

# Roles of eosinophils and catecholamines in pathophysiology of exercise-induced asthma

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To study the roles of eosinophils and catecholamines in the pathophysiology of exercise-induced asthma (EIA), an exercise challenge test was performed in 22 asthmatic children, using a bicycle ergometer, and the changes in lung function, plasma norepinephrine (NE), epinephrine (E) and eosinophil cationic protein (ECP) levels with exercise were evaluated. Subjects were classified into three groups from the results of lung function test, respectively: EIA-negative group, EIA-positive groups with good recovery and poor recovery. Eosinophil counts before exercise in the EIA-positive groups were significantly higher than those in the EIA-negative group. The ECP levels rose with exercise in all groups. Five minutes after exercise, ECP values fell to the pre-exercise level in the EIA-negative group, but rose to higher levels in EIA-positive groups. Plasma E levels rose with exercise and showed 4-fold increase in the EIA-negative group, whereas only 1.5-fold increase of plasma E levels was observed in the EIA-positive group with poor recovery. From these findings, a possible contribution of sympathoadrenal hypofunction and a probable involvement of eosinophils in the pathophysiology of EIA have been suggested.

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Exercise-induced asthma (EIA) has been documented in patients with bronchial asthma in up to 50% (1). The mechanism remains uncertain. Several investigators have reported blunted rise of norepinephrine (NE) and epinephrine (E) levels in the peripheral blood of EIA patients with exercise (2–4). It has also been reported that  $\beta$ -adrenoceptor agonists are potent stabilizers of human mast cells (5), and that E inhibits IgE-induced histamine release from human lung fragments (6). The impaired initial mobilization of E from the adrenal medulla may thus be important in the pathogenesis of EIA (7).

With the concept of airway inflammation, the role of eosinophils in asthma has been a focus of attention in these ten years. Increases of eosinophil numbers and eosinophil degranulation products, such as eosinophil cationic protein (ECP), major basic protein (MBP) and eosinophil protein X (EPX) in peripheral blood as well as in the bronchial alveolar lavage (BAL) fluids in patients with a late asthmatic response (LAR) have been reported (8–12). Venge et al. have found rises of

both eosinophil count and ECP levels during exercise in blood of asthmatic patients (13). These studies, however, have been performed in adult asthmatic patients and the role of eosinophils in EIA in asthmatic children has not been studied.

We therefore performed exercise challenge tests on 22 asthmatic children, and investigated the roles of eosinophils and the relationship between the activation of eosinophils and catecholamines in the pathogenesis of EIA.

## Material and methods

### Subjects

Twenty-two asthmatic children, 9 to 15 years of age (14 boys and 8 girls), were studied. They had been suffering from asthma for 6 to 12 years. All of them had allergies for house dusts and mites, confirmed by positive skin prick tests or high RAST score for these allergens, and had histories of EIA more than once prior to this study. Blood IgE levels and some other additional values are shown in Tab. 1. Medications were prohibited for 24 h

Table 1. Various pre-exercise parameters in the three groups. Eos: eosinophils; ECP: eosinophil cationic protein; E: epinephrine; NE: norepinephrine. All values are expressed as means  $\pm$  1 SD. In these parameters, a statistical significance was observed only in the numbers of eosinophils by one-way analysis of variance and two-tailed Student's *t*-test. A single asterisk denotes  $p < 0.05$ , double asterisks,  $p < 0.01$  in comparison with EIA-negative group.

|                     | EIA-positive    |                 | EIA-negative   |
|---------------------|-----------------|-----------------|----------------|
|                     | Poor-recovery   | Good-recovery   |                |
| Number              | 7               | 5               | 7              |
| Age (yr)            | 11.7 $\pm$ 1.8  | 11.6 $\pm$ 2.4  | 11.1 $\pm$ 2.0 |
| Sex                 | M4, F3          | M4, F1          | M4, F3         |
| IgE (U/ml)          | 674 $\pm$ 670   | 526 $\pm$ 359   | 392 $\pm$ 291  |
| WBC/mm <sup>3</sup> | 5400 $\pm$ 520  | 4940 $\pm$ 1000 | 5730 $\pm$ 710 |
| Eos/mm <sup>3</sup> | 815 $\pm$ 423** | 560 $\pm$ 311*  | 331 $\pm$ 56   |
| ECP (ng/ml)         | 3.1 $\pm$ 1.2   | 3.9 $\pm$ 1.0   | 3.1 $\pm$ 1.2  |
| E (pg/ml)           | 50.3 $\pm$ 10.9 | 45.7 $\pm$ 8.6  | 50.5 $\pm$ 9.9 |
| NE (pg/ml)          | 599 $\pm$ 289   | 436 $\pm$ 178   | 480 $\pm$ 217  |

