

Bronchial hyperresponsiveness in preschool children with allergic rhinitis

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ABSTRACT

Background: Nonasthmatic subjects with allergic rhinitis often have bronchial hyperresponsiveness (BHR), characteristic of asthma. The presence and degree of atopy is suggested to be important for BHR in patients with asthma. We aimed to assess BHR to methacholine (direct stimulus) and to adenosine 5'-monophosphate (AMP; indirect stimulus) in preschool children with allergic rhinitis and to investigate their relationship with the degree of atopy.

Methods: Methacholine and AMP bronchial challenges were performed in preschool children with allergic rhinitis ($n = 96$), using a modified auscultation method. The end point concentration, resulting in audible wheezing and/or oxygen desaturation, was determined for each challenge. The degree of atopy was assessed using serum total IgE levels, the number of positive skin-prick tests, and atopic scores (sum of graded wheal size).

Results: BHR to methacholine (end point concentration, ≤ 8 mg/mL) and to AMP (end point concentration, ≤ 200 mg/mL) was observed in 32 (33.3%) and 26 (27.1%) subjects, respectively. No significant relationship was observed between BHR to methacholine and any atopy parameter. In contrast, the atopic scores were higher in the AMP-BHR⁺ group compared with the AMP-BHR⁻ group, and a significant association was found between the degree of atopic scores and the frequency of BHR to AMP (score for trend, $p = 0.006$). Such a relationship was not observed for serum total IgE levels and the number of positive SPTs.

Conclusion: BHR to methacholine and BHR to AMP were detected in a significant proportion of preschool children with allergic rhinitis. The degree of atopy in terms of atopic scores seems to be an important factor for BHR to AMP but not for BHR to methacholine.

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Although bronchial hyperresponsiveness (BHR) is a characteristic feature of asthma, it can be frequently detected in patients having allergic rhinitis without asthma.¹ Not only patients with perennial allergic rhinitis but also those with seasonal allergic rhinitis show BHR. Even in seasonal allergic rhinitis patients with grass pollen sensitivity, BHR is frequently observed during off-season as well as during the pollen season.² Bronchial challenge with methacholine or histamine, which exerts its effect directly on airway smooth muscle, is the most commonly used and better standardized test for quantifying BHR.³ The reported prevalence of BHR to methacholine in school-age children and adults with allergic rhinitis ranges from 37 to 61%.^{4–6} In contrast, adenosine 5'-monophosphate (AMP) causes bronchoconstriction indirectly through degranulation of primed mast cells and consequent release of proinflammatory mediators.⁷ Several clinical studies have shown that a significant proportion of adult patients with allergic rhinitis also display BHR to AMP.^{8,9}

Allergic rhinitis may develop in the first years of life and may be an early manifestation of an atopic predisposition.¹⁰ This early onset was confirmed in a European study on 1402 children aged 3–5 years, in whom the prevalence of allergic rhinitis was found to be 16.8%.¹¹ Recently, we showed an increased prevalence of BHR to methacholine in preschool children with allergic rhinitis,¹² which appeared to be similar to those observed in school-age children and adults.^{4–6} However, no information is available on BHR to AMP in preschool children with allergic rhinitis.

In epidemiological studies, atopy has constituted a risk for BHR in children and adults.^{13,14} Several studies have suggested that not only the presence but also the degree of atopy is important in BHR to methacholine or AMP in patients with asthma.^{15,16} Previous studies

have reported the relationship between BHR to methacholine and the degree of atopy in patients with allergic rhinitis,^{5,6} but limited information exists on such a relationship in preschool children.¹² On the other hand, the relationship of BHR to AMP with the degree of atopy has not been studied in patients with allergic rhinitis. In this study, we performed methacholine and AMP bronchial challenges in preschool children with allergic rhinitis using a modified auscultation method and analyzed their relationship with the degree of atopy.

METHODS

Nonasthmatic children with allergic rhinitis aged 4–6 years were recruited consecutively from the allergy clinic of Seoul National University Hospital, Seoul, Korea. The diagnosis of allergic rhinitis was made based on the patients' history, rhinologic examination, and skin-prick test (SPT). They had recurrent symptoms of sneezing, rhinorrhea, and nasal stuffiness or itching, apart from the common cold, during the previous year. Pallor or swelling of the nasal mucosa and turbinates was a frequent finding. All patients had atopy defined by a positive reaction to SPT.

