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Cutaneous Histamine Levels and Histamine Releasability from the Skin in Atopic Dermatitis and Hyper-IgE-Syndrome

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Summary. We determined the histamine content in the skin of 22 adults with atopic dermatitis, one patient with hyper-IgE-syndrome, and 20 controls by the enzymatic double isotope assay. In addition, we performed a pilot study of histamine degradation in the skin. We tested, furthermore, the releasability of histamine from skin sections of patients with atopic dermatitis and healthy controls upon challenge with acetylcholine, anti-IgE, and compound 48/80. Histamine was also determined in 13 plasma specimens and was always < 1 ng/ml. The mean $\pm$ SEM histamine concentration in the skin was 196 ± 30 ng/mg protein in controls and 262 ± 68 ng/mg protein in atopic dermatitis (no statistically significant difference). One control and three patients with atopic dermatitis exhibited a slight, the hyper-IgE patient a marked, elevation of the skin histamine content. No gross differences in the degradation rate of histamine were observed between patients and controls. Acetylcholine and 48/80 induced the same histamine release in both groups; with anti-IgE, almost the double amount of histamine was released from the skin of atopic dermatitis patients as compared to controls. These findings suggest an enhanced releasability of histamine upon immunologic challenge in atopic dermatitis.

Key words: Atopic dermatitis – Hyper-IgE-syndrome – Skin histamine content – Histamine releasability

Introduction

In atopic dermatitis, the participation of histamine in the pathogenetic chain may be suspected from the intensive itching and partial effectiveness of classic (H_1) antihistamines in the suppression of symptoms. However, only a few studies have been concerned with the direct determination of histamine in the skin of affected individuals. Therefore, we measured the histamine content in the skin of normal controls and patients with atopic dermatitis with the highly sensitive and specific enzymatic double isotope assay [1]. Furthermore, a pilot study of enzymatic degradation of histamine in the normal and affected skin was performed. Plasma histamine concentration and serum IgE levels were also determined. However, observed tissue levels of histamine are a result of its local production, release, and catabolism. For example, a high elimination rate of histamine from the skin can results in normal concentrations even in the case of high histamine release from the mast cells [2]. Thus, it was postulated by Lichtenstein et al. [3] and Ring [4] that the state of atopy is characterized by an increased releasability of mast cells. Although this hypothesis has not yet been experimentally proved in the skin, an enhanced histamine releasability from basophils was shown by Ring and O'Connor [5] in patients with atopic dermatitis.

A study of the dynamic components is, therefore, necessary. In the present study, we determined the releasability of histamine from skin sections of patients with atopic dermatitis and healthy controls by means of well-established releasing substances: acetylcholine [6, 7], anti-IgE [6, 8], and compound 48/80 [6, 9, 10].

Patients

Twenty-two adult inpatients of the Department of Dermatology, Düsseldorf University, were included in the study (14 males, eight females, age 15 – 71 years). The severity of the disease was graded on a scale from 1 (minimum) to 4 (maximum) (score 1 – one patient; score 2 – ten patients; score 3 – eight patients; score 4 – three patients). A 42-year-old male patient with the hyper-IgE-syndrome [11] and excessive symptomatology, including frequent skin infections resulting in scar formation, had a score of 5. He died of his disease 1 year after the present evaluation. The patients did not take any drugs prior to skin biopsy and no external steroid preparations were applied. Skin biopsies, approximately 0.5 cm^2 in size, were taken

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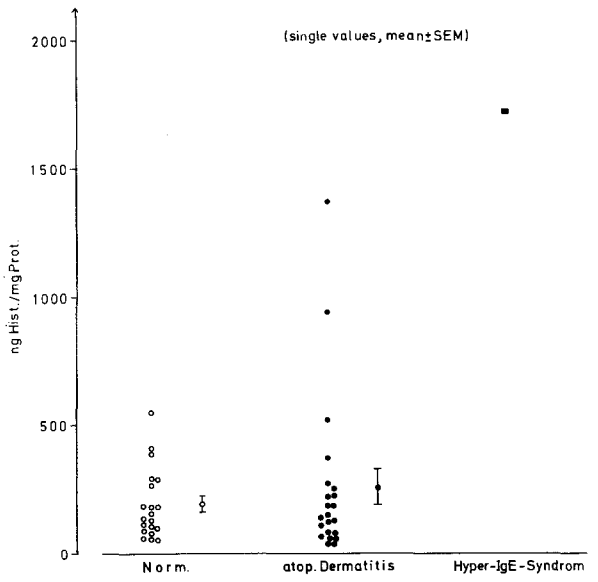

