

Defective inhibition of sodium on basophil histamine release in patients with allergic rhinitis and bronchial asthma

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Defective inhibition of sodium on basophil histamine release in patients with allergic rhinitis and bronchial asthma. A. Tedeschi, M. Cottini, C. Salmaso, N. Milazzo, A. Miadonna. ©ERS Journals Ltd 1996.

ABSTRACT: The aim of this study was to evaluate whether Na⁺ exerts its inhibitory effect on basophil histamine release induced by immunoglobulin E (IgE)-dependent (anti-IgE) and IgE-independent (N-formyl-methionyl-leucyl-phenylalanine (FMLP), interleukin-3 (IL-3)) stimuli in patients with allergic rhinitis (n=24) and allergic bronchial asthma (n=10).

Peripheral blood leucocytes were stimulated with anti-IgE, FMLP and IL-3 in the presence of high and low Na⁺ concentrations, and histamine release was measured using a fluorometric method.

In standard Na⁺-containing medium, spontaneous and stimulated histamine release was higher in allergic patients (n=34) (both rhinitic and asthmatic) than in healthy subjects (n=41). Na⁺ removal from extracellular medium and its isosmotic substitution with choline chloride or with N-methyl-D-glucamine led to a significant increase of anti-IgE-, FMLP- and IL-3-induced histamine release in normal subjects, but not in allergic patients. The increase in Na⁺ concentration in the extracellular medium was accompanied by a dose-dependent decrease of anti-IgE- and FMLP-induced histamine release in normal subjects, but not in allergic patients. The behaviour of atopic and healthy subjects was different and not related to the basophil responsiveness to activating signals. The incubation of basophils from healthy subjects with sera from allergic patients did not have a significant influence on the inhibitory effect of Na⁺.

Basophils from healthy subjects and atopic patients respond differently when stimulated in a low Na⁺ medium. The reduced sensitivity to the inhibitory effect of Na⁺ may contribute to basophil dysfunction in patients with respiratory allergy. *Eur Respir J., 1996, 9, 2070–2076.*

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Human basophils bear high affinity immunoglobulin E (IgE) receptors on their membrane and can release potent inflammatory mediators, such as histamine and leukotriene C₄, following antigen binding to specific IgE on the cell membrane [1]. It has been demonstrated that IgE-mediated histamine release from human basophils is not correlated with membrane IgE density [2], and that the intrinsic capacity of the basophil to respond to activating signals is important in determining the amounts of mediators released [3]. To define the responsiveness to activating agents, the term "releasability" was coined several years ago by LICHTENSTEIN and co-workers [4].

Although the microenvironment can influence mediator release from basophils, in particular through the action of cytokines, the data collected so far indicate that the intrinsic ability of the cells is an important parameter conditioning mediator release. Basophil releasability is increased in patients with allergic rhinitis and asthma [5–7], and it has been suggested that it may contribute to the pathogenesis of allergic disease. LICHTENSTEIN and co-workers [8] showed that the severity of symptoms in allergic patients during the ragweed season correlated with basophil sensitivity to antigen stimulation

in vitro. A correlation has been found between basophil number in peripheral blood and bronchial hyperresponsiveness as assessed by methacholine challenge in asthmatic patients [9]; therefore, the enhanced basophil releasability that has been observed in patients with respiratory allergy may be of clinical significance.

Although, in the last few years, much progress has been made in the understanding of the biochemical pathways involved in mediator secretion from human basophils, the causes of the enhanced basophil releasability have not yet been defined. It has been demonstrated that Ca²⁺ plays an important role in signal transduction and basophil histamine release [10–11]; however, other cations, such as Na⁺ and K⁺, participate in the regulation of basophil histamine release [12–13]. Na⁺ influx inhibits histamine release, whereas K⁺ efflux has an enhancing effect [13]. The inhibition by Na⁺ profoundly affects basophil releasability, since anti-IgE-nonreleasing basophils are converted into releasing basophils when they are suspended in a low Na⁺ medium [14]. After removal of Na⁺ from extracellular medium, basophils from normal subjects acquire the capacity to release histamine on challenge with a weak agonist, such as interleukin-3

(IL-3) [15]. The inhibitory effect of Na⁺ is altered in patients with allergic rhinitis [16], and this impairment coexists with an enhanced releasability. Furthermore, a defect in Na⁺ metabolism and Na⁺ transport across biological membranes in allergic patients has been postulated by some authors [17–19]. SKONER *et al.* [17] found a reduction of Na⁺, K⁺ adenosine triphosphatase enzyme activity in the platelets of allergic patients, due to a circulating inhibitor. TRIBE *et al.* [18] showed that patients with airway hyperresponsiveness had increased Na⁺ influx into peripheral blood leucocytes, stimulated by a serum-borne factor. It is not clear how these biochemical alterations could influence the expression of allergic manifestations, but it has been hypothesized that they could cause an increase of airway reactivity and an altered responsiveness or function of a large number of cell types.