To eliminate those subjects with hidden asthma, the medical records were carefully reviewed and subjects were excluded if they showed any history of symptoms suggestive of asthma or when this disease had ever been diagnosed by a doctor. In addition, a questionnaire concerning asthma-related symptoms (wheezing, nocturnal coughing, exercise-induced respiratory symptoms, and persistent cough with colds)¹⁷ was administered. Subjects were included in the study only when their parents responded negatively to all four questions.

SPTs were performed according to the guidelines of the European Academy of Allergology and Clinical Immunology¹⁸ using 13 common aeroallergens: house-dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), animal danders (cat epithelia and dog epithelia), pollens (mugwort, rye grass, ragweed, hazel, alder, and oak), molds (*Aspergillus fumigatus* and *Alternaria alternata*), and cockroach (*Blattella germanica*) (Allergopharma, Reinbeck, Germany). Histamine and isotonic saline were used as positive and negative controls, respectively. All SPTs were performed on the volar sides of both forearms with 26G, sterile, disposable needles. Positive SPTs were defined by a mean wheal diameter ≥ 3 mm larger than the negative

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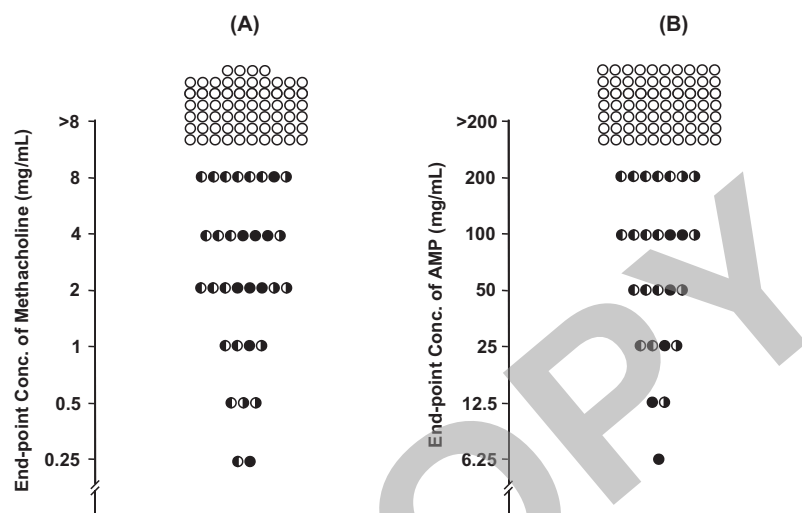
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Figure 1. End point concentrations of (A) methacholine and (B) adenosine 5'-monophosphate (AMP) in subjects with allergic rhinitis. ○, end point concentration attained due to wheeze detection alone; ●, that attained due to oxygen desaturation alone; ●, that attained due to both wheeze detection and oxygen desaturation; ○, no response.



control at 15 minutes after application. In addition to the number of positive SPTs, the wheal size of the positive SPT was also assessed. Wheal diameters were graded so that a wheal of 3.0–4.4 mm scored 1, 4.5–6.7 mm scored 2, 6.8–10.1 mm scored 3, and ≥ 10.2 mm scored 4; all of the graded scores were summed up to give an atopic score for each subject.