before the studies with the exception of two patients who had taken theophylline on the morning of the study. None of the subjects had received corticosteroids for at least one month prior to this study. All children and/or their parents gave informed consent.

#### Exercise provocation test

Exercise challenge tests were done according to the guidelines of the American Academy of Allergy (14). A bicycle ergometer was used with a loading of 13–15 kpm/kg for 6 min. After exercise, the heart rates of these subjects were over 180/min except for one boy, whose heart rate was 140/min at maximum. The record of this boy and also those of other two children who had showed low forced vital capacity (FVC) before challenge test were excluded from the results. All exercise challenges were performed in the morning. The ambient temperature and relative humidity ranged from 23 to 27°C, and from 50 to 60%.

#### Blood sampling and respiratory function test

Before the start of exercise, a 21-gauged indwelling needle was inserted to the antecubital vein filled with heparinized saline. After insertion, a subject rested in the supine position for 20–30 min. Three or 4 ml of heparinized blood was drawn just before, just after, and 5 and 15 min after exercise. The blood sample were centrifuged immediately at 10,000  $\times$  g at 4°C for 5 min. The plasma was divided into two portions, 0.5 ml of plasma was frozen immediately at –40°C for later ECP assay and 1 ml at –70°C until the day of catecholamine assay. White blood cell and eosinophil count, and

IgE levels were determined from the blood sample taken just before the exercise.

Pulmonary function tests were performed just before, 5 and 15 min after exercise in duplicate. Values of FVC, forced expiratory volume in 1 s (FEV<sub>1.0</sub>) and peak expiratory flow (PFR) were recorded.

#### Plasma catecholamine assay

Plasma catecholamine levels were measured by the method of Goldstein (15), with slight modifications. Briefly, 0.8 ml of 1 M Tris-HCl, pH 8.6, 15 mg of acidified alumina (Woelm Pharma, Eschwege, Germany), 40  $\mu$ l of 5 mM metabisulfite (Nacalai Tesque, Kyoto, Japan) and 1 mg of dihydroxybenzylamine (DHBA) (Aldrich, Milwaukee, U.S.A.) were added to 0.8 ml of plasma. Cate-