The aim of this study was to evaluate the effect of Na⁺ on basophil histamine release induced by IgE-dependent and IgE-independent stimuli in patients with allergic rhinitis and asthma. We have found that the inhibitory activity of Na⁺ on basophil histamine release is impaired in patients with either allergic rhinitis or bronchial asthma and that the defective inhibition concerns both IgE-dependent and IgE-independent stimuli.

Subjects and methods

Patients and control populations

The patients were selected among the subjects followed by the out-patient allergy clinic, Ospedale Maggiore Policlinico, Milan and by the Allergy Unit, USSL 12, Bergamo. Normal healthy subjects were used as controls and were recruited from the hospital staff and the faculty students; informed consent was obtained from all patients and control subjects before venous blood collection. Twenty four patients with allergic rhinitis (12 males), 10 patients with mild allergic bronchial asthma (7 males) and 41 healthy subjects (23 males) were included in the study. The mean age (\pm SEM) of the allergic patients was 28 ± 2 yrs and the mean age of the control subjects was 37 ± 1 yrs. Respiratory allergy was diagnosed on the basis of a detailed clinical history and skin-prick tests. The allergens involved were grass pollen ($n=25$), *Dermatophagoides pteronyssinus* ($n=13$), birch pollen ($n=2$) and *Parietaria judaica* ($n=2$). Seven patients reacted to two or more allergens. Neither allergic nor control subjects were taking any medication at the time of blood collection.

Sample collection and preparation of leucocyte suspensions

Leucocyte suspensions were prepared by dextran sedimentation of peripheral venous blood (60–80 mL), anticoagulated with 0.01 M ethylenediamine tetra-acetic acid (EDTA) and mixed with 6% dextran in saline solution (Solplex 70; Sifra, Verona, Italy) and 30 mM dextrose (Sigma Chemical Co., St Louis, USA). The cells were allowed to sediment for 60–90 min at room temperature, according to LICHTENSTEIN and OSLER [20]. The leucocyte-rich plasma was centrifuged at $300 \times g$ for 15 min

at 4°C, and the cell button was washed twice in Tyrode's buffer, pH 7.4, containing (mM): NaCl 140; dextrose 5.5; KCl 2.2; NaH₂PO₄ 0.36; NaHCO₃ 12. The cells were then suspended in Tyrode's buffer with 1.8 mM CaCl₂ and 0.5 mM MgCl₂. These suspensions in Tyrode's buffer were utilized to evaluate histamine release in control conditions. To investigate the effects of Na⁺ on histamine release, NaCl was replaced with the chloride salt of choline, a nonpermeating Na⁺ analogue. When the NaCl concentration was reduced, the concentration of choline chloride was increased correspondingly to maintain a constant 140 mM of the two cations together. Leucocyte suspensions were also prepared with isotonic N-methyl-D-glucamine (Sigma Chemical Co. Ltd) solutions. The osmolarity of the solutions employed was checked with an osmometer, and ranged 270–300 mOsm·L⁻¹.

Basophil concentration in leucocyte suspensions was evaluated by alcian blue staining [21], and subsequent counting in a Fuchs-Rosenthal chamber (Walter Schrenk, Hofheim am Taunus, Germany). When cell viability was assessed by trypan blue (provided by Farmacia Ospedale Maggiore Policlinico, Milan, Italy) exclusion, all the leucocyte suspensions were more than 95% viable.

Evaluation of the effect of serum on the inhibitory effect of Na⁺

To investigate whether the effect of Na⁺ on basophil histamine release is influenced by a soluble serum factor, basophils from healthy subjects were incubated with sera from allergic patients for 60 min at 37°C, following the method used for passive sensitization [22]. The sera were taken from allergic patients who had already shown an impaired inhibitory effect of Na⁺ on IgE mediated basophil histamine release. Control incubation with buffer was performed. After incubation, leucocyte suspensions were centrifuged at $300 \times g$ for 15 min at 4°C, washed in Tyrode's buffer, and resuspended in high and low Na⁺ solutions. Basophil stimulation with anti-IgE and IL-3 was then carried out.