Methacholine and AMP challenge tests were performed using a modification of the method described by Springer *et al.*¹⁹ These challenges were performed from December through February, which corresponds to the pollen-free winter season when the levels of house-dust mites have been found to be the lowest and least changing in our country.²⁰ At the time of testing, all subjects had been free of acute respiratory infection for a period of 4 weeks. The methacholine test was designed to be the first test performed and to be followed by the AMP test with an interval of at least 7 days. Intranasal corticosteroids were withheld for at least 2 weeks, and all other medications were withheld for 3 days before each challenge. Fresh solutions of methacholine (0.03–8 mg/mL) and AMP (0.39–200 mg/mL) were made up in a phosphate buffer solution. All children were required to have normal chest auscultation and an arterial oxygen saturation of $\geq 98\%$ at baseline. Chest and tracheal auscultation was performed using a regular pediatric stethoscope by two pediatricians, and oxygen saturation was measured using pulse oximetry (Radical; Masimomet Co., Irvine, CA). The solutions were delivered during quiet tidal breathing *via* a nebulizer (DeVilbiss 644; DeVilbiss, Somerset, PA) driven by compressed air at a flow rate of 4.5 L/min; the unit was calibrated to give a mean output of 0.36 mL/min. Inhalation was performed using a face mask and continued for 2 minutes of tidal breathing, beginning with the buffer solution. This was followed by doubling concentrations of methacholine or AMP every 5 minutes. At the end of each inhalation period, the trachea and both lungs were auscultated repeatedly during quiet breathing for ~ 3 minutes. The two examiners assessed the wheezing independently, which was considered to occur when their findings coincided. Arterial oxygen saturation was monitored continuously during and after each period of inhalation. The procedure was continued until either an end point or the maximal concentration was reached. The end point was defined as the concentration of methacholine or AMP resulting in audible wheezing and/or a fall in oxygen saturation of at least 5% from baseline (desaturation).

Blood samples were collected from all subjects for measurement of total eosinophil counts and total IgE levels. The number of eosinophils was counted using an automated hematology analyzer (Coulter Counter STKS; Beckman Coulter, Fullerton, CA) in a sample of blood containing ethylenediaminetetraacetic acid. Serum total IgE levels

were measured using the Coat-A-Count Total IgE IRMA (Diagnostic Products, Los Angeles, CA).

Parents gave written informed consent for their children to participate in the study. The study protocol was approved by the Institutional Review Board of Seoul National University Hospital.

Statistical Analysis

Data are presented as the mean $\pm$ SD or as geometric means with a range of 1 SD. Subjects were considered to have BHR to methacholine or to AMP when they displayed end point concentrations of methacholine ≤ 8 mg/mL¹⁹ or of AMP ≤ 200 mg/mL.²¹ The variables or frequencies were compared between the two groups using the Student's two-tailed *t*-test or chi-square test. The score test for trend was used to evaluate the potential relationships between the degree of each atopy parameter (serum total IgE, number of positive SPTs, and atopic score) and the frequency of BHR. A value of $p \leq 0.05$ was considered significant.

RESULTS

A total of 111 children with allergic rhinitis were enrolled in this study. Fifteen of these children were subsequently excluded because of oxygen saturation $< 98\%$ before challenges ($n = 5$), failure to undergo methacholine or AMP challenges according to the schedule ($n = 6$), and incomplete data due to other causes ($n = 4$). Complete data were therefore available for 96 subjects.

Regarding the SPT, all patients had a positive response to at least one of the allergens. Among the individual allergens, house-dust mites were the most prevalent (*D. farinae*, 92.7%, and *D. pteronyssinus*, 90.6%). The mean wheal size was greatest for house-dust mites (*D. pteronyssinus*, 7.8 ± 3.5 mm, and *D. farinae*, 6.9 ± 2.4 mm; data on other allergens not shown).

Thirty-two subjects (33.3%) responded to methacholine by one or both of the criteria at a concentration of ≤ 8 mg/mL (Fig. 1 A). Twenty-six subjects (27.1%) responded to AMP by one or both of the criteria at a concentration of ≤ 200 mg/mL (Fig. 1 B). BHR to both methacholine and AMP was detected in 17 subjects (17.7%). Fifteen subjects with BHR to methacholine failed to respond to AMP, and nine subjects with BHR to AMP failed to respond to methacholine.

