

Regulation of histamine release from human basophil leucocytes: role of H_1 , H_2 and H_3 receptors

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A novel class of histamine receptors (H_3), controlling histamine synthesis and release, was described in rat and human brain and peripheral nerve endings. The present study was undertaken to evaluate whether H_3 receptors contribute to the regulation of histamine release from human basophils. Basophil leucocytes were incubated with a H_3 antagonist (thioperamide; concentrations ranging from 1 nM to 10 μ M) or with a H_3 agonist ((R) α -methyl-histamine; concentrations ranging from 1 to 100 nM), and subsequently were stimulated with optimal doses of anti-IgE and formyl-methionyl-leucyl-phenyl-alanine (f-met peptide). No significant modifications of histamine release were observed after incubation either with the H_3 agonist or with the H_3 antagonist. By contrast, a H_2 antagonist (cimetidine; concentrations ranging from 1 to 100 μ M) exerted a dose-dependent enhancing effect on anti-IgE- and, to a lesser extent, on f-met peptide-induced histamine release. A H_1 antihistamine (chlorpheniramine; concentrations ranging from 100 nM to 1 μ M), at the highest concentration employed, displayed an inhibitory activity on IgE-dependent and IgE-independent histamine release. Exogenous histamine was shown to exert a dose-dependent inhibitory effect on two-staged anti-IgE-induced histamine release. Taken as a whole, these results suggest that H_3 receptors are not involved in the regulation of histamine release from human basophils; by contrast, H_2 receptors participate in controlling histamine release from human basophils, as previously demonstrated by other authors.

Key words: basophil leucocytes; histamine receptors; histamine release.

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Antigen-induced histamine release from human basophils was shown to be reduced by extracellular histamine. It has been postulated that released histamine acts as a feed-back inhibitor of histamine release, and that this control mechanism is mediated by H_2 receptors (3, 12, 13, 19). H_2 antagonists, as cimetidine and burimamide, can in fact enhance histamine release from stimulated basophils by preventing the inhibitory effect of extracellular histamine; moreover, the specific H_2 agonist, dimaprit, mimics the blocking effect of extracellular histamine on histamine release (14, 19). Recently, Arrang et al. (1, 2) and Ishikawa et al. (6) demonstrated the presence of a novel class of histamine receptors in the brain and in peripheral nerve terminals, both in rats and in humans.

These receptors, which were named H_3 , were shown to be involved in the feed-back control of histamine synthesis and release from histaminergic nerve endings.

The aim of the present study was to investigate whether H_3 receptors are involved in the regulation of histamine release from human basophil leucocytes.

MATERIAL AND METHODS

Preparation of leucocyte suspensions

Basophil leucocytes were obtained from peripheral blood drawn from 30 healthy subjects (mean

age 28 years). Leucocytes were separated by dextran sedimentation, according to the method of Lichtenstein et al. (8). The blood samples, anticoagulated with 0.01 M EDTA, were mixed with 6 % dextran in saline solution Macrodex, Baxter, Trieste, Italy) and allowed to sediment for 60–90 min; the leucocyte-rich plasma was aspirated, centrifuged and the cell button was washed twice in Tyrode's buffer (pH 7.4) containing 135 mM NaCl, 5.5 mM dextrose, 2.7 mM KCl, 0.36 mM NaH_2PO_4 and 12 mM NaHCO_3 . The cells were then suspended in Tyrode's buffer also containing 1.8 mM CaCl_2 and 0.5 mM MgCl_2 . Basophil leucocytes were stained with Alcian blue (5) and counted in duplicate in a Fuchs-Rosenthal chamber; a concentration of 4×10^4 basophils/ml was obtained adjusting the volume of Tyrode's buffer.

Histamine release from basophil leucocytes

Basophil leucocyte stimulation was carried out in 12×75 mm polyethylene tubes. Briefly, 2×10^4 basophils, suspended in 0.5 ml Tyrode's buffer with Ca^{2+} and Mg^{2+} , were added to the tubes containing 0.5 ml anti-IgE (final dilution 5×10^{-3}) or formyl-methionyl-leucyl-phenylalanine (f-met peptide; final concentration 10 μM). Both agents were purchased from Sigma Chemical (St. Louis, Mo, USA). After incubation for 30 min at 37°C , the leucocyte suspensions were centrifuged at 2,000 g for 30 min at 4°C and the supernatant precipitated with an equal volume of 4 % HClO_4 . After another centrifugation step at 2,000 g for 15 min at 4°C , the supernatant was collected and utilized for histamine analysis. Histamine concentration was measured by a fluorometric method, according to the technique of Siraganian (15). Spontaneous histamine release was evaluated by incubating the cells (0.5 ml cell suspension diluted with 0.5 ml Tyrode's buffer with Ca^{2+} and Mg^{2+}) for 30 min at 37°C in the absence of any stimulus; total histamine content was obtained adding 0.5 ml 4 % HClO_4 to 0.5 ml cell suspension. The percentage of histamine release was calculated as follows:

$$\% \text{ release} = \frac{e - s}{t - s} \times 100$$

where e represents the fluorometric reading for experimental supernatant, s the mean value of spontaneous histamine release, and t the total histamine content. Every sample was analysed in duplicate and the mean value considered.

