

Regulation of human basophil activation

III. Impairment of the inhibitory effect of Na⁺ on IgE-mediated histamine release in patients with allergic rhinitis

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We recently observed that external Na⁺ inhibited the IgE-dependent human basophil histamine release (HR) in normal subjects. In this article we report differences in the Na⁺ effect on basophil HR between normal subjects (n = 16) and age matched patients with allergic rhinitis (AR) (n = 18). As expected, in vitro anti-IgE-stimulated basophils from the group with AR released greater amounts of histamine than basophils from the normal group. However, removal of external Na⁺ (and replacement by N-methyl-D-glucamine) abolished this difference between the two groups. HR in the normal group increased to the same high level as that of the group with AR. By contrast, the release of histamine in the group with AR was not further increased by Na⁺ removal. Although high releasers were more frequent in the group with AR, the absence of effect after Na⁺ removal was not due to the high basal release level (in the presence of Na⁺) because no effect after Na⁺ removal was also observed with medium releasers. These results strongly suggest that increased basophil HR in populations with AR, and possibly in other allergic populations, is linked to a defect in the inhibitory effect of Na⁺. (J ALLERGY CLIN IMMUNOL 1992;90:52-8)

Key words: Immediate hypersensitivity, allergic rhinitis, basophils, histamine release, Na⁺

The presence of specific IgE in serum and on cell membranes is widely recognized as a major way to differentiate allergic from normal subjects.¹ Also, other antibodies of the IgG class are suspected to play an important role in allergic symptoms.² However, presence and concentrations of specific anaphylactic antibodies do not always correlate with the severity of the allergic disorders.³ Thus, besides an alteration of the IgE response, it has been proposed that immediate hypersensitivity is due to an increase in the capacity of the effector cells to release mediators, that is, "releasability,"⁴ and to a hyperresponsiveness of the target organs.⁵ Many authors have reported that IgE-mediated HR in some allergic diseases was indeed increased compared with HR in normal subjects.⁶

Abbreviations used

HR: Histamine release

AR: Allergic rhinitis

However, releasability had no biochemical basis. This finding was mainly due to the difficulties in obtaining large amounts of purified basophils for biochemical studies. We recently demonstrated an inhibition by Na⁺ influx of IgE-mediated human basophil HR in normal subjects.⁷ It was thus tempting to determine whether the Na⁺ inhibitory effect was altered in basophils from patients suffering an allergic disease with enhanced releasability. Indeed, we report that the increased IgE-mediated HR of basophils from patients with AR is linked to a decrease of the inhibitory effect of Na⁺.

MATERIAL AND METHODS

Patients

Eighteen patients (eight male and 10 female patients), with a mean age (± 1 SD) of 41.7 ± 14.9 years, were selected according to a clinical history of AR confirmed by in vitro leukocyte HR in response to the relevant allergen.

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The involved allergens were grass pollens ($n = 10$) and *Dermatophagoides pteronyssinus* ($n = 2$), and animal danders, cat ($n = 6$), mouse, and rat ($n = 3$). The study was performed when the subjects were free of symptoms. Some patients were sensitized to two allergens, and their basophils were tested accordingly. Control subjects consisted of 16 individuals (nine male and seven female subjects; mean age, 37.9 ± 12.1 years) without clinical history of allergy. Both allergic and normal subjects were volunteers, and informed consent was obtained. For ethical reasons, skin tests to multiple allergens were not performed on healthy subjects to confirm their nonallergic status. Indeed, we are not certain that such treatment is without consequence on further immune or allergic response. Neither allergic nor control subjects received any medication for at least 1 month before the study.

Reagents

Goat Fc-specific, antihuman IgE (Nordic Immunological Laboratories, Tilburg, The Netherlands), N-methyl-D-glucamine (Sigma Chemical Co., St. Louis, Mo.), fura-2 (Calbiochem, La Jolla, Calif.), and allergen extracts (*D. pteronyssinus*, mixed grass pollens, and animal danders: cat, mouse, and rat, Laboratoire des Stallergènes, Joinville-le-Pont, France) were purchased as indicated.

