EFFECT OF MITE ALLERGEN ON Na/H EXCHANGE IN PERITONEAL MAST CELLS

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The ability of mast cells to take part in an IgE-mediated allergic reaction depends on the presence of receptors for IgE on their surface [3, 7]. Contact between allergen and cell-bound IgE serves as the beginning of the IgE-mediated allergic reaction, which leads to release of histamine and other mediators from mast cells [11]. A key question in the study of triggering of the secretory process of the cell in response to an immune stimulus is that of the molecular mechanisms of transmembrane transmission of the signal from the surface inside the cell. An essential role in this triggering is played by ion transport. It is known, for instance, that Na/H exchange is involved in histamine secretion by mast cells stimulated by a calcium ionophore [5]. However, there is no information on the mechanisms of transmembrane signal transmission in IgE-mediated activation of mast cells by an allergen and on the role of Na/H exchange in this reaction.

The aim of this investigation was to study the mechanisms of involvement of Na/H-exchange in the IgE-mediated allergic reaction of mast cells, using an allergen from the mite *Dermatophagoides farinae.* We know that mites present in house dust can induce allergic diseases, and that the main role in sensitization is played by mites of the genus *Dermatophagoides* [2, 13].

EXPERIMENTAL METHOD

The pH-sensitive probe BCECF-AM [2,7-*bis*-carboxyethyl-5(6)-carboxyfluorescein], obtained from "Molecular Probes," EIPA (ethylisopropylamiloride), from "Merck," PMA (phorbol-12-myristate-13-acetate) from "Serva," HEPES (the Na-salt) and digitonin, from "Sigma," and staurosporin (an inhibitor of protein kinase C), isolated from fungi of the genus *Streptomyces,* from Calbiochem, were used in the work.

The mite allergen and allergoid were obtained by N. A. Perovaya, on the staff of the I. Mechnikov Institute of Vaccines and Sera. The mite allergen was extracted from a culture of the house dust mite *D. farinae.* Modification of the allergen was carried out by the method in [6]. Formation of the allergoid was monitored by gel-chromatography, isoelectric focusing, and microdot EIA.

Sera from patients with bronchial asthma, with sensitization to the house dust mite *D. farinae,* sera from healthy blood donors with a low titer of specific IgE (i.e., sera from clinically healthy individuals), and placental serum were used in the experiments. The test object consisted of peritoneal mast cells obtained from rats by the method in [12]. The composition of the control buffer was: 10 mM HEPES, 145 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$,

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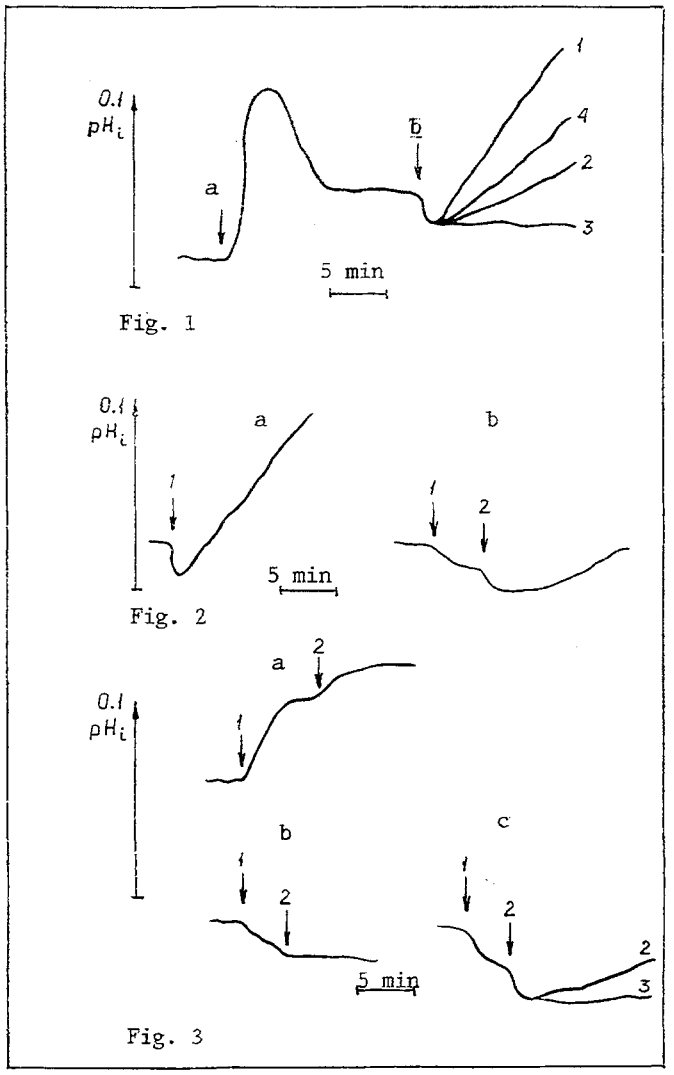

