

The effect of inhaled ipratropium bromide alone and in combination with oral terfenadine on bronchoconstriction provoked by adenosine 5'-monophosphate and histamine in asthma

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The aim of this study was to investigate the effect of terfenadine, an antihistamine, 180 mg orally, the anticholinergic drug, ipratropium bromide (IB), 0.5 mg nebulized aerosol, the combination of these two drugs, and placebo tablets and aerosol on histamine- and adenosine 5'-monophosphate (AMP)-induced bronchoconstriction in a randomized, double-blind fashion. Airway response was evaluated as FEV₁. After placebo, the geometric mean (GM) provocative concentration causing a 20% fall in FEV₁ from the postsaline baseline value (PC₂₀) for histamine and AMP was 0.63 and 5 mg/ml, respectively. Terfenadine displaced the FEV₁ concentration-response curves obtained with both histamine (GM PC₂₀ values increasing to 26.92 mg/ml) and AMP (GM PC₂₀ values increasing to 26.7 mg/ml) to the right. IB had a small, but significant, protective effect against the fall in FEV₁ produced by histamine and AMP, the GM PC₂₀ values increasing to 1.69 and to 12.6 mg/ml, respectively. Terfenadine and IB in combination produced protection against histamine and AMP that was more than the production produced by either drug alone, the GM PC₂₀ values increasing to 54.76 and 47.7 mg/ml, respectively. There was no correlation between degree of bronchodilatation induced by active treatments and concentration ratios for AMP or histamine. These data suggest that histamine release and vagal reflexes both contribute to AMP-induced bronchoconstriction in clinical asthma in man. (J ALLERGY CLIN IMMUNOL 1991;87:939-47.)

The mechanism by which adenosine and its related nucleotide, AMP, cause bronchoconstriction when it is inhaled by atopic^{1,2} and nonatopic^{1,3} subjects with asthma remains to be clarified. Adenosine has been demonstrated to potentiate the release of the preformed mediators β -hexosaminidase and histamine from immunologically activated rodent⁴ and human⁵ mast cells in vitro, probably through an interaction with specific cell-surface receptors of the A₂ subtype.^{4,5} Additional support for mast cell involvement in AMP-induced

Abbreviations used

AMP: Adenosine 5'-monophosphate
PC₂₀: Provocation concentration required to produce a 20% fall in FEV₁ from the postsaline baseline value
IB: Ipratropium bromide
GM: Geometric mean

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bronchoconstriction is gained from the finding that sodium cromoglycate and nedocromil sodium, inhibitors of mast cell activation,⁶ are highly effective in protecting the airways against AMP in both atopic⁷ and nonatopic⁸ subjects with asthma. The possibility that histamine is implicated as the mast cell product most likely involved in causing bronchoconstriction provoked by AMP is suggested by demonstrating that selective H₁-receptor antagonists are particularly effective inhibitors of the response.^{2,3,9} Once it is released, histamine provokes bronchoconstriction both directly by stimulating H₁ receptors on airway smooth

TABLE I. Characteristics of subjects studied

Subject No.	Sex	Age (yr)	Baseline FEV ₁ (% pred)	PC ₂₀ histamine (mg/ml)	PC ₂₀ AMP (mg/ml)
1	M	65	104	0.20	0.02
2	F	23	97	0.54	0.39
3	M	35	93	3.11	45.04
4	F	52	82	0.61	4.85
5	M	50	85	0.22	0.54
6	M	44	72	1.03	1.90
7	F	24	88	0.27	3.61
8	M	28	87	1.56	23.92
Mean		40.1	88.5	0.61*	6.18*
SEM		± 5.3	+ 3.4	(0.2-3.1)	(0.5-45.0)

Pred, Predicted.

