Relationship between airway sensitivity to adenosine 5’ monophosphate and the shape of the concentration–response curve to methacholine in subjects with allergic rhinitis

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The objective of this study was to determine differences in airway sensitivity to adenosine 5’-monophosphate (AMP) between allergic rhinitis subjects with plateau and those without evidence of plateau on the concentration–response curves to methacholine.

A total of 51 adults (38 subjects with allergic rhinitis and 13 healthy controls) were challenged with increasing concentrations of methacholine and AMP. The methacholine challenge was terminated when there was a 40% or more decrease in forced expiratory volume in 1 sec (FEV1), whereas the AMP challenge was stopped when FEV1 had fallen by more than 20%.

A plateau for methacholine was detected in all 13 healthy controls and in 27 patients with allergic rhinitis (AR-plateau group), whereas 11 subjects with allergic rhinitis did not exhibit a plateau (AR-non-plateau group). The median (range) PC20 AMP (provocative concentration required to produce a 20% fall in FEV1) value for the AR-non-plateau group was 440 mg ml⁻¹ (33–400), compared with 400 mg ml⁻¹ (121–400) in the AR-plateau group (P=0.03) and 400 mg ml⁻¹ in the healthy control group (P=0.007). The proportion of subjects who showed bronchoconstriction in response to AMP was higher in the AR-non-plateau group (73%) than in the AR-plateau group (30%) (P=0.03). However, three subjects with allergic rhinitis who had normal sensitivity to methacholine and plateau showed bronchoconstriction in response to AMP.

We conclude that, in subjects with allergic rhinitis, the absence of plateau on the concentration–response curves to methacholine is associated with a higher prevalence and degree of bronchoconstriction in response to AMP. However, the two bronchoconstrictor stimuli were not identifying the same abnormalities of the airways.

Key words: allergic rhinitis; adenosine 5’-monophosphate; methacholine.

Introduction

It is widely appreciated that asthma is an inflammatory disease of the airways associated with airway hyperresponsiveness and variable airflow obstruction (1). Methacholine challenge has been widely used for the detection and quantitation of airway responsiveness (2), and the response to this bronchoconstrictor agent is commonly expressed as the provocative concentration (PC20) or dose (PD20) causing a 20% fall in forced expiratory volume in 1 sec (FEV1). However, airway hyper-responsiveness can be defined as the tendency of the airways to narrow too easily and too much in response to a wide variety of provoking stimuli (3). Airway narrowing that occurs too easily (airway sensitivity) is assessed by measuring the interpolated PC20, but this measure does not assess excessive bronchoconstriction (4,5). Thus, complete description of the dose–response curve requires at least two parameters, one for sensitivity (PC20) and one for maximal response (plateau). A plateau on the concentration–response curve to inhaled methacholine is a feature of healthy subjects, whereas it is very infrequently detected in asthmatics (6,7).

Multiple investigations have shown that increased sensitivity to methacholine is a common feature in non-asthmatic subjects with allergic rhinitis (8–10). Furthermore, when exposed to high concentrations of methacholine, an appreciable number of subjects with allergic rhinitis show a maximal response plateau, but plateau is not detected in a significant proportion (about 35%) of patients (11,12).

Besides methacholine, an important proportion of subjects with allergic rhinitis also have increased sensitivity to inhaled adenosine 5’-monophosphate (AMP) (13,14). Methacholine-induced bronchoconstriction is likely to be
due primarily to a direct effect of the agonist on its receptors on airway smooth muscle. In contrast, the underlying mechanism of bronchoconstriction induced by AMP is mainly indirect, involving mast cell mediator release (15–17). A recent study (14) showed that although methacholine and AMP sensitivity are significantly related, the two bronchoconstrictor agents do not always identify the same individuals with allergic rhinitis, suggesting that sensitivity to the two bronchoconstrictor agents is not reflecting the same abnormalities of the airways.

