

Mast cell mediator release in nonasthmatic subjects after endobronchial adenosine challenge

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Background: Adenosine 5'-monophosphate (AMP) has been shown to cause bronchoconstriction in atopic subjects but to have no effect on nonatopic nonasthmatic subjects. Endobronchial AMP challenge has previously been shown to cause mast cell mediator release in asthmatic subjects, but it is unknown whether a similar response occurs in atopic nonasthmatic and nonatopic nonasthmatic control subjects who have no response to inhalation AMP challenge.

Objective: This study examined the change in mast cell-derived products after endobronchial saline challenge and AMP challenge in subjects with and without a positive inhalation response to AMP.

Methods: Inhalation challenge with AMP challenge was performed in normal, atopic nonasthmatic, and atopic asthmatic subjects. Levels of mast cell mediators were measured after endobronchial adenosine challenge and after placebo endobronchial saline challenge.

Results: There were significant increases in histamine, tryptase, protein, and prostaglandin D₂ levels ($P = .02$, $P = .02$, $P = .01$, and $P = .01$, respectively) after AMP challenge compared with after saline challenge in nonatopic nonasthmatic subjects. There was no significant increase in any mediator in either of the other 2 groups.

Conclusion: This study suggests dissociation between mediator release and bronchoconstriction in response to AMP. (*J Allergy Clin Immunol* 2004;114:34-9.)

Key words: Mast cell, adenosine, bronchoscopy, endobronchial challenge

Adenosine is a naturally occurring purine nucleoside with a ubiquitous presence in human tissues and functions intracellularly as a mediator and extracellularly as an autocrine mediator.¹ Increased levels have been reported in the bronchoalveolar lavage (BAL) fluid of asthmatic subjects compared with that of control subjects, suggesting

Abbreviations used

AMP: Adenosine 5'-monophosphate

BAL: Bronchoalveolar lavage

PGD₂: Prostaglandin D₂

that there is an imbalance in the usual homeostatic mechanisms of purine metabolism in asthma.² Inhalation of adenosine 5'-monophosphate (AMP), which is rapidly dephosphorylated to adenosine in vivo,³ causes bronchoconstriction in asthmatic and atopic nonasthmatic subjects but not in nonatopic nonasthmatic subjects.⁴ The related nucleoside guanosine and the major metabolite inosine have no effect, suggesting that this is a specific receptor-mediated effect.⁵

Adenosine is believed to act indirectly through mast cell mediator release. Treatment with the antihistamines astemizole and terfenadine (H₁ receptor antagonists) and the mast cell stabilizers sodium cromoglycate and nedocromil sodium^{1,6,7} attenuate the in vivo response to inhaled AMP. Pretreatment with ipratropium bromide also attenuates the response to inhaled AMP, suggesting that the vagal system also contributes to the bronchoconstrictive response.⁸ Nasal challenge with adenosine has been shown to cause release of mast cell-derived products in atopic subjects.⁹ A previous endobronchial AMP challenge study demonstrated mast cell mediator release in asthmatic subjects,¹ but it remains unclear whether this effect is specific to asthma or whether it occurs in all subjects and it is the subsequent response to released mast cell products, which differs between subjects.

The aim of this study therefore was to examine the response to endobronchial AMP challenge in normal, atopic nonasthmatic, and asthmatic subjects to determine whether mast cell mediator release was specific to subjects with bronchoconstriction after challenge with inhaled AMP.

METHODS

Subjects

Ethical approval was granted by the Research Ethics Committee of the Queen's University of Belfast. All subjects provided written informed consent. All subjects were nonsmokers and had not

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received any antihistamines, long-acting β_2 -agonists, theophyllines, leukotriene antagonists, or inhaled or oral steroids in the preceding 6 months. Asthmatic patients were recruited if they (1) had a prior clinical diagnosis of asthma and a history of intermittent shortness of breath or wheeze, (2) were atopic (reacted to at least one allergen on skin prick testing), or (3) had an FEV₁ of at least 60% of predicted value. All other subjects had no symptoms suggestive of asthma; atopic nonasthmatic subjects had at least one positive skin prick test response, as above.