*Geometric mean and range.

muscle and postcapillary venules and, to a lesser degree, by activating cholinergic reflex pathways.¹⁰

Enhancement of mast cell mediator release may not be the only mechanism accounting for the bronchoconstriction provoked by inhaled purines. There is some evidence to incriminate neural reflexes in the response. In rabbit airways, adenosine has been demonstrated to potentiate the constrictor response to transmural nerve stimulation by an A₂-receptor mechanism.¹¹ Pauwels and Van der Straeten¹² have demonstrated in another animal model, the inbred rat, an inhibitory effect of atropine on bronchoconstriction provoked by intravenous adenosine. In subjects with asthma, Okayama et al.¹³ have reported some reversal of an established adenosine bronchoconstriction with inhaled atropine and lidocaine, although one study has failed to demonstrate a definite inhibitory effect of inhaled IB when it is administered before adenosine challenge.¹⁴

To examine and define the relative contribution of cholinergic mechanisms and histamine release to the airway response provoked by inhaled AMP, we have investigated the effect of prior administration of the specific muscarinic-receptor antagonist, IB, and the potent and selective H₁-histamine receptor-antagonist, terfenadine, alone and in combination on the airway response to inhaled AMP in atopic asthma.

METHODS

Subjects

Eight subjects (five men) with a mean age of 40.1 ± 5.3 years (mean ± SEM) participated in the study. All subjects were nonsmokers with atopic asthma as defined by positive skin prick tests (>2 mm wheal response) to six common aeroallergens: house dust, *Dermaophagoides pteronyssinus*, *D. farinae*, mixed grass pollen, tree pollen, and cat fur (Bencard Allergy Unit, Brentford, Middlesex, U.K.).

All patients had baseline FEV₁ >70% of their predicted values of >1.5 L, and none were receiving oral corticosteroids or theophylline (Table I). Inhaled bronchodilators were stopped for 8 hours before each visit to the laboratory, although subjects were allowed to continue inhaled corticosteroids as usual. Patients were not studied within 4 weeks of an upper respiratory tract infection or exacerbation of their asthma, and all visits to the laboratory were performed at the same time of day. All subjects gave written informed consent, and the study was approved by the Southampton University and Hospitals Ethical Subcommittee.

Bronchial provocation

Pulmonary function was measured as FEV₁ by means of a dry-wedge spirometer (Vitalograph Ltd., Buckinghamshire, U.K.), the higher of two consecutive measurements being recorded. On each challenge day, histamine acid phosphate (BDH Chemicals Ltd., Poole, Dorset, U.K.) and AMP (Sigma Chemical Co., St. Louis, Mo.) were freshly prepared in 0.9% sodium chloride to produce a range of doubling concentrations of 0.03 to 32 mg/ml (0.1 to 104 mmol/L) and 0.39 to 400 mg/ml (4.48 to 1151.6 mmol/L), respectively. The solutions were administered as aerosols generated from a starting volume of 3 ml in a disposable Inspiron minnebulizer (C. R. Bard International Ltd., Sunderland, U.K.) driven by compressed air at 8 L/min. Under these conditions, the nebulizer generates an aerosol with a mass-median particle diameter of 4.7 μm.¹⁵ Subjects inhaled the aerosolized solutions in five breaths from end tidal volume to full inspiratory capacity via a mouthpiece.¹⁶

Study design

The study was divided into two phases. In the first phase, subjects attended the laboratory on two separate occasions at least 48 hours apart to undertake concentration-response studies with inhaled histamine and AMP. On each occasion, after 15 minutes of rest, three baseline measurements of FEV₁ were recorded at 3-minute intervals. Subjects then inhaled nebulized 0.9% sodium chloride, and FEV₁ mea-

surements were repeated at 1 and 3 minutes, the higher value being recorded. If FEV₁ did not fall by >10% of the baseline value, bronchial provocation was performed with inhaled histamine or AMP. After administration of each concentration of agonist, FEV₁ was measured at 1 and 3 minutes, the higher of the two values being recorded. Increasing doubling concentrations of histamine (0.03 to 32 mg/ml) or AMP (0.39 to 400 mg/ml) were inhaled at 5-minute intervals until FEV₁ had fallen by >20% of the postsaline baseline value or the highest concentrations of agonist had been administered. The percentage decrease in FEV₁ was plotted against the cumulative concentration of agonist administered on a logarithmic scale and that concentration of agonist producing a PC₂₀ from the postsaline baseline value determined by linear interpolation.