Leukocyte HR

HR was measured as previously described.⁸ After sedimentation with dextran, the leukocyte-rich supernatant was twice washed in HEPES-buffered isotonic solution (NaCl, 140 mmol/L; KCl, 2.6 mmol/L; HEPES, 10 mmol/L; glucose, 5.5 mmol/L, pH 7.4) or in "low Na⁺" saline solution in which Na⁺ was isoosmotically replaced with N-methyl-D-glucamine and pH was readjusted to 7.4 with HCl. Osmolarity was checked with an osmometer, and the Ca⁺⁺ concentration (approximately 1 μ mol/L) was measured in saline solution before any Ca⁺⁺ addition with the fura-2 method.⁹ Na⁺ concentration in low Na⁺ saline solution was lower than 4 mmol/L, as measured by indirect potentiometric method. Leukocytes were finally suspended at 30 to 40 basophils per cubic millimeter, either in low Na⁺ or in 140 mmol/L of Na⁺ saline solution. The leukocyte-rich cell suspensions were then aliquoted (400 μ l) in plastic tubes containing 50 μ l of anti-IgE antiserum or specific allergen at defined concentrations (or buffer alone as control) and 50 μ l of CaCl₂ (2 mmol/L, final concentration) or buffer alone. After an incubation in a water bath (37° C for 30 minutes), the tubes were centrifuged (700 g for 20 minutes), and 300 μ l of each supernatant was added to 300 μ l of HClO₄, 0.8N. Total histamine content was assessed by adding 300 μ l of HClO₄, 0.8N to 300 μ l of cell suspension. After centrifugation, the HClO₄ extracts were kept at 4° C until histamine determination was performed with an automated spectrofluorometric assay.¹⁰ Percentages of HR were calculated as follows:

$$100 \times (\text{test} - \text{control}) / (\text{total} - \text{control})$$

where "test," "control," and "total" are the histamine contents to be measured in the supernatant, in the control (with-

out stimulus), and in the "total histamine" tubes, respectively. None of the reagents used interfered with the histamine assay.

Statistical studies

Statistical comparisons were performed with the Wilcoxon's rank-sum test. It has been suggested⁶ that parametric tests for statistical studies cannot be used for releasability comparison studies because there is a great variability in release in atopic as well as in normal subjects. However, reevaluation of our data with Student's *t* test yielded similar conclusions with that of the Wilcoxon's test. Statgraphics software (STSC, Inc., Rockville, Md.) was used for calculations.

RESULTS

Mean HR from basophils induced by anti-IgE (1 μ g/ml) was approximately 50% higher in allergic patients compared with that in normal subjects (Fig. 1) ($64.8\% \pm 4.9\%$ versus $40.4\% \pm 5.4\%$, respectively; $p < 0.01$). This increased HR in allergic patients has been previously reported,¹¹⁻¹⁵ particularly in patients with AR.^{16, 17} We also observed the usual variability in leukocyte HR⁸ in both groups (from 12% to 72% in the normal subjects and from 28% to 100% in the group with AR). Thus, it is clear that, although there is a significant difference between the two groups, the release level is not a sufficient criterion to categorize a given subject as allergic or normal. Since we recently demonstrated that Na⁺ exerted an inhibitory effect on leukocyte HR,⁷ we tested whether the absence of the inhibitory effect of Na⁺ could be responsible (1) for the high release observed in some subjects (allergic or nonallergic) and/or (2) for the higher release in the allergic group taken as a whole. To answer these questions, the leukocytes were stimulated by anti-IgE in saline solution with or without 140 mmol/L of Na⁺ (in the latter case Na⁺ was replaced by 140 mmol/L of the nonpermeant Na⁺ substitute, N-methyl-D-glucamine) in the presence of 2 mmol/L of Ca⁺⁺ or without Ca⁺⁺. In low Na⁺ medium and 2 mmol/L of Ca⁺⁺, HR was enhanced in normal patients as we previously observed (Fig. 2) (from $40.4\% \pm 5.4\%$ in 140 mmol/L of Na⁺ to $63.4\% \pm 5.5\%$ in low Na⁺; $p < 0.001$). (For clarity, release levels and statistical significance are given in the text only for the maximal release, i.e., with anti-IgE at 1 μ g/ml.) In contrast, no statistically significant difference was observed in the group with AR after Na⁺ removal ($61.7\% \pm 5.7\%$ in 140 mmol/L and $66.7\% \pm 8.0\%$ in low Na⁺). It is important to note that the levels of HR in low Na⁺ and 2 mmol/L of Ca⁺⁺ from the basophils of normal subjects ($63.4\% \pm 5.5\%$) and groups with AR ($66.7\% \pm 8.0\%$) were not significantly different. Thus, the re-

