Bronchial Responsiveness to Adenosine 5′-Monophosphate (AMP) and Methacholine Differ in Their Relationship with Airway Allergy and Baseline FEV_1

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Bronchial hyperresponsiveness (BHR) and inflammation are central hallmarks of asthma. Studies in patients with asthma suggest that BHR to adenosine 5′-monophosphate (AMP) is a better marker of bronchial inflammation than BHR to methacholine. The association between markers of airway inflammation and BHR to methacholine and AMP in a population of young adults, with mild symptoms if any, was evaluated. A total of 230 subjects who participated in a follow-up study on occupational allergy were included. Before exposure to occupational allergens, subjects completed a questionnaire on respiratory symptoms and were tested for atopy, blood eosinophilia ($\geq$ 275/mm^3), and BHR to methacholine and AMP ($\geq$ 15% fall in FEV_1). Risk estimates were expressed as prevalence ratios (PR) and 95% confidence intervals (95% CI). Dose–response slopes (DRS) for methacholine and AMP were compared between healthy control subjects, self-reported allergic rhinitis, and allergic asthma. BHR to AMP was associated with allergic rhinitis (PR 2.51, 95% CI: 1.22;5.17), allergic asthma (PR 4.38, 95% CI: 1.98;9.66), with atopy (PR 3.87, 95% CI: 1.76;8.52), and blood eosinophilia (PR 3.57, 95% CI: 1.48;8.77), but not with baseline FEV_1. BHR to methacholine was inversely related to prechallenge FEV_1 (PR 0.97, 95% CI: 0.96;0.99). For both methacholine and AMP the geometric mean DRS increased along the axis asymptomatic–allergic rhinitis–allergic asthma, but for AMP the increase was the strongest. In this population study among young adults, BHR to AMP refers to allergic background of airway lability and BHR to methacholine is related to a diminished airway caliber.

Keywords: adenosine; bronchial hyperresponsiveness; methacholine; airway allergy

Nonspecific bronchial hyperresponsiveness (BHR) is a central hallmark of asthma. Histamine and methacholine are the most commonly used triggers to mimic BHR in the laboratory. It has now been well-established that an ongoing inflammatory process in the airway wall is one of the most prominent underlying factors determining the expression of BHR. The association between BHR and bronchial inflammation in asthma is supported by the observation that BHR increases with allergen exposure and is reduced by allergen avoidance and antiinflammatory treatment (1–4). However, a clear dose–response relationship with the severity of BHR and sputum inflammatory markers has not been established (5–7).

In the past decade, adenosine 5′-monophosphate (AMP) has been introduced as a bronchoconstrictive stimulus. Whereas histamine and methacholine act by a direct effect on the airway smooth muscle, AMP-induced bronchoconstriction occurs predominantly indirectly by stimulation of adenosine A_{2B} receptors on mast cells that facilitate the release of inflammatory mediators from mast cells (8–10). Results of clinical studies suggest that BHR to AMP reflects allergic airway wall inflammation more accurately than BHR to methacholine. Living at high altitude, as an allergen avoidance measure, improves AMP responsiveness in individuals with asthma, yet not methacholine responsiveness (11). More severe AMP responsiveness has further been shown to be associated with enhanced peak flow variability and higher symptom scores, whereas antiinflammatory treatment reduces BHR to AMP in patients with asthma to a greater extent than BHR to methacholine (3, 11, 12). Furthermore, in contrast to methacholine, AMP responsiveness is associated with indirect parameters of airway inflammation such as exhaled nitric oxide, eosinophils in peripheral blood as well as sputum, and ECP (11, 13, 14).

BHR may also occur in allergic rhinitis, suggesting similar lower airway pathology in allergic rhinitis and asthma (14–16). This may be due to airway wall inflammation, as this has been shown to occur in allergic rhinitis as well, although less intense than in asthma (7, 16). A recent study by Polosa and coworkers has shown that sputum eosinophilia in allergic rhinitis correlates with BHR to AMP but not with BHR to methacholine, although most subjects were hyperresponsive to methacholine as well (14).

Until now, studies on AMP responsiveness have only been conducted in well-defined patients with a proven allergic sensitization. For the first time, we have studied determinants of methacholine and AMP responsiveness in a population of young adults who were mostly asymptomatic. We focused on self-reported airway allergy, including symptoms of the whole respiratory tract, that is, rhinitis, conjunctivitis, and asthma.