In the second phase of the study, subjects attended the laboratory on eight occasions at the same time of day, 1 week apart, to undertake four concentration-response studies with each agonist. Terfenadine, 180 mg, or matched placebo was administered orally 3 hours before measurement of the baseline FEV₁, the highest of three measurements being recorded. Subjects then received either nebulized isotonic IB (0.5 mg) or nebulized vehicle consisting of 0.9% sodium chloride. The aerosol solutions were generated from a starting volume of 3 ml in an Inspiron mininebulizer driven by compressed air at 8 L/min and inhaled to dryness by deep tidal breathing during a 7- to 8-minute time period. The same nebulizer was used for all studies on all subjects. Thus, on the four visits required for the histamine and AMP challenges, each subject received (1) oral and aerosol placebos, (2) oral terfenadine, 180 mg, and aerosol placebo, (3) oral placebo and inhaled IB, 0.5 mg, and (4) the combination of oral terfenadine and inhaled IB. The treatments were randomized separately for each of the two agonists and administered double blind. For each agonist, the four experiments were performed during the 4-week period after determination of the PC₂₀ value. The challenge procedure used was identical to the challenge described during the control period.

Data analysis

Figures refer to the mean $\pm$ SEM unless it is otherwise stated, and the $p < 0.05$ level of significance was accepted. Pre- and posttreatment baseline values of FEV₁ before bronchial challenges were compared within each study day by Wilcoxon's signed-rank test for paired data and between study days by two-factor analysis of variance, followed by the Newman-Keul's procedure.

The fall in FEV₁ after each concentration of agonist was expressed as a percentage of the higher of the two postsaline baseline FEV₁ recordings. Since the postsaline FEV₁ values after terfenadine or IB alone and in combination were significantly higher than values after placebo, the constrictor responses of the agonists were expressed as percentage fall from the postdrug baseline value. The percentage fall in FEV₁ was plotted against the cumulative concentration of bronchoconstrictor agonist on a logarithmic scale, and the PC₂₀ was determined by linear interpolation.

The repeatability of the challenge procedure with inhaled histamine and AMP in these patients was determined according to the method of Altman and Bland¹⁷ of plotting the difference against the mean of the logarithmically transformed PC₂₀ values obtained on the placebo-treatment and open-study days. The mean and standard deviation of the difference between these values were then derived and used to calculate the coefficient of repeatability between the results of the 2 study days.

The slopes of the AMP and histamine concentration-response curves were determined by least-squares linear regression analysis and compared with postplacebo and posttreatment study days with the Student's t test for paired data. After terfenadine alone and in combination with IB, a 20% fall in FEV₁ was not obtained in three subjects (Nos. 2, 3, and 8) and six subjects (Nos. 2, 3, 5, 6, 7, and 8) respectively, at the highest concentration of histamine administered, and therefore, a minimal estimate was obtained by calculating the cumulative PC₂₀ on the next doubling concentration beyond the highest administered. Because of these censored data, the results were compared for significance with Wilcoxon's signed-rank test. AMP and histamine PC₂₀ values were logarithmically transformed to normalize their distribution and compared by means of two-factor analysis of variance. Concentration ratios for the protective effect of terfenadine and IB alone and in combination against bronchoprovocation with each agonist were calculated by dividing the PC₂₀ value obtained after administration of the active drugs by the value obtained after administration of placebo. The relative potency of the active drugs in protecting against bronchoconstriction induced by the two agonists was analyzed by comparing the concentration ratios with the Wilcoxon's signed-rank test.