All subjects attended on 2 occasions. At the screening visit, clinical assessment, skin prick testing, and AMP inhalation challenge were performed, and informed consent was obtained. At the subsequent visit (at least 72 hours after screening visit), bronchoscopy and endobronchial AMP challenge were performed. On each occasion, medication was withheld for at least 8 hours before the study visit.

Skin prick testing

Skin prick testing was performed by using a standardized puncture technique¹⁰ with allergen preparations of house dust mite, cat and dog protein, and grass pollen (Dome-Hollister-Stier). A positive reaction was taken as a wheal size of 3 mm or larger.

Inhalational challenge

Spirometry was performed according to American Thoracic Society Guidelines¹¹ with a Vitalograph spirometer. AMP (Sigma-Aldrich Ltd) was freshly prepared in 0.9% saline in doubling concentrations ranging from 0.391 to 400 mg/mL. The AMP provocation test was performed by using the 2-minute tidal breathing method of Cockcroft et al¹² with a Medix Turbonebuliser with an output of 0.13 mL/min. PC₂₀ AMP was calculated by means of linear interpolation.

Bronchoscopy

AMP (Sigma-Aldrich Ltd) was freshly made up on the morning of the bronchoscopy in 0.9% saline from a stock solution of 400 mg/mL. At bronchoscopy, subjects were given intravenous midazolam (maximum dose, 14 mg) to achieve mild sedation, and the hypopharynx was anesthetized with lignocaine spray. Vocal cord and tracheal anesthesia was achieved with 4 mL of 4% lignocaine introduced transcricoidally. Oxygen was routinely applied at 2 L/min through nasal cannulae. Heart rate, electrocardiogram, and oxygen saturations were monitored throughout the procedure. The bronchoscope (240 IT Olympus Optical Co Ltd) was introduced orally, and 2-mL aliquots of 2% lignocaine were used as necessary to anesthetize the airways below the carina to suppress coughing. An initial BAL was performed in the lingula (2 × 60 mL of prewarmed isotonic saline). The BAL formed the basis of another study and will not be discussed further here. The subsequent endobronchial challenge protocol was based on that described by Polosa et al,¹ with minor modifications. In brief, the starting dose of AMP for endobronchial challenge was 1/10 of PC₂₀ AMP with quadrupling concentrations up to a maximum of 400 mg/mL. In subjects with no response to adenosine, the starting dose was 40 mg/mL, increasing to a maximum of 400 mg/mL. A maximum of 3 aliquots of AMP was administered in each challenge. Baseline and postchallenge lavage volume was standardized at 20 mL. The analogue response (adapted from Polosa et al¹) to endobronchial AMP challenge was recorded by using the scale shown in Table I. Subjects remained under observation for a period of at least 2 hours after the procedure.

Processing of samples

A total cell count was measured with a modified Neubauer haemocytometer and was expressed as the number of cells × 10⁵ per milliliter of BAL fluid. Cell viability was assessed by means

TABLE I. Analogue score for recording the response seen after instillation of saline (placebo challenge) and AMP (active challenge)

Visual analogue score (grade)	Reaction observed
0	No reaction
1	Subject coughs after instillation of AMP (no coughing after saline challenge)
2	Subject coughs/immediate pallor then hyperemia/increased secretion after instillation of AMP
3	Bronchoconstriction observed after instillation of AMP

of Trypan blue exclusion staining. Viable cells are expressed as a percentage of total cell numbers. Samples were centrifuged at 200g for 10 minutes at 4°C to separate any debris and were then stored at -70°C for subsequent analysis.

Mediator assays

Histamine. Histamine was measured by using a commercially available radioimmunoassay (Immunotech). The limit of detection quoted for this assay is 0.2 nmol/L.

Total protein. Total protein concentrations were measured in BAL samples by using the commercially available BioRad kit (BioRad Laboratories Ltd). The detection limit for the assay is quoted as 2 µg/mL.

Prostaglandin D₂. Prostaglandin D₂ (PGD₂) was measured by using a commercially available ELISA (Cayman Chemicals). The limit of detection for the assay is 80% of the maximum binding and is calculated from the standard curve.

