

# Airway responsiveness to adenosine 5'-monophosphate in smokers and nonsmokers with atopic asthma

Ytske Oosterhoff, MD, Maarten A. M. Jansen, Dirkje S. Postma, MD, PhD,  
and Gerard H. Koëter, MD, PhD Groningen, The Netherlands

Airway hyperresponsiveness is a characteristic feature in subjects with asthma. The severity of airway responsiveness can be assessed with a variety of inhaled stimuli. Inhalation of adenosine 5'-monophosphate (AMP) has been demonstrated to induce airway constriction indirectly via stimulation of purinoceptors on inflammatory cells, notably mast cells, or via neural reflex mechanisms<sup>1, 2</sup>; whereas methacholine is a stimulus that induces airway constriction mainly by direct stimulation of the muscarinic receptors on airway smooth muscle cells. Hypersensitivity to allergens has been shown to determine the degree of AMP airway responsiveness in atopic subjects, because it has been demonstrated that allergen exposure temporarily increases AMP airway responsiveness.<sup>3</sup> Because it has been found as well that the sensitivity to AMP in subjects with nonatopic asthma was increased to the same degree as in those with atopic asthma,<sup>4</sup> it is likely that other factors are involved in determining the degree of airway responsiveness to AMP.

Recently, we found that smoking was associated with increased airway responsiveness to AMP in nonatopic subjects with chronic obstructive pulmonary disease. This suggests that inflammatory changes in the airways elicited by smoking determine the AMP sensitivity in chronic obstructive pulmonary disease.<sup>5</sup> In this study we investigated the additional influence of smoking on airway responsiveness to AMP in atopic asthma.

## METHODS

Sixteen nonsmokers and 12 smokers with atopic asthma and five nonatopic healthy smokers, aged 18 to

### Abbreviations used

AMP: Adenosine 5'-monophosphate  
FEV<sub>1</sub>: Forced expiratory volume in 1 second  
PC<sub>20</sub>: Provocative concentration causing a 20% fall in FEV<sub>1</sub>

45 years, were selected on basis of previously described criteria.<sup>5</sup> The study was approved by the hospital medical ethics committee, and written informed consent was obtained before the start of the study.

From 4 weeks before the start of the study, subjects were allowed to use inhaled bronchodilators only. Treatment with inhaled corticosteroids, cromoglycate, and theophylline was stopped. Inhaled bronchodilators, if used, were withdrawn at least 8 hours before each provocation test.

Subjects attended the laboratory on two visits at the same time of day. On the first day a methacholine provocation test was performed, which was followed, after 2 to 7 days, by an AMP provocation test. Inhalation provocation tests were performed with doubling concentrations of 0.03 to 256 mg/ml (0.15 to 1308 mmol/L) methacholine chloride or 0.04 to 320 mg/ml (0.11 to 913 mmol/L) AMP (Sigma Chemical Co., St. Louis, Mo.) according to a 2-minute tidal breathing method.<sup>5</sup> The challenge was discontinued when forced expiratory volume in 1 second (FEV<sub>1</sub>) had fallen by 20% or more of the prechallenge level or when the highest concentration had been administered. Provocative concentration causing a 20% fall in FEV<sub>1</sub> (PC<sub>20</sub>) values were calculated by linear interpolation between the last two data points of the logarithmic concentration-response curve. If at the highest concentration administered FEV<sub>1</sub> had fallen less than 20% of the prechallenge level, the doubling concentration of the highest concentration was used as the PC<sub>20</sub> value; if FEV<sub>1</sub> had fallen more than 15% at the highest concentration administered, the PC<sub>20</sub> value was calculated by linear extrapolation of the logarithmic concentration-response curve.

PC<sub>20</sub> values were analyzed after base 2 logarithmic transformation. After verification of normal distributions with the Kolmogorov-Smirnov goodness of fit test, differences in parameters between groups were ana-

From the Department of Pulmonology, University Hospital, Groningen, The Netherlands.

Supported by a grant from The Nederlands Astma Fonds (grant no. 89.15).

Reprint requests: D. S. Postma, MD, PhD, Department of Pulmonary Medicine, University Hospital, P.O. Box 30001, 9700 RB Groningen, The Netherlands.