Relationship between PC₂₀ AMP, and PC₂₀ histamine values and the respective concentration ratios after terfenadine or IB alone and in combination were investigated by least-squares linear regression analysis of log-transformed values.

Relationship between concentration ratios and the degree of bronchodilatation after active treatment was investigated by least-squares linear regression analysis.

RESULTS

There were no significant differences in the mean baseline or postsaline values of FEV₁ between study days. However, the mean baseline FEV₁ values after administration of terfenadine, 180 mg, IB, 0.5 mg, and the two drugs in combination, were significantly higher than after placebo administration (Tables IIA, IIB, IIC, and IID).

After placebo administration, terfenadine, 180 mg, IB, 0.5 mg, and the combination of the two drugs, both AMP and histamine provoked concentration-dependent falls in FEV₁ with slopes that did not depart significantly from parallel. For the eight subjects studied, after placebo, the GM (range) PC₂₀ values for histamine and AMP were 0.63 (0.09 to 4.51) and 5

TABLE IIA. Baseline FEV₁ values (liter) after placebo treatment

Subject No.	Histamine study day		AMP study day	
	Oral P	Inhaled P	Oral P	Inhaled P
1	2.70	2.55	2.55	2.35
2	3.60	3.75	3.75	3.60
3	3.35	3.35	3.55	3.60
4	2.20	2.15	2.10	2.10
5	2.75	2.75	2.70	2.70
6	2.15	2.25	1.90	1.85
7	2.60	2.60	2.55	2.50
8	3.65	3.60	3.70	3.65
Mean	2.88	2.88	2.85	2.83
SEM(±)	0.21	0.22	0.26	0.26

P, Placebo.

TABLE IIB. Baseline FEV₁ values (liter) after terfenadine treatment

Subject No.	Histamine study day		AMP study day	
	Oral T	Inhaled P	Oral T	Inhaled P
1	2.80	2.85	2.80	2.65
2	3.80	3.80	3.65	3.70
3	3.65	3.65	3.60	3.65
4	2.35	2.45	2.20	2.25
5	2.75	2.85	3.00	3.10
6	2.80	2.75	2.15	2.05
7	3.05	3.00	3.35	3.25
8	3.80	3.80	4.00	3.95
Mean	3.13	3.14	3.09	3.08
SEM(±)	0.20	0.19	0.24	0.25

T, Terfenadine; P, placebo.

(0.6 to 35.8) mg · ml⁻¹, respectively (Figs. 1 and 2) (Table III). The challenge procedure in this group of patients was found to be repeatable for both histamine and AMP, there being a coefficient of repeatability of 1.4 doubling concentration for histamine and of one doubling concentration for AMP, respectively. The inhalation test was repeatable to within a single doubling dilution in six of the eight subjects receiving histamine and in seven of the eight subjects receiving AMP.

When terfenadine is compared with placebo, terfenadine administered before challenge displaced the FEV₁ concentration-response curves obtained with both histamine and AMP to the right (Figs. 1 and 2). For histamine, a minimum estimate of the GM (range) PC₂₀ after terfenadine administration was 26.92 (3.94 to 64.00) mg/ml, which was significantly higher ($p < 0.01$) than that achieved with the same agonist after placebo administration, 0.63 mg/ml (range 0.09 to

4.51) (Table III). The GM (range) PC₂₀ AMP after placebo administration of 5 mg/ml (0.6 to 35.8) also increased to 26.7 mg/ml (2.7 to 160.6) after terfenadine administration ($p < 0.01$) (Table III). Thus, when expressed as concentration ratios, terfenadine afforded a 43.5-fold and 5.3-fold protection of the airways against the FEV₁ response to inhaled histamine and AMP, respectively which was significantly different ($p < 0.01$).