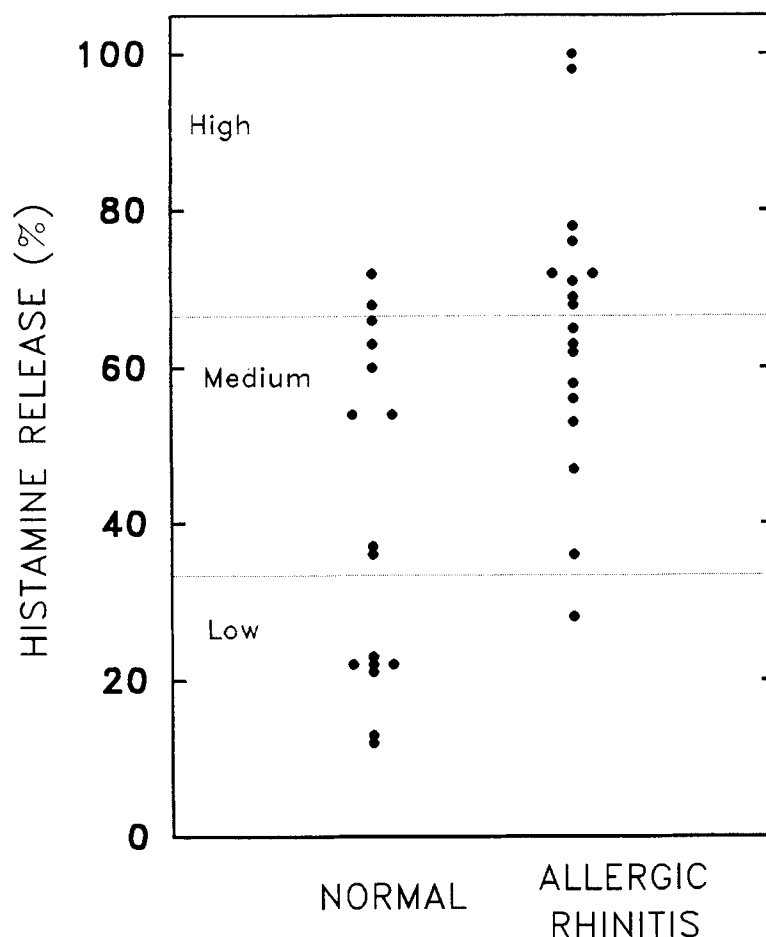


FIG. 1. Anti-IgE-induced HR from leukocytes from normal subjects ($N = 16$) and from patients with AR ($N = 18$) was performed in standard saline solution (140 mmol/L of Na^+ and 2 mmol/L of Ca^{++}) in the presence of anti-IgE, 1 $\mu\text{g}/\text{ml}$. After incubation (30 minutes at 37°C), histamine was measured in the supernatants. Data in Figs. 1 through 4 were obtained from experiments performed in parallel with the same subjects.

leasability status of basophils from patients with AR, taken as a whole, was identical with that of basophils from normal subjects incubated in the absence of Na^+ . The inhibitory effect of Na^+ could, however, be demonstrated in patients with AR but only in low Ca^{++} (from $16.2\% \pm 3.7\%$ in low Na^+ to $1.6\% \pm 1.4\%$ in 140 mmol/L of Na^+ ; $p < 0.001$). An unexpected result was observed in low Na^+ and low Ca^{++} . The release was significantly lower in the group with AR than in the normal group ($16.2\% \pm 3.7\%$ versus $29.8\% \pm 4.6\%$, respectively; $p < 0.05$). We then studied whether the effect of Na^+ could be evidenced after stimulation by the specific allergens (cat danders and grass pollens). Of course, no comparison with a normal group could be made. However, again no inhibitory effect of Na^+ was clearly evidenced at 2 mmol/L of Ca^{++} , even at moderate release with

suboptimal allergen concentration (Fig. 3). Inhibition by Na^+ in low Ca^{++} was also observed.