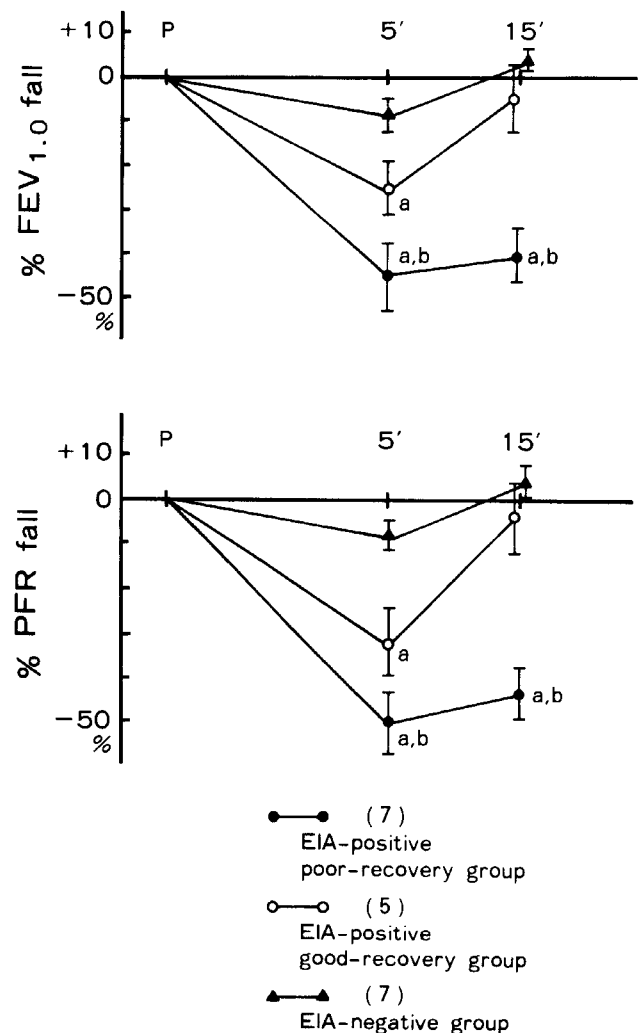


Fig. 1. Percent fall of PFR and FEV<sub>1.0</sub> with exercise. The values are given as mean  $\pm$  1 SD. Methods used for statistical analysis are described in the text. P: pre-exercise, 5' and 15': 5 and 15 min after exercise; a) values in EIA-positive groups were significantly different from those in EIA-negative groups ( $p < 0.01$ ); b) values in EIA-positive group with poor-recovery were different from those in good-recovery group ( $p < 0.05$ ).

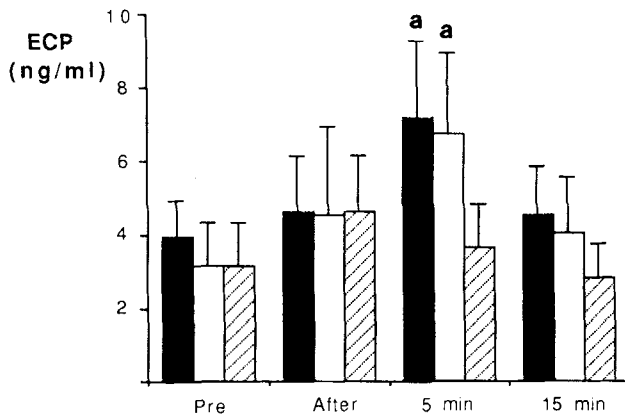


Fig. 2. Changes of plasma ECP levels in EIA-positive group with good recovery (closed bar,  $n = 5$ ) and with poor recovery (open bar,  $n = 7$ ), and in EIA-negative group (hatched bar,  $n = 7$ ) by exercise. The data represent means  $\pm 1$  SD. Methods used for statistical analysis are described in the text. Pre: pre-exercise. After: just after exercise, 5 min and 15 min: 5 and 15 min after exercise; a) values in EIA-positive groups were significantly different from those in EIA-negative groups ( $p < 0.01$ ).

cholamines and DHBA were adsorbed on the alumina by shaking for 20 min. After being washed three times with cold water, they were eluted by 0.1 M perchloric acid. Then, the eluent was injected onto high performance liquid chromatography with electrochemical detection (BAS LC-4B, BAS Japan, Tokyo, Japan).

Plasma ECP levels were determined with  $^{125}$ I-labelled ECP radio-immunoassay kit (Pharmacia), kindly donated by Sandoz Pharma, Japan.

#### Statistical analysis

Experimental results were expressed as means  $\pm$  SD. One-way analysis of variance (ANOVA) were used for statistical analysis. When a  $p$  value of  $F$  of ANOVA was observed to be significant between groups, then comparisons between each group were done by the two-tailed Student's  $t$ -test. A  $p$  value of  $< 0.05$  was considered to be significant.

#### Results

On the basis of the percent fall of PFR and  $FEV_{1.0}$  after exercise, respectively, the subjects were divided into three groups: EIA-negative group ( $n = 7$ ), and EIA-positive groups with good recovery ( $n = 5$ ) and with poor recovery ( $n = 7$ ) (Fig. 1). EIA-positive group with poor recovery showed significantly larger percent falls of  $52.1 \pm 5.8$  and  $46.3 \pm 8.4$  in PFR and  $FEV_{1.0}$  in comparison with those in good-recovery group ( $33.3 \pm 6.9$  and  $26.8 \pm 6.7$ ,  $P < 0.05$ ) 5 min after exercise. Wheezing was audible for all in these two EIA-positive groups. At 15 min after exercise, PFR and  $FEV_{1.0}$

in the EIA-positive group with good recovery returned to their pre-exercise level, but those in poor-recovery group remained at the same level and wheezing was still audible. Those who had taken theophylline in the morning of the exercise provocation test belonged to EIA-positive group with poor recovery, respectively.