Inhaled IB 15 minutes before challenge had a small, but significant, protective effect against the fall in FEV₁ produced by histamine and AMP (Figs. 1 and 2). The GM PC₂₀ histamine increased from 0.63 mg/ml after placebo to 1.69 mg/ml (0.38 to 17.34) after IB administration ($p < 0.01$). The same dose of inhaled IB was equally effective in protecting against the AMP-provoked fall in FEV₁, the GM PC₂₀ AMP increasing from 5 after placebo to 12.6 mg/ml (1.5

TABLE IIC. Baseline FEV₁ values (liter) after IB treatment

Subject No.	Histamine study day		AMP study day	
	Oral P	Inhaled IB	Oral P	Inhaled IB
1	2.55	2.60	2.75	2.85
2	3.60	3.80	3.50	3.65
3	3.30	3.40	3.45	3.60
4	2.10	2.30	2.35	2.35
5	2.50	3.10	2.45	2.90
6	2.25	2.70	1.90	2.15
7	2.55	2.75	2.90	3.25
8	3.65	3.85	3.70	3.90
Mean	2.81	3.06	2.88	3.08
SEM(±)	0.22	0.20	0.23	0.22

P, Placebo.

TABLE IID. Baseline FEV₁ values (liter) after combined terfenadine plus IB treatment

Subject No.	Histamine study day		AMP study day	
	Oral T	Inhaled IB	Oral T	Inhaled IB
1	2.60	2.55	2.65	2.90
2	3.85	3.85	3.70	3.70
3	3.70	3.75	3.75	4.10
4	2.25	2.40	2.45	2.65
5	2.85	3.00	3.10	3.20
6	2.80	2.90	2.45	2.70
7	3.05	3.30	3.05	3.15
8	3.75	4.00	3.90	4.15
Mean	3.11	3.22	3.13	3.32
SEM(±)	0.21	0.22	0.21	0.20

T, Terfenadine.

to 95.5). Thus, IB produced a 2.7-fold and 2.5-fold protection of the airways against histamine and AMP, respectively, which was not significantly different.

When two active treatments were combined, a displacement of the FEV₁ concentration-response curves to the right was achieved with both histamine and AMP (Figs. 1 and 2). For histamine, a minimum estimate of the GM PC₂₀ after treatment combination was 54.76 mg/ml (23.25 to 64.00), which was significantly higher than that achieved after placebo ($p < 0.01$), terfenadine ($p < 0.05$), or IB ($p < 0.01$) (Table III). Similarly, the drug combination increased the PC₂₀ AMP to 47.7 mg/ml (14.0 to 211.0), which was significantly higher than that achieved after placebo, ($p < 0.001$), terfenadine ($p < 0.05$), or IB administration alone ($p < 0.01$) (Table III). Thus, in these eight subjects, treatment combination achieved a 88.6-fold and 9.6-fold protection of the airways against

histamine and AMP-respectively, which was significantly different ($p < 0.01$). There was no correlation between degrees of bronchodilatation induced by active treatments and concentration ratios for AMP or histamine.

DISCUSSION

This study confirmed our previous findings that inhaled AMP causes concentration-related bronchoconstriction in subjects with asthma¹⁻³ and that the potent, selective H₁-histamine receptor-antagonist, terfenadine, is an effective inhibitor of this response.^{2, 3, 9} In this study, we have used the specific muscarinic receptor-antagonist, IB, alone and in combination with terfenadine, to explore the relative contribution of cholinergic pathways to the antihistamine-resistant component of AMP-induced bronchoconstriction. Alone, inhaled IB, in a dose that in a previous study