As stated above, there was great variability in both groups (Fig. 1). Thus, it was not clear whether the absence of the Na^+ effect in the group with AR was related to the higher frequency of high releasers or to the AR status. We thus examined these data by dividing the subjects into three groups according to the release in standard conditions (140 mmol/L of Na^+ and 2 mmol/L of Ca^{++}): low (0% to 33% of HR with 1 $\mu\text{g}/\text{ml}$ of anti-IgE), medium (34% to 66%), and high (67% to 100%) releasers. As depicted in Fig. 4, removal of Na^+ -enhanced anti-IgE-induced HR in the three normal groups. By contrast, the HR was not increased in the allergic groups, whatever the basal release in standard medium. The results in AR and normal medium releasers are particularly demonstra-

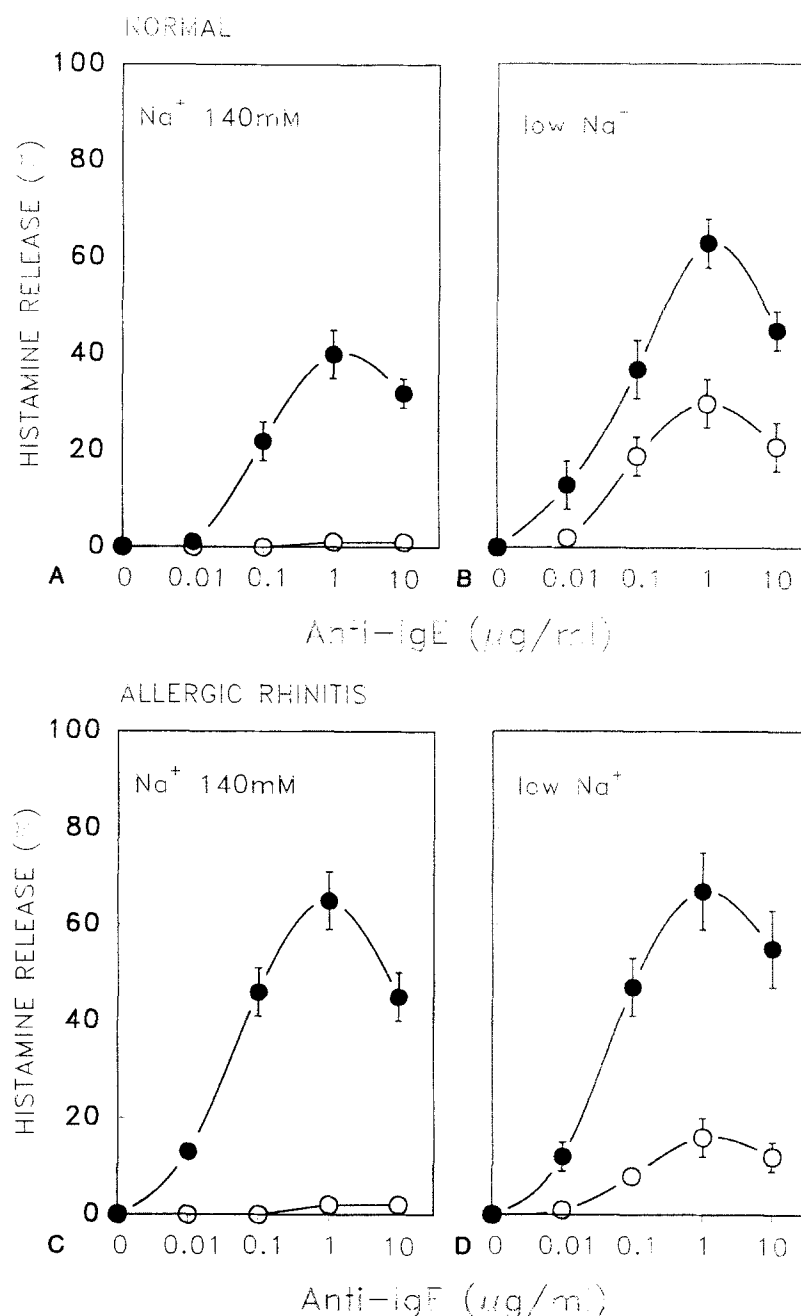


FIG. 2. Effect of external Na⁺ on leukocyte HR in normal subjects and in patients with AR. **A** and **B**, Anti-IgE-induced HR from human leukocytes from normal subjects. **C** and **D**, From patients with AR. **A** and **C**, Performed in saline solution containing 140 mmol/L of 140 Na⁺. **B** and **D**, 140 mmol/L of *N*-methyl-D-glucamine as