Subjects were divided into two groups according to the presence of BHR to methacholine (Table 1) and the presence of BHR to AMP (Table 2). The methacholine-BHR⁺ and methacholine-BHR⁻ groups did not differ in terms of clinical characteristics including blood eosinophils or atopy parameters (Table 1). No differences in clinical characteristics between the AMP-BHR⁺ group and the AMP-BHR⁻

Table 1 The clinical characteristics and atopy parameters of the two groups classified according to the presence of bronchial hyperresponsiveness (BHR) to methacholine

	BHR ⁺ (n = 32)	BHR ⁻ (n = 64)
Age (yr)	5.4 ± 0.7	5.5 ± 0.7
Sex (M/F)	20/12	40/24
Blood eosinophils (/μL)	554.4 ± 320.9	402.5 ± 332.5
Baseline O ₂ saturation (98%/99%/100%), n	2/12/18	3/20/41
Atopy parameters		
Serum total IgE (IU/mL)	234.4 (74.1–741.3)	177.8 (70.8–446.7)
Number of positive SPT	3.31 ± 1.93	2.89 ± 1.46
Atopic score	7.38 ± 3.99	6.22 ± 3.50

Mean ± SD or Geometric mean (range of 1 SD).
SPT = skin-prick test.

Table 2 The clinical characteristics and atopy parameters of the two groups classified according to the presence of bronchial hyperresponsiveness (BHR) to adenosine 5'-monophosphate

	BHR ⁺ (n = 26)	BHR ⁻ (n = 70)
Age (yr)	5.4 ± 0.6	5.5 ± 0.7
Sex (M/F)	16/10	46/24
Blood eosinophils (/μL)	625.0 ± 386.5*	389.3 ± 291.5
Baseline O ₂ saturation (98%/99%/100%), n	3/9/14	4/22/44
Atopy parameters		
Serum total IgE (IU/mL)	245.5 (95.5–631.0)	182.0 (66.1–501.2)
Number of positive SPT	3.46 ± 1.82	2.87 ± 1.54
Atopic score	9.04 ± 4.18 [†]	5.70 ± 3.06

Mean ± SD or geometric mean (range of 1 SD).
**p* < 0.05, compared with the BHR⁻ group.
†*p* < 0.05, compared with the BHR⁻ group.
SPT = skin-prick test.

group were evident except for blood eosinophils, which were significantly higher in the AMP-BHR⁺ group. Among the atopy parameters, atopic scores were significantly higher in the AMP-BHR⁺ group than in AMP-BHR⁻ group, whereas serum total IgE levels and the number of positive SPTs were not different between the two groups (Table 2).

To further evaluate the association between the degree of atopy and BHR, subjects were stratified according to each atopy parameter (serum total IgE levels [≤110, 111–280, and >280 IU/mL], the number of positive SPT [1–2, 3–4, and ≥5], and atopic score [1–4, 5–7, and ≥8]) and the frequency of BHR to methacholine (Fig. 2) or BHR to AMP (Fig. 3) was assessed. Score tests for trend showed that BHR to methacholine was not predicted by any of the atopy parameters (serum total IgE, *p* = 0.790; number of positive SPT, *p* = 0.575; atopic score, *p* = 0.393; Fig. 2). In the analysis of BHR to AMP according to the different levels of each atopy parameter, a significant association was found between the atopic scores and BHR to AMP (score test for trend, *p* = 0.006) but not between serum total IgE or the number of positive SPTs and BHR to AMP (*p* = 0.237 and *p* = 0.264, respectively; Fig. 3).

DISCUSSION

Epidemiological evidence consistently shows the frequent coexistence of asthma and allergic rhinitis in patients.²² Therefore, to investigate BHR in patients with allergic rhinitis, excluding the subjects with concomitant or undetected asthma is essential. For this purpose, our patients were carefully screened for symptoms suggestive of

asthma by scrutinizing their medical records. In addition, a questionnaire concerning the presence of asthma-related symptoms was administered.