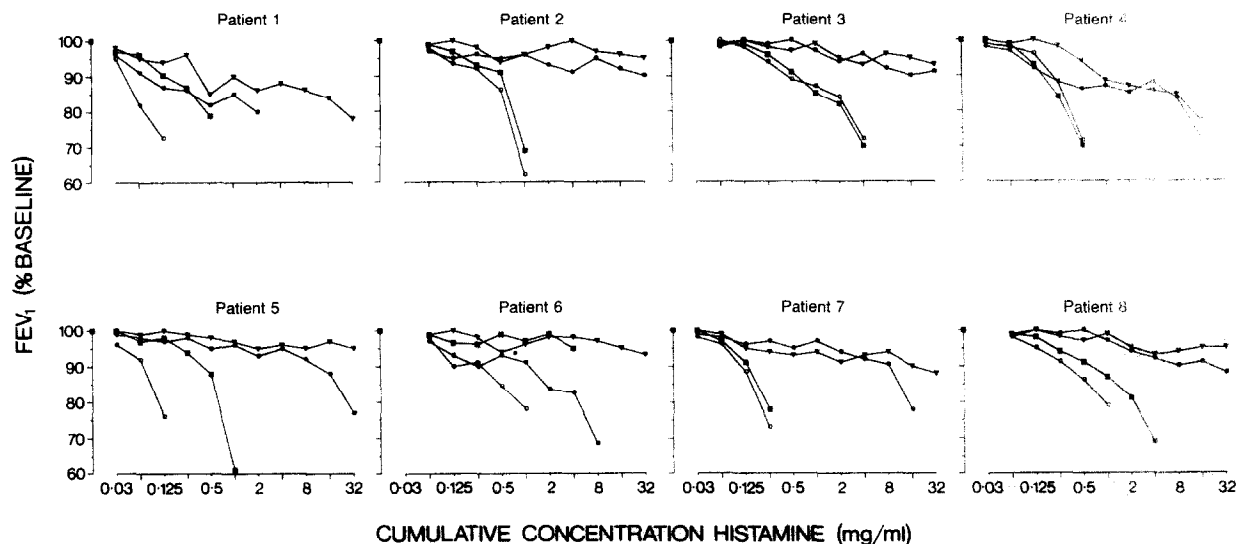


FIG. 1. Effect of placebo (○), terfenadine, 180 mg (●), IB, 0.5 mg (■), and the combination (▼) on the concentration-related falls in FEV₁ produced by inhaled histamine in eight subjects with asthma.

produced a mean 197-fold protection of asthmatic airways against the concentration-related fall in FEV₁ by methacholine,¹⁴ afforded a significant 2.5-fold protection to the airways against the constrictor effect of AMP. This finding suggests that cholinergic pathways contribute a small, but significant, component of the bronchoconstriction induced by inhaled AMP in asthma. Terfenadine and IB in combination produced protection against AMP that was greater than that produced by either drug alone. We have previously suggested that a major component of bronchoconstriction provoked by adenosine and AMP occurs through the enhanced release of preformed mediators, principally histamine, from preactivated airway mast cells.¹⁸ Indeed, the potent and selective H₁-histamine receptor-antagonists, terfenadine and astemizole, have been demonstrated to reduce greatly the bronchospastic response of asthmatic airways to inhaled AMP^{2, 3, 9} and are without significant anticholinergic activity.¹⁹ However, because the total protection against AMP-induced bronchoconstriction could not be achieved by increasing terfenadine dosage from 180 to 600 mg, alternative antihistamine-resistant pathways are likely to be involved.³

One possibility is that vagal reflexes may directly contribute to the bronchoconstrictor action of inhaled purines in asthma, as suggested by Pauwels and Van der Straeten¹² and Okayama et al.¹³ IB is a potent muscarinic receptor antagonist in the airways. However, the ability of an anticholinergic agent to attenuate bronchoconstriction provoked by different stimuli in asthma, such as histamine,²⁰⁻²² allergen,^{23, 24} cold