J ALLERGY CLIN IMMUNOL 1993;92:773-6

Copyright © 1993 by Mosby-Year Book, Inc.

0091-6749/93 \$1.00 + .10 1/54/49179

TABLE I. Subject characteristics

|                                | Nonsmokers with asthma | Smokers with asthma | Control subjects |
|--------------------------------|------------------------|---------------------|------------------|
| Number                         | 16                     | 12                  | 5                |
| Age (yr)                       | 28.8 ± 7.1             | 25.0 ± 4.3          | 24.0 ± 3.3       |
| Medication use                 | 16/16                  | 6/12*               | 0                |
| Atopy†                         | 4.7 ± 2.8              | 5.8 ± 2.4           | 0                |
| Pack years                     | NA                     | 5.2 ± 2.4           | 2.3 ± 1.4‡       |
| No. of cigarettes              | 0                      | 15.8 ± 5.8          | 17.6 ± 8.1       |
| FEV <sub>1</sub> (% predicted) | 87.6 ± 3.4             | 90.8 ± 16.2         | 102.9 ± 9.3§     |

Values are expressed as means ± SD.

NA, Not assessed.

\* $p = 0.002$  versus nonsmokers with asthma.

†Number of positive skin tests.

‡ $p = 0.02$  versus smokers with asthma.

§ $p = 0.02$  versus nonsmokers with asthma.

lyzed by using Student's *t* tests for unpaired observations. Nonnormal distributed variables were compared by means of Mann-Whitney U tests. A two-tailed *p* value of less than 0.05 was considered significant. Data are presented as means (±SD). All analyses were performed with the SPSS/PC<sup>+</sup> V4.01 software package (SPSS Inc., Chicago, Ill.).

## RESULTS

Subject characteristics are listed in Table I. Between the smokers and nonsmokers with asthma no significant difference was found in mean values of age, baseline percent predicted FEV<sub>1</sub>, and number of positive skin test results. The only difference was medication use, which was more frequent in the nonsmoking group. All 16 nonsmokers with asthma used inhaled bronchodilators (β<sub>2</sub>-adrenergics or ipratropium bromide), whereas only 6 of the 12 smokers with asthma needed bronchodilator therapy ( $p = 0.002$ ). Besides, 11 of the 16 nonsmokers with asthma used inhaled corticosteroids or cromolyns, in contrast to only 2 of the 12 smokers with asthma ( $p = 0.007$ ).

Percent predicted FEV<sub>1</sub> values were higher in the control subjects as compared with both groups of subjects with asthma, reaching significance with the nonsmokers with asthma ( $p = 0.02$ ). The number of pack years smoked was significantly lower in the control group as compared with the group of smokers with asthma ( $p = 0.045$ ), but the mean daily number of cigarettes smoked was not different.

Mean baseline percent predicted FEV<sub>1</sub> values were not significantly different within the three groups before the two different provocation tests. Geometric mean PC<sub>20</sub> methacholine values did not differ significantly between the two groups of

subjects with asthma (Fig. 1). The geometric mean value in the nonsmoking group was 0.56 mg/ml and in the smoking group, 1.0 mg/ml, representing a difference of 0.8 doubling concentrations. The geometric mean PC<sub>20</sub> value in the smoking control group was significantly higher as compared with both groups of subjects with asthma (122.8 mg/ml;  $p < 0.001$ ). Two subjects in the control group did not respond when the highest concentration of methacholine (256 mg/ml) was administered. The geometric mean PC<sub>20</sub> AMP was significantly higher in smokers with asthma than in nonsmokers with asthma (15.1 and 3.8 mg/ml, respectively,  $p = 0.02$ ) (Fig. 1), representing a difference of 2.0 doubling concentrations. The geometric mean PC<sub>20</sub> AMP value of the control group was significantly higher compared with both groups of subjects with asthma (357 mg/ml;  $p < 0.001$ ). Three subjects in the control group did not respond when the highest concentration of methacholine was administered (320 mg/ml).