an Na⁺ substitute in the presence of Ca²⁺ (●) or no Ca²⁺ (○). After incubation (30 minutes at 37° C), histamine was measured in supernatants. Results are means of net percent of HR ± SEM (N = 16, normal patients; N = 18, allergic patients).

tive. Indeed, the moderate release in these groups is well suited to reveal any modulation thereof. As depicted in Fig. 4, the release in normal medium releasers was enhanced by Na⁺ removal from

52.8% ± 4.5% to 70% ± 4.2%; $p < 0.05$), whereas the release in the corresponding group with AR was not significantly different, with or without Na⁺, from 55% ± 3.5% to 46.2% ± 12.6%, respectively.

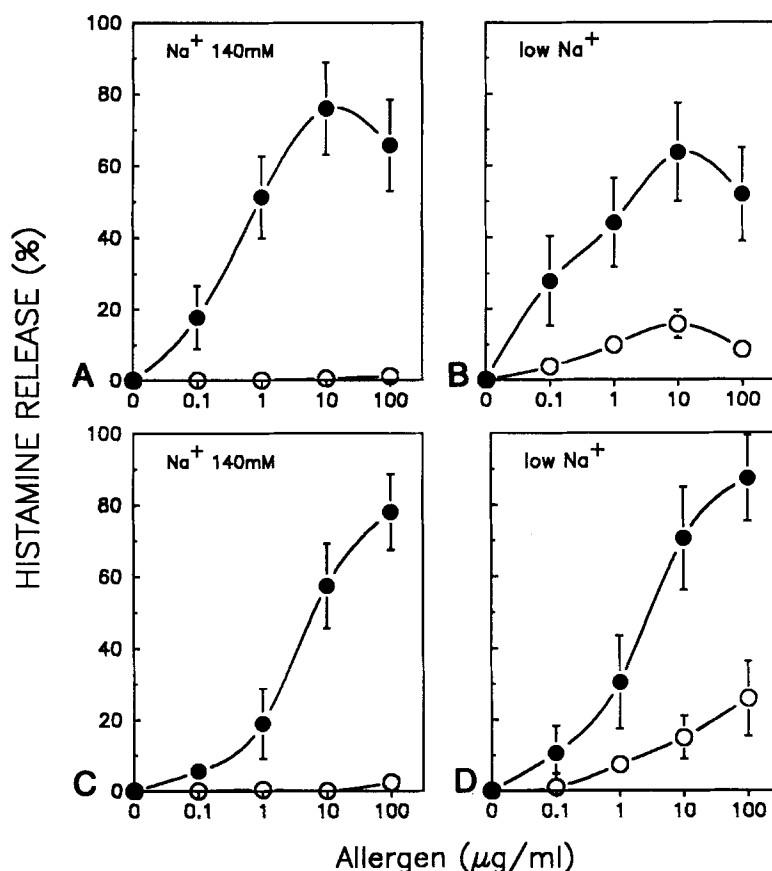


FIG. 3. Effect of external Na^+ on allergen-induced HR from human leukocytes from allergic patients. **A** and **B**, Grass pollen. **C** and **D**, Cat danders. **A** and **C**, Performed in saline solution containing 140 mmol/L of Na^+ . **B** and **D**, Or performed in low Na^+ in presence of Ca^{++} (●) or no Ca^{++} (○). After incubation (30 minutes at 37° C), histamine was measured in supernatants. Results are means of net percent of HR $\pm$ SEM (**A** and **B**, N = 10; **C** and **D**, N = 6).