Histamine release from leucocyte suspensions

Leucocyte stimulation was performed in 12 by 75 mm polyethylene tubes (LP Italiana, Milan, Italy). Briefly, $2-4 \times 10^4$ basophils, suspended in 0.5 mL Tyrode's buffer or choline chloride solution, were added to prewarmed tubes (37°C) containing 0.5 mL of the same solution with polyclonal goat anti-human IgE, ϵ -chain specific, (Sigma Chemical Co. Ltd, final dilutions of $1/5 \times 10^5$, $1/5 \times 10^4$ and $1/5 \times 10^3$, corresponding to protein concentrations of 0.1, 1 and 10 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively), N-formyl-methionyl-leucyl-phenylalanine (FMLP) (Sigma Chemical Co. Ltd, concentrations ranging 10^{-8} – 10^{-6} M) or recombinant human IL-3 (purchased from Amersham, Aylesbury, UK; concentrations ranging 0.1–10 $\text{ng}\cdot\text{mL}^{-1}$). After mixing and incubating for 60 min at 37°C, the reaction was stopped by cooling the tubes at 4°C and by centrifuging at $1,000 \times g$ for 20 min at the same temperature. After centrifugation, the supernatants were aspirated, mixed with an equal volume of 6% HClO₄ and centrifuged at $2,000 \times g$ for 15 min at 4°C. Histamine concentration in the supernatants was measured using an automated fluorometric technique [23]. Spontaneous histamine release

was evaluated by measuring histamine concentration in the supernatant of unstimulated cells; total histamine content was obtained by adding 0.5 mL 6% HClO₄ to 0.5 mL cell suspension. Net histamine release induced by secretagogues was calculated as percentage of total histamine content after subtraction of spontaneous release.

Every experiment was carried out in duplicate, and the average difference between replicate determinations was less than 10%. None of the reagents employed in this study interfered with the fluorometric determination of histamine concentration or modified cell viability, as assessed by trypan blue exclusion.

Statistical analysis

The InStat statistical software package (Graphpad software, San Diego, CA, USA) was used for statistical analysis. The results were expressed as the mean \pm SEM. Significant differences were assessed with the two-tailed Student's t-test for paired and unpaired data. Correlation was evaluated by linear regression, calculated according to the least squares method. A p-value lower than 0.05 was considered to be significant.

Results

The mean percentage of spontaneous histamine release during a 60 min leucocyte incubation in standard Tyrode's buffer was 4.0 \pm 1.0% in allergic patients and 1.5 \pm 0.1% in control subjects, with a significant difference between the two populations (p<0.01). As no difference was found between rhinitics and asthmatics, the data from these two populations were pooled. Na⁺ removal from extracellular medium caused significant increase of spontaneous histamine release in control subjects (4.0 \pm 0.5%; p<0.001) but not in allergic patients (5.5 \pm 0.8%; NS).

Basophil stimulation with anti-IgE caused a dose-dependent histamine release, the highest response occurring when anti-IgE was used at the dilution of 1/5 \times 10³ both in allergic patients (36 \pm 4%) and in healthy subjects (26 \pm 3%) (fig. 1). The results from rhinitics and

Table 1. – Basophil histamine release induced by anti-IgE (1/5 \times 10³), FMLP (10⁻⁶ M) and IL-3 (10 ng \cdot mL⁻¹) in high and low Na⁺ solutions

	Allergics		Controls	
	High Na ⁺	Low Na ⁺	High Na ⁺	Low Na ⁺
Anti-IgE	57 \pm 12	56 \pm 8	24 \pm 11	51 \pm 12 ^{††}
FMLP	37 \pm 13	42 \pm 13	38 \pm 6	48 \pm 6*
IL-3	22 \pm 11	26 \pm 12	0	18 \pm 4**

Leucocytes from patients with respiratory allergy (n=5) and from healthy subjects (n=5) were suspended in isotonic solutions containing 140 mM NaCl or 140 mM N-methyl-D-glucamine. Results are expressed as net percentage of histamine release \pm SEM. Na⁺ removal led to a significant increase of basophil histamine release in normal subjects, but not in allergics. NS: not significant; IgE: immunoglobulin E; FMLP: N-formyl-methionyl-leucyl-phenylalanine; IL-3: interleukin-3. *, **, ††: p<0.05, p<0.01, p<0.005 low vs high Na⁺.

asthmatics were pooled, since no significant difference was found between the two populations. A trend towards a higher histamine release was found in allergic patients than in control subjects at all anti-IgE concentrations; statistical analysis revealed that the difference between allergic and control subjects was significant at the anti-IgE dilutions of 1/5 \times 10⁵ (p<0.001) and 1/5 \times 10³ (p=0.05). The behaviour of the two populations was clearly different when basophils were suspended in a medium where NaCl had been isosmotically replaced with choline chloride. In a low Na⁺ medium, a marked increase of anti-IgE-induced histamine release with respect to the high Na⁺ medium was found in healthy subjects (anti-IgE diluted 1/5 \times 10³: 59.2 \pm 3 vs 26 \pm 3%; p<0.001), but not in the patients (33 \pm 4% vs 36 \pm 4%; NS) (fig. 1).

