

Responses of plasma vasoactive intestinal polypeptide to methacholine and exercise loading in children and adolescents with bronchial asthma

Ohzeki T, Ishitani N, Hanaki K, Motozumi H, Ohtahara H, Fukushima K, Nakai S, Kishida M, Kobayashi S, Shiraki K. Responses of plasma vasoactive intestinal polypeptide to methacholine and exercise loading in children and adolescents with bronchial asthma.

Responses of plasma vasoactive intestinal polypeptide (VIP) to methacholine inhalation and to exercise loading were studied in asthmatic patients to clarify a significant role of the peptide. The mean of basal VIP in asthmatics was not significantly different from the normals. The levels were increased after FEV (1.0) (forced expiratory volume in a second) decreased to 80% of the baseline following methacholine inhalation and were returned to values similar to the baseline when FEV (1.0) recovered to 100%. Thirty minutes after the exercise, mean VIP was significantly lower in patients with the lowest FEV (1.0). These results suggest that VIPergic system contributes, at least partly, to relax constricted bronchioles in some patients with bronchial asthma.

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It is well known that vasoactive intestinal polypeptide (VIP) is found in various tissues including intestine (1,2), brain and peripheral nerves (3). However, the peptide was firstly discovered as a smooth-muscle-relaxant in the lung (4–6) with various effects (7–9). VIP has been reported to mediate non-adrenergic neurons (10,11) and to reduce the bronchial hypersensitivity (12–14).

It has been postulated that bronchial hyperresponsiveness of asthma is due to a loss of bronchial relaxation mediated by VIP (15). Additionally, VIP may possibly cause a change in the pulmonary blood flow (7) or the secretion of mucosa (9). A recent study (15) clarified that contents of VIP in the lung tissue of adults with bronchial asthma were low. VIP has been considered as one of the candidates for a pathogenetic factor and for a therapeutic agent of bronchial asthma.

It is of value to elucidate roles of VIP in asthmatic patients especially in childhood, when the disorder is one of the most frequent and important diseases. However, the significance of VIP in children with asthma has not been completely clarified.

In this study, we measured plasma VIP levels of asthmatic children and adolescents after methacholine inhalation and after exercise loading to evaluate significant roles of the peptide in bronchial asthma.

Subjects and methods

Plasma VIP values before and after methacholine inhalation and exercise loading were determined in patients with bronchial asthma (Table). The

Table 1. Subjects in the study.

		n	age*
[Methacholine inhalation]	boys	12	11.2 (6–17)
	girls	14	11.4 (7–16)
	total	26	11.3 (6–17)
[Exercise loading]	boys	14	11.6 (9–17)
	girls	11	11.5 (7–16)
	total	25	11.6 (7–16)

* Mean ages and the ranges (parentheses) are demonstrated.

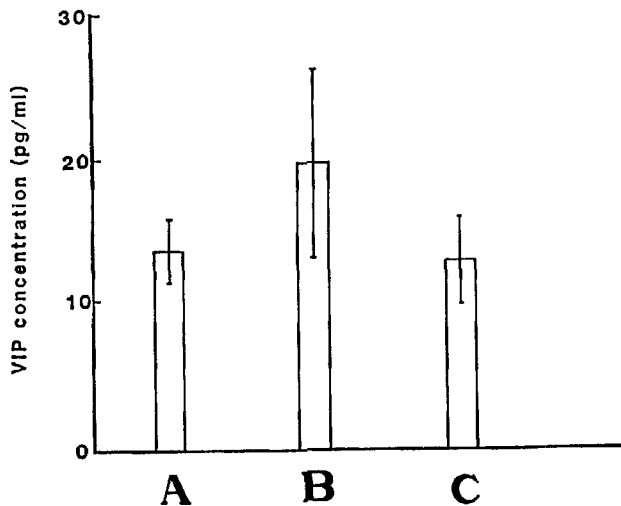


Fig. 1. Plasma VIP levels (mean $\pm$ SE) before methacholine inhalation (A), when values of FEV (1.0) were decreased to 80% of the baseline (B), and at the time of the recovery (C). VIP: 1 pg/ml = 0.303 pmol/L.

examinations were performed during the attack-free periods lasting at least 4 weeks and antiasthmatic agents (methylxanthine derivatives, beta-adrenergic stimulants) had not been administered for at least 2 weeks before the tests.