METHODS

Subjects

The study population comprised 290 volunteers who participated in a follow-up study on occupational allergy among bakery apprentices (n = 110) and newly applied laboratory animal workers (n = 180). At baseline, before entering their practice year subjects completed a questionnaire on respiratory symptoms, were tested for allergic sensitization to common and occupational allergens, and underwent bronchial challenges to methacholine and AMP. Peripheral blood was withdrawn for eosinophil count. For this study, we included only subjects who completed at least one of the two bronchial challenges. The ultimate study population consisted of 230 subjects. The 60 excluded subjects all

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completed a questionnaire, and did not differ from the remaining study population in symptom prevalence.

The Medical Ethics Committee of the University of Wageningen approved the protocol, and written informed consent was obtained from participating subjects.

Questionnaire

Symptoms of airway allergy were defined as allergic rhinitis/conjunctivitis: "itchy or watery eyes" and/or "sneezing or a runny nose" when exposed to any allergen, and allergic asthma; "asthma" or "chest tightness" when exposed to any allergen. The definitions excluded each other, that is, subjects reporting both symptoms were categorized as having asthma.

Skin Prick Test (SPT)

SPTs were performed on the forearm with five common allergens: grass or birch pollen, house dust mite, and cat or dog fur (ALK Benelux, Houten, The Netherlands). Allergens of the work environment were tested as well: rat, mouse, guinea pig, and rabbit in laboratory animal workers and wheat, rye, and amylase in bakers. A SPT was considered positive if the mean wheel diameter exceeded the negative control with 3 mm.

Eosinophil Count

Eosinophils were counted in a 1:11 dilution using a Bürk counting chamber. Eosinophilia was defined as a count $\geq$ 275 eosinophils/mm^3, as this value is significantly associated with symptoms of airway allergy in a population study (17).

Bronchial Challenge

Bronchial challenges to methacholine and AMP were performed at the same time of day with an interval of 2–14 d. Salbutamol was stopped 8 h before the test and salmeterol and antihistaminics were stopped 48 h before the test. Subjects with a baseline $FEV_1 < 65\%$ of the predicted value were excluded. Challenges were performed using a breath actuated dosimeter (Jaeger GmbH, Germany) driven by compressed air at 20 psi. We used a modification of the standardized protocol, which was described in detail earlier (18). Briefly, inhaled doses were quadrupled from 0.02 to 38.4 mg (90.5 μmol) for AMP and from 0.01 to 2.4 mg (10.0 μmol) for methacholine. FEV_1 was measured 2 min after each dose step by a pneumotachometer (Jaeger GmbH), and the higher of two acceptable measurements was selected to create dose–response curves.

Statistical Analysis

For each challenge we calculated the dose needed to cause a 15% fall in FEV_1 (PD_{15}) by linear interpolation between the last two points. Pearson correlation coefficient was calculated to compare the outcome of the two challenges. Determinants of BHR to either stimulus were assessed by calculation of prevalence ratios (PR) with 95% confidence intervals (95% CI), using PROC PHREG by the statistical package SAS 6.11 (19). To test for independence of AMP and methacholine we subsequently performed analyses with BHR to the alternate stimulus in the model.

The result of the bronchial challenges was also expressed by dose–response slope (DRS), calculated as the maximum percentage fall in FEV_1 divided by the cumulative dose agent in micromoles (20, 21). The distribution of DRS values was skewed, but normalized after log-transformation. Before log-transformation DRS values of 0.001 were added to eliminate DRS values of 0.00. Differences in geometric mean values for DRS among allergic rhinitis/conjunctivitis, allergic asthma, and asymptomatic control subjects were tested using linear regression (PROC REG), as this allows adjustment for potential confounding by age, sex, and smoking. β Values then represent the difference between the log-transformed DRS values, and after involution, the ratio between the geometric mean DRS values under comparison.

