade III), L-isoproterenol, and histamine dihydrochloride were obtained from Sigma Chemical Co. (St. Louis, Mo.), formoterol from Ciba-Geigy Co., HBSS from Gibco Laboratories (Grand Island, N. Y.), and Nembutal from Abbott Laboratories.

Statistical analysis

All the values were represented as mean $\pm$ standard error of the mean. Statistical analysis was performed by a one-way analysis of variance and Student's *t* test. When significant differences were evident (as indicated by the *F* test), comparison of the two values was made with a two-tailed Student's *t* test for paired or unpaired observations; *p* values <0.05 were considered statistically significant.

RESULTS

SG_{aw} was monitored at regular intervals for as long as 96 hours after challenge. The baseline values of SG_{aw} in the saline-challenge and antigen-challenge groups were 0.95 ± 0.19 and $0.88 \pm 0.14 \text{ cm } H_2O^{-1} \text{ s}^{-1}$, respectively. There was no significant difference in the baseline SG_{aw} between the two groups. As illustrated in Fig. 2, the markedly decreased SG_{aw} during the IAR ($13.9\% \pm 3.9\%$ of baseline) recovered gradually and returned to baseline 4 hours after antigen challenge. The LAR phenomena were observed 6 to 8 hours after antigen challenge ($52.7\% \pm 7.7\%$ and $65.7\% \pm 8.1\%$, respectively; $p < 0.02$, as compared with that of saline challenge) and persisted for about 2 to 4 hours. When an LAR is defined as a 25% reduction in SG_{aw} 6 or 8 hours after antigen challenge, the LAR was observed in all the antigen-challenge group. No significant changes in SG_{aw} were found after saline challenge.

The baseline values of SG_{aw} in the saline-, formoterol-, and isoproterenol-pretreated groups were 0.98 ± 0.16 , 0.72 ± 0.14 , and $1.04 \pm 0.10 \text{ cm } H_2O^{-1} \text{ s}^{-1}$, respectively. There were no significant differences in the baseline SG_{aw} among the three groups. The LAR defined as a 25% reduction in SG_{aw} was observed in 12 of 15 saline-pretreated animals. Formoterol, administered by inhalation, significantly attenuated the IAR (5 minutes), inhibited the prolonged asthmatic response after the IAR (2 hours), and blocked the LAR phenomena (6 to 8 hours and 10 hours) (Fig. 3). In contrast, isoproterenol did not inhibit the LAR, although it significantly attenuated