Similar results were obtained when N-methyl-D-glucamine was used as Na⁺ substitute in the extracellular medium (table 1).

Basophil incubation with the synthetic peptide FMLP, at concentrations ranging 10⁻⁸–10⁻⁶ M, resulted in a dose-dependent histamine release, with maximum release at the concentration of 10⁻⁶ M (fig. 2). A trend towards a higher histamine release was observed in allergic patients, with a significantly greater release at the concentration of

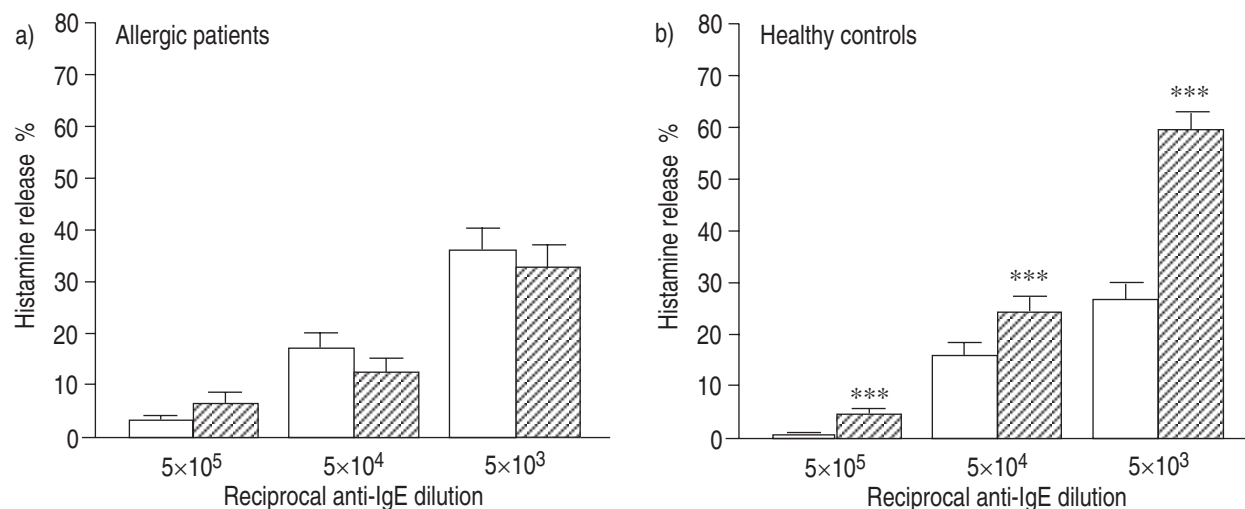


Fig. 1. – Anti-IgE-induced histamine release from basophils of: a) patients with allergic rhinitis (n=24) and allergic asthma (n=10) and; b) healthy controls (n=41) suspended in high (□) and low (▨) Na⁺ solutions. The results are expressed as mean \pm SEM. ***: p<0.001 vs release obtained in high Na⁺. IgE: immunoglobulin E.