Polosa et al. (18) have recently demonstrated that, in subjects with allergic rhinitis, airway sensitivity to AMP is more strongly related to airway inflammation than is that to methacholine. Furthermore, we have found that, in non-asthmatic subjects with allergic rhinitis, the absence of plateau on the concentration–response curve to methacholine is associated with increased numbers of eosinophils in induced sputum (unpublished data). In asthmatics, airway inflammation is also related with the level of plateau on the concentration–response curves to methacholine, but not with the sensitivity to this bronchoconstrictor agent (19). In addition, inhaled corticosteroids have been shown to reduce the maximal response to methacholine with little effect on the sensitivity (20). Taken together, these observations confirm the role of inflammatory mechanisms in determining both the maximal response plateau to methacholine and the sensitivity to AMP.

In this study we have tested the hypothesis that bronchoconstriction in response to AMP identifies those subjects with allergic rhinitis who had no plateau. To that end, we investigated differences in airway sensitivity to AMP between allergic rhinitis subjects with plateau and those without evidence of plateau on the concentration–response curves to methacholine.

**Materials and methods**

**SUBJECTS**

The study population comprised 40 subjects with allergic rhinitis and 13 healthy controls. Subjects with allergic rhinitis were recruited consecutively from our outpatient clinic, whereas healthy subjects were recruited from volunteers in our institution and among students. All 53 subjects were life-long non-smokers, and none had history of chronic bronchitis, emphysema or respiratory tract infections during the 4 weeks before the study. The subjects’ baseline FEV1 was more than 80% predicted. Current or ex-smokers, pregnant women and patients with significant renal, hepatic or cardiovascular disease were specifically excluded.

Subjects with allergic rhinitis were defined as those individuals with a characteristic history of perennial or seasonal allergic rhinitis (rhinorrea, sneezing, nasal itch, nasal obstruction) and who also had skin sensitization to perennial or seasonal allergens. No subject had a present or past history of asthma (wheezing, dyspnoea, chest tightness, chronic cough or exercise wheeze).

Healthy subjects had no history of asthma, allergic rhinitis, atopic eczema or other relevant disease, and were receiving no medication. Three were atopic as defined by a skin wheal response > 3 mm to at least one allergen.

The study protocol had been approved by the local ethics committee, and written informed consent was obtained from all participants.

**STUDY DESIGN**

Allergic rhinitis subjects with only seasonal symptoms and skin sensitization to pollen allergens were studied during a period of natural pollen exposure (April-June), whereas those with perennial symptoms were studied during a period of maximal exposure to mites (October-December).

The study was of an open design. On a screening day prior to the study, the inclusion and exclusion criteria were examined and spirometry and skin prick test were performed. On the second visit (2–30 days after initial evaluation), complete methacholine concentration–response curves were obtained, followed after 7–11 days (visit 3) by an AMP inhalation test. Each subject attended the laboratory at the same time of the day during each visit (+2 h) and baseline FEV1 varied by less than 10%.

**SPIROMETRY**

Expiratory flows were measured with a calibrated dry rolling seal spirometer (Model 2130, Sensormedics Co., Yorba Linda CA, U.S.A.) according to standardized guidelines (21). Baseline FEV1 and forced vital capacity (FVC) were measured until three reproducible recordings were obtained. Highest values were used for analyses. Manoeuvres were accepted as technically satisfactory if the variation of the two best FEV1 values was below 5%, if the back-extrapolated volume was lower than 100 ml or 5% FEV1 and if the expiratory time was at least 6 sec. Reference values were those of the European Community for Coal and Steel (22).

**SKIN PRICK TEST**

In subjects with allergic rhinitis, atopic status was measured by skin prick tests using 13 common allergens applied to the forearm. The allergens (ALK-Abelló, Madrid, Spain) tested were house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), household pets (cat and dog), pollens (mixed grass, *Pollenus orientalis*, olive, mixed weed and *Parietaria judaica*) and moulds (alternaria, *Aspergillus fumigatus*, cladosporium and penicillium). Histamine and glycerinated saline were used as positive and negative controls. In healthy subjects, skin-prick testing was performed with the six most common aeroallergens found in the Valencia area (*Dermatophagoides pteronyssinus*, mixed grass, olive, *Parietaria judaica*, and cat and dog dander). After 20 min wheal size was recorded as the long axis and its perpendicular. A skin test was considered positive if the mean wheal diameter was at least 3 mm.
INHALATION CHALLENGE TESTS