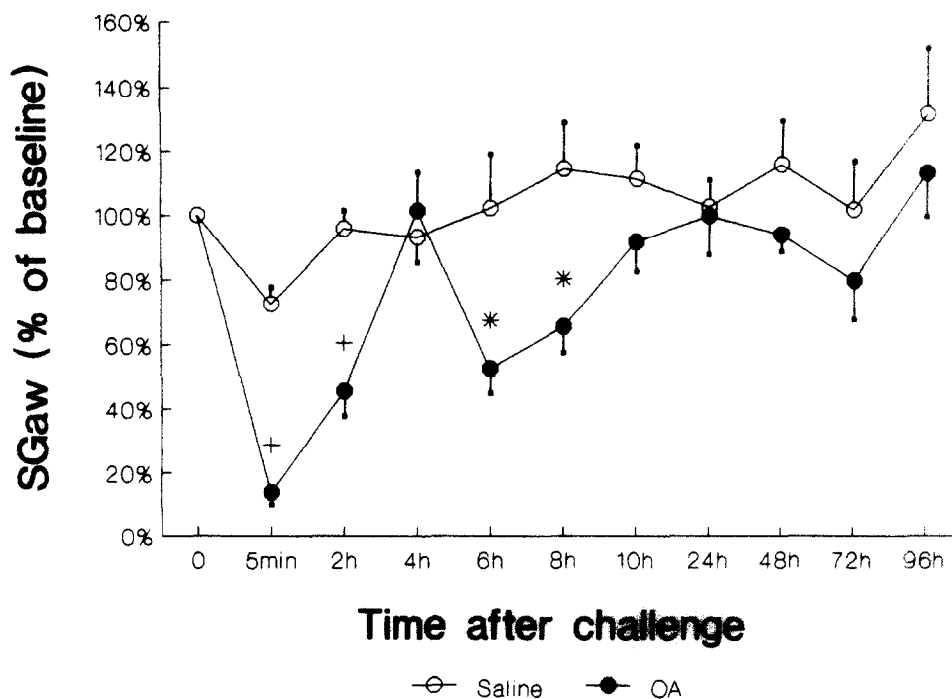


FIG. 2. Changes of SG_{aw} in sensitized guinea pigs after saline (N = 8)- or antigen (OA; N = 10)-inhalation challenge; **p* < 0.01 and **p* < 0.02 compared with that of the saline-challenged group. IAR (5 minutes) and LAR (6 to 8 hours) were observed after OA challenge.

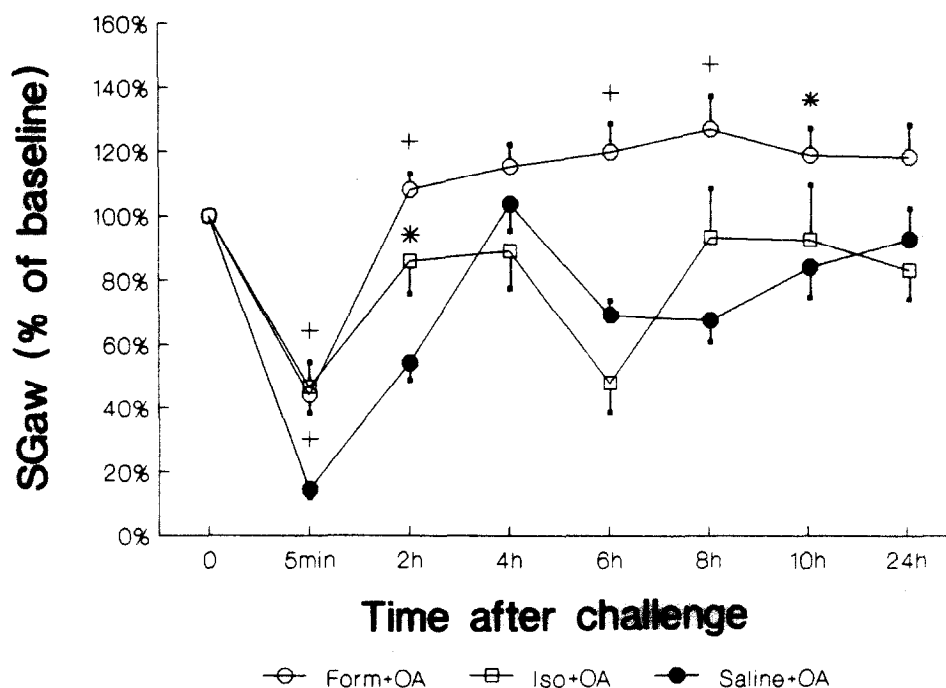


FIG. 3. Effects of formoterol and isoproterenol on changes of SG_{aw} in guinea pigs after OA challenge. Animals inhaled an aerosolized solution of 10 µg/ml of formoterol (Form + OA; N = 13), 1 mg/ml of isoproterenol (Iso + OA; N = 8), or saline (Saline + OA; N = 15) 15 minutes before challenge; **p* < 0.01 and **p* < 0.02 compared with that of the saline-pretreated group. LAR was completely prevented by formoterol, but not by isoproterenol.

