

Effect of oral terfenadine on the bronchoconstrictor response to inhaled histamine and adenosine 5'-monophosphate in non-atopic asthma

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ABSTRACT Inhaled adenosine 5'-monophosphate (AMP) causes bronchoconstriction in atopic asthma, probably after *in vivo* conversion to adenosine. It has been suggested that adenosine potentiates preformed mediator release from mast cells on the mucosal surface of the airways by interacting with specific purinoceptors, without affecting the release of newly generated mediators. The airway response of nine non-atopic subjects with "intrinsic" asthma to inhaled AMP and the influence of the oral, selective H₁ histamine receptor antagonist terfenadine on this response was investigated. The geometric mean provocation concentrations of histamine and AMP required to produce a 20% fall in FEV₁ (PC₂₀) were 1.82 and 13 mmol/l. In subsequent placebo controlled time course studies the FEV₁ response to a single inhalation of the PC₂₀ histamine was ablated after pretreatment with oral terfenadine 180 mg. This dose of terfenadine caused an 80% inhibition of the bronchoconstrictor response to the PC₂₀ AMP when measured as the area under the time course-response curve and compared with the response to PC₂₀ AMP preceded by placebo. Terfenadine 600 mg failed to increase protection against AMP further, but both doses of terfenadine delayed the time at which the mean maximum fall in FEV₁ after AMP was achieved. Terfenadine 180 mg had no effect on methacholine induced bronchoconstriction in the same subjects. These data suggest that inhaled AMP may potentiate the release of preformed mediators from preactivated mast cells in the bronchial mucosa of patients with intrinsic asthma.

Introduction

Adenosine is a naturally occurring purine nucleoside formed from the cleavage of adenosine 5'-monophosphate (AMP) by 5'-nucleotidase.¹ Its physiological effects are due to stimulation of cell surface purinoceptors associated with adenylate cyclase, to cause either a decrease (A₁) or an increase (A₂) in intracellular levels of cyclic 3'5'-AMP.² When inhaled by atopic subjects with asthma adenosine causes concentration related bronchoconstriction, which reaches maximum 3-5 minutes after challenge and gradually subsides over 30-60 minutes.³ We have recently reported that the adenosine nucleotide AMP

also provokes bronchoconstriction in subjects with allergic asthma, probably after *in vivo* conversion to adenosine.⁴ The response of asthmatic airways to adenosine is selectively antagonised by the competitive adenosine receptor antagonist theophylline,^{5,6} and potentiated by the adenosine uptake inhibitor dipyridamole,⁷ suggesting that it is likely to be occurring through stimulation of specific cell surface purinoceptors.

Recent work suggests that the bronchoconstrictor effect of adenosine depends on the release of spasmogenic mediators from activated bronchial mast cells.⁸⁻¹⁰ Thus adenosine and synthetic analogues with some specificity for A₂ purinoceptors potentiate mediator release from preactivated rodent¹¹ and human⁸ mast cells, although this effect appears to be restricted to the release of preformed and not newly generated mediators.¹¹

Within the secretory granules of human mast cells histamine is the only preformed mediator known to

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contract airway smooth muscle. In subjects with atopic asthma the potent and selective H₁ histamine receptor antagonists terfenadine and astemizole almost completely inhibit the bronchoconstrictor response to inhaled AMP, while reducing the immediate allergen induced response by only 50%.¹² Thus in asthma associated with atopy the airways response to AMP probably results from the augmentation of histamine release from activated mast cells on the surface of the bronchial mucosa.

In 1947 Rackemann introduced the term intrinsic asthma to describe asthmatic patients in whom a causative external allergen could not be implicated.¹³ The role of inflammatory cells and their mediators in the pathogenesis of intrinsic asthma, however, has been little investigated. In this study we have investigated the airways response of patients with intrinsic asthma to inhaled AMP and the influence of H₁ histamine receptor blockade with the H₁ anti-histamine terfenadine on this response.