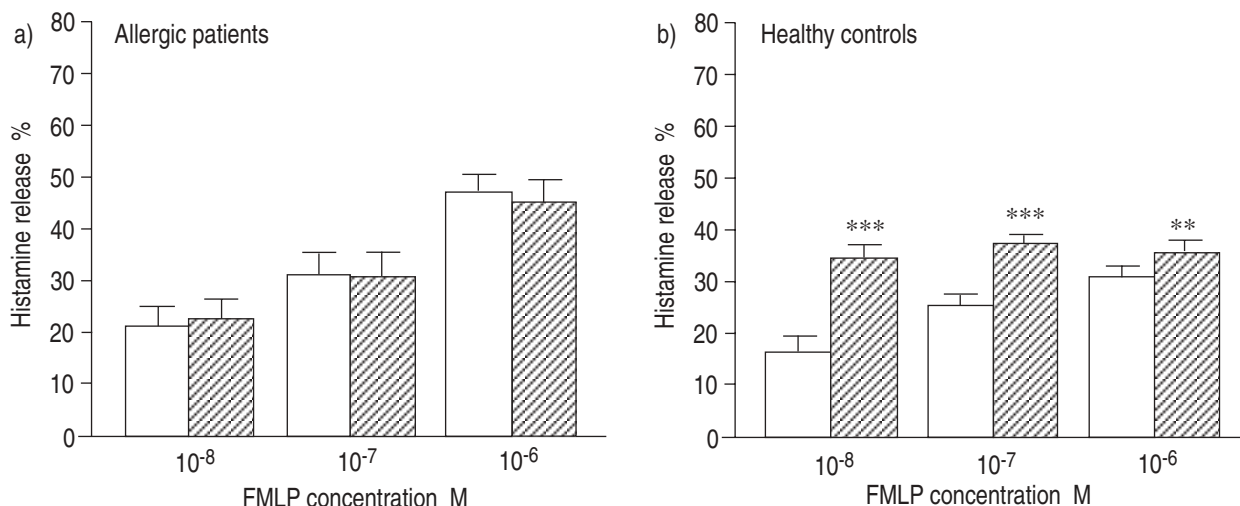


Fig. 2. — FMLP-induced histamine release from basophils of: a) patients with allergic rhinitis (n=24) and allergic asthma (n=10) and; b) healthy controls (n=41) suspended in high (□) and low (▨) Na⁺ solutions. The results are expressed as mean±SEM. ***: p<0.001 vs release obtained in high Na⁺; **: p<0.01 vs release obtained in high Na⁺. FMLP: N-formyl-methionyl-leucyl-phenylalanine.

10⁻⁶ M (47±4 vs 31±2%; p<0.01). When leucocytes were suspended in a low Na⁺ solution, a significant increase of FMLP-induced histamine release was observed in the controls (10⁻⁷ M FMLP: 25±2% in high Na⁺ vs 36±2% in low Na⁺; p<0.001), but not in the allergic patients (32±4% in high Na⁺ vs 31±5% in low Na⁺; NS). The effect of Na⁺ removal in normal subjects was more marked at the lowest FMLP concentration used.

The increase in Na⁺ concentration in the extracellular medium was accompanied by a dose-dependent inhibition of anti-IgE- and FMLP-induced histamine release in normal subjects, but not in allergic patients (fig. 3). In normal subjects, histamine release was maximal at 0 mM NaCl and minimal at the physiological concentration of 140 mM NaCl. Conversely, in allergic patients anti-IgE- and FMLP-induced histamine release at 0 mM NaCl was not significantly different from the values obtained at 140 mM NaCl.

In standard Tyrode's buffer, IL-3 (10 ng·mL⁻¹) provoked a histamine release >5% of total histamine content in 10 out of 34 (29%) allergic patients and in 2 out of 16 (12%) healthy subjects. Mean histamine release induced by 10 ng·mL⁻¹ IL-3, a concentration which has been shown to be optimal for histamine release [15], was 3.2±0.8% in healthy subjects and 5.7±2.1% in allergic patients, with a trend towards a higher response in the latter population (fig. 4). Removal of extracellular Na⁺ caused a significant increase of histamine release in normal subjects, but not in allergic patients (histamine release induced by 10 ng·mL⁻¹ in low Na⁺ was 43±4%; p<0.001 in healthy subjects and 12±2%; NS in allergic patients).

The subjects were divided into three groups according to the intensity of basophil response. Basophils releasing 0–20% of total histamine content were considered low releasers; an intermediate response ranged 20–50%; whereas, above 50% the response was considered high.