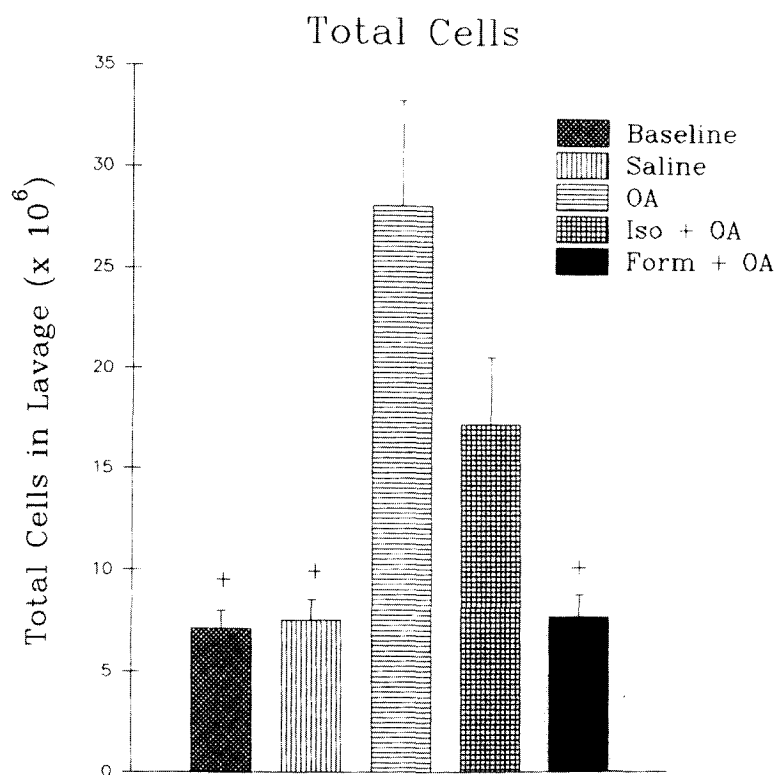


FIG. 4. Total cell counts in BALF 24 hours after saline or OA challenge. Each group consisted of eight to nine guinea pigs; $^*p < 0.01$ compared with the value in the OA-challenged group. Number of total cells in BALF significantly increased in OA-challenged group, compared with baseline of sensitized, but not challenged, or the saline-challenged group. Formoterol completely inhibited the antigen-induced cellular increase, but not by isoproterenol.

the IAR and prevented the prolonged asthmatic response after the IAR (2 hours).

BAL was performed 24 hours after challenge. Some animals were killed for the BAL after completion of the measurement of pulmonary function (e.g., 9/15 of the saline-OA challenge, 8/13 of the formoterol-OA challenge, and 8/8 of the isoproterenol-OA challenge groups). The animals in the saline-OA and isoproterenol-OA challenged groups demonstrated the LAR. As illustrated in Fig. 4, the cellular components in BALF markedly increased in the antigen (OA)-challenged group compared with the baseline of the sensitized, but not challenged group, or the saline-challenged group. For example, macrophages significantly increased from 5.4 ± 0.6 ($\times 10^6$ cells; baseline group) to 15.1 ± 2.4 , eosinophils from 1.4 ± 0.3 to 11.2 ± 4.3 , neutrophils from 0.1 ± 0.03 to 1.3 ± 0.5 , and lymphocytes from 0.19 ± 0.04 to 0.4 ± 0.1 (Fig. 5). Formoterol inhibited the antigen-induced influx of inflammatory cells into the airway completely. After formoterol, the numbers of macrophages, eosinophils, and neutrophils in BAL were significantly lower than those in the OA-challenged group (Fig. 5). The number of lymphocytes was also lower than that in the OA-chal-

lenged group, but not statistically significant (Fig. 5). Isoproterenol failed to inhibit the increase of the cellular components in BALF after antigen challenge.

The bronchial responsiveness to histamine after antigen challenge increased 24 hours after challenge and returned to baseline 72 hours after challenge (Table I). The PC_{50} value at 24 hours (20.4 ± 2.6 $\mu\text{g/ml}$) was significantly lower than that of the prechallenge baseline (26.2 ± 2.6 $\mu\text{g/ml}$; $p < 0.05$, with paired Student's t test). Formoterol significantly decreased the bronchial responsiveness to histamine 24 hours after antigen challenge. The PC_{50} value at 24 hours (46.8 ± 7.1 $\mu\text{g/ml}$) was significantly higher than that of the baseline (29.8 ± 1.8 $\mu\text{g/ml}$; $p < 0.05$), that of the saline-pretreated control (20.4 ± 2.6 $\mu\text{g/ml}$; $p < 0.01$), or that of the isoproterenol-pretreated group (24.7 ± 2.3 $\mu\text{g/ml}$; $p < 0.02$).