Tryptase. Tryptase was measured by using the UniCAP system (Pharmacia & Upjohn Ltd), which is based on ImmunoCAP technology. The detection limit for this assay is quoted as 1 µg/L.

Statistical methods

Statistical analysis was performed by using SPSS version 11.0 (Statistical Package for Social Sciences). All data were tested for normality of distribution by using the Shapiro-Wilk W test. For nonparametric data, values are quoted as medians and interquartile ranges. For parametric data, values are quoted as means and SDs. Endobronchial response between groups was compared with the χ^2 test. Comparisons between more than 2 groups were performed by using Kruskal-Wallis analysis. Post hoc multiple comparisons were then performed to demonstrate the underlying statistical differences (Stats Direct). Nonparametric correlations were performed by using the Spearman signed-rank test. Subjects who inhaled the maximum dose of 400 mg/mL AMP and whose FEV₁ did not decrease by 20% were given an assigned value for PC₂₀ of 640 mg/mL for subsequent analysis. Mediator levels of less than the level of detection were given a value 0.01 below the quoted level of detection. Statistical significance was accepted at a P value of less than .05.

RESULTS

Twenty-one subjects underwent the full endobronchial challenge protocol (Table II). There were no significant differences between the groups in terms of age and baseline spirometry values. PC₂₀ AMP of asthmatic subjects was significantly less when compared with those

TABLE II. Demographic details of subjects

	Normal subjects	Atopic subjects	Asthmatic subjects
No.	8	6	7
Age (y), median (IQR)	22 (21.0-24.8)	21.5 (18.8-23.0)	26.0 (21.0-30.0)
Male subjects	5	3	2
FEV ₁ % predicted mean (SD)	100.3 (8.1)	99.8 (14.2)	90.0 (14.8)
FVC % predicted mean (SD)	97.6 (10.2)	99.2 (15.4)	95.6 (16.4)
PC ₂₀ AMP (mg/mL), median (IQR)	640	432.0 (144.8-640)	3.6 (2.3-7.9)

IQR, Interquartile range.

of either atopic or healthy subjects ($P < .01$ and $P < .01$, respectively; Table II).

No subject had any visual response to the placebo saline challenge (reaction 0). All subjects had a visual response of greater than 0 to the AMP challenge. The analogue response to AMP in subjects divided by group is shown in Fig 1. The difference in bronchoconstriction observed between healthy and asthmatic subjects was significant ($\chi^2 = 6.23$; $P = .01$).

There was no significant difference in the percentage volume return or total cell numbers between presaline and pre-AMP challenges and between postsaline and post-AMP challenges in any group and no significant differences in percentage volume returns or total cell numbers between groups at any stage (data not shown).

Mediator levels at the various time points are given in Table III. None of the mediator concentrations differed significantly between presaline and pre-AMP values. Histamine, tryptase, protein, and PGD₂ concentrations increased significantly after endobronchial AMP challenge in healthy subjects when postsaline and post-AMP concentrations were compared (Fig 2). The response in asthmatic and atopic nonasthmatic subjects was variable, with demonstrable release in some subjects but not in others but with no overall significant increase in mediator levels (Table III).

When all subjects were analyzed together, there were significant correlations between the change (post-AMP–postsaline levels) in tryptase and histamine concentrations ($r = 0.73$; $P < .01$) and between the change (post-AMP–postsaline levels) in histamine and protein concentrations ($r = 0.63$; $P = .02$). There was no correlation between the change in PGD₂ concentration and changes in any other mediator.

There was a significant inverse correlation between the change in tryptase and histamine concentrations (post-AMP–postsaline) and the PC₂₀ AMP for asthmatic subjects ($r = -0.77$, $P = .04$ and $r = -0.87$, $P = .01$, respectively; data not shown), but no correlation was found between the change in protein or PGD₂ concentrations and the PC₂₀ AMP in this group.