air,^{25, 26} and exercise,^{27, 28} is variable. In the present study, the known pharmacologic specificity of IB and its capacity to protect the airway response to inhaled AMP in seven of eight subjects with asthma implicate vagally mediated pathways in the causation of AMP-induced bronchoconstriction. By using a large dose of IB, we have demonstrated significant 2.5-fold protection against the bronchoconstrictor effects of AMP. Similarly, Mann et al.¹⁴ demonstrated that a similar dose of IB afforded a significant 1.7-fold protection to the airways against the concentration-related fall in FEV₁ by adenosine, although they concluded that the small effect observed on the adenosine response represented functional antagonism secondary to the bronchodilatory effect of the drug. In the present study, the lack of correlation between bronchodilation and bronchoprotection does not support this view. Similarly, previous studies with a variety of different bronchoconstrictor stimuli failed to demonstrate any clear relationship between the bronchodilator effect and the protective effect of anticholinergic drugs in bronchial asthma,^{20, 22, 23, 25} indicating that airway responsiveness to an antimuscarinic agent is different with regard to bronchodilator and bronchoprotective effects.

Although our findings support the view that vagally mediated pathways are involved in the pathophysiology of the bronchospastic response of inhaled adenosine in asthma, the exact stimulus prompting vagal reflex activation remains to be clarified. Possibilities include a direct effect of purine nucleosides in the stimulation of cholinergic reflexes and indirect activation through the release of mast cell mediators, such

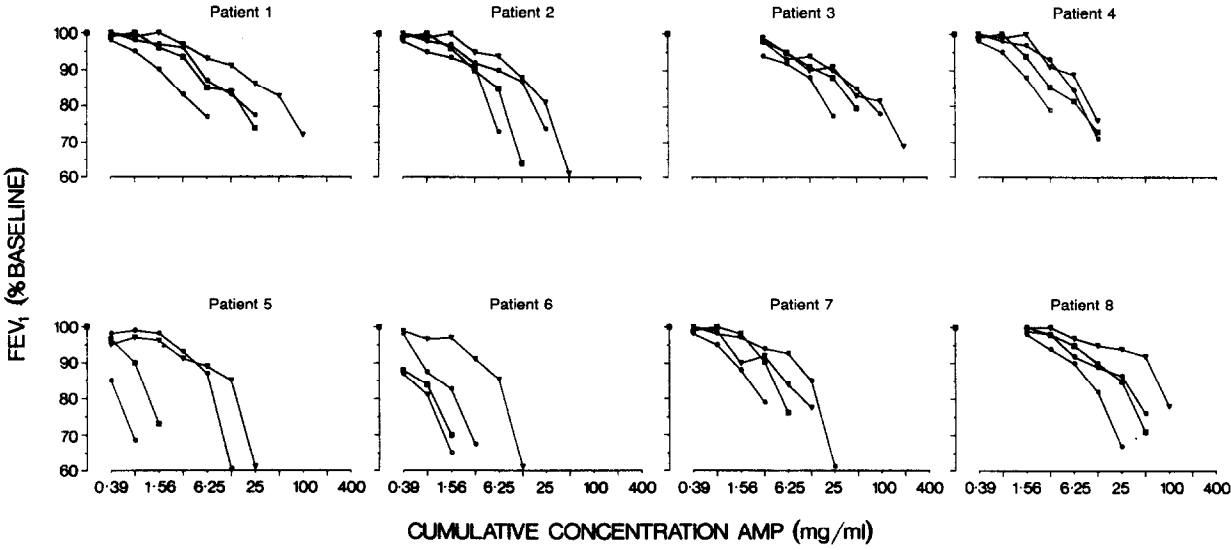


FIG. 2. Effect of placebo (○), terfenadine, 180 mg (●), IB, 0.5 mg (■), and the combination (▼) on the concentration-related falls in FEV₁ produced by inhaled AMP in eight subjects with asthma.