## DISCUSSION

This study demonstrates a significantly higher sensitivity to AMP provocation in nonsmokers with asthma as compared with smokers with asthma, whereas the baseline FEV<sub>1</sub>, the sensitivity to methacholine provocation, and the atopic state were comparable between the groups. However, the use of therapy to reduce symptoms was remarkably higher in the group of nonsmokers with asthma as compared with the group of smokers with asthma. This suggests that inflammatory factors related to disease activity rather than smoking determine the PC<sub>20</sub> AMP in asthma.

In contrast to findings in this study in young

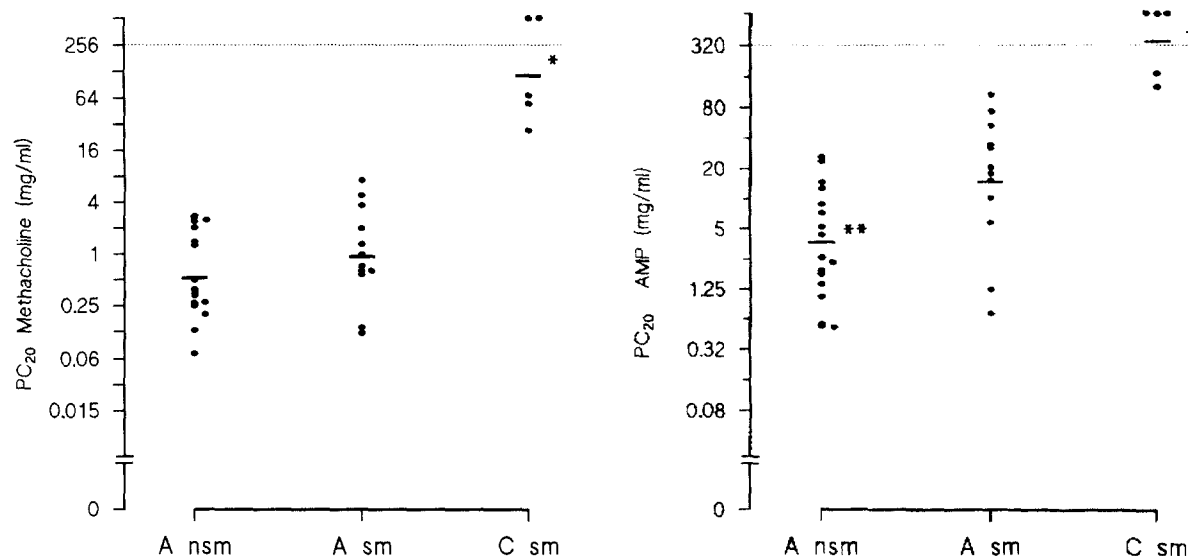


**FIG. 1.** PC<sub>20</sub> methacholine (left) and PC<sub>20</sub> AMP (right) values of nonsmokers with atopic asthma (A nsm), smokers with atopic asthma (A sm), and nonatopic control subjects who smoke (C sm). One unit in PC<sub>20</sub> represents one doubling dilution. Individual values are represented by dots. Mean values, expressed as geometric means, are represented by horizontal bars. The dashed line indicates the highest concentration administered. \* $p < 0.001$  compared with both groups of subjects with asthma; \*\* $p = 0.02$  compared with the group of smokers with asthma.

subjects with asthma, smoking has been found to determine the AMP sensitivity in elderly subjects with increased methacholine or histamine airway responsiveness. AMP airway responsiveness was found to be higher in smokers than in nonsmokers with chronic obstructive pulmonary disease, along with comparable values of baseline FEV<sub>1</sub> and sensitivity to methacholine provocation.<sup>5</sup> Correspondingly, preliminary results from a study by Joyce et al.<sup>6</sup> showed that among subjects with airway hyperresponsiveness to histamine, current smokers were more sensitive to AMP provocation than former smokers. Smoking itself did not induce AMP airway responsiveness, because healthy smokers without airway responsiveness to methacholine were not sensitive to AMP provocation,<sup>5</sup> which confirms findings in this study. Results of this study do not exclude that smoking is of influence on airway responsiveness to AMP in young subjects with atopic asthma; however, other factors seem to contribute to the airway responsiveness to AMP to a larger extent. Conversely, it can be considered that the results of this study show that smoking decreased sensitivity to AMP (e.g. by increased degranulation of mast cells in individuals who smoke).