Methods

SUBJECTS

Nine patients, seven of them women, with a mean age of 56 (SEM 5) years, participated in the study. All subjects were non-smokers with intrinsic (non-atopic) asthma as defined by negative responses to prick skin tests (<2 mm weal response) with 10 common allergens (house dust, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, mixed grass pollen, tree pollen, cat fur, dog hair, feathers, *Candida albicans*, and *Aspergillus fumigatus*—Bencard, Brentford, Middlesex), no history of occupational asthma or diseases associated with atopy, and serum IgE concentrations within the normal range (<81 IU/ml). An eosinophil count was performed on a venous blood

sample. All patients had a baseline forced expiratory volume in one second (FEV₁) of over 60% of predicted values or >1.5 l, and none was receiving oral corticosteroids, theophylline, or sodium cromoglycate on a regular basis (table 1). Bronchodilators were not inhaled for eight hours before each visit to the laboratory, although patients were allowed to continue inhaling steroids as usual. No one was studied within four weeks of an upper respiratory tract infection or exacerbation of their asthma. Subjects gave informed consent and the study was approved by the Southampton University and hospitals ethical committee.

BRONCHIAL PROVOCATION

Airway calibre was measured before and during the provocation as the better of two consecutive FEV₁ measurements by means of a dry wedge spirometer (Vitalograph, Buckinghamshire). On each challenge day histamine acid phosphate (BDH Chemicals, Poole), AMP (Sigma Chemical Co, St Louis, USA), and methacholine (Sigma, Poole, Dorset) were made up in 0.9% sodium chloride to produce a range of doubling concentrations of 0.03–8 mg/ml (0.1–26 mmol/l), 0.04–100 mg/ml (1.11–287.9 mmol/l), and 0.03–16 mg/ml (0.16–81.74 mmol/l). The solutions were administered as aerosols generated from a starting volume of 3 ml in a disposable Inspiron mini nebuliser (CR Bard International, Sunderland) driven by compressed air at 8 l/min.⁻¹ In these conditions the nebuliser generates an aerosol with a mass median particle diameter of 4.7 µm.¹⁴ Subjects inhaled the aerosolised solutions in five breaths from end tidal volume to full inspiratory capacity via a mouthpiece.¹⁵

STUDY DESIGN

The study was divided into four phases.

Table 1 Patients' characteristics

Patient No	Sex	Age (y)	Duration of asthma (y)	Smoking history	Baseline FEV ₁ (% predicted)	Serum IgE (U/ml)*	Eosinophil count (blood) (× 10 ⁹ /l)	Histamine PC ₂₀ (mg/ml)	AMP PC ₂₀ (mg/ml)	Treatment
1	F	51	2	Nil for 15 y	102	40	0.3	0.8	15	S
2	F	62	12	Never	107	24	0.1	1.2	65	T,B
3	F	70	12	Nil for 16 y	97	10	0.2	0.2	5.5	S,Bf
4	F	32	2	Never	83	13	0.2	0.3	1.0	S,Bf
5	F	41	8	Never	74	15	0.3	0.3	3.5	S
6	F	68	42	Never	80	18	0.2	1.8	28.8	S,B
7	M	57	2	Never	87	10	0.6	0.4	0.2	S,B
8	M	46	46	Never	46	23	0.1	0.6	1.0	S,Bf
9	F	78	20	Never	78	5	0.1	0.2	7.2	S,B
Mean (SEM)		56 ± 5	16 ± 5		83.8 ± 6.0	17.6 ± 3.5	0.2 ± 0.1	0.6† (0.2–1.8)	4.5† (0.2–28.8)	

*Normal = <81 U/ml.

†Geometric mean (range).

S—salbutamol; T—terbutaline; B—beclomethasone dipropionate; Bf—beclomethasone dipropionate 250 µg/actuation.

Study 1

Subjects attended the laboratory at the same time of day on two separate occasions, at least 48 hours apart, to undergo concentration-response studies with inhaled histamine and AMP.