Methacholine (16) in normal saline containing 10 different concentrations (39/78/156/313/625/1250/2500/5000/10000/20000 μ g/ml) was prepared. DeVilbiss type 646 nebrizer (DeVilbiss Co., Somerset, PA, USA) was used to inhale at the speed of 5 L/min for 2 min. After inhalation of normal saline, methacholine of the lowest concentration (39 μ g/ml) was administered. Forced expiratory volume in 1 second (FEV 1.0) was measured before and after the inhalation using Autospiror DISCOM 12 (CHEST Co., Tokyo, Japan). The concentration was increased until FEV (1.0) was decreased below 80% of the baseline value. Plasma VIP levels were determined before the inhalation, at the time when FEV (1.0) was decreased, and after it returned to the baseline level.

Exercise loading (17) was performed using a treadmill (Nihon Kodan Co., Tokyo, Japan) at the speed of 6 km/h, an inclination of 10%, for 6 min. The patients were divided into 3 groups according to the maximum fall of FEV (1.0) after the exercise. In group 1, the fall of FEV (1.0) was less than 15%. The falls of FEV (1.0) in Groups 2 and 3 were 15–30% and more than 30%, respectively. Plasma samples for determination of VIP were obtained at 3 occasions (at the end of the exercise loading, 5 min and 30 min after the first sampling).

Venous blood was obtained with venipuncture at the median-cephalic or median basic veins. The samples of one milliliter were collected into ice-cooled tubes containing heparin (50 KIU/tube,

Novo Co., Denmark) and aprotinin (500 KIU/tube, Sigma Co., St. Louis, MO, USA) and centrifuged immediately at 3500 rpm for 15 min at 4°C. The plasma samples were stored at -80°C until assay.

Iodination of porcine VIP (Peninsula Lab., Belmont, CA, USA) was performed following the modified technique of Hunter & Greenwood (18) using chloramine-T described elsewhere (19). Antiserum against VIP (Peninsula Lab., Belmont, CA, USA) was used for radioimmunoassay of the peptide and separation of bound and free was performed with dextran-coated charcoal. Intra- and interassay variations were 7.0% and 13.0%, respectively.

Statistical analysis was performed using STAX (STAX, Tokyo, Japan) statistical software and NEC PC-9801 (NEC, Tokyo Japan). Results are shown as the mean $\pm$ SE. Data were assessed by the unpaired Wilcoxon test. $P < 0.05$ was considered significant.

Consent was obtained from the patients and, at least, one of the parents.

Results

Mean of the basal levels of the asthmatic patients ($n = 26$) before the examination (13.9 ± 2.3 pg/ml) were not significantly different from the normal children (mean $\pm$ SE: 14.5 ± 3.9 pg/ml).

When FEV (1.0) decreased to the 80% of the baseline after methacholine inhalation, plasma VIP was elevated to 19.5 ± 6.6 pg/ml though no