DISCUSSION

Basophil HR after anti-IgE^{6, 17} or concanavalin A¹⁶ challenge has been reported to be enhanced in patients with AR compared with that in nonallergic subjects. This has also been demonstrated in atopic dermatitis.¹³ However, in chronic urticaria, less HR is obtained than that in a control group.¹⁸ This is believed to be due to in vivo desensitization to IgE-dependent stimulus. In asthma, allergic or nonallergic, these data appear more heterogeneous, since, according to some studies, there is no enhanced release,^{19, 20} whereas other studies reported an increased release^{11, 12, 15} after anti-IgE challenge. Possible differences¹² in technical procedures and patient selections could explain these discrepancies.

The releasability concept was proposed to explain differences in release between atopic and normal subjects that could not be explained by membrane IgE densities. Thus, it has been clearly demonstrated that

the latter was a function of plasma IgE concentration but was not at all correlated with the extent of the IgE-mediated release.⁴ Releasability was demonstrated to be linked to the ability to respond to dimeric cross-links.²¹ Thus, high responders were demonstrated to release to dimeric as well as to trimeric IgE. In contrast, basophils from low responders released only to trimeric IgE. Moreover, the trimer IgE concentration for 25% HR was lower in high releasers.²¹ This finding strongly suggested that, in basophils with enhanced releasability, a lower degree of Fcε receptor cross-linking is sufficient for the release. Releasability was then defined as the property to respond to "weak" stimuli. Thus, an intrinsic parameter, crucial for the release level, was postulated. Extrinsic factors have also been postulated to explain releasability. Thus, some authors suggested heterogeneity between IgE molecules, with some IgE supposedly being more "efficient" in sensitizing target cells.^{11, 22, 23} Other au-

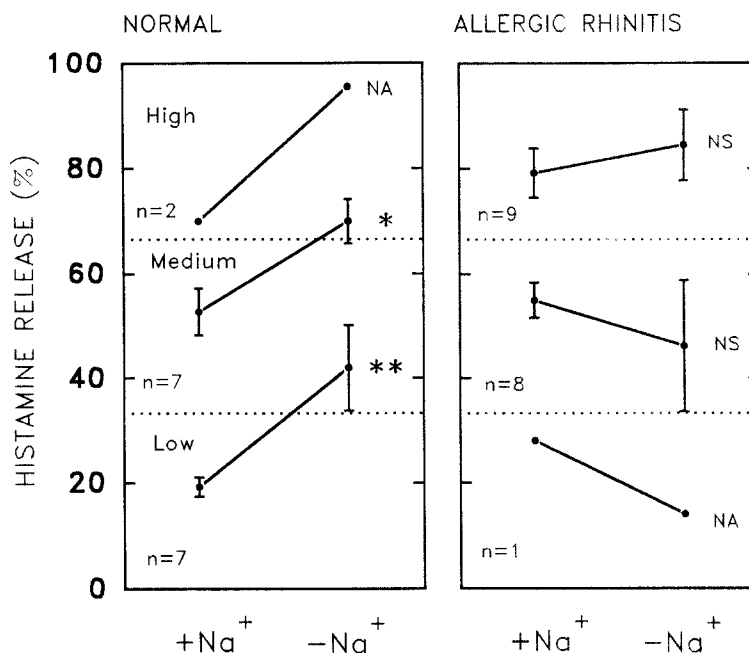


FIG. 4. Effect of external Na⁺ on leukocyte HR in low, medium, and high releasers. Subjects with AR and normal subjects were divided into three groups according to HR level at 1 μ g/ml of anti-IgE (see Fig. 1). For each group, HR in presence of Na⁺ was compared with HR in absence of Na⁺; ** $p < 0.01$; * $p < 0.05$; NS, not significant; NA, not applicable (sample too small for statistical study).