After isoproterenol, PC_{50} had a tendency to decrease after antigen challenge but did not reach statistical significance ($p < 0.07$).

DISCUSSION

Guinea pigs have been used as a model of human asthma because similarities exist between the two species in the response to a variety of contractile and

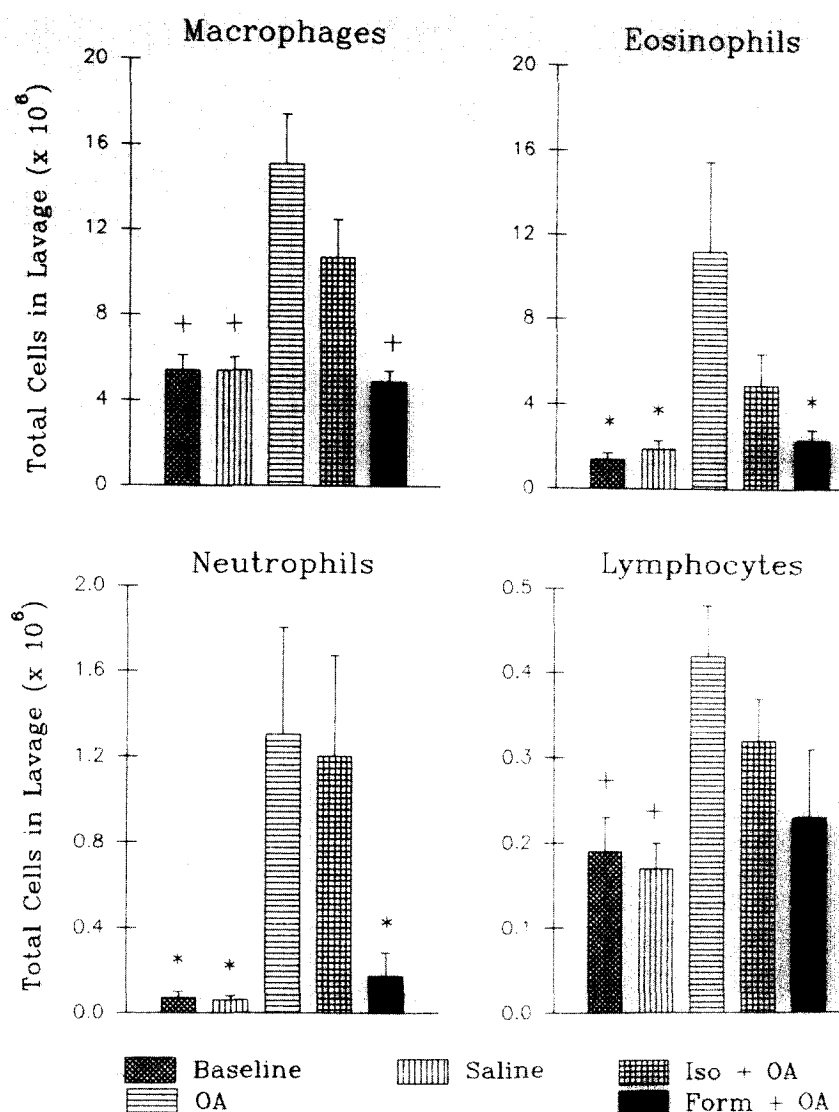


FIG. 5. Differential cell counts in BALF 24 hours after saline or antigen challenge. Each group consisted of eight to nine animals; * $p < 0.01$ and * $p < 0.05$ compared with the value in OA-challenged group. All cellular components in BALF significantly increased in OA-challenged group compared with that of baseline or of saline-challenged group. Formoterol completely inhibited antigen-induced cellular influx (except lymphocytes), although isoproterenol did not inhibit airway inflammation.