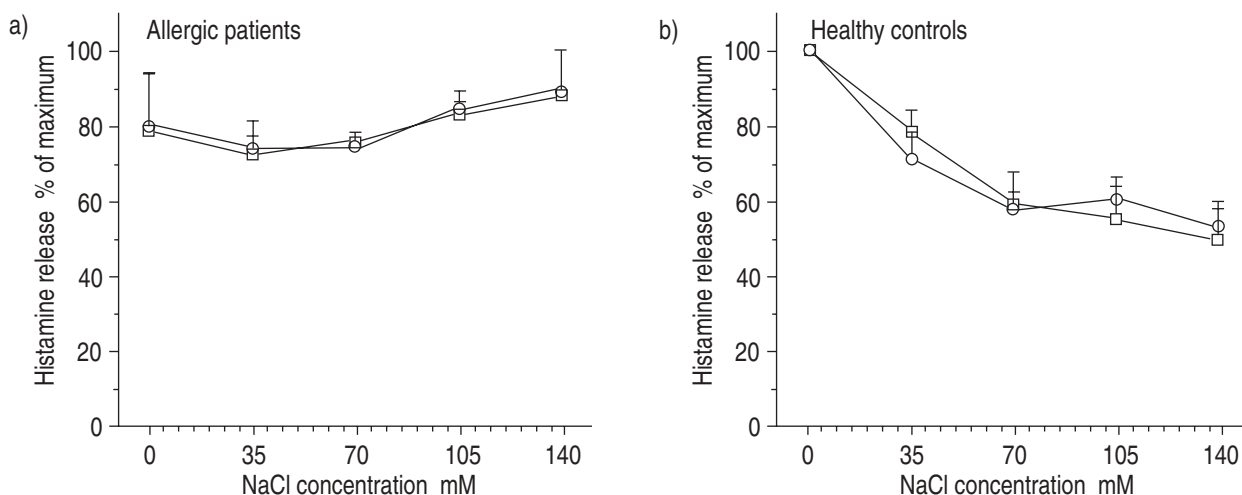


Fig. 3. — Effects of different extracellular NaCl concentrations on anti-IgE- (1/5×10³; —□—) and FMLP- (10⁻⁶ M; —○—) induced histamine release in: a) allergic patients (n=3) and; b) healthy controls (n=8). In low Na⁺ solutions, NaCl was isosmotically replaced with choline chloride. The results are expressed as mean percentage of maximum release. For the controls, histamine release was significantly (p<0.05) lower at all NaCl concentrations than in the presence of 140 mM choline chloride (0 mM NaCl). In contrast, in allergic patients histamine release in low Na⁺ was not significantly different from histamine release in the presence of NaCl at any concentration. IgE: immunoglobulin E; FMLP: N-formyl-methionyl-leucyl-phenylalanine.

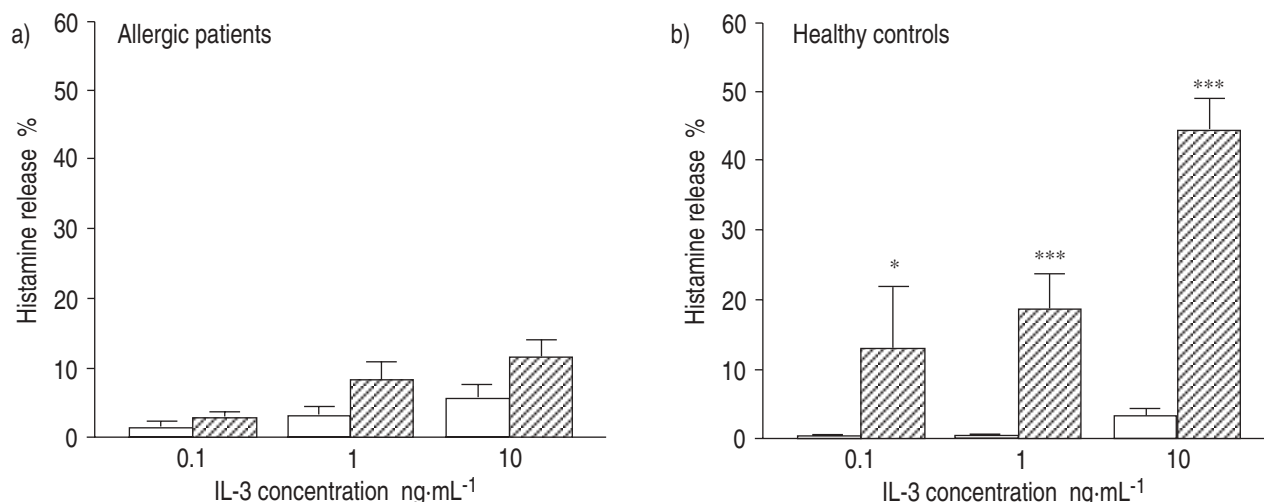


Fig. 4. – IL-3-induced histamine release from basophils of: a) patients with allergic rhinitis (n=24) and allergic asthma (n=10) and; b) healthy controls (n=16) suspended in high (□) and low (▨) Na⁺ solutions. The results are expressed as mean±SEM. ***: p<0.001 vs release obtained in high Na⁺; *: p<0.05 vs release obtained in high Na⁺. IL-3: interleukin-3.

A higher percentage of high releasers was found among the allergics than among the controls (29 and 12%, respectively). Na⁺ removal did not cause an increase in histamine release in allergic patients independently of the basophil responsiveness. Conversely, in control subjects Na⁺ removal caused a significant increase of histamine release induced by anti-IgE (1/5×10³), which was unrelated to the basophil responsiveness (fig. 5). Similar findings were obtained with FMLP.