Fig. 1. Changes in pH_i of mast cells measured with the fluorescent probe BCECF: a) addition of serum to mast cells suspended in control buffer (without albumin); b) addition of mite allergen (protein concentration 0.06 mg/ml) to mast cells treated with serum from patient with bronchial asthma, sensitized to house dust mite *D. farinae* (1), serum from a clinically healthy blood donor (2), and placental serum (3), addition of mite allergoid (protein concentration 0.06 mg/ml) to mast cells treated with serum from patient with mite sensitization (4).

Fig. 2. Effect of mite allergen on activity of Na/H-exchange in mast cells incubated with serum of patient with sensitization to mites. A) In absence of EIPA (30 μM), B) in presence of EIPA (30 μM): 1) addition of EIPA, 2) addition of mite allergen.

Fig. 3. Effect of staurosporin on changes in pH_i of mast cells treated with serum from patient with mite sensitization and activated by mite allergen. A) Addition of 10 nM PMA (1), of mite allergen (2); B) addition of 0.2 μM staurosporin (1), of 10 nM PMA (2); C) addition of staurosporin (1), of mite allergen after treatment of cells with 0.2 μM staurosporin (2), of mite allergen after treatment of cells with 1 μM staurosporin (3).

1 mM $MgCl_2$, 5 mM glucose, and 0.1% bovine serum albumin, pH 7.4, 37°C. In the experiments 10 μl of mite allergen or allergoid, with a protein concentration of 0.06 mg/ml, was added to 1 ml of suspension.

Intracellular pH was measured by means of the fluorescent pH-sensitive probe BCECF by the method in [9]. Mast cells in the control buffer were incubated with 1-2 μM BCECF-AM (which can pass through the cell membrane) at 37°C for 30 min with constant mixing. The cell suspension was then centrifuged for 10 min at 1000*g* and resuspended in 1 ml of buffer solution. Immediately before measurement 40 μl of the suspension ($2 \cdot 10^6$ mast cells) was added to 1 ml of buffer solution (without albumin), containing 100 μl serum. Fluorescence of the samples was measured on "Hitachi 650-60" spectrofluorometer at 37°C. The wavelengths of excitation and emission were 505 and 535 nm and the width of the slits 5 and 2.5 nm respectively. To determine the intracellular pH the mast cells were subjected to lysis by 50 μM digitonin, and changes in pH were recorded with the aid of a microelectrode and pH-meter ("Beckman") with an error of ± 0.01 unit.

EXPERIMENTAL RESULTS

The intracellular pH (pH_i) of the intact mast cells was 6.92 ± 0.01 ($n = 37$), sufficiently close to the value determined previously by ourselves and other workers [4, 10]. Addition of mite allergen to the cells caused very small changes in pH_i: a temporary fall of 0.02 pH unit was followed by alkalification of the cytoplasm, exceeding the basal level by 0.015 unit 15 min after addition of the allergen.

On the addition of sera from different donors to the mast cell suspension alkalification of the cytoplasm was observed. The kinetics of this alkalification is illustrated in Fig. 1a. After a rapid initial rise of 0.09 unit there was gradual fall of pH_i, and after 5-10 min pH_i was established at a new level, 0.05 unit higher than the pH value before addition of the serum to the cell suspension. No further change in pH_i was observed. There is evidence that the new value of pH_i is maintained in the cell for not less than 1-1.5 h [1]. Changes in pH_i during the action of sera from different donors on the cells were of the same type. Alkalification of the cytoplasm was probably connected with an increase in the rate of entry of sodium into the cell, evidently as a result of activation of Na/H exchange. This is supported by evidence obtained previously with the aid of labeled sodium on different types of cells [8]. The serum was shown to stimulate the inward flow and to promote accumulation of ^{22}Na in the cells, but had no effect on the rate of its departure from the cells.