RESULTS

Table 1 shows general characteristics of the study population. Subjects reaching a 15% fall in FEV_1 during the first test refused the second challenge more often than subjects without a positive test (16.7% versus 7.6%). Since methacholine challenge was more often the first test (86.4%), nonparticipation to the second test particularly affected AMP challenges. A PD_{15} methacholine was more often determined than a PD_{15} AMP. Twice a positive threshold occurred in 23 of 206 subjects (11.2%), and twice a negative threshold in 130 of 206 (63.1%) subjects. In subjects reaching a 15% fall in FEV_1 in both tests, the geometric mean value for PD_{15} was 1.2 μmol for methacholine and 14.0 μmol for AMP. Thus on a molar basis, AMP was 12 times less potent than methacholine in causing a 15% fall in FEV_1. PD_{15} values of AMP and methacholine correlated significantly with each other, as did the DRS values ($R_{PD15} = 0.52$, $p < 0.05$ and $R_{DRS} = 0.50$, $p < 0.001$, respectively).

Table 2 shows the results of univariate and multiple regression analyses, adjusted for sex, age, and smoking status. BHR to methacholine was associated with a low baseline FEV_1 expressed as percentage of predicted, whereas BHR to AMP was not. The latter was more strongly associated with allergy, indicated by both upper and lower respiratory symptoms, a positive SPT, and eosinophilia. The association between allergic sensitization and BHR to either stimulus increased with the number of positive SPTs, but in each case AMP yielded a higher risk estimate than methacholine.

Current smoking increased the risk of BHR to AMP more strongly than the risk of BHR to methacholine. Excluding subjects with nasal and/or bronchial corticosteroid treatment

TABLE 1. CHARACTERISTICS OF THE STUDY POPULATION (N = 230)

Age, yr	24.5 Range (17.5–48.5)
Female	115 (50.0%)
Baseline FEV_1, %predicted	
First challenge (n = 230)	105.8 Range (68.5–141.1)
Second challenge (n = 206)	104.8 Range (74.7–140.5)
Bronchial challenge test	
PD_{15} methacholine $\leq$ 10.0 μmol (2.4 mg) (n = 229)	77 (33.6%)
PD_{15} AMP $\leq$ 90.5 μmol (38.4 mg) (n = 207)	34 (16.4%)
Allergic rhinitis/conjunctivitis (n = 225)48 (21.4%)	48 (21.4%)
Allergic asthma (n = 225)	23 (10.3%)
$\geq$ 1 positive SPT (n = 221)	84 (38.0%)
Eosinophils > 275/ml (n = 211)	11 (5.2%)
Current smoker (n = 230)	58 (25.2%)
Passive smoker (n = 133)	53 (39.8%)
Nasal or bronchial corticosteroid medication (n = 229)	7 (3.1%)
Occupational allergy (n = 230)	8 (3.5%)

Definition of abbreviations: AMP = adenosine 5′-monophosphate; PD_{15} = provocative dose needed to cause a 15% fall in FEV_1; SPT = skin prick test.

did not change the results, and neither did exclusion of subjects with occupational allergy (work-related airway symptoms plus a positive SPT to work-related allergen).

Including BHR to methacholine in the analysis for BHR to AMP and vice versa did not markedly change the results, nor did defining BHR to methacholine as a $PD_{15} < 7.6$ μmol, 12 times less than the maximum cumulative dose of AMP (90.5 μmol).

Figure 1 shows DRS values for both challenge tests in allergic rhinitis/conjunctivitis, allergic asthma, and asymptomatic control subjects. In this analysis, we included only subjects who completed both challenges to avoid bias by nonparticipation to the second test with respect to the test result of the first one. Both for methacholine and AMP the DRS increased along the symptom axis asymptomatic–rhinitis/conjunctivitis–asthma, but for AMP the increase was the greatest. Compared with asymptomatic control subjects, DRS–methacholine was 2.5 times greater in allergic asthma and 1.5 times greater in allergic rhinitis/conjunctivitis; for DRS–AMP this was, respectively, 4.4 and 2 times. Consequently, the difference in DRS–AMP was greater between the two symptom groups.

DISCUSSION

In this population study among bakery apprentices and newly applied laboratory animal workers before the start of their practice year, a PD_{15} methacholine was associated with a reduced baseline FEV_1 and not with allergic determinants. In contrast, a PD_{15} AMP was associated with symptoms of allergic rhinitis/conjunctivitis and allergic asthma, as well as allergic sensitization and eosinophilia, whereas it was not associated with a low level of lung function.