Fig. 1. Histamine content in the skin

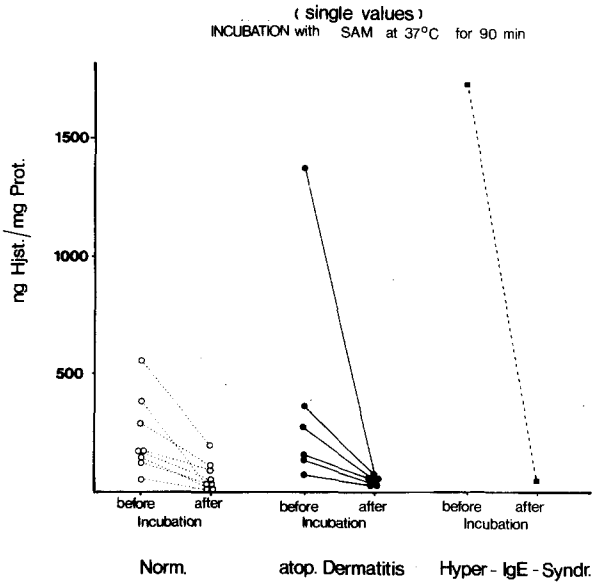

Fig. 2. Histamine degradation in skin homogenates in vitro

from the most severely affected areas after local anesthesia with 2% mepivacaine (1 ml). Unaffected skin was not examined for ethical reasons. Normal skin was obtained from 20 patients undergoing surgery for minor benign tumors (12 males, eight females, age 18–67 years). No skin from the head was used. A small specimen of the skin was submitted for routine histologic examination, the major part was immediately frozen and kept at −20° C until use for a maximum of 5 weeks.

Materials and Methods

The skin samples were minced with fine scissors and homogenized with an Ultra-Turrax homogenizer in Hank's saline in an ice bath. Residual tissue histamine was extracted by boiling for 5 min, the specimens were centrifuged at 300 × *g* for 10 min, and the connective tissue was discarded. The histamine content of the supernatants was determined by the enzymatic double isotope assay of Beaven et al. [1] using S-adenosyl-1-methyl-^{14}C-methionine (sp. act. 62 mCi/mmol, Amersham, England) and histamine N-methyl-transferase prepared from guinea pig brains. The histamine catabolic activity of the skin homogenates was measured following the assumption of Francis et al. [12] that N-methylation is the only important catabolic pathway in the skin. Skin homogenates of seven patients and eight controls were incubated with S-adenosyl-methionine (100 nmol/l, Serva, Heidelberg, Federal Republic of Germany) for 90 min at 37° C. The pre- and postincubation histamine concentrations were determined as described above.

For determination of histamine release, the fresh skin specimens were minced with fine scissors in Hank's balanced salt solution (HBSS) without Ca^{2+} and Mg^{2+}, pH 7.4, in an ice bath. The obtained skin sections of approximately 1 mm^3 were divided into four aliquots and incubated for 10 min at 37° C with HBSS containing Ca^{2+} and Mg^{2+} for spontaneous release and acetylcholine perchlorate (Merck, Darmstadt, FRG) (10^{-8} M), anti-IgE (Pharmacia, Freiburg, FRG) (3 µg/ml), and compound 48/80 (Sigma Chem. Co., St. Louis, USA) (10^{-7} M). All incubations were done in duplicate. The incubation was stopped by cooling the samples in ice. The tubes were centrifuged for 10 min at 300 × *g* and the histamine content was measured in the supernatants and sediments. The histamine released by the challenging substances was expressed as a percent of total histamine content and corrected for spontaneous release by HBSS. The protein content was measured with the method of Lowry et al. [13]. Serum IgE determinations were carried out with a commercial PRIST kit (Pharmacia, Freiburg, FRG).