Bronchial challenges were performed using a modified auscultation method that set the two criteria (wheeze detection and oxygen desaturation) for determining the end point. For both methacholine and AMP challenges, the incidence of wheeze detection at the end point of a positive challenge was higher than that of oxygen desaturation. This is consistent with previous findings^{19,23} and suggests that wheeze detection may be a more sensitive indicator of induced bronchoconstriction than oxygen desaturation. BHR to methacholine was detected in 33.3% (32/96) of our subjects. This frequency is similar to that of our previous work,¹² which investigated a different series of preschool children with allergic rhinitis. On the other hand, 26 subjects (27.1%) exhibited BHR to AMP. This frequency is comparable with those^{8,9} reported in adult patients with allergic rhinitis when differences in challenge methodologies and the criteria for BHR (200 mg/mL versus 400 mg/mL) are considered. The frequencies of BHR to methacholine and BHR to AMP reported here are considered significant because a low proportion of healthy controls were found to have BHR to methacholine (15.6%) and AMP (9.4%) in our previous work using the same challenge method.²⁴

We previously reported that neither the number of SPT responses nor the serum total IgE level was significantly different between the methacholine-BHR⁺ and -BHR⁻ groups among preschool children with allergic rhinitis.¹² We extended these observations in the present study by providing data regarding atopic scores and showed that no differences exist in any quantitative measure of atopy between the two groups. Mete *et al.*⁶ showed that patients who have allergic rhinitis with a higher number of positive SPT responses, particularly those with five or more, are more likely to have BHR to methacholine. Because the vast majority of our subjects had four or less SPT responses, the current study most likely lacked the contrast to show an effect of the number of SPT responses on BHR. Cuttitta *et al.*⁵ observed that BHR was significantly associated with higher total IgE levels in older children with allergic rhinitis. This discrepancy may be explained by the age-dependent relationship between serum total IgE and BHR to methacholine: *i.e.*, a stronger relationship in older children compared with younger children <5 years of age.²⁵ The analyses among stratified groups according to levels of each atopy parameter also revealed no significant association with BHR to methacholine. Our findings suggest that the degree of atopy is not important in the pathogenesis of BHR to methacholine in preschool children with allergic rhinitis.

In contrast to BHR to methacholine, atopic scores were significantly higher in the AMP-BHR⁺ group than in the AMP-BHR⁻ group. Furthermore, a significant association was found between the degree of atopic scores and the frequency of BHR to AMP. Such a relationship was not observed for serum total IgE levels and the number of positive SPTs. The present study is the first one on the association between BHR to AMP and the degree of atopy in preschool children with allergic rhinitis. Recently, we have reported similar findings in school-age children with allergic rhinitis.²⁶ In the latter study, however, BHR to AMP showed a good relationship with total serum IgE as well as with atopic scores. The discrepancy between school-age children and preschool children with regard to the association of BHR to AMP with serum total IgE may be explained by the ambiguous relationship between BHR and serum total IgE in younger children.²⁵

Both the presence and the degree of atopy have been suggested to be critically important in airway responses to AMP, compared with those to methacholine, in patients with asthma. Ludviksdottir *et al.*²⁷ showed that patients with atopic asthma are significantly more responsive to AMP than those with nonatopic asthma, whereas methacholine responsiveness did not differ between these two groups. Fowler and Lipworth¹⁵ found that patients with asthma who were responsive to AMP had a higher mean number of SPT responses than unresponsive patients, whereas this was not the case for methacholine

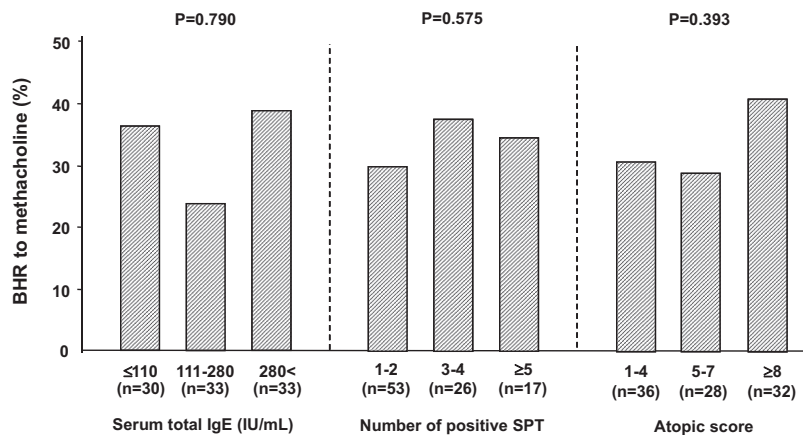


Figure 2. Frequency of bronchial hyperresponsiveness (BHR) to methacholine according to the levels of serum total IgE (left panel), the number of positive skin-prick tests (SPTs) (middle panel), and the atopic score (right panel). The *p* values calculated by test for trend.