Inhalation provocation tests were performed according to a 2-min tidal breathing method (23) with the nose clipped. Subjects were instructed to withhold their treatment for at least 2 weeks (nasal topical corticosteroids and nasal topical cromoglycate) and 3 days (anti-histamines) prior to each challenge. The solutions were administered at room temperature as aerosols generated from a starting volume of 2 ml in Hudson 1720 nebulizers (Temecula, CA, U.S.A.). The mean ± standard deviation (SD) outputs of two nebulizers were determined by weighing each nebulizer before and after 2-min nebulizations on six occasions. These were 0.16 ± 0.03 for the methacholine nebulizer and 0.15 ± 0.02 for the AMP nebulizer.

Methacholine chloride and AMP (Sigma Chemical Co., St. Louis, MO, U.S.A.) were dissolved in normal saline to produce doubling concentrations, range 0–39–200 mg ml⁻¹ for methacholine and 1.56–400 mg ml⁻¹ for AMP, and immediately used for bronchial challenge. The first nebulization administered in each challenge was normal saline, and the post-saline FEV₁ was used as the baseline for the calculation of subsequent percentage fall in FEV₁.

After challenge with saline, doubling concentrations of methacholine chloride or AMP were inhaled. Because of the effect of a deep inspiration on subsequent airway tone (24), only one measurement for FEV₁ was performed 60–90 sec after inhalation of each concentration unless the forced expiratory manoeuvre was judged to be technically unsatisfactory. The methacholine challenge was stopped when there was a 40% or more decrease in FEV₁ when the highest concentration had been inhaled, or if unpleasant side effects or dyspnoea compelled the patient to stop. The AMP challenge was continued until a concentration of 400 mg ml⁻¹ had been given or until a 20% decrease in FEV₁ was achieved. Two inhalations of salbutamol (200 µg) from a metered-dose inhaler were then administered to each subject, and the FEV₁ was measured 15 min later. Further doses of salbutamol were given if necessary until the FEV₁ returned to >90% of the post-saline value.

DATA ANALYSIS

A concentration–response curve was obtained from each challenge by plotting the percentage change in FEV₁ from the post-saline value against the logarithm of the concentration of agonist. The methacholine concentration–response curves were characterized by their sensitivity (PC₂₀) and, if possible, by their maximal response plateau level. Methacholine PC₂₀ was calculated from the log concentration–response curves by linear interpolation of the two adjacent data points. A plateau response was considered to be present if three or more data points for the highest concentrations of methacholine fell within a 5% response range. The level of the maximal FEV₁ response was obtained by averaging the data points on the plateau (25).

The AMP concentration–response curves were characterized by their PC₂₀.

A methacholine PC₂₀ value of 200 mg ml⁻¹ was assigned to 11 patients with allergic rhinitis and to eight healthy subjects in whom FEV₁ dropped less than 20% even when the highest concentration of methacholine was used. Further, a PC₂₀ value for AMP could not be calculated in 22 subjects with allergic rhinitis and 13 healthy subjects. On these occasions the PC₂₀ value was censored to the highest concentration of AMP given (400 mg ml⁻¹).

All summary statistics are expressed as means ± SEM except for PC₂₀ values. To evaluate normality of distributions the Kolmogorov–Smirnov test was used. Because the PC₂₀ values were not normally distributed even after logarithmic transformation, statistical analysis was performed with the non-parametric Kruskal–Wallis test for differences among the groups. When the results of this test indicated significant difference, each pair was examined by means of the Mann-Whitney U-test. The distributions of all other variables were not significantly different from a standard normal distribution; hence, Student's t-test and one-way analysis of variance (ANOVA) followed by a post-hoc t-test for multiple comparisons were applied. Categorical variables were analysed with the Fisher's exact test. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS for Windows, release 6.01; SPSS Inc., Chicago, IL, U.S.A.). Statistical significance was assumed for P<0.05.