TABLE III. Histamine and AMP PC₂₀ values after each drug treatment

Subject No.	P	Histamine study days			P	AMP study days		
		Terfenadine	IB	IB plus terfenadine		Terfenadine	IB	IB plus terfenadine
1	0.09	3.94	0.85	50.73	5.4	33.2	29.7	114.7
2	1.06	64.00	1.28	64.00	6.3	33.0	11.5	48.5
3	4.51	64.00	3.97	64.00	35.8	160.6	95.5	211.0
4	0.57	20.03	0.48	23.25	4.2	18.3	12.5	18.5
5	0.14	52.71	1.16	64.00	0.6	14.4	1.9	28.3
6	1.55	8.99	17.34	64.00	1.2	2.7	1.5	14.0
7	0.23	28.12	0.38	64.00	4.2	26.8	8.7	17.5
8	1.75	64.00	4.11	64.00	25.2	76.3	62.2	178.3
GM	0.63	26.92	1.69	54.76	5.0	26.7	12.6	47.7
(range)	(0.1-4.5)	(3.9-64)	(0.4-17.3)	(23.3-64)	(0.6-35.8)	(2.7-160.6)	(1.5-95.5)	(14.0-211.0)

P, Placebo.

as histamine and prostaglandins. There is some evidence to suggest that histamine released into the airways could influence underlying vagal airway tone. In anesthetized dogs, interruption of vagal neurotransmission results in a significant reduction in histamine-induced bronchoconstriction.²⁹ In man, intravenous atropine has been reported to reduce the airway response to inhaled histamine in asthma.³⁰ More recent studies with atropine^{20, 31, 32} and inhaled hexamethonium bromide³³ have demonstrated that cholinergic and ganglionic blockade also inhibited the airway response to inhaled histamine, again implicating the vagal pathway in the response to this mediator. In the present study, IB produced a small but significant 2.7-fold protection of the airways against histamine,

compatible with observations made by other investigators. Therefore, a possibility is that reflex vagal bronchoconstriction in AMP-induced bronchoconstriction may be due to the effects of endogenous histamine on muscarinic receptors. Recently, Peachell et al.³⁴ have demonstrated that, in immunologically activated human lung mast cells, adenosine not only enhances histamine release but also potentiates prostanoïd production. In addition, Phillips and Holgate³⁵ and Crimi et al.³⁶ have demonstrated that cyclooxygenase blockade significantly inhibits part of the contractile response to purines in subjects with asthma. Considering that the effects on airway caliber of prostanoïds are known to be mediated in part by vagal reflexes,³⁷ the findings of the present study might be

interpreted as endogenous prostanoid production from mast cells by adenosine, producing some of their bronchoconstrictive effect by activating cholinergic pathways.

Thus, although vagal reflex activation may be an important, if variable, component of AMP-induced bronchoconstriction, most of its effects are likely to be mediated by a direct action between histamine released from activated mast cells and H_1 receptors on smooth muscle. The factors underlying the cholinergic component of AMP-induced bronchoconstriction have not been determined and may involve a direct effect of purine nucleotides and/or nucleosides on airway neural receptors and contributions made by mast cell-derived mediators, such as histamine and prostaglandins, whose effects also include vagal nerve stimulation.

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Characteristics of CR3-mediated aggregation in human eosinophils: Effect of priming by platelet-activating factor

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We have used double-color fluorescence-activated cell sorter analysis to characterize the homotypic aggregation response of human eosinophils (EOs). With this method, we demonstrate for the first time that EOs are able to form stable aggregates. The aggregation response induced by the phorbol ester, phorbol myristate acetate (PMA), was low and was not primed by platelet-activating factor (PAF). In contrast, the aggregation response induced by opsonized particles was markedly enhanced after priming with PAF. Additional experiments with several blocking monoclonal antibodies indicate that the CR3 receptor present on human EOs mediates the homotypic aggregation induced by PMA and by small opsonized particles through a putative "cell-adhesion" site on CR3, which binds to its counter structure on the opposing cell. The signal that initiates this binding event is generated after PMA addition or activation of the iC3b-binding site and is not sensitive for priming by PAF. The priming by PAF of the aggregation response induced by opsonized particles is restricted to an action on the iC3b-binding site, possibly by enhancing the affinity for its ligand. (J ALLERGY CLIN IMMUNOL 1991;87:947-54.)

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