On day 1, after 15 minutes' rest, three baseline measurements of FEV₁ were made at three minute intervals. The subjects then inhaled nebulised 0.9% sodium chloride and the FEV₁ was measured at 1 and 3 minutes, the higher value being recorded. Provided that the FEV₁ did not fall by more than 10% of the baseline value, a histamine concentration-response study was carried out. After administration of each histamine concentration FEV₁ was measured at 1 and 3 minutes and the higher value recorded. Increasing concentrations of histamine were inhaled at five minute intervals until the FEV₁ had fallen by over 20% of the postsaline baseline value or the highest concentration had been administered. The percentage decrease in FEV₁ was plotted against the cumulative concentration of histamine administered on a logarithmic scale and the provocation concentration of histamine required to produce a 20% fall in FEV₁ from the postsaline FEV₁ (PC₂₀ histamine) derived by linear interpolation. On day 2 a bronchial provocation test with AMP was undertaken in a similar manner and the PC₂₀ value for AMP obtained.

Study 2

Patients attended the laboratory at the same time of day on four occasions, at least 48 hours apart, to undertake time course studies with inhaled histamine and AMP. These were carried out three hours after they had received oral terfenadine 180 mg or matched placebo, randomised separately for each of the two agonists and administered double blind. On each occasion three baseline measurements of FEV₁ were made at three minute intervals after 15 minutes' rest. Nebulised 0.9% sodium chloride was then administered and repeat FEV₁ measurements were made at 1 and 3 minutes. If the FEV₁ did not fall by more than 10% of the baseline value, the previously determined PC₂₀ histamine or AMP was administered and measurements of FEV₁ were recorded at regular intervals up to 45 minutes after the challenge. On the two occasions when inhaled histamine was given after oral placebo and terfenadine, a concentration-response study was performed with increasing doubling concentrations of histamine acid phosphate administered by skinprick, the doses ranging from 4 to 128 mg/ml (13–416 mmol/l). The total weal circumference at 10 minutes with each concentration of histamine was measured by computer assisted planimetry and integrated to obtain weal area.¹⁶

Study 3

The PC₂₀ AMP was administered three hours after subjects had received a higher dose of terfenadine (600

mg) and the changes in FEV₁ were again followed for 45 minutes as described above.

Study 4

Patients attended the laboratory on two further occasions at the same time of day one week apart, to perform a concentration-response study with methacholine, three hours after they had received oral terfenadine 180 mg or matched placebo, randomised and administered double blind.

DATA ANALYSIS

Values are means with standard errors in parentheses unless otherwise stated and $p < 0.05$ is accepted as significant. Baseline FEV₁ values after treatment with terfenadine were compared with those after placebo by means of Student's *t* test for paired data. FEV₁ at each agonist concentration and time interval was expressed as a percentage of the postsaline value. Since postsaline FEV₁ values after terfenadine were significantly higher than those after placebo, the agonist constrictor response was expressed as a percentage of the postdrug baseline.¹⁷ The slopes of the histamine and AMP concentration-response curves were determined by least squares linear regression analysis and compared by Student's *t* test to determine whether the curves departed significantly from parallel. In the time course studies the following three indices were selected to characterise the percentage fall in FEV₁-time curves: maximum fall in FEV₁, rate of fall in FEV₁ to maximum, and the overall bronchoconstrictor response determined by integrating the area under the curve (AUC) by trapezoid integration. The inhibition of bronchoconstriction achieved by terfenadine was determined by subtracting the area of the FEV₁-time course curve after active treatment from that after placebo, and expressing the result as a percentage of the placebo response. The measurements obtained from the time course study were compared by two factor analysis of variance and the Newman-Keuls procedure. The total skin weal areas with histamine for each concentration on terfenadine and on placebo days were compared by Student's *t* test for paired data. The concentration-response curves with methacholine on the different treatment days, expressed as the PC₂₀ values, were compared by means of Student's *t* test for paired data.

Results

There were no significant differences in baseline or postsaline FEV₁ values on any of the study days.