Results

BASELINE CHARACTERISTICS

Fifty-one of the 53 subjects completed the study. Two subjects with allergic rhinitis stopped the methacholine challenge prematurely because of dyspnoea and cough. Data of these two patients have not been included in the analysis because no comment can be made regarding the presence or absence of a plateau. A maximal response plateau on the concentration–response curve to methacholine was detected in all 13 healthy controls and in 27 patients with allergic rhinitis (AR-plateau group), whereas 11 subjects with allergic rhinitis showed concentration–response curves without evidence of plateau (AR-non-plateau group).

The anthropometric, clinical and pulmonary function data at baseline for the subjects who completed the study are shown in Table 1. The three groups did not differ with respect to age, sex and baseline pulmonary function. Moreover, both groups with allergic rhinitis were similar with respect to duration of symptoms and prevalence of skin sensitization to perennial or seasonal allergens. As expected, the PC₂₀ methacholine values were significantly lower (P<0.001) in the AR-non-plateau group than in either the AR-plateau group or the healthy control group (Table 1). Although the PC₂₀ values were lower in the AR-plateau group than in the healthy control group, this difference was not significant (P=0.14). Mean baseline FEV₁ values were not significantly different within the three groups before the two different provocation tests.
SENSITIVITY TO AMP IN THE THREE GROUPS

The median (range) PC_{20} AMP value for the AR-non-plateau group (Fig. 1) was 44.0 (3.3–400.0), compared with 400.0 (12.1–400.0) in the AR-plateau group (P=0.03) and 400.0 (1.2–200.0) in the healthy control group (P=0.007). No significant differences were detected between the AR-plateau group and the healthy control group (P=0.13).

The proportion of subjects who showed bronchoconstriction in response to AMP was higher in the AR-non-plateau group (eight out of 11) than in the AR-plateau group (eight out of 27) (P=0.03). Both groups with allergic rhinitis had a greater prevalence of bronchoconstriction in response to AMP than healthy controls (P<0.05). Furthermore, in the AR-plateau group, the level of plateau (Fig. 2) was significantly higher in subjects who showed bronchoconstric-

Table 1. Demographics and functional characteristics

<table>
<thead>
<tr>
<th>Allergic rhinitis</th>
<th>Non-plateau group</th>
<th>Plateau group</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>11</td>
<td>27</td>
<td>13</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>28.3±2.9</td>
<td>31.7±1.8</td>
<td>36.0±3.5</td>
</tr>
<tr>
<td>Male/female</td>
<td>5/6</td>
<td>17/10</td>
<td>5/8</td>
</tr>
<tr>
<td>Duration of symptoms (yrs)</td>
<td>7.1±2.0</td>
<td>8.6±1.5</td>
<td>—</td>
</tr>
<tr>
<td>Perennial/seasonal</td>
<td>5/6</td>
<td>13/14</td>
<td>—</td>
</tr>
<tr>
<td>FEV_1 (% predicted)</td>
<td>105.2±3.8</td>
<td>108.1±1.8</td>
<td>111.8±2.5</td>
</tr>
<tr>
<td>FEV_1/FVC %</td>
<td>84.2±1.5</td>
<td>85.4±1.1</td>
<td>86.6±1.0</td>
</tr>
<tr>
<td>PC_{20} methacholine (mg ml(^{-1}))*</td>
<td>2.5 (0.6–7.1)</td>
<td>70.3 (1.2–200.0)</td>
<td>200.0 (11.6–200.0)</td>
</tr>
<tr>
<td>Prechallenge FEV_1 (l)</td>
<td>3.71±0.24</td>
<td>4.04±0.17</td>
<td>3.84±0.25</td>
</tr>
<tr>
<td>Methacholine</td>
<td>3.74±0.21</td>
<td>4.05±0.18</td>
<td>3.79±0.23</td>
</tr>
<tr>
<td>AMP</td>
<td></td>
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</tbody>
</table>

FEV_1: forced expiratory volume in 1 sec; FVC: forced vital capacity; PC_{20}: provocative concentration of agonist required to produce a 20% fall in FEV_1.

Values are means ± SEM.

*Data presented as median with range in parentheses.
striction in response to AMP than in those unresponsive to this bronchoconstrictor agent (31·0 ± 1·5% vs. 17·5 ± 1·3%, \( P < 0·001 \)).