Various parameters at pre-exercise in the three groups are shown in Tab. 1. All parameters were the same in the three groups except for the eosinophil count, which in EIA-positive groups were significantly higher than those in EIA-negative group. The doses and durations of medication, and the histories of recent asthma attacks and their severities were not different among three groups (data not shown).

The changes of plasma ECP levels with exercise are shown in Fig. 2. The ECP levels rose just after exercise in all groups. At 5 min after exercise, the ECP levels in the EIA-positive groups were even higher, but fell to the pre-exercise levels 15 min after exercise, whereas those in the EIA-negative group were same as the pre-exercise level at 5 and 15 min after exercise.

Just after exercise, although the plasma E level in EIA-negative group showed approximately 4-fold increase, only 2.5-fold and 1.5-fold increase in EIA-positive groups with good and poor recovery were observed. Plasma E level of poor-recovery group was significantly lower than that of good-recovery group just after exercise ( $p < 0.05$ ). At 5 and 15 min after exercise, plasma E levels in three groups were not different (Fig. 3).

Plasma NE levels in two EIA-positive groups showed about 2-fold increase just after exercise,

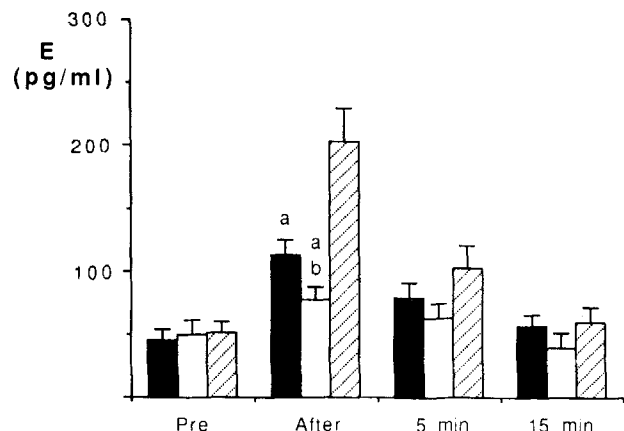


Fig. 3. Changes of plasma epinephrine levels in EIA-positive group with good-recovery (closed bar,  $n = 5$ ) and with poor-recovery (open bar,  $n = 7$ ), and in EIA-negative group (hatched bar,  $n = 7$ ) by exercise. The data represent means  $\pm 1$  SD. Pre: pre-exercise. After: just after exercise, 5' and 15': 5 and 15 min after exercise; a) values in EIA-positive groups were significantly different from those in EIA-negative groups ( $p < 0.01$ ); b) values in EIA-positive group with poor-recovery were different from those in good-recovery group ( $p < 0.05$ ).

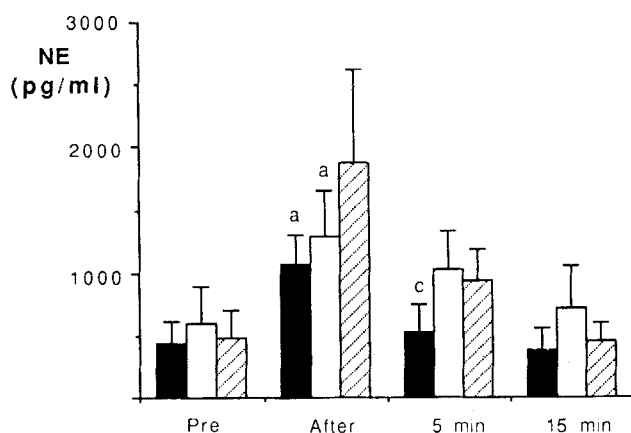


Fig. 4. Changes of plasma norepinephrine levels in EIA-positive group with good-recovery (closed bar,  $n=5$ ) and with poor-recovery (open bar,  $n=7$ ), and in EIA-negative group (hatched bar,  $n=7$ ) by exercise. The data represent means  $\pm$  1 SD. pre: pre-exercise. After: just after exercise, 5' and 15': 5 and 15 min after exercise; a) values in EIA-positive groups were significantly different from those in EIA-negative groups ( $p < 0.01$ ); c) value in EIA-positive group with good-recovery was different from those in poor-recovery group and EIA-negative group ( $p < 0.05$ ).

but 4-fold increase in NE level was observed in EIA-negative group. 5 min after exercise, the NE levels in EIA-positive with good recovery were lower than other groups. Then, the NE levels of all groups returned to the pre-exercise state (Fig. 4).