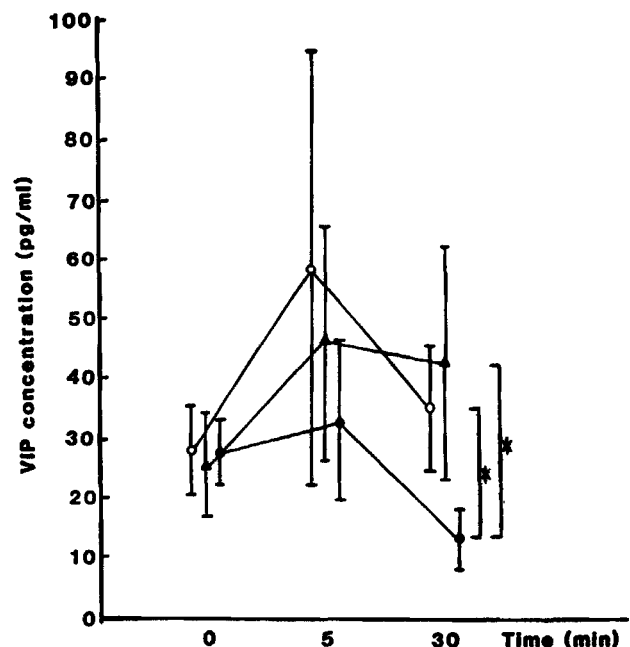


Fig. 2. Plasma VIP levels (mean $\pm$ SE) in the group 1 (○), 2 (▲) and 3 (●) after the exercise loading showing a significant (*: $p < 0.05$) decrease of VIP in the group 3.

statistical significance was demonstrated. After FEV (1.0) returned to the basal level, the mean was lowered (12.9 ± 3.0 pg/ml), which was similar to the value before the examination (Fig. 1).

At the end of the exercise loading, plasma levels of the peptide in the 3 groups were in the similar range. The plasma levels were elevated in all groups five minutes after the end of the exercise. The mean value was the highest in Group 1 (58.4 ± 36.3 pg/ml) and the values were 46.2 ± 19.8 pg/ml and 33.0 ± 14.5 pg/ml in Groups 2 and 3, respectively. The mean of VIP levels in Group 1 (43.0 ± 19.5 pg/ml) and in Group 2 (35.2 ± 10.2 pg/ml) remained in higher ranges 30 min after. However, the mean of Group 3 (13.5 ± 5.0 pg/ml) was significantly ($p < 0.05$) lower compared with Groups 1 and 2 (Fig. 2).

Discussion

Recently, Ollerenshaw et al. (15) clearly documented that VIP contents in the lung tissues of asthmatic patients were markedly decreased and suggested that the lowered VIP might contribute, at least partly, to the onset of asthma. However, they have not completely elucidated that the lower content of VIP in patients with asthma was the cause of the disease or the result (19). Our present study was designed to demonstrate more clearly the role of VIP in asthmatics especially in childhood and adolescence.

Plasma concentrations of VIP in the asthmatic children were elevated after methacholine inhalation and subsequent decrease of FEV (1.0) (16). The plasma levels returned to the baseline after the constriction was relieved. The results may indicate the possibility that, in the attack of asthma, plasma VIP levels rose probably due to consequence of increased VIPergic activity to dilate the constricted airways. However, this provocation test with methacholine made constriction of airway to the same degree (20% fall of FEV 1.0) and could not differentiate the severity of hyperresponsiveness in each patient.

The exercise loading test (17) was performed for the purpose to classify the patients into groups according to responses of their respiratory tract. After the exercise was ended, plasma VIP levels exhibited no difference between the 3 groups probably because bronchial constriction did not yet occur at this time. After 30 minutes, the levels of VIP were significantly ($p < 0.05$) decreased in patients whose mean fall of FEV (1.0) was larger (Group 3) than the other two groups (Groups 1 and 2). These results are consistent with contribution of a relaxant effect of VIP in the attack of asthma (15). The patients in Group 3 were considered to be

with the more severe respiratory derangement due to the decrease in VIPergic responsiveness as compared to the other 2 groups.

It has been demonstrated that VIP has an antagonistic effect to histamine induced bronchial constriction (12–14). In addition, the peptide has been known to have effects on the artery (7), gland secretion (8), and ion transport (8) in the respiratory tract. It was reported that the dominant neurogenic component of airway relaxation might not be adrenergic nor cholinergic, but probably VIPergic (6, 10, 19). The non-adrenergic system, such as VIPergic neurons, antagonize the bronchoconstriction.

The peptide was probably secreted from the lung in these patients (15) although possibility that the increased VIP was derived from organs other than the respiratory tract was not completely excluded.

The results in the study demonstrate that plasma VIP levels were elevated in response to decrease of FEV (1.0) after methacholine inhalation and exercise in asthmatics and that VIP was markedly low in patients with the largest fall of the respiratory function after exercise. It is suggested that VIP may contribute, at least partly, to relax the respiratory tract constriction in some patients with bronchial asthma.

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