When we analyzed the individual responses to both bronchoconstrictor agents, it was evident that AMP and methacholine hyper-responsiveness was not always detected in the same individuals with allergic rhinitis. Three subjects of the AR-non-plateau group failed to respond to AMP. In addition, bronchoconstriction in response to AMP was detected in three subjects with allergic rhinitis who had normal sensitivity to methacholine (PC_{20} > 8 mg ml\(^{-1}\)) and a plateau.

**Discussion**

The results of the present study indicate that non-asthmatic subjects with allergic rhinitis without evidence of plateau on the concentration–response curves to methacholine have a higher prevalence and degree of bronchoconstriction in response to inhaled APM than allergic rhinitis subjects with plateau. Furthermore, the results also show that the presence of airway hyper-responsiveness to methacholine (increased sensitivity and absence of plateau) is not necessarily accompanied by bronchoconstriction in response to AMP, and that inhaled AMP causes airway narrowing in a significant proportion of allergic rhinitis subjects without airway hyper-responsiveness to methacholine. These findings confirm that the two bronchoconstrictor agents are not identifying the same abnormalities of the airways in subjects with allergic rhinitis.

Our study shows that bronchoconstriction in response to inhaled AMP is detected in 42% of subjects with allergic rhinitis, and this is in keeping with our previous observations (14). By contrast, Phillips et al. (13) measured airway responsiveness to AMP in 10 atopic non-asthmatic subjects. In this study, seven subjects (70%) had a PC_{20} value \( \leq 400 \text{ mg ml}^{-1} \). There are several possible explanations for this apparent discrepancy. In the study by Phillips et al. (13), the nebulizer output was 0·48 ml min\(^{-1}\) and subjects inhaled the aerosol solutions in five consecutive breaths from end tidal volume to full inspiratory capacity. In contrast, the nebulizer used in the present study had an output of 0·15 ml min\(^{-1}\) and subjects inhaled the aerosol by tidal breathing for 2 min. Furthermore, it is important to emphasize that subjects in the study by Phillips et al. (13) were not selected on the presence of allergic rhinitis, but on the basis of the presence of atopy.

At present, airway responsiveness is usually measured in concentration–response curves as the provocative concentration of histamine or methacholine causing a fall of 20% in FEV\(_1\), which is called the sensitivity (PC_{20}). Thus, the terms airway hyper-responsiveness and hypersensitivity are used interchangeably. However, some studies (5) have focused on the importance of characterizing the entire methacholine concentration–response curve not only by sensitivity, but also by the maximal airway narrowing response value (plateau). In our study, methacholine responsiveness was characterized by the sensitivity and the magnitude of airway narrowing. A maximal response plateau on the concentration–response curve was detected in 27 (71%) of our patients with allergic rhinitis and this is in keeping with previous reports (9,11,12). However, our subjects with allergic rhinitis were tested during a period of maximal natural allergenic exposure and previous studies have demonstrated that natural allergenic exposure causes the loss of plateau in a significant number of subjects with asthma (26) or allergic rhinitis (27). This may explain the high prevalence of allergic rhinitis without evidence of plateau in this study. In addition, airway sensitivity to inhaled AMP increases during periods of natural allergen exposure in asthmatics (28), but the effect of allergen exposure on AMP sensitivity has not been evaluated in subjects with allergic rhinitis.

Although the association between airway inflammation and bronchial hyper-responsiveness in asthma is a widely accepted concept (29), the pathogenesis of bronchial hyper-responsiveness associated with allergic rhinitis is still debated. However, inflammatory changes such as eosinophil accumulation (18,30–32) and enhanced collagen deposition in the lower airways (33) are seen in both allergic rhinitis and asthma, although it occurs to a lesser degree in patient with allergic rhinitis who have no symptoms of asthma. One of our reasons for undertaking this study was the hypothesis that if both the maximal response plateau to methacholine and sensitivity to AMP depend, at least in part, on the presence of inflammatory cells in the lower airways (18,19), bronchoconstriction in response to AMP might be detected in subjects with allergic rhinitis who had no plateau. In the current study, the proportion of subjects who showed bronchoconstriction in response to AMP was significantly higher in allergic rhinitis subjects without plateau than in those with plateau, and subjects with allergic rhinitis who had no plateau were more sensitive to AMP than those who achieved a plateau. Therefore, lack of a plateau was an indication of greater sensitivity to AMP. However, an important proportion of subjects did not conform to this relationship. Eight of our 27 (30%) rhinitics with plateau had bronchoconstriction in response to AMP. In addition, there were subjects (27%) who were insensitive to AMP who had no plateau despite decreases in FEV\(_1\) > 40%. Thus, our hypothesis that bronchoconstriction in response to AMP identifies those subjects with allergic rhinitis who had no plateau was not confirmed. On the contrary, our results support the proposal that, at least in patients with allergic rhinitis, methacholine and AMP responsiveness are not reflecting the same abnormalities of the airways.