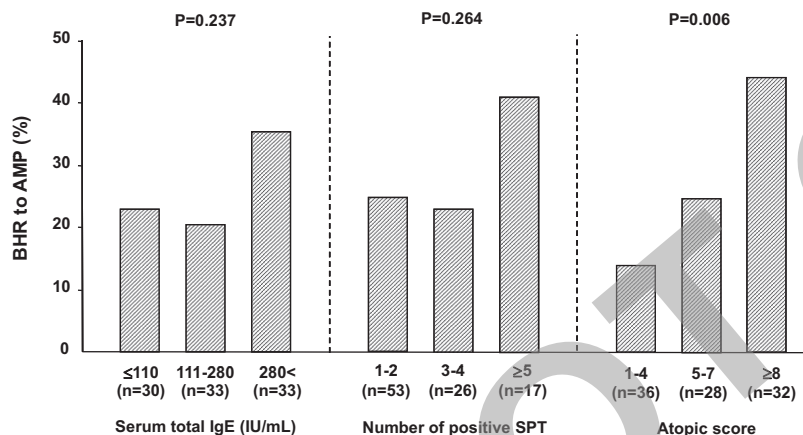


Figure 3. Frequency of bronchial hyperresponsiveness (BHR) to adenosine 5'-monophosphate (AMP) according to the levels of serum total IgE (left panel), the number of positive skin-prick tests (SPTs) (middle panel), and the atopic score (right panel). The *p* values calculated by test for trend.

responsiveness. In preschool children with asthma we also have reported a good relationship between the AMP end point concentration and the degree of atopy reflected in serum total IgE and atopic scores.²⁸ Our results suggest that even in population selected for allergic rhinitis, the degree of atopy may be an important factor for BHR to AMP.

This is the first study to compare the relationship of BHR to methacholine and BHR to AMP with atopy using a modified auscultation method for bronchial challenges in preschool children with allergic rhinitis. The establishment of a relationship between atopic scores and BHR to AMP emphasizes the potential importance of a quantitative approach to atopy in patients with allergic rhinitis. In patients with asthma, BHR to AMP reflects underlying airway inflammation more accurately than BHR to methacholine.²⁹ It was also shown that in nonasthmatic subjects with allergic rhinitis, airway responsiveness to AMP is more strongly related to sputum eosinophilia than that to methacholine.⁸ This is in keeping with our observation of significantly higher blood eosinophils in the AMP-BHR⁺ group than in the AMP-BHR⁻ group. Our results may raise the hypothesis that the degree of atopy may be a marker of airway inflammation in patients with allergic rhinitis.

We acknowledge the limitation of this study. We can not exclude the possibility that the lack of correlation between BHR to methacholine and atopy might be caused by a statistical error. Although it is hard to verify the optimal number of subjects that provide sufficient statistical power, considering that BHR to AMP showed a significant relationship with atopic scores within the same population, the lack of association is not likely because of a type II error. In asthmatic subjects, several factors are known to be associated with severe BHR, *i.e.*, pet ownership at homes,³⁰ exposure to second-hand smoke,^{31,32} a history of atopic dermatitis,³³ and parental asthma.³² These factors

may also act as confounders in subjects with allergic rhinitis but data on the former three variables were not available. As for parental asthma, the proportion of subjects with parental asthma was higher in the methacholine-BHR⁺ group than in methacholine-BHR⁻ group, and it also was higher in the AMP-BHR⁺ group than in AMP-BHR⁻ group. Although we admit that parental asthma might have affected BHR, their confounding effects may be equal to both BHR to methacholine and BHR to AMP.

In summary, not only BHR to methacholine but also BHR to AMP were detected in a significant proportion of preschool children with allergic rhinitis. The degree of atopy in terms of atopic scores seems to be an important factor for BHR to AMP, but not for BHR to methacholine. To the best of our knowledge, this is the first study of BHR to AMP and its relationship with a quantitative measure of atopy in preschool children with allergic rhinitis.

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