relaxant substances and in the putative involvement of chemical mediators in antigen-induced pulmonary response.¹⁸ Sensitized guinea pigs clearly demonstrate an LAR. Iijima et al.⁶ demonstrated the LAR in guinea pigs sensitized to *Ascaris suum*, and Hutson et al.⁷ in guinea pigs sensitized by inhalation of aerosolized OA, under cover of mepyramine to avoid an immediate fatal anaphylaxis. In this study, we developed a model of the LAR in guinea pigs similar to that described by Hutson et al.⁷ The LAR defined as a 25% reduction in SG_{aw} was observed in 88% of the antigen-challenged animals (10/10 in the OA-challenged group and 12/15 in the saline-pretreated OA-challenged group). There are, however, differences in the

time of occurrence of the LAR between the two studies. The LAR occurred 13 to 24 hours after antigen challenge in the model of Hutson et al.,⁷ whereas we observed the LAR 6 to 8 hours after the challenge, which is similar to the LAR observed in human asthma. The so-called "late" LAR, which has been reported in the model of Hutson et al.⁷ to occur more than 24 hours after antigen challenge was not observed in our model. Although it is hard to determine the source of these differences, it might be, in part, due to the differences in the methods of sensitization and challenge between the two models.

A question has been raised if airway conductance, as measured with a whole-body plethysmograph,

TABLE I. The effect of formoterol on the antigen-induced bronchial hyperresponsiveness

Pretreatment	PC ₅₀ (μg/ml of histamine)		
	Saline (N = 8)	Isoproterenol (N = 8)	Formoterol (N = 8)
Before	26.2 ± 2.6	28.4 ± 2.5	29.8 ± 1.8
24 hr after	20.4 ± 2.6*	24.7 ± 2.3	46.8* ± 7.1†
72 hr after	27.3 ± 3.9	27.9 ± 3.1	37.9 ± 5.3

* $p < 0.05$ compared with the prechallenge baseline with paired Student's t test.

† $p < 0.01$ and $p < 0.02$ compared with the saline- and isoproterenol-pretreated groups, respectively (with unpaired Student's t test).

whereas the animal breathes through the upper airways, truly reflects the patterns of pulmonary response of the lower airways. Johns et al.¹⁹ observed the LAR 18 to 24 hours after antigen challenge in 28% of guinea pigs with a whole-body plethysmograph according to the method described by Hutson et al.,⁷ whereas the LAR was observed in only 10% of animals with a measurement of lung resistance through a tracheostomy. The values of SG_{aw}, as measured by our system, have been previously demonstrated to be reasonably accurate and reliable when these values are compared with values measured by a pleural catheter technique.¹⁶ We agree, however, that our method may be measuring changes in total respiratory tract conductance, including the upper and lower airways. The increase in cellular constituents in BALF after antigen challenge strongly supports the significant participation of the pulmonary component to the total respiratory tract involvement in our system. Therefore, we propose that our guinea pig model is a useful animal model to investigate the mechanisms of the LAR phenomena.

In our guinea pig model, we demonstrated an LAR (6 to 8 hours) and an increase in nonspecific bronchial responsiveness after antigen challenge, both of which are associated with a cellular influx into the airway. The change in bronchial responsiveness to histamine after antigen challenge was smaller than we expected. We suspect that we failed to find much difference in PC₅₀ after antigen challenge because the animals used in this study were very sensitive to histamine and the baseline PC₅₀ was very low. The main components of the cellular influx in our model are eosinophils and macrophages. The cell component predominantly responsible for airway inflammation after antigen challenge is likely to depend on species, antigens used, and the method of sensitization. de Monchy et al.²⁰

demonstrated the increased numbers of eosinophils in human BALF during the LAR (6 to 7 hours) after house dust-mite inhalation. Metzger et al.²¹ demonstrated increases in eosinophils, neutrophils, and lymphocytes in human BALF 48 hours after antigen challenge (mainly *Alternaria*). Murphy et al.²² described that, in rabbits, the LAR and the subsequent increase in airway reactivity are dependent on the presence of neutrophils, since the chemical depletion of neutrophils decreased the LAR and airway reactivity. In guinea pigs, as reported by Hutson et al.,⁷ leukocytes (especially eosinophils) probably play an important role in the development of the airway inflammation. This is consistent with our findings.