We do not believe that our findings can be explained by measurement errors, since the results were obtained after carefully addressing such aspects of methodology as study design and challenge methods. Firstly, there were no significant differences between the baseline airway calibre prior to bronchial challenge on any of the study days. Thus, effects caused by differing baseline airway calibre on the subsequent determination of PC_{20} could be eliminated. In addition, challenges were carried out at the same time of the day, thus ruling out a possible influence of circadian variations on airway responsiveness. Secondly, inhalation challenges were not performed randomly and there is a
slight possibility that the methacholine challenge might have influenced the AMP test. However, this seems to be unlikely, since the study days were separated by 7–11 days. Thirdly, none of the subjects was being treated with medications that could have affected the response to bronchoconstrictor agents. Moreover, by subjecting each volunteer to all provocations, it was possible to make a direct comparison of the effects of each agonist in the same subjects.

In our study, estimation of the airway sensitivity to both bronchoconstrictor agents was complicated because 11 (41%) allergic rhinitis patients with plateau and eight (61%) healthy subjects had PC20 methacholine values above the upper limit of measurement. In addition, 19 (70%) allergic rhinitis subjects with plateau, three (27%) allergic rhinitis patients without plateau and 13 (100%) healthy controls had PC20 AMP values above the upper limit of measurement. In addition, 19 (61%) healthy subjects had PC20 methacholine values beyond a 40% fall from baseline FEV1. The degree of responsiveness was characterized only by the sensitivity, maximal response and position of the concentration–response curve to both bronchoconstrictor agents. This should be addressed in future studies.

Another reason for careful interpretation of the study results is that a proportion of subjects who showed FEV1 falls >40% without evidence of plateau might have plateau beyond a 40% fall from baseline FEV1. The degree of airway narrowing induced in this study was similar to that in other reports (9,34), but it is possible that a greater prevalence of plateaus would have been seen had we allowed FEV1 to decrease by >40%. In addition, AMP responsiveness was characterized only by the sensitivity, and therefore no comment can be made regarding the relationship between the shape of the concentration–response curve to both bronchoconstrictor agents. This should be addressed in future studies.

The present results have clinical implications. It can be assumed that airway hyperresponsiveness in subjects with allergic rhinitis represents a latent phase of asthma that becomes clinically active over the course of time. The presence of airway hypersensitivity to methacholine in ragweed-allergic rhinitis has been shown to carry a substantially increased risk of the development of asthma over subsequent years (8). However, this study could not be confirmed by data from a 4-year follow-up in 66 patients with perennial or seasonal allergic rhinitis (35). Our results indicate that methacholine and AMP responsiveness are not identifying the same individuals with allergic rhinitis, and it is tempting to speculate that in subjects with allergic rhinitis, the presence of bronchoconstriction in response to AMP might be an indication of increased susceptibility to the development of asthma. Further prospective studies are needed to clarify the prognostic value of AMP responsiveness in subjects with allergic rhinitis.

In summary, although subjects with allergic rhinitis who did not exhibit a plateau in their methacholine response are more sensitive to inhaled AMP than those who attain a plateau, inhaled AMP causes airway narrowing in a significant proportion of subjects with allergic rhinitis and normal responsiveness to methacholine. These findings suggest that the two bronchoconstrictor agents are not identifying the same abnormalities of the airways.

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**References**


Sensitivity to Adenosine 5’ Monophosphate and the Concentration–Response Curve