Effect of extracellular histamine on anti-IgE-induced histamine release from human basophils

Histamine release from human basophils can be divided in two stages, as demonstrated by Lichtenstein (10). The first stage ("activation") is antigen dependent and Ca^{2+} independent, whereas the second stage ("release") is Ca^{2+} dependent. The first was shown to be affected by agents influencing cyclic AMP levels, whereas the second stage was modified by deuterium oxide (affecting the microtubule system) and metabolic inhibitors (11). Leucocyte stimulation was carried out in two stages, and effects of extracellular histamine on the first stage of anti-IgE-induced histamine release were evaluated (exogenous histamine was shown to increase the cyclic AMP level of human leucocytes).

Leucocytes were divided in aliquots and suspended in Tyrode's buffer without Ca^{2+} , containing different histamine concentrations (0, 0.01, 0.1, 1 and 5 μM). After incubation for 10 min at 37°C in the presence of histamine and optimal concentrations of anti-IgE, the cells were washed twice in Ca^{2+} -free Tyrode's buffer, and incubated for 30 min at 37°C in Ca^{2+} -containing Tyrode's buffer. Histamine release in the cell supernatants was then evaluated as described.

Study of the effects of histamine receptor antagonists and (R) α methyl-histamine on histamine release from stimulated basophil leucocytes

Leucocyte suspensions were incubated for 10 min at 37°C with a H_1 antagonist (chlorpheniramine, concentration range 100 nM – 10 μM), a H_2 antagonist (cimetidine, concentration

range 1 – 100 μM) and a H_3 antagonist (thioperamide, concentration range 1 nM – 10 μM). Chlorpheniramine was purchased from Schering Corp. (Kenilworth, NJ, USA) and cimetidine from Smith Kline & French, Philadelphia, PA, USA); thioperamide was a generous gift of Dr. J.M. Arrang (Unité de Neurobiologie, Centre Paul Broca de l'INSERM, Paris, France). At the concentrations used in the experiments, none of the antihistamines interfered with the fluorometric histamine determination or induced a direct histamine release. After incubation for 10 min at 37°C with the antihistamine, the cells were exposed to the activating agents as described.

The effect of the H_3 selective agonist (R) α methyl-histamine (kindly provided by Dr. J. Arrang) was also investigated. The cells were incubated for 10 min at 37°C with (R) α methyl-histamine, at concentrations ranging from 1 to 100 nM (concentrations which were proven to inhibit histamine release from histaminergic nerve endings). The leucocytes were subsequently stimulated with anti-IgE and f-met peptide. The fluorimetric reading for (R) α methyl-histamine was subtracted from each sample under examination.

Statistical analysis

Results were expressed as mean $\pm$ SEM. The effects of antihistamines, histamine and (R) α -methyl-histamine on histamine release were ana-

lysed by two-tailed Student's test for paired data (data in the absence and in the presence of histamine antagonists and agonists were compared). *P* values < 0.05 were regarded as significant.

RESULTS

Basophils of six blood samples were used for all experiments with histamine agonists and antagonists. In some cases the number of basophils obtained from the blood samples was not sufficient to evaluate the effects of all drugs. Therefore, basophils from different subjects were used for different drugs and basophils from some subjects were used on different occasions. A non-significant difference in mean basal value of histamine release was found among the different sample groups examined. When basophils of one subject were used on different occasions, basal values of histamine release were highly reproducible and variability did not exceed 5 % of the observed value.

Thioperamide, in the studied concentration range, did not significantly modify the anti-IgE- and f-met peptide-induced histamine release. Mean percent anti-IgE-induced histamine release was 27.3 ± 3.0 without any drug, 27.4 ± 2.8 in the presence of 1 nM thioperamide, and 27.4 ± 3.2 with 10 μM thioperamide. Mean percent f-met peptide-induced histamine release was 30.9 ± 5.7 without any drug, 27.9 ± 4.9 with 1 nM thioperamide, and

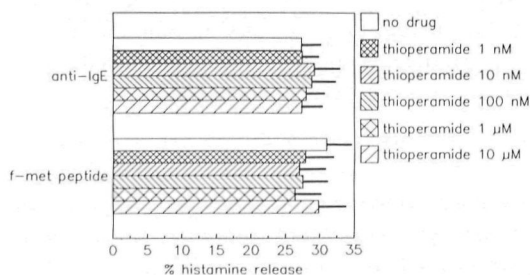


Fig. 1. Washed leucocytes from normal donors were incubated with the concentrations of thioperamide shown 10 min before addition of anti-IgE (1/5000) or f-met peptide (10 μM). Results represent the mean $\pm$ SEM of 10 different experiments.