Study 1

The concentration of inhaled histamine required to produce a 20% fall in FEV₁ from the postsaline baseline (PC₂₀ histamine) ranged from 0.2 to 1.8 mg/ml (0.7–6.0 mmol/l), with a geometric mean of 0.6 mg/ml

Table 2 Slopes of agonist concentration-response curves

Subject No	Histamine		AMP	
	Slope	r	Slope	r
1	-21.6	-0.97	-23.9	-0.92
2	-8.5	-0.81	-8.8	-0.81
3	-17.7	-0.89	-30.1	-0.99
4	-19.3	-0.85	-10.3	-0.97
5	-12.0	-0.93	-5.5	-0.26
6	-19.8	-0.98	-9.6	-0.98
7	-15.7	-0.78	-39.9	-0.74
8	-8.8	-0.72	-8.7	-0.78
9	-28.6	-0.99	-14.3	-0.80
Mean	-16.8	-0.88	-16.5	-0.81
(SEM)	(2.2)	(0.03)	(3.2)	(0.07)

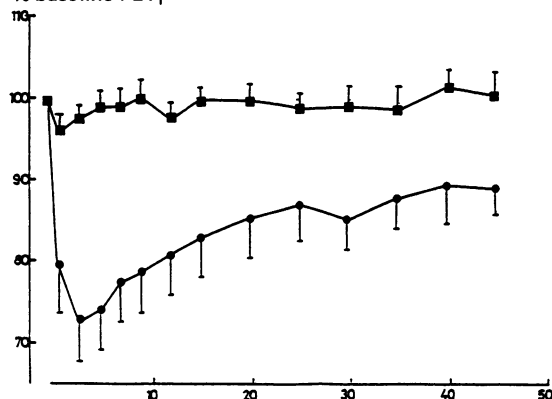
(1.8 mmol/l) table 1. The PC₂₀ AMP ranged from 0.2 to 28.8 mg/ml (0.6–83 mmol/l) with a geometric mean of 4.5 mg/ml (13 mmol/l). There was no significant difference in the slopes of the concentration-response curves with histamine and AMP, mean values being -16.8 (2.2) and -16.5 (3.2) respectively ($p = 0.94$; table 2). Thus, when expressed in molar terms, AMP was 8.4 (0.4–46) times less potent than histamine in causing bronchoconstriction in this group of subjects.

Study 2

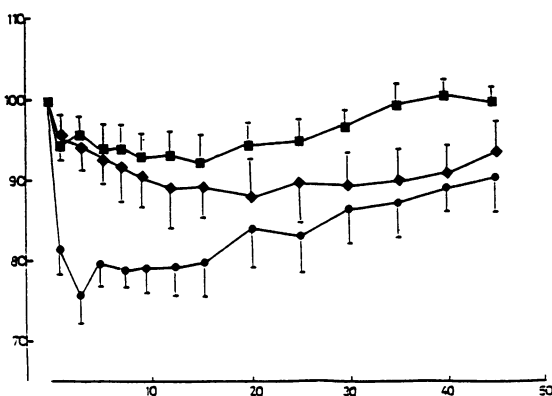
Mean baseline FEV₁ values after administration of terfenadine 180 mg (2.2 and 2.3 l on the two study days) were significantly greater than the values obtained after placebo (2.1 l on both days; $p < 0.02$ —table 3). The mean FEV₁ after terfenadine 600 mg (2.3 l) was not significantly greater than that obtained after terfenadine 180 mg.

Administration of the PC₂₀ histamine after placebo caused a rapid fall in FEV₁ in all subjects reaching a mean maximum of 69.7% (4.4%) of the postsaline FEV₁ at 3.7 (0.3) minutes. The FEV₁ then gradually recovered, although 45 minutes after the challenge it was still significantly below baseline (10.9% (2.9%); $p < 0.01$). After terfenadine 180 mg the FEV₁ response to challenge with the PC₂₀ histamine was

% baseline FEV₁



(a)



(b)

Time (min)

Fig 1 (a) Effect of oral placebo (●) and terfenadine 180 mg (■) on the reduction in FEV₁ after a single dose (PC₂₀) of inhaled histamine. (b) Effect of oral placebo (●), terfenadine 180 mg (■), and terfenadine 600 mg (◆) on the reduction in FEV₁ produced by a single dose (PC₂₀) of inhaled AMP. Each point represents the mean and SEM for nine subjects.