Results

The cutaneous histamine levels are summarized in Fig. 1. The mean values ± SEM of the histamine content in the skin were 196 ± 30 ng histamine/mg protein (range 55–551) for the healthy probands and 262 ± 68 ng histamine/mg protein (range 39–1375) for the atopic dermatitis patients. The patient with the hyper-IgE-syndrome had a skin histamine concentration of 1,726 ng histamine/mg protein. The original and log-transformed data were analyzed using the Kolmogoroff-Smirnov test, the *F* test, the Mann-Whitney test, and the Student's *t* test. Three of the 22 patients with atopic dermatitis and one of he 20 controls had histamine levels in the skin higher than 2 SD above the mean of controls. There was no significant difference on statistical analysis between the values from patients with atopic dermatitis and non atopics. Plasma histamine levels of 13 patients and 11 healthy probands were all below 1 ng/ml. Serum IgE levels were elevated in 20 of 22 patients. There was no correlation between skin histamine leevel, severity of the the disease, and serum IgE level. Extensive histamine degradation occurred after incubation of the skin homogenates with S-adenosyl-methionine from patients with atopic dermatitis, hyper-IgE-syndrome, and healthy probands (Fig. 2). No significant differences in the remaining histamine content were found between the groups.

The results of the histamine release experiments are summarized in Table 1. The histamine release from skin

Table 1. Releasability of histamine from skin sections of patients with atopic dermatitis and controls. Values represent mean ± SEM and are corrected for spontaneous release (% of total histamine released by challenging substance minus % spontaneous release)

	HBSS	ACh	anti-IgE	48/80
Controls	31.6 ± 4.2 ($n = 9$)	7.0 ± 3.5 ($n = 7$)	16.1 ± 5.6 ($n = 9$)	17.5 ± 5.0 ($n = 8$)
Patients	25.9 ± 4.0 ($n = 9$)	5.1 ± 3.2 ($n = 6$)	31.8 ± 5.7 ($n = 9$)	15.7 ± 4.6 ($n = 9$)
Significance	$P < 0.05$	ns	$P < 0.05$	ns

Releasing substances: Hank's balanced salt solution (HBSS) = spontaneous release, acetylcholine perchlorate (ACh) (10^{-8} M), anti-IgE (3 µg/ml), and compound 48/80 (10^{-7} M)

sections incubated with HBSS containing Ca^{2+} and Mg^{2+} was only defined as spontaneous release. The spontaneous release was 31.6% ± 4.2% (mean ± SEM) of the total histamine content for the controls and 25.9% ± 4.0% for the patients with atopic dermatitis ($P < 0.05$).

The histamine release from skin sections challenged with acetylcholine was only slightly greater than the spontaneous histamine release. No significant difference was noted for the the corrected values between the control and atopic dermatitis groups (7.0% ± 3.5% vs 5.1% ± 3.2%). Compound 48/80 induced in both controls and atopic dermatitis patients a histamine release of 17.5% ± 5.0% and 15.7% ± 4.6%, respectively. The difference between the groups was not statistically significant.

In striking contrast, the challenge of the skin sections with anti-IgE yielded a histamine release in atopic dermatitis patients which was almost twice the value of controls (31.8% ± 5.7% for patients, 16.1% ± 5.6% for controls, $P < 0.05$). No strict correlation between IgE levels and anti-IgE-induced histamine release was observed.

Discussion

In atopic dermatitis, itching is the predominant symptom, and thus one might expect elevated values of histamine in the skin dur to increased synthesis or storage. On the other hand, lowering of the histamine content by continuous release from mast cells is also conceivable. Direct determinations of histamine content and metabolism in tissues were previously hampered by methodological difficulties yielding widely differing results [2]. The skin content of histamine in atopic dermatitis was tested by Johnson et al. [14]. They found increased values in patients, but they concluded that histamine did not seem to be of major significance in the cutaneous alterations in the disease. However, the dinitrofluorobenzene method of Lowry used in this study is neither very specific nor sensitive [2].