Basophils from control subjects were incubated with sera from allergic patients, washed and resuspended in high and low Na⁺ solutions. This procedure failed to influence basophil behaviour significantly (table 2). Na⁺ substitution with N-methyl-D-glucamine led to a significant increase of histamine release regardless of previous basophil incubation with buffer or allergic sera.

Discussion

It has been demonstrated that Na⁺ downregulates IgE-mediated histamine release from basophils of healthy subjects [12, 13] and that the inhibitory effect of Na⁺ is impaired in patients with allergic rhinitis [16]. The present results indicate that the defective inhibition of Na⁺ is present in allergics (both patients with allergic rhinitis and asthmatics), and that it concerns basophil histamine release induced by IgE-dependent (anti-IgE) and IgE-independent (FMLP, IL-3) stimuli. These data also show that basophils from patients with respiratory allergy are more prone to release histamine spontaneously or in response to anti-IgE, FMLP and IL-3 than basophils from healthy subjects. This finding may have a clinical relevance, since it has been demonstrated that the severity of allergic symptoms is correlated with basophil sensitivity to antigen stimulation *in vitro* [8].

Initially, it was supposed that basophil releasability was almost exclusively linked to intrinsic factors, but evidence has been collected in recent years that extrinsic factors, such as IgE, cytokines and other soluble factors, can influence mediator release from basophils. We have found, in fact, that IgE may have a different sensitizing capacity [24], and this may influence the efficiency of the basophil/IgE system. This observation has been confirmed and extended by MACDONALD *et al.* [25], who have shown that atopic subjects have a type of IgE (designated IgE+), which has a particular ability to activate basophils. According to these authors, the presence of IgE+ is almost entirely restricted to atopic subjects and correlates with disease severity. IL-3, interleukin-5 (IL-5) and granulocyte/macrophage colony-stimulating factor (GM-CSF) have been identified as the cytokines with the strongest activity on basophil histamine release [26–28]. Furthermore, chemokines and histamine-releasing factors secreted by mononuclear cells and other inflammatory cells can directly induce histamine release from basophils [29–32]. Indeed, the ability of basophils to release histamine may be the consequence both of intrinsic cellular properties and extrinsic factors, such as IgE, cytokines and histamine-releasing factors. The control mechanisms of basophil histamine release are

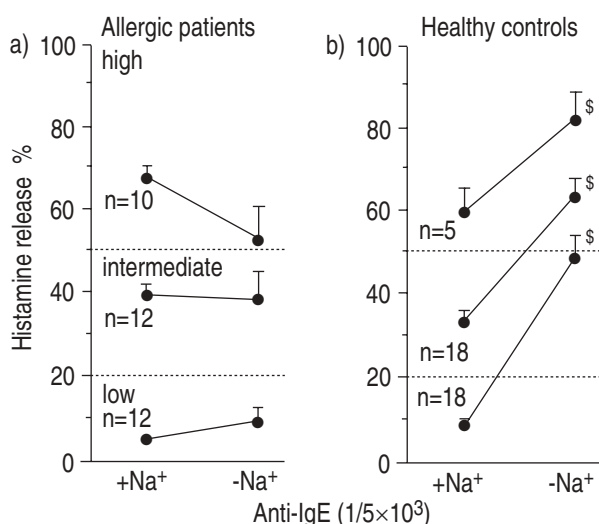


Fig. 5. – Effect of extracellular Na⁺ on basophil histamine release induced by an optimal dose of anti-IgE (dilution 1/5×10³, protein content 10 µg·mL⁻¹) in: a) allergic patients (n=34) and; b) healthy subjects (n=41). The subjects were divided into three groups (low, intermediate and high releasers) according to the basophil responsiveness (see table 2). For each group, histamine release in high Na⁺ was compared to histamine release in low Na⁺. The results are expressed as mean±SEM. \$: p<0.0001 vs release obtained in high Na⁺.

Table 2. – Effect of the incubation with sera from allergic patients on histamine release from basophils of healthy subjects suspended in high and low Na⁺ solutions

	Incubation with buffer				Incubation with allergic serum			
	High Na ⁺	Low Na ⁺	Difference	p-value	High Na ⁺	Low Na ⁺	Difference	p-value
Anti-IgE	9.8±2.5	22.2±23.9	12.4±1.2	<0.01	10.3±1.3	21.2±1.2	10.9±1.3	<0.0001
IL-3	1.5±0.6	8.2±1.0	6.7±0.8	<0.01	0.1±0.1	3.1±0.8	2.9±0.8	<0.006