thors proposed that in vivo activation by histamine-releasing factors was responsible for increased releasability, as evidenced by increased spontaneous release in atopic patients, particularly in the presence of deuterium oxide.^{24, 25}

A previous article from Hook and Siraganian²⁶ demonstrated that replacement of external Na⁺ with a nonpermeant Na⁺ substitute (choline chloride) enhanced HR. However, the mechanisms of this effect was not further explored. We recently demonstrated that external Na⁺ at physiologic concentration inhibits HR from human basophils.⁷ This inhibition was observed with anti-IgE, calcium ionophore A23187, and f-met peptide. However, for calcium ionophore A23187 and f-met peptide, this effect was observed in <1 mmol/L of external Ca⁺⁺. An effect of external Na⁺ was observed at physiologic concentrations (1 to 2 mmol/L) only with anti-IgE. We thus studied if a failure of the inhibitory effect of Na⁺ could explain the increased anti-IgE-induced HR previously observed in allergic patients, particularly in patients with AR.

Our data indicate that removal of external Na⁺ allows basophils from a normal group to release histamine at the same high level as basophils from a group with AR. By contrast, the HR from basophils of the

population with AR was not enhanced by this treatment. Since high releasers are more frequent in the allergic population, we then considered whether the absence of Na⁺ effect in this population was due to the high percentage of high releasers or to AR as a variate. Indeed, one possibility was that no effect of Na⁺ removal was observed in the allergic group because the HR was already maximal and thus could not be further enhanced. It is clearly indicated in Fig. 4 that Na⁺ removal increases the release in the normal group but not in the group with AR, independently of the basal release in the presence of Na⁺. These results suggest a better definition of the "releasability" concept. Indeed, in both groups with AR and normal populations, there is a great variability between the donors; high releasers are present as well in a non-allergic population. Superimposed on this basal releasability could be a second type of releasability that, as demonstrated by our data about AR, would be linked to the allergic status. In this hypothesis, only this second type of releasability, which is linked to a defective Na⁺ inhibition, appears to be enhanced in the group with AR.

In conclusion, we propose that there is a failure in the inhibitory effect of Na⁺ on IgE-mediated basophil HR in patients with AR. It remains unknown if this

defect could be extended to other allergic diseases with enhanced releasability. However, this model could add to our understanding of the biochemical basis of immediate hypersensitivity and allow for the screening of new antiallergic drugs.