DISCUSSION

The major finding of this study is evidence for mast cell mediator release after endobronchial challenge in normal nonatopic subjects who previously exhibited no response

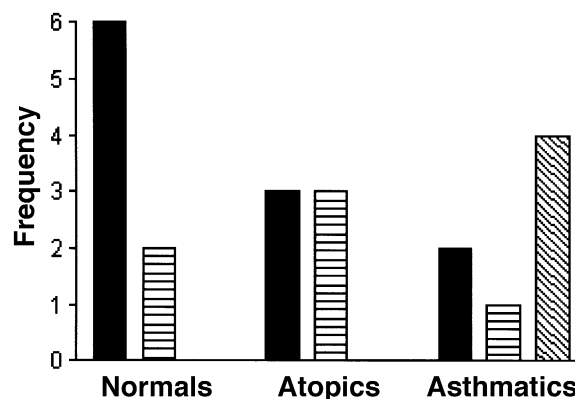


FIG 1. The frequency of the analogue response (grade 1 [solid bars], grade 2 [horizontally striped bars], and grade 3 [diagonally striped bar]) to endobronchial AMP challenge in normal, atopic, and asthmatic subjects.

to inhalation of AMP. Because tryptase is a mast cell–specific protease,¹³ its increase after challenge and correlation with histamine suggests that the cellular source is the mast cell and not other histamine-containing cells, such as the basophil.

Mast cell mediator release in normal subjects occurred at a dose of AMP that did not cause bronchoconstriction when inhaled. AMP has previously been thought to cause mast cell mediator release from immunologically primed cells and to have no action in normal subjects, who can inhale high doses without bronchoconstriction. These results suggest that stimulation with AMP causes mast cell mediator release in healthy nonatopic subjects and suggests dissociation between this response and bronchoconstriction. The concomitant increase in protein levels may reflect either increased permeability of tracheobronchial vessels caused by AMP itself¹⁴ or the action of vasoactive mediators, such as histamine.¹⁵

There are a number of possible explanations for the dissociation of endobronchial mediator release and bronchoconstriction. It may be that it is the release of mediators specifically within airway smooth muscle rather than the airway lumen that causes bronchoconstriction. In resected lung specimens, Ammit et al¹⁶ found that bronchial rings that displayed responsiveness to allergen *ex vivo* contained more mast cells within the smooth muscle than those that did not. In postmortem studies investigators¹⁷ have found increased numbers of mast cells in smooth muscle in those who have died from