Leucocytes from healthy subjects (n=4) were incubated for 60 min at 37°C with buffer or with sera from allergic patients (n=4), washed, resuspended in high (140 mM NaCl) and low (140 mM N-methyl-D-glucamine) Na⁺ solutions, and stimulated with anti-IgE and IL-3. The results are expressed as net percentage histamine release and represent the mean±SEM of seven experiments, since leucocytes from three normal subjects were incubated with sera of two different allergic patients. A defective inhibition by Na⁺ on basophil histamine release had previously been found in the allergic patients selected for the experiments. The incubation with sera from allergic patients failed to significantly influence the inhibitory effect of Na⁺ on histamine release from normal basophils. IgE: immunoglobulin-E; IL-3: interleukin-3.

numerous and complex: on the basis of present knowledge, it is difficult to ascribe the enhanced basophil releasability of patients with respiratory allergy to a single factor, either intrinsic or extrinsic.

A great variability of basophil response to activating agents was observed both in allergic patients and healthy subjects. To evaluate whether the failure to find an inhibitory effect of Na⁺ in allergic patients was related to the higher frequency of high releasers, the patients and the control subjects were divided into three groups according to the magnitude of basophil response, and the behaviour was examined in a low Na⁺ medium. Different responses were found in controls and allergics which were independent of the basophil histamine releasability. Therefore, the defective inhibition by Na⁺ is typical of the allergic status and is unrelated to the intensity of basophil response. This failure in the inhibitory effect of Na⁺ may be one of the factors responsible for the enhanced basophil releasability in allergics.

It has been demonstrated that the inhibitory effect of Na⁺ is linked to its intracellular concentration, and the mechanism of action is probably related to the modulation of membrane potential and intracellular pH [13, 15, 33]. The defective inhibition of Na⁺ on basophil histamine release in allergic patients could be due to an intrinsic basophil abnormality or to the effect of an extrinsic (soluble) factor. To further investigate this point, basophils from healthy subjects were incubated with sera from atopic patients and then stimulated in high and low Na⁺ solutions. The incubation with sera from allergic patients failed to influence the behaviour of normal basophils in a significant way. These data apparently suggest that the reduced sensitivity to the inhibitory effect of Na⁺ in allergic patients is due to an intrinsic cellular abnormality rather than to a soluble serum factor. This finding should, however, be interpreted cautiously, since we cannot ignore the fact that serum freezing, storage and thawing may have altered the capacity to influence basophil behaviour in a low-Na⁺ medium. The reduced sensitivity to the inhibitory effect of Na⁺ in allergics could, in fact, be the result of cellular priming, possibly due to cytokines or other soluble factors with a consequent active status of receptors. Another explanation of the defective Na⁺ inhibition could be an alteration in the mechanisms in either of Na⁺ loading or Na⁺ depletion in basophils from allergic patients.

Some alterations regarding Na⁺ transport and metabolism have been observed in allergic patients, SKONER

et al. [17] have documented a reduction of Na⁺, K⁺ adenosine triphosphate (ATPase) enzyme activity in the platelets of allergic patients, due to a circulating inhibitor, and this in turn could influence the intracellular concentration of these monovalent cations. TRIBE *et al.* [18] have shown that patients with airway hyperreactivity have an increased Na⁺ influx into peripheral blood leucocytes, stimulated by a serum-borne factor. An increased Na⁺ influx and an inhibited Na⁺, K⁺ ATPase could have profound effects on Na⁺ homeostasis and, in particular, on Na⁺/Ca²⁺ exchanges. These alterations could influence the functional activity of several cell types, including basophils. As yet, little is known about ion pathways through the basophil membrane. Preliminary data indicate that IgE-dependent activation of human basophils leads to the opening of nonselective cation channels [34]. Furthermore, it seems that Na⁺/Ca²⁺ exchange is operating in human basophils, and that IL-3 exerts its enhancing effect on IgE-mediated histamine release by promoting the exchange of intracellular Na⁺ with extracellular Ca²⁺ [35]. Further studies are needed in order to investigate how the nonselective cation channels and the Na⁺/Ca²⁺ exchanger operate in basophils from healthy subjects and atopic patients.

In conclusion, our data indicate that basophils from patients with allergic rhinitis or allergic bronchial asthma release more histamine than basophils from healthy subjects either spontaneously or on challenge with anti-immunoglobulin-E, N-formyl-methionyl-leucyl-phenylalanine and interleukin-3. Furthermore, at physiological concentrations, Na⁺ exerts an inhibitory effect on basophil histamine release in healthy subjects, but not in atopic patients. The impairment of the inhibitory effect of Na⁺ may be one of the causes of the enhanced basophil histamine release in patients with respiratory allergy.

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