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REFERENCES

- Ishizaka T, De Bernardo R, Tomioka H. Identification of basophil granulocyte as a site of allergic histamine release. *J Immunol* 1972;108:1000-8.
- Parish WE. The clinical relevance of heat-stable short-term sensitizing anaphylactic IgG antibodies (IgG S-TS) and of related activities of IgG4 and IgG2. *Br J Dermatol* 1981;105:223-31.
- Lichtenstein LM, Ishizaka K, Norman PS, Sobokta AK, Hill BM. IgE antibody measurements in ragweed hay fever. Relationship to clinical severity and the results of immunotherapy. *J Clin Invest* 1973;52:472-82.
- Conroy MC, Adkinson NF Jr, Lichtenstein LM. Measurements of IgE on human basophils: relation to serum IgE and anti-IgE-induced histamine release. *J Immunol* 1977;118:1317-21.
- Mathé AA, Hedqvist P, Holmgren A, Svanborg N. Bronchial hyperreactivity to prostaglandin F₂-alpha and histamine in patients with asthma. *Br Med J* 1973;1:193-6.
- Marone G, Casolaro V, Ayala F, Melillo G, Condorelli M. Releasability in allergic disorders. In: Mellilo G, Norman PS, Marone G, eds. *Clinical immunology: respiratory allergy*, vol 2. Toronto, Philadelphia: BC Decker, 1990:67-77.
- Beauvais F, Shimahara T, Inoué I, Hiéblot C, Burtin C, Benveniste J. Regulation of human basophil activation. II. Histamine release is potentiated by K⁺ efflux and inhibited by Na⁺ influx. *J Immunol* 1992;148:149-54.
- Beauvais F, Bidet B, Descours B, Hiéblot C, Burtin C, Benveniste J. Regulation of human basophil activation. I. Dissociation of cationic dye binding from histamine release in activated human basophils. *J ALLERGY CLIN IMMUNOL* 1991;87:1020-8.
- Gryniewicz G, Poenie M, Tsien RY. A new generation of Ca²⁺ indicators with greatly improved fluorescence properties. *J Biol Chem* 1985;260:3440-50.
- Ruff F, Saindelle A, Dutripon E, Parrot JL. Continuous automatic fluorometric evaluation of total blood histamine. *Nature* 1967;214:279-81.
- Assem ESK, Attalah NA. Increased release of histamine by anti-IgE from leucocytes of asthmatic patients and possible heterogeneity of IgE. *Clin Allergy* 1981;11:367-74.
- Gaddy NK, Busse WW. Enhanced IgE-dependent basophil histamine release and airway reactivity in asthma. *Am Rev Respir Dis* 1984;134:969-74.
- Marone G, Giuliano R, Lembo G, Ayala F. Human basophil releasability. II. Changes in basophil releasability in patients with atopic dermatitis. *J Invest Dermatol* 1986;87:19-23.
- Plaut M, Kazimierzczak W, Lichtenstein LM. Abnormalities of basophil "releasability" in atopic and asthmatic individuals. *J ALLERGY CLIN IMMUNOL* 1986;78:968-73.
- Casolaro V, Galeone D, Giacommo A, Sanduzzi A, Melillo G, Marone G. Human basophil/mast cell releasability. V. Functional comparisons of cells obtained from peripheral blood, lung parenchyma, and bronchoalveolar lavage in asthmatics. *Am Rev Respir Dis* 1989;139:1375-82.
- Busse WW, Swenson CA, Sharpe GA, Koschat M. Enhanced basophil histamine release to concanavalin A in allergic rhinitis. *J ALLERGY CLIN IMMUNOL* 1986;78:90-7.
- Casolaro V, Spadaro G, Marone G. Human basophil releasability. VI. Changes in basophil releasability in patients with allergic rhinitis and bronchial asthma. *Am Rev Respir Dis* 1990;142:1106-11.
- Kern F, Lichtenstein LM. Defective histamine release in chronic urticaria. *J Clin Invest* 1976;57:1369-77.
- Findlay SR, Lichtenstein LM. Basophil "releasability" in patients with asthma. *Am Rev Respir Dis* 1980;122:53-9.
- Peters SP, Tung RS, Chatham M, Bleecker ER, Lichtenstein LM. Inhibitory and enhanceability of basophil histamine release in asthmatic and normal subjects. *Int Arch Allergy Appl Immunol* 1985;76:344-9.
- MacGlashan DW Jr, Peters SP, Warner J, Lichtenstein LM. Characteristics of human basophil sulfidopeptide leukotriene release: releasability defined as the ability of the basophil to respond to dimeric cross-links. *J Immunol* 1986;136:2231-9.
- Conroy MC, Adkinson NF Jr, Lichtenstein LM. Passive sensitization of human basophils: evidence for heterogeneity in the IgE molecule. *Int Arch Allergy Appl Immunol* 1979;60:106-9.
- MacDonald SM, Lichtenstein LM, Proud D, et al. Studies of IgE-dependent histamine releasing factors: heterogeneity of IgE. *J Immunol* 1987;139:506-12.
- Tung R, Lichtenstein LM. In vitro histamine release from basophils of asthmatic and atopic individuals in D₂O. *J Immunol* 1982;128:2067-72.
- Sampson HA, Broadbent KR, Bernhisel-Broadbent J. Spontaneous release of histamine from basophils and histamine-releasing factor in patients with atopic dermatitis and food hypersensitivity. *N Engl J Med* 1989;321:228-32.
- Hook WA, Siraganian RP. Influence of anions, cations, and osmolarity on IgE-mediated histamine release from human basophils. *Immunology* 1981;34:723-31.