Clinical studies in subjects with atopic asthma suggest that BHR to AMP reflects bronchial inflammation in asthma more accurately than BHR to methacholine (3, 11–13). The one study that compared methacholine and AMP challenge in allergic rhinitis has also shown that BHR to AMP is a better marker for the bronchial inflammation than methacholine (14). In contrast to these studies, our study population was mostly asymptomatic at the time of the study, even if a history of allergy was present. It may well be that this reflects an ongoing bronchial inflammation despite the relative absence of symptoms, or the presence of only upper airway symptoms. Alternatively, AMP responsiveness may be considered a more sensitive marker than methacholine to pick up mild allergic airway inflammation.

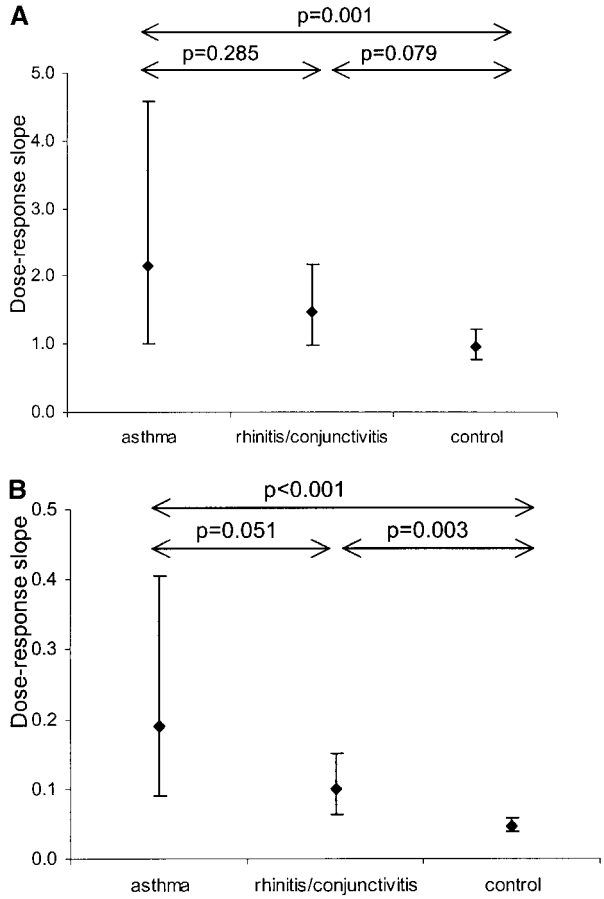

Figure 1. (*A, B*) Differences in dose–response slopes between subjects with symptoms of allergic asthma, allergic rhinitis/conjunctivitis, and control subjects without symptoms.

We did not measure any direct marker of airway inflammation in sputum, bronchial lavage fluid, or exhaled air. Nevertheless, our observation of a positive association between blood eosinophilia and BHR to AMP and not with BHR to methacholine may suggest that AMP responsiveness is associated with allergic airway inflammation. This is supported by a recent observation of Van den Berge and coworkers that AMP respon-

TABLE 2. PREVALENCE RATIOS AND 95% CONFIDENCE INTERVALS FOR BRONCHIAL HYPERRESPONSIVENESS TO METHACHOLINE AND AMP

	PD_{15} Methacholine ⩽ 10.0 μmol					PD_{15} AMP ⩽ 90.5 μmol				
		Univariate Regression		Adjusted for Age, Sex, Smoking			Univariate Regression		Adjusted for Age, Sex, Smoking	
	N	PR	(95% CI)	PR	(95% CI)	N	PR	(95% CI)	PR	(95% CI)
Allergic rhinitis/conjunctivitis	19	1.26	(0.75;2:12)	1.26	(0.74;2.16)	14	2.70	(1.34;5.42)†	2.51	(1.22;5.17)*
Allergic asthma	12	1.68	(0.91;3.12)	1.79	(0.96;3.33)	9	3.54	(1.64;7.65)†	4.38	(1.98;9.66)‡
⩾ 1 positive SPT	34	1.34	(0.85;2.12)	1.36	(0.85;2.16)	23	4.08	(1.89;8.82)†	3.87	(1.76;8.52)‡
Eosinophils ⩾ 275/mm^3	6	1.72	(0.75;3.98)	1.59	(0.69;3.70)	6	3.82	(1.57;9.28)†	3.57	(1.48;8.77)†
FEV_1, % predicted	77	0.97	(0.96;0.99)†	0.97	(0.96;0.99)†	34	0.99	(0.96;1.02)	1.00	(0.97;1.02)
Current smoking	26	1.50	(0.94;2.41)	—		16	2.65	(1.35;5.20)†	—	
Passive smoking	14	0.96	(0.49;1.89)	—		4	0.86	(0.25;2.95)	—	
Female	43	1.28	(0.81;2.00)	—		17	1.03	(0.53;2.02)	—	
Age	77	1.00	(0.96;1.04)	—		34	1.03	(0.98;1.09)	—	