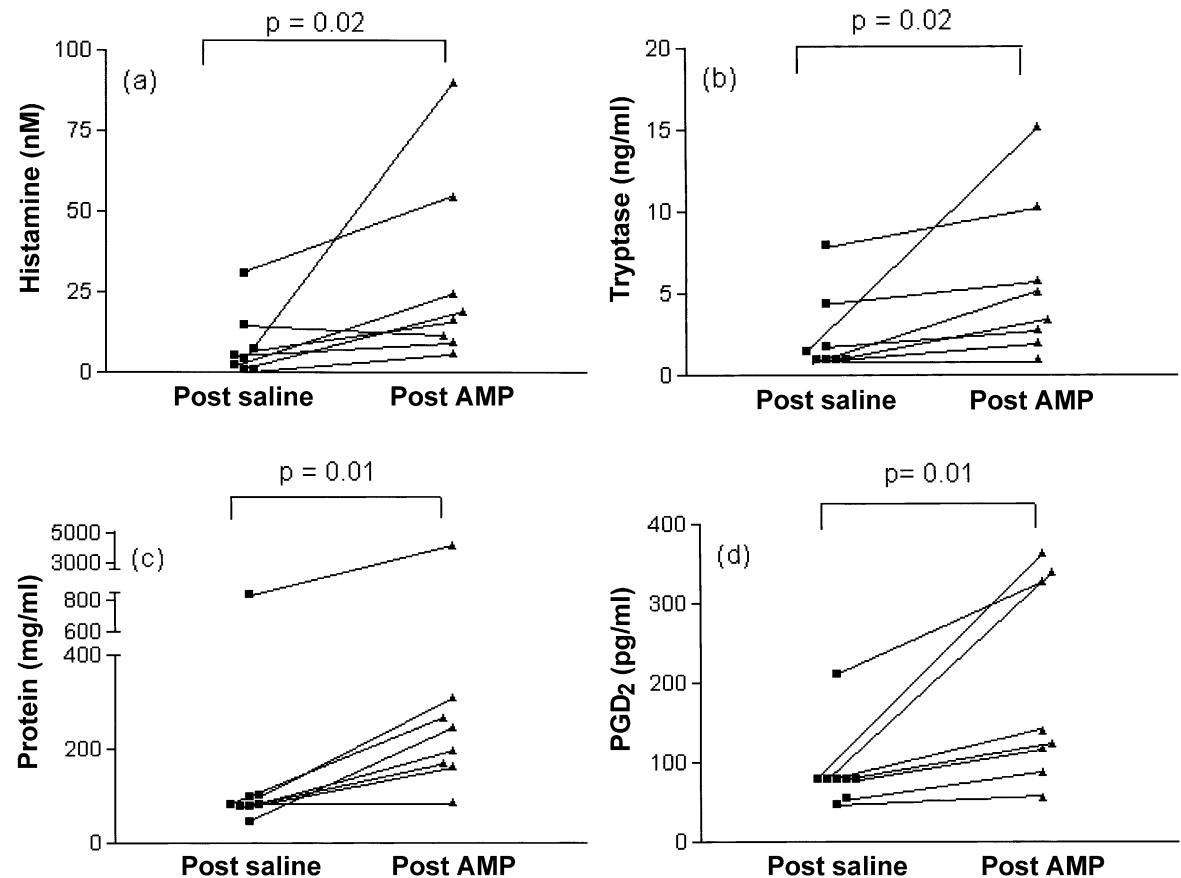


FIG 2. Levels of histamine (a), tryptase (b), protein (c), and PGD₂ (d) in healthy subjects after endobronchial saline challenge and after endobronchial AMP challenge.

TABLE III. Mediator levels in normal, atopic, and asthmatic subjects before and after saline endobronchial challenge and before and after AMP endobronchial challenge

	Presaline			Postsaline			Pre-AMP			Post-AMP		
	N	AT	AS	N	AT	AS	N	AT	AS	N	AT	AS
Histamine (nmol/L)	8.4 (4.8-46.2)	31.4 (1.6-13.2)	44.9 (19.8-87.0)	5.1 (1.6-13.2)	39.0 (15.1-58.0)	44.4 (22.0-74.4)	8.9 (3.2-31.9)	34.5 (13.3-96.7)	24.2 (4.2-62.8)	17.9 (10.1-47.3)	31.5 (14.7-46.5)	38.7 (11.0-90.7)
Tryptase (ng/mL)	1.9 (1.8-7.4)	8.9 (3.7-13.7)	5.1 (1.5-10.6)	1.3 (1.0-3.7)	6.4 (1.6-21.4)	5.0 (1.0-10.0)	1.6 (1.0-26.2)	6.6 (1.8-19.7)	3.0 (1.0-14.2)	4.3 (2.2-9.2)	3.4 (2.4-8.9)	13.7 (1.0-18.9)
Protein (mg/mL)	175.9 (76.-431.2)	264.4 (173.1-516.6)	179.0 (114.1-379.5)	84.2 (79.6-104.4)	112.3 (63.4-232.5)	248.1 (221.8-683.1)	185.8 (89.6-563.7)	166.3 (68.7-536.8)	146.1 (62.5-503.3)	222.4 (166.8-300.1)	151.8 (114.9-403.5)	220.7 (114.8-568.6)
PGD ₂ (pg/mL)	129.6 (81.0-207.5)	134.6 (97.1-209.4)	244.1 (141.6-303.3)	81.0 (62.5-81.0)	141.0 (100.4-198.0)	153.5 (108.9-605.6)	82.4 (81.0-148.5)	128.1 (95.4-244.7)	142.2 (101.4-517.5)	131.9 (95.2-337.0)	105.1 (98.0-791.8)	517.9 (77.6-918.8)

Results are shown as median (interquartile range).
N, Normal control subjects; AT, atopic nonasthmatic subjects; AS, atopic asthmatic subjects.