The LAR and the accompanying rise in nonspecific bronchial responsiveness are inhibited by pretreatment with sodium cromoglycate or corticosteroids, but not by short-acting β_2 -agonists.^{9, 10} β_2 -agonists were believed to treat only the symptoms of asthma without affecting the underlying inflammatory processes. In this study, however, formoterol completely suppressed the appearance of the LAR and the antigen-induced airway inflammation, although isoproterenol, a conventional short-acting β_2 -agonist, failed to do either.

Both formoterol and isoproterenol attenuated but did not completely suppress the IAR. It might be speculated that the IAR (14.4% ± 2.5% of baseline SG_{aw}) observed in our model was too severe to be completely protected by β_2 -agonists alone. Formoterol also decreased the antigen-induced increase in bronchial hyperresponsiveness, as measured by histamine. This is based on the finding that formoterol inhibited the antigen-induced cellular influx into the airway, and the effect of formoterol on bronchial responsiveness might be due to the inhibition of airway inflammation. After isoproterenol, the PC₅₀ value was lower than the prechallenge value, but was not statistically significant ($p < 0.07$). This finding might be derived from the low baseline value of PC₅₀ because of animals highly sensitive to histamine, as previously described.

Formoterol, by inhalation, was reported to decrease the airway responsiveness to methacholine in children with asthma, even at 12 hours,²³ and improve the bronchial hyperresponsiveness to histamine and cold air after long-term administration.²⁴ Palmqvist et al.²⁵ reported that formoterol, administered before antigen challenge, attenuated the LAR in subjects with asthma. These clinical observations support our findings. Recently, salmeterol, a new long-acting β_2 -agonist, has been reported to inhibit the LAR and the antigen-induced rise in nonspecific bronchial responsiveness in subjects with asthma.²⁶ It is interesting that long-acting β_2 -agonists, such as formoterol and salmeterol, but not short-acting β_2 -agonists, can inhibit

the LAR and the accompanying rise in bronchial responsiveness. The pertinent question is whether the inhibitory effects of formoterol on the LAR and airway inflammation are due to the persisting long action of β_2 -agonists stimulation. If mast cell activation continues for several hours after antigen challenge and mediators released from mast cells attract inflammatory cells into the airway,²⁷ the persisting action of formoterol in suppressing mediator release may have an important advantage in inhibition of the LAR and airway inflammation. There are studies that β_2 -agonists significantly attenuated the antigen-induced LAR when they were administered at a frequency to maintain their maximum effect.^{28, 29} In contrast, formoterol may have beneficial effects on the airway inflammation by means other than its long-acting bronchodilator action. Indeed, salmeterol is reported to inhibit mediator release from activated macrophages in the presence of β -blockers.³⁰ Additional studies are necessary to clarify the exact mechanisms of the inhibitory effects of formoterol on the antigen-induced LAR and airway inflammation.

In summary, we demonstrated that the antigen-induced LAR and the increased bronchial responsiveness associated with the airway inflammation in guinea pigs were inhibited by formoterol, a new long-acting β_2 -agonist. It is suggested that formoterol has inhibitory effects on the underlying inflammatory processes in asthma in addition to its prolonged bronchodilation, although the exact mechanisms of formoterol in inhibiting the LAR phenomena remain unknown.