The habit of smoking itself may have influenced our patient selection and airway responsiveness to methacholine. First, a so-called "healthy smoker

effect" may be present. It has previously been reported that individuals with severe asthma more frequently experience bronchial obstruction on exposure to cigarette smoke.<sup>7</sup> As a consequence, subjects with more severe disease will more often refrain from smoking. This may have influenced the selection of subjects in the different groups in our study. Second, in subjects with allergic rhinitis it has been demonstrated that smoking and atopy have a combined effect to increase airway responsiveness to methacholine.<sup>8</sup> It must therefore be realized that smoking may have increased the degree of methacholine responsiveness in our smokers atopic asthma, resulting in a relatively lower PC<sub>20</sub> value. This may explain why the PC<sub>20</sub> methacholine values in both groups of subjects with asthma are comparable.

The discrepancy in AMP sensitivity between the groups of smokers and nonsmokers with asthma cannot be explained by differences in therapy used, because antiinflammatory therapy had been discontinued for 4 weeks before the study. Antiinflammatory therapy is known to reduce AMP sensitivity to a great extent.<sup>9</sup> However, nonsmokers with asthma used more antiinflammatory therapy as maintenance treatment than smokers with asthma. Thus results would have been opposite if therapy still influenced airway responsiveness 4 weeks after discontinuation.

In conclusion, this study demonstrates that in contrast to findings in elderly subjects with increased airway responsiveness to methacholine, smoking does not increase sensitivity to AMP in young subjects with atopic asthma. The nonsmokers with atopic asthma had significantly lower airway responsiveness to AMP as compared with the smokers with atopic asthma, along with comparable FEV<sub>1</sub>, airway responsiveness to methacholine, and atopy, as reflected in the number of positive skin test results. Because the only difference between the nonsmokers and the smokers with asthma was the use of therapy to reduce symptoms, the results suggest that airway responsiveness to AMP is a more sensitive measurement of disease activity than airway responsiveness to methacholine in subjects with atopic asthma.

## REFERENCES

1. Pauwels R, Joos G, Van der Straeten M. Bronchial hyperresponsiveness is not bronchial hyperresponsiveness is not bronchial asthma. *Clin Allergy* 1988;18:317-21.
2. Ng WH, Polosa R, Church MK. Adenosine bronchoconstriction in asthma: investigations into its possible mechanism of action. *J Clin Pharmacol* 1990;30:89S-98S.
3. Aalbers R, Kauffman HF, Koëter GH, Postma DS, De Vries K, De Monchy JGR. Dissimilarity in methacholine and adenosine 5'-monophosphate responsiveness 3 and 24 h after allergen challenge. *Am Rev Respir Dis* 1991;144:352-7.
4. Phillips GD, Holgate ST. Absence of a late-phase response or increase in histamine responsiveness after bronchial provocation with adenosine 5'-monophosphate in atopic and non-atopic asthma. *Clin Sci* 1988;75:429-36.
5. Oosterhoff Y, de Jong JW, Jansen MAM, Koëter GH, Postma DS. Airway responsiveness to adenosine 5'-monophosphate in chronic obstructive pulmonary disease is determined by smoking. *Am Rev Respir Dis* 1993;147:553-8.
6. Joyce H, Yap JCH, Pride NB. Bronchial responses to inhaled hypertonic saline and adenosine in middle aged smokers and ex-smokers [Abstract]. *Thorax* 1991;646:747P.
7. Stankus RP, Menon PK, Rando RJ, Glindmeyer H, Salvaggio JE, Lehrer SB. Cigarette smoke-sensitive asthma: challenge studies. *J ALLERGY CLIN IMMUNOL* 1988;82:331-8.
8. Buczko GB, Zamel N. Combined effect of cigarette smoking and allergic rhinitis on airway responsiveness to inhaled methacholine. *Am Rev Respir Dis* 1984;129:15-6.
9. O'Connor BJ, Ridge SM, Barnes PJ, Fuller RW. Greater effect of inhaled budesonide on adenosine 5'-monophosphate-induced than on sodium-metabisulfite-induced bronchoconstriction in asthma. *Am Rev Respir Dis* 1992;146:560-4.