## Discussion

Several factors, such as airway cooling, osmotic change of airways, increase of chemical mediator release from mast cells, and bronchoconstriction through a vagal reflex have been proposed and thought to be responsible for the initiation of EIA (16–18). These factors may influence each other. Airway cooling or drying may stimulate an irritant receptor in the airways, consequently leading to a bronchoconstriction through vagal efferent fibers (19). Hyperosmolarity evoked by airway drying may cause a release of chemical mediators from mast cells (20, 21).

All these phenomena may be inhibited by E. It has been shown to inhibit IgE-induced histamine release from human lung fragments (6), and it may also inhibit cholinergic neurotransmission (22). Therefore, a blunted rise of epinephrine, which was noted in EIA-positive groups in the present study, seems to aggravate bronchoconstriction induced by exercise as reported before (2–4).

We have shown two subpopulations in EIA-positive children featured by the different manner of recovery. Since percent falls of PFR and FEV<sub>1.0</sub> after exercise were sufficient enough for both

groups to cause asthmatic reactions, and kinetics of plasma ECP and catecholamine levels in both groups were similar, but quite different from those in EIA negative group, these responses were certainly induced by exercise and the underlying mechanism for EIA may be common in two groups. If that is the case, the difference between two EIA-positive groups may reflect the severity of the bronchoconstriction at initial phase of EIA, and/or result from the difference in the reactivity of sympathoadrenal pathway to exercise. Because less increase of plasma E level was observed in poor-recovery group just after exercise.

An increase of eosinophil number in peripheral blood is a known characteristic of asthma (23), and have been reported as an index of the severity and activity of asthma (10, 24). Eosinophil granule proteins, which are considered to be toxic for bronchial epithelial tissues, have also been investigated as markers of the inflammatory status in asthmatic patients. An increase of blood ECP levels in the early asthmatic response after an allergen challenge, and increased levels of ECP, eosinophil peroxidase (EPO) and eosinophil-derived neurotoxin in the peripheral blood of asthmatic patients have also been reported (10, 24, 25).

Our results, which demonstrated a difference in the eosinophil counts in the peripheral blood before exercise, and a difference in the kinetics of ECP levels during exercise between EIA-positive and negative children suggest that eosinophil may also be involved in the pathophysiology of EIA.

Although the precise mechanism, which evoked ECP release from eosinophils by exercise are still unknown, it may be possible that eosinophils, an increase of which observed in EIA-positive groups before exercise, had been activated before and/or during exercise and resulted in higher ECP levels after exercise. Histamine release from human basophil and rat mast cells stimulated by eosinophil granule-derived cationic proteins have already been reported (26, 27). This may lead to the bronchoconstriction after exercise. Similar results have also been reported in reaction for exercise in adult asthmatic patients by Venge et al. (13).

Possibly, the E levels in blood regulate the release of ECP from eosinophils, since the greater increase of the E levels in blood in EIA-negative group was followed by the rapid decrease of the ECP levels, and the ECP levels in EIA-positive groups kept rising after the blunted rise of the E levels. The finding that an administration of a  $\beta_2$ -agonist, salbutamol, resulted in a significant fall in both blood eosinophil count and ECP concentrations in normal subjects supports this possibility (28). On the other hand, EPO release from eosinophils and superoxide production by eosinophils

were not inhibited by a co-incubation with  $\beta_2$ -agonists *in vitro* (29). For the implication of this discrepancy, a further controlled study is needed.

Our study suggests a possible contribution of sympathoadrenal hypofunction and a probable involvement of eosinophils in the pathophysiology of EIA.

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