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REFERENCES

1. O'Byrne PM, Dolovich J, Hargreave FE. State of art: late asthmatic responses. *Am Rev Respir Dis* 1987;136:740-51.
2. Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE. Allergen-induced increase in nonallergic bronchial reactivity. *Clin Allergy* 1977;7:503-13.
3. Cartier A, Thomson NC, Frith PA, Roberts R, Hargreave FE. Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway caliber. *J ALLERGY CLIN IMMUNOL* 1982;70:170-7.
4. Shampain MP, Behrens BL, Larsen GL, Henson PM. An animal model of late pulmonary responses to *Alternaria* challenge. *Am Rev Respir Dis* 1982;126:493-8.
5. Abraham WM, Delehunt JC, Yerger L, Marchette B. Characterization of a late-phase pulmonary response after antigen challenge in allergic sheep. *Am Rev Respir Dis* 1983;128:839-44.
6. Iijima H, Ishii M, Yamauchi K, et al. Bronchoalveolar lavage and histologic characterization of late asthmatic response in guinea pigs. *Am Rev Respir Dis* 1987;136:922-9.
7. Hutson PA, Church MK, Clay TP, Miller P, Holgate ST. Early and late-phase bronchoconstriction after allergen challenge of nonanesthetized guinea pigs. *Am Rev Respir Dis* 1988;137:548-57.
8. 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.
9. 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.
10. Hutson PA, Holgate ST, Church MK. The effect of cromolyn sodium and albuterol on early and late-phase bronchoconstriction and airway leukocyte infiltration after allergen challenge of nonanesthetized guinea pigs. *Am Rev Respir Dis* 1988;138:1157-63.
11. Lofdahl CG, Svedmyr N. Formoterol fumarate, a new β_2 -adrenoceptor agonist. *Allergy* 1989;44:264-71.
12. Graff-Lonnevig V, Browaldh L. Twelve hours' bronchodilating effect of inhaled formoterol in children with asthma: a double-blind cross-over study versus salbutamol. *Clin Exp Allergy* 1990;20:429-32.
13. Maesen FPV, Smeets JJ, Gubbelmans HLL, Zweers PGMA. Bronchodilator effect of inhaled formoterol vs salbutamol over 12 hours. *Chest* 1990;97:590-4.
14. McAlpine LG, Thomson NC. Prophylaxis of exercise-induced asthma with inhaled formoterol, a long-acting β_2 -adrenergic agonist. *Respir Med* 1990;84:293-5.
15. Malo JL, Cartier A, Trudeau C, Ghezzi H, Gontovnick L. Formoterol, a new inhaled β_2 -adrenergic agonist, has a longer blocking effect than albuterol on hyperventilation-induced bronchoconstriction. *Am Rev Respir Dis* 1990;142:1147-52.
16. Gerlach RF, Medinsky MA, Hobbs CH, et al. Effect of four-week repeated inhalation exposure to unconjugated azodicarbonamide on specific and non-specific airway sensitivity of the guinea pig. *J Appl Toxicol* 1989;9:145-53.
17. Pennock BE, Cox CP, Rogers RM, Cain WA, Wells JH. A noninvasive technique for measurement of changes in specific airway resistance. *J Appl Physiol* 1979;46:399-406.
18. Kallos P, Kallos L. Experimental asthma in guinea pigs revisited. *Int Arch Allergy Appl Immunol* 1984;73:77-85.
19. Johns K, Sorkness R, Graziano F, Castleman W, Lemanske RF. Contribution of upper airways to antigen-induced late airway obstruction responses in guinea pigs. *Am Rev Respir Dis* 1990;142:138-42.
20. de Monchy JGR, Kauffman HF, Venge P, et al. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. *Am Rev Respir Dis* 1985;131:373-6.
21. Metzger WJ, Richerson HB, Worden K, Monick M, Hunninghake GW. Bronchoalveolar lavage of allergic asthmatic patients following allergen bronchoprovocation. *Chest* 1986;89:477-83.
22. Murphy KR, Wilson MC, Irvin CG, et al. The requirement for polymorphonuclear leukocytes in the late asthmatic response and heightened airway reactivity in an animal model. *Am Rev Respir Dis* 1986;134:62-8.
23. Becker AB, Simons FER, McMillan JL, Faridy T. Formoterol, a new long-acting selective β_2 -adrenergic receptor agonist: double-blind comparison with salbutamol and placebo in children with asthma. *J ALLERGY CLIN IMMUNOL* 1989;84:891-5.
24. Von Berg A, Berdel D. Efficacy of formoterol-metered aerosol in children. *Lung* 1990;168(S):90-8.
25. Palmqvist M, Balder B, Lowhagen O, Melander B, Svedmyr N, Wahlander L. Late asthmatic reaction prevented by inhaled