Since the action of the allergen on the cell was mediated through IgE, experiments to investigate the effect of the mite allergen on pH_i were carried out on mast cells treated with serum of patients with sensitization to mites. As the control we used serum from blood donors with a low concentration of specific IgE and placental serum, not containing IgE. Addition of the mite allergen (0.06 mg/ml) to mast cells incubated for 15 min with serum of a patient with mite allergy led to biphasic changes in pH_i: an initial temporary fall of 0.02 pH unit was followed by alkalification of the cytoplasm, which exceeded the basal level by 0.085 unit. In mast cells activated by donor's serum with a low concentration of specific IgE, in response to injection of the mite allergen pH_i fell, and by the 15th minute after addition of the allergen it was increased by 0.09 unit compared with the basal level (Fig. 1).

It can be postulated on the basis of the results that the response of cells to mite allergen depended on the specific IgE concentration in the sera, with which the mast cells had been treated beforehand: the higher the titer of specific IgE in the serum, the greater the degree of alkalification of the cell cytoplasm, caused by the mite allergen. Incubation of mast cells with placental serum completely abolished the effect of the mite allergen. This can be explained by the absence of IgE in the placental serum due to the inability of immunoglobulins of this class to pass through the placental barrier.

The modified form of mite allergen (allergoid), with reduced allergenicity but preserving its immunogenic properties at a sufficiently high level, also led to biphasic changes in pH_i of the mast cells incubated beforehand with serum of a donor sensitized to the domestic dust mite *D. farinae*. The allergoid (0.06 mg/ml) caused pH_i to fall by

0.02 unit, but later it increased to 1.03 pH unit above the basal level after 15 min (Fig. 1). Thus the rise of pH_i caused by the allergoid was much less than that caused by the allergen. Probably the incomplete response of the mast cells to allergoid was due, on the one hand, to a decrease in the number of antigenic determinants in the allergoid molecule, and on the other hand, to the inaccessibility for IgE of the remaining determinants, hidden within the aggregated, enlarged protein molecules of the allergoid [6].

Alkalification of the cytoplasm of the mast cells in response to mite allergen may evidently be connected with activation of Na/H exchange. In fact, preliminary incubation for 5 min of mast cells treated with serum of a patient with mite allergy, with 30 μM EIPA (a blocker of Na/H exchange and a derivative of the diuretic amiloride) inhibited the increase in pH_i induced by the mite allergen (Fig. 2).

An important role in transmembrane signal transmission in cells is known to be played by protein kinase C. We found that phorbol ester (PMA, 10 nM), which penetrates into the cell and imitates the action of the endogenous protein kinase C activator 1,2-diacylglycerol, causes an increase in pH_i of the mast cells without initial acidification of the cytoplasm (Fig. 3a). The increase in pH_i was connected with activation of Na/H exchange, for EIPA (30 μM) blocked this effect. Addition of the mite allergen against the background of PMA led to a further increase in pH_i (Fig. 3A). This may probably be evidence of additional activation of protein kinase C by the mite allergen.

To assess the role of protein kinase C in activation of Na/H exchange in mast cells we used staurosporin, a selective inhibitor of this enzyme. In our experiments sturosporin in a concentration of 0.2 μM did not lead to any increase in pH_i caused by PMA, and blocked the effect of the mite allergen only partially (Fig. 3B, C). An increase in the staurosporin concentration to 1 μM led to complete inhibition of the effect of the mite allergen on mast cells treated with the serum of a patient with mite allergy.

Thus the mite allergen, by interacting with mast cells treated with the serum of a patient with bronchial asthma, sensitized to the domestic dust mite *Dermatophagoides farinae,* causes changes in the intracellular pH. Na/H-exchange, in whose activation protein kinase C is evidently involved, participates in the regulation of pH_i of the peritoneal mast cells.

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