asthma compared with control specimens obtained from lung resections. Brightling et al¹⁸ recently demonstrated that patients with asthma have more mast cells in bronchial smooth muscle than subjects with eosinophilic bronchitis (who have eosinophilic airway inflammation as seen in asthmatic subjects but who, unlike asthmatic subjects, have no airway hyperresponsiveness). These authors have suggested that the infiltration of mast cells into the smooth

muscle and the subsequent interaction between these cells is crucial in the development of disordered airway function that is pathognomonic of asthma. Subjects with eosinophilic bronchitis have been found to have higher concentrations of histamine and PGD₂ in sputum than asthmatic subjects, supporting the hypothesis that microlocalization of mast cells is related to the development of asthma.¹⁹ Thus it may be the site of mast cell

mediator release, rather than the magnitude of the response, that is more relevant in determining whether bronchoconstriction occurs.

An additional explanation may relate to mast cell functional heterogeneity, which has been previously demonstrated in human mast cells.^{20,21} These functional differences could potentially cause a dissociation between mast cell mediator release and airway response because it is possible that the response of mast cells in the bronchial airways may differ from the response of mast cells in other compartments, notably those in bronchial smooth muscle.

The findings of this study are in contrast to previous work by Polosa et al,⁹ who did not find evidence of mast cell mediator release after nasal AMP challenge in nonatopic subjects. These differences may reflect either mast cell heterogeneity, with BAL mast cells being more responsive to AMP than nasal mast cells, or the substantially lower dose of AMP used in their study (6.5 mg). In our study nonatopic subjects received higher doses of endobronchial AMP than either of the other 2 groups. It is possible that the mast cell mediator release may be a consequence of the high dose of AMP administered. It would be of interest to measure mast cell mediator release after endobronchial challenge with the same intermediate dose of AMP in all groups. This would help to establish whether BAL mast cells from control subjects are less responsive to endobronchial AMP than those of atopic subjects.

No significant change in mediator levels after AMP challenge in both atopic and asthmatic subjects was detected in this study. The number of subjects in each group was small, but given the previously positive study of Polosa et al,¹ who used similar numbers of asthmatic subjects, and the fact that in small numbers of normal subjects a significant change in mediator levels was observed, it seems unlikely that the small numbers alone provide an explanation for this negative finding.

Only one asthmatic subject in this study received all 3 aliquots of doses of endobronchial AMP. In all other subjects, the challenge was terminated early because endobronchial AMP caused coughing that was believed to be distressing to the subject. Polosa et al¹ specifically chose subjects who had a PC₂₀ AMP value of greater than 6.25 mg/mL, whereas subjects in this study were more hyperresponsive (median PC₂₀ AMP, 3.6 mg/mL; interquartile range, 2.3-7.9 mg/mL). Polosa et al¹ attempted 10 bronchoscopies and were able to complete 8. The most hyperresponsive subject they studied had a PC₂₀ of 7.7 mg/mL and was taking only inhaled salbutamol. This challenge was terminated by the investigators because of coughing and respiratory distress after instillation of AMP. From both studies, it would seem that more hyperresponsive subjects are less tolerant of endobronchial AMP challenge and that the most likely explanation for the failure to see significant mast cell mediator release in this study is related to the small doses of endobronchial AMP administered.

In the atopic nonasthmatic group, as anticipated, there was a variable response, with no overall significant

increase in mediator release. This is perhaps unsurprising in view of the variable response to AMP on inhalation challenge.²² One of the aims of this study was to investigate the relationship between airway hyperresponsiveness to AMP and the subsequent release of mast cell mediators. There was no correlation between mediator changes and PC₂₀ AMP in the atopic subjects. This may either reflect the heterogeneity of response displayed within that population or that the airways response to inhaled AMP is dependent on other factors in addition to mast cell mediator release. The negative correlation observed between changes in histamine and tryptase levels and PC₂₀ AMP in the asthmatic population would seem to indicate that more hyperresponsive subjects had a greater release of histamine and tryptase, despite receiving less endobronchial AMP.

In conclusion, this study has shown that normal nonatopic subjects respond to endobronchial AMP with mast cell mediator release in vivo at a dose that fails to cause bronchoconstriction when inhaled, indicating a dissociation between these phenomena.

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