- salbutamol and formoterol [Abstract]. *J ALLERGY CLIN IMMUNOL* 1989;83:244.
26. Twentyman OP, Finnerty JP, Harris A, Palmer J, Holgate ST. Protection against allergen-induced asthma by salmeterol. *Lancet* 1990;336:1338-42.
 27. Durham SR, Lee TH, Cromwell O, et al. Immunologic studies in allergen-induced late-phase asthmatic reactions. *J ALLERGY CLIN IMMUNOL* 1984;74:49-60.
 28. Eiser N. Effect of histamine H₁-receptor antagonism and β_2 -adrenergic stimulation on late asthmatic responses to inhaled antigen [Abstract]. *Am Rev Respir Dis* 1990;141:874.
 29. Van Bever HP, Desager KN, Stevens WJ. The effect of inhaled fenoterol, administered during the late asthmatic reaction to house dust mite (*Dermatophagoides pteronyssinus*). *J ALLERGY CLIN IMMUNOL* 1990;85:700-3.
 30. Baker AJ, Fuller RW. Anti-inflammatory effect of salmeterol on human alveolar macrophages [Abstract]. *Am Rev Respir Dis* 1990;141:394.

Antigenic and allergenic analysis of psyllium seed components

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*The outer portions (husk) of psyllium seeds are a concentrated source of natural fiber used in some bulk-fiber laxatives and cereals. They are known to elicit respiratory allergic reactions after inhalation or ingestion among sensitized individuals. Antigenic and allergenic characterization of three psyllium-seed fractions (husk, endosperm, and embryo) was conducted with crossed immunoelectrophoresis (CIE), crossed radioimmuno-electrophoresis, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis to determine the source of psyllium allergenicity. Homologous CIE demonstrated psyllium endosperm and embryo extracts contained seven and four antigens, respectively. Husk extracts were too gelatinous to react by CIE. However, heterologous CIE profiles of endosperm or embryo extracts, reacted with antihusk antibodies, resulted in antigen-antibody precipitin peaks that matched the heavy staining precipitin lines of homologous reactions for endosperm and embryo, respectively. These results indicated that commercial-grade husk, endosperm, and embryo contained similar antigens. Extracts of all three seed components contained antigens that bound IgE antibodies in the sera of 11 psyllium RAST-positive individuals, as determined by crossed radioimmuno-electrophoresis. The few prominent husk protein/peptide bands resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis were common in either embryo or endosperm. Immunoblots revealed common IgE reactive bands in all three seed fractions. Microscopic examination of the powdered commercial-grade psyllium (95% pure) revealed it contained endosperm and embryo particles. These immunologic, biochemical, and microscopic findings suggest that other contaminating seed components are primarily responsible for the allergenicity of commercial-grade psyllium-husk powder rather than the husk itself. (*J ALLERGY CLIN IMMUNOL* 1992;89:866-76.)*

Key words: Psyllium, psyllium husk, allergenicity, laxatives, cereals, fiber

The outer coat (husk) of psyllium seeds (*Plantago ovata*) is a concentrated source of natural soluble fiber used in some bulk-fiber laxatives and cereals because

Abbreviations used

CIE: Crossed immunoelectrophoresis
CIE: Crossed radioimmuno-electrophoresis
SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
BSA: Bovine serum albumin

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of its laxative properties and, recently, its demonstrated effect in reducing serum cholesterol.¹⁻⁴ This fiber has the potential for eliciting respiratory allergic reactions in some sensitive individuals after inhalation