Juhlin [15] found increased values in patients with atopic dermatitis using a combined electrophoretic and fluorometric histamine assay. Interestingly, there was no significant difference between affected and normal-looking skin. In our study, we used the very sensitive and specific enzymatic double isotope assay of Beaven et al. [1] for histamine determination. Although 3 of 22 patients showed values above the mean + 2SD of controls, there was no significant difference between controls and patients in the statistical analysis. The rather scattered values of the histamine content in normal skin are in accordance with data in the literature [2]. In contrast to the patients with atopic dermatitis, one patient with the hyper-IgE-syndrome had a histamine concentration 8.8 times above the mean of controls. This value is not due to a defect of histamine-methyl-transferase activity, since extensive histamine degradation took place. The same applies for the other patients with elevated histamine levels. Although our results do not point to a gross defect in the histamine catabolism, further studies are necessary to detect or exclude subtle differences in enzyme activity. Such tests have not yet been carried out in the skin of atopic patients. Our findings of almost identical histamine concentrations in the skin of patients with atopic dermatitis and normal controls do not, of course, exclude the possibility of a role of this biogenic amine in the pathogenesis of atopic dermatitis. It is conceivable that in the skin and in other organs there is an abnormal response to physiologic concentrations of the substance [16–18]. Moreover, a tissue may have a higher rate of turnover of histamine and yet the actual histamine content may be low [2]. The inflammatory vasodilatation might exert a washout effect which masks a high histamine release. Therefore, we performed a study of histamine release upon immunologic and pharmacologic stimuli since an enhanced releasability of mast cells in atopy was previously postulated by Lichtenstein et al. [3] and Ring [4]. Ring and O'Connor [5] were able to demonstrate that the basophils of patients with atopic dermatitis released more histamine than the basophils of controls upon challenge with methacholine and iothalamate. We could also show an enhanced cholinergic release from the mast cells of actively sensitized animals and from the adenoids of atopic patients [19]. Our present findings do not suggest a cholinergic release mechanism operating in the skin of

both healthy controls and atopic dermatitis patients. However, a relatively high spontaneous release was noted in our experiments, which can be explained by the fact that the preparation of sections of the firm skin tissue leads inevitably to some damage of mast cells. Unfortunately, there is as yet no technique available for obtaining purified mast cells from human skin. A similar high spontaneous release was observed in animal [20] and human [21] tissues having a consistency less than that of skin.

There was a statistically significant, but only slightly higher, histamine release from the skin of controls as compared to atopic dermatitis patients; this small difference is probably without biologic relevance.

Compound 48/80 induced a quantitatively similar release in both controls and patients. Since the release mechanism of this agent is a nonspecific pharmacologic membrane effect [6, 22], this finding is not surprising; it corresponds to the in vivo results of Voorhorst et al. [23]. The most important point in this study, therefore, is the finding that with anti-IgE there was almost twice the amount of histamine released from atopic dermatitis skin specimens than from normal skin. Furthermore, anti-IgE released twice as much histamine as did compound 48/80 from the skin of atopics, whereas these two substances released almost identical amounts of histamine from normal skin. These findings suggest a particular lability of the mast cells from atopic dermatitis patients to immunological stimuli.

From these results it seems that in atopic dermatitis there is an enhanced histamine-releasing tendency of cutaneous mast cells specifically upon immunologic challenge. The hitherto postulated generally enhanced releasability [3, 4] was not observed in our experiments, since spontaneous release was even higher in controls and pharmacologically mediated release by compound 48/80 was identical in both groups.

A second conclusion that can be drawn from our results is that a high cutaneous turnover rate of histamine may be present in the diseased skin in atopic dermatitis, i.e., a high release and a fast elimination resulting in a normal actual histamine concentration.

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