Table 3 Baseline FEV₁ values (l)

Subject No	Histamine study days		AMP study days		
	Placebo	Terfenadine 180 mg	Placebo	Terfenadine 180 mg	Terfenadine 600 mg
1	2.7	2.6	2.6	2.8	2.6
2	2.9	2.7	2.7	2.6	3.0
3	2.2	2.1	2.2	2.3	2.3
4	2.2	2.8	2.3	2.9	2.7
5	2.1	2.8	2.0	2.7	2.9
6	1.0	1.2	1.3	1.2	1.3
7	2.2	2.5	2.4	2.8	3.0
8	1.5	1.4	1.4	1.4	1.3
9	2.0	2.3	2.0	2.2	2.0
Mean	2.1 (0.2)	2.2 (0.2)	2.1 (0.1)	2.3 (0.1)	2.3 (0.2)

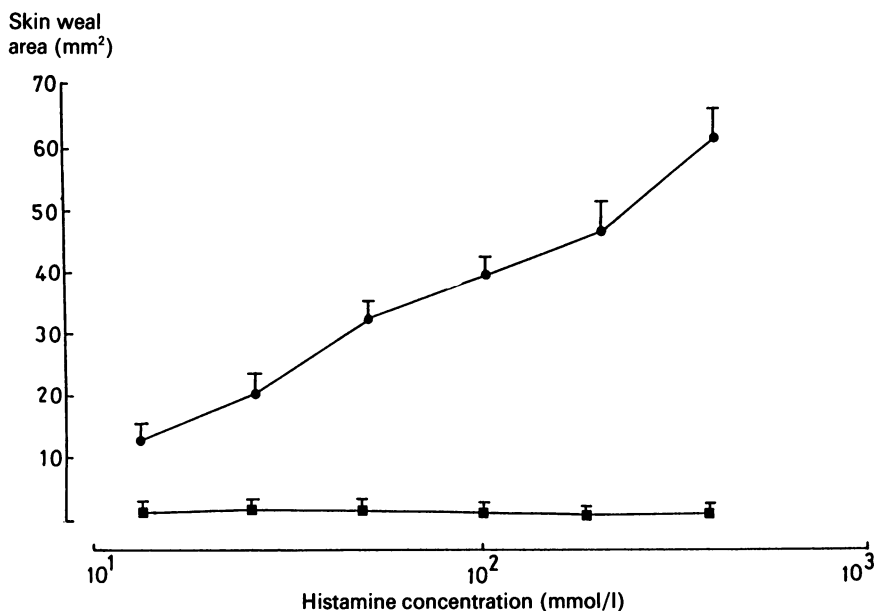


Fig 2 Effect of oral placebo (●) and terfenadine 180 mg (■) on the skin weal response to prick testing with histamine, 4–128 mg/ml (13–416 mmol/l). Each point represents the mean and SEM for nine subjects.

ablated (fig 1a). This dose of terfenadine also inhibited the weal response to prick skin testing with histamine, causing a 97.9% (2.1%) inhibition of the maximum histamine concentration used, 128 mg/ml (416 mmol/l) (fig 2).

The PC₂₀ AMP caused a rapid decrease in FEV₁ after placebo, reaching a mean maximum value of 72.1% (3.2%) of the postsaline baseline value at 6.6 (1.8) minutes. The rate of recovery of FEV₁ was similar to that after histamine. After terfenadine 180 mg, FEV₁ fell to 88.1% (2.5%) of the baseline value at 9.8 (3.7) minutes (fig 1b) and the bronchoconstrictor response to AMP measured as AUC was inhibited by 80.8% (18.0%) by comparison with that after placebo ($p < 0.01$). The AUCs following terfenadine 180 mg for histamine and AMP provocation did not differ significantly.