Definition of abbreviations: AMP = adenosine 5′-monophosphate; CI = confidence interval; PD_{15} = provocative dose needed to cause a 15% fall in FEV_1; PR = prevalence ratios; SPT = skin prick test.

* $p < 0.05$.
† $p < 0.01$.
‡ $p < 0.001$.

siveness is associated with an elevated number of eosinophils in sputum as well as peripheral blood (22). One may argue that the presence of occupational allergy in our population might have led to an overrepresentation of active airway inflammation in work-related allergy with continuing exposure. However, exclusion of the eight subjects with occupational allergy did not change the results.

Another point of concern might be the overlap between allergic asthma and nonallergic asthma or chronic obstructive pulmonary disease (COPD), in which AMP responsiveness has also been shown to be increased, and associated with sputum eosinophilia as well (23). However, in our study, the majority of airway obstruction will be due to allergic asthma, as 98% of the subjects were under 40 yr of age. Moreover, our definition of airway allergy was based on symptoms specific to allergens. Taken together, it is less likely that COPD or nonallergic asthma plays an important role in BHR.

In our study, methacholine responsiveness was not clearly associated with symptoms of airway allergy. Like others, we found an inverse relationship between methacholine responsiveness and baseline airway caliber (6). For AMP, we did not find such an association. This could have been the result of selective nonparticipation of subjects with lung function impairment to AMP challenge. We excluded this kind of bias, as the percentage predicted value of baseline FEV_1 prior to methacholine challenge was equal for subjects who did not participate in AMP challenge and subjects who did and were not hyperresponsive to this agent (106.1% versus 104.3%).

Another explanation for the association between BHR to methacholine and baseline FEV_1 refers to chronic airway inflammation and remodeling that are considered to affect airway caliber (6, 13, 24). Our results confirm the notion that methacholine responsiveness may be considered a marker of chronic airway obstruction with subsequent airway remodeling.

Our finding of an increasing association between BHR to AMP and allergic symptoms of upper and lower airways is also supported by the increasing dose–response slope along the symptom axis asymptomatic–allergic rhinitis/conjunctivitis–allergic asthma. Although this increase was present for both methacholine and AMP, it was far more pronounced for the latter. In this analysis, we included only subjects who completed both tests to prevent bias by selective dropout of subjects with a positive threshold to the first test. Our results provide further evidence for the hypothesis that rhinitis and asthma have more in common than only the presence of allergy.

An important issue in comparing bronchial responsiveness to different stimuli is whether they represent different phenomena or just reflect a difference in potency of the agents to cause bronchoconstriction. It is known that AMP is a less potent trigger in causing bronchoconstriction than methacholine. In clinical asthma, AMP has been described 4–6 times less potent (25) and in allergic rhinitis even 20–25 times less potent (8, 14). On a molar base, we found AMP 12 times less potent than methacholine in causing a 15% fall in FEV_1. We measured BHR to AMP at a ninefold increased dose compared with methacholine, and used this to define BHR. Reanalyzing the data with a more stringent definition of BHR to methacholine (12 times less the maximum cumulative dose of AMP) did not change the results. Moreover, AMP and methacholine responsiveness were not strongly correlated with each other, and adjusting for each other in the regression model did not affect the results. These observations strengthen the hypothesis that AMP and methacholine responsiveness represent different phenomena that contribute to allergic airway obstruction.

We conclude that in this population of young adults, AMP and methacholine responsiveness is present in individuals with both upper and lower respiratory symptoms. AMP responsiveness seems to refer to the allergic mechanism of airway obstruction, whereas methacholine responsiveness is more strongly related to a diminished airway caliber. Follow-up studies are needed to determine whether bronchial responsiveness to AMP is a stronger risk factor than methacholine for the new onset or deterioration of allergic airway disease.

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