Study 3

High dose terfenadine (600 mg) inhibited the bronchoconstrictor response to AMP by 60.6% (18.7%) by comparison with the AUC after placebo ($p < 0.01$) and this did not differ significantly from the inhibition achieved by terfenadine 180 mg ($p = 0.5$). At all times after five minutes from challenge, however, terfenadine 600 mg afforded less protection than terfenadine 180 mg (fig 1b). The mean time taken to achieve the maximum fall in FEV₁ with AMP increased from 6.6 (1.8) minutes after placebo to 9.8 (3.7) minutes after terfenadine 180 mg and 16.6 (3.8) minutes after terfenadine 600 mg). The greater bronchoconstriction

observed with the higher dose of the antihistamine was due to five subjects in whom terfenadine 600 mg produced only 27.4% (17.4%) inhibition of the AUC, compared with 102.3% (23%) inhibition in the remaining four subjects.

Study 4

There was no shift in the methacholine concentration-response curves after terfenadine 180 mg ($p = 0.5$). PC₂₀ methacholine values ranged from 0.1 to 2.9 mg/ml (0.3–14.7 mmol/l) after placebo with a geometric mean of 0.4 mg/ml (2.1 mmol/l) and from 0.1–3.0 mg/ml (0.5–15.3 mmol/l) after terfenadine 180 mg, with a geometric mean value of 0.4 mg/ml (2.1 mmol/l) (table 4).

Table 4 Provocation concentrations of methacholine producing a 20% fall in FEV₁ (PC₂₀ from baseline (mg/ml))

Subject No	Placebo	Terfenadine 180 mg
1	1.6	1.1
2	2.1	3.0
3	0.2	0.2
4	0.6	0.5
5	0.4	0.4
6	0.2	0.1
7	2.9	1.7
8	0.2	0.1
9	0.1	0.1
Geometric mean (range)	0.4 (0.1–2.9)	0.4 (0.1–3.0)

Discussion

This study shows that AMP administered by inhalation to patients with intrinsic (non-atopic) asthma causes bronchoconstriction with a time course similar to that observed with inhaled adenosine in subjects with atopic asthma.³ We have further shown that, in intrinsic asthma, bronchoconstriction provoked by AMP is inhibited to a major degree by the histamine H₁ receptor antagonist terfenadine. The inhibitory effect of this selective histamine H₁ receptor antagonist suggests that release of histamine from activated mast cells in the bronchi has a central role in producing the constrictor airway effects of AMP and, by implication, adenosine, as previously suggested in atopic asthma.¹²

By constructing cumulative concentration-response curves for AMP and histamine and showing that these did not depart significantly from parallel, we were able to define the position of the curves as PC₂₀ values and use these to derive an index of relative potency for the two bronchoconstrictor agonists. In the patients studied AMP was 8.4 times less potent than histamine, on a molar basis, in causing bronchoconstriction, compared with a fourfold difference in potency when the same comparison was made between these two agonists in a group of atopic asthmatic subjects.⁶ In a previous study no difference in responsiveness to adenosine between atopic and non-atopic asthmatic subjects was found.¹⁸

In this group of non-atopic asthmatic subjects terfenadine 180 mg produced a significant degree of bronchodilatation, similar to that seen in atopic asthma,¹⁹ but it failed to protect the airways against the bronchoconstrictor effect of methacholine. This suggests that in both forms of the disease the airways are under some degree of histamine tone.

After terfenadine 180 mg the bronchoconstrictor response to inhaled AMP was greatly attenuated. The same dose of terfenadine completely inhibited both the bronchoconstrictor response to a dose of inhaled histamine sufficient to cause a mean maximum fall in FEV₁ to 69.7% of baseline, and the skin weal response to histamine 128 mg/ml (416 mmol/l—figure 2). The specificity of this dose of terfenadine in producing H₁ histamine receptor blockade is supported in these non-atopic subjects by its failure to protect against bronchoconstriction induced by methacholine. These findings are in agreement with those of two previous studies, which showed a 35 fold protection of the airways against the bronchoconstrictor action of inhaled histamine but no protection against methacholine.^{19,20} We propose therefore that the attenuation of AMP provoked bronchoconstriction by terfenadine is due to its action as an antagonist of H₁ histamine receptors and argue for a central role of histamine release in the airways response to this inhaled purine

derivative in individuals with non-atopic asthma. These results are in agreement with those of a previous study, in which terfenadine and chemically unrelated and potent H₁ histamine receptor antagonist, astemizole, inhibited the bronchoconstrictor response to AMP in subjects with atopic asthma;¹² but they would appear to contradict the findings of another study, which showed no significant increase in plasma concentrations of histamine or neutrophil chemotactic factor after AMP challenge.¹⁰ These latter findings may have been due to lack of sensitivity of the histamine assay or to the selection of subjects with such a high degree of non-specific bronchial reactivity that very little histamine release would be needed before bronchoconstriction occurred.

Histamine is the only known preformed spasmogenic mediator present in the secretory granules of human lung mast cells, so the inhibitory effect of histamine H₁ receptor antagonists on the airway response to AMP indicates that this nucleotide (and by implication adenosine) causes bronchoconstriction by potentiating ongoing mediator release from activated mast cells in the bronchial mucosa. In atopic asthma the number of mast cells recovered by bronchoalveolar lavage is increased, and their spontaneous release of histamine is greater than from mast cells recovered from normal lung.²¹ The ability of AMP to provoke an antihistamine sensitive bronchoconstriction in intrinsic asthma suggests that these cells are already activated in the airways—although, as previously discussed, the level of mast cell activation in the two disease forms may differ. Recently Marquardt *et al* have reported that adenosine and related synthetic analogues potentiate degranulation of murine interleukin-3 dependent, bone marrow derived mast cells when stimulated for mediator release with the calcium ionophore A23187 or antigen,¹¹ but do not affect the release of newly generated mediators. Some support for a similar mechanism operating for human lung mast cells is provided by the observation that adenosine and its non-hydrolysable analogues have no effect as secretagogues of lung mast cells per se, but are able to potentiate ongoing IgE dependent histamine release.⁸

In the patients with intrinsic asthma we studied, terfenadine 180 mg inhibited the airways response to inhaled AMP by 80.8%—compared with 86.6% when the same dose of terfenadine was studied in atopic asthmatic subjects.¹² Since terfenadine and its metabolites are competitive antagonists for histamine at its H₁ receptors, it is possible that the reduction in FEV₁ with AMP challenge that remained after treatment with terfenadine 180 mg was due to incomplete antagonism of endogenously released histamine. No further inhibitory activity against inhaled AMP, however, was observed after we increased the dose of

terfenadine to 600 mg, suggesting that the terfenadine resistant response represents a non-histamine component. The mean time to maximum bronchoconstriction with AMP was delayed from 3.7 minutes after placebo to 16.6 minutes after the higher dose of terfenadine, suggesting that inhaled AMP might also enhance the release of newly formed bronchoconstrictor mediators such as prostaglandin D₂ and leukotriene C₄, since their release from activated mast cells is delayed beyond that of histamine.^{22 23}

The higher dose of terfenadine resulted in less inhibition of the bronchoconstrictor response to inhaled AMP (60.6%) than did terfenadine 180 mg (80.8%), although this difference was not significant. It is difficult to account for this observation. Compliance is unlikely to have been a problem since the two subjects with the greatest bronchoconstrictor response after terfenadine 600 mg showed complete inhibition of the skin weal response to prick testing with histamine at a concentration of 416 mmol/l.

In conclusion, the data presented here are consistent with the suggestion that most of the bronchoconstrictor response to inhalation of AMP in non-atopic asthmatic subjects is due to histamine release in the airways. We suggest that adenosine and its nucleotide AMP cause bronchoconstriction in these non-atopic subjects by potentiating ongoing release of preformed mediators from activated airway mast cells. Our data would also be consistent with an additional effect of these purine derivatives, possibly augmentation of the release of newly generated mediators, either from mast cells or from other mediator secreting cells in the airways.

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