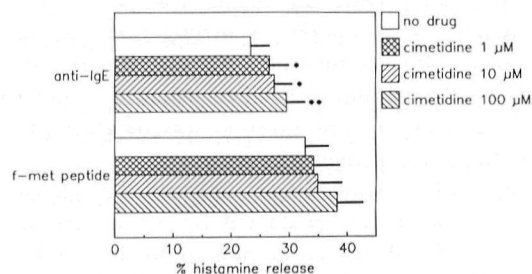


Fig. 2. Washed leucocytes were incubated with cimetidine, at concentrations ranging from 1 μM to 100 μM , 10 min before addition of anti-IgE (1/5000) or f-met peptide (10 μM). Results represent the mean $\pm$ SEM of 15 different experiments. Significantly different from control values in the absence of drug: * *P* < 0.001; ** *P* < 0.0001.

29.8±4.6 with 10 μ M thioperamide (Fig. 1). A significant enhancement of anti-IgE-induced histamine release was observed when the cells were incubated with cimetidine. Mean percent anti-IgE-induced histamine release was 23.4±3.1 in basal conditions, 26.5±3.2 ($P < 0.001$) with 1 μ M cimetidine, and 29.5±3.4 ($P < 0.0001$) with 100 μ M cimetidine. A dose-related enhancing effect of cimetidine on histamine release was also observed when basophil leucocytes were stimulated by f-met peptide: mean percent histamine release was 32.8±4.5 in control conditions, 34.1±4.6 with 1 μ M cimetidine, and 38.3±5.1 with 100 μ M cimetidine (Fig. 2). However, the effect was not statistically significant at all the concentrations used. The H_1 antihistamine, chlorpheniramine, at the highest dose employed (10 μ M), displayed an inhibitory effect both on IgE-mediated and IgE-independent histamine release. Mean percent anti-IgE-induced histamine release was 25.5±4.1 with no drug and 19.4±3.7 ($P < 0.05$) after incubation with 10 μ M chlorpheniramine. There was a dose-related inhibitory trend of chlorpheniramine on histamine release induced by f-met peptide, although not significant at all the doses used. Mean percent histamine release was 35.4±5.3 with no drug and 30.6±5.5 with 10 μ M chlorpheniramine (Fig. 3).

When leucocyte suspensions were incubated with different histamine concentrations (from

0.01 to 5 μ M), during the first stage of histamine release, a significant and dose-dependent reduction of anti-IgE-induced histamine release was found. By contrast, basophil exposure to the H_3 agonist (R) α methyl-histamine did not modify basophil response to activating agents (Fig. 4).

DISCUSSION

Extracellular histamine has an inhibitory effect on anti-IgE-induced histamine release from human basophils, as already observed by Bourne et al. (3, 4) and Lichtenstein et al (12). Conversely, (R) α methyl-histamine, a selective H_3 agonist, is devoid of any appreciable effect on histamine release. Accordingly, also the H_3 selective antagonist thioperamide does not modify anti-IgE- and f-met peptide-induced histamine release. This suggests that H_3 receptors are not involved in the regulation of histamine release from human basophils.

An inhibitory effect on IgE-mediated histamine release is found when basophil leucocytes are pre-incubated with the highest dose of the H_1 antagonist chlorpheniramine (10 μ M). This is in keeping with Lichtenstein et al. (13), who demonstrated that several H_1 antihistamines, namely at high concentrations (0.1 - 1 mM), have an inhibitory activity on histamine release. The H_2 antagonist cimetidine enhances anti-IgE- and, to a lesser extent, f-met-peptide-induced histamine release. The effect is dose-

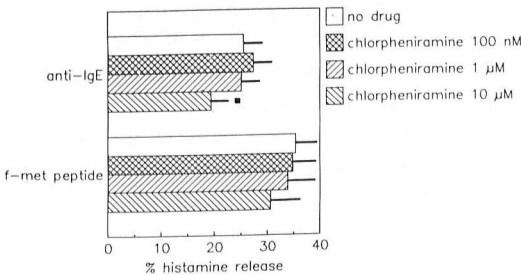


Fig. 3. Washed leucocytes from normal donors were incubated with chlorpheniramine, at concentrations ranging from 100 nM to 10 μ M, 10 min before addition of anti-IgE (1/5000) or f-met peptide (10 μ M). Results represent the mean±SEM of 12 different experiments. Significantly different from control values in the absence of drug: ■ $P < 0.05$.

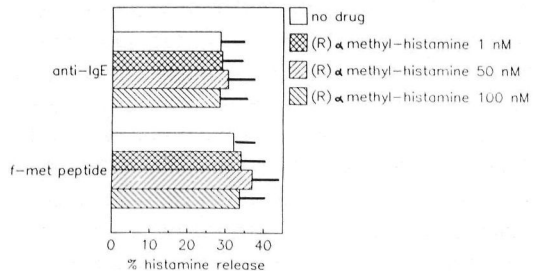


Fig. 4. Washed leucocytes from normal donors were incubated for 10 min with the H_3 agonist (R) α methyl-histamine, at concentrations ranging from 1 nM to 100 nM, and then stimulated with anti-IgE (1/5000) or f-met peptide (10 μ M). Results represent the mean±SEM of 10 different experiments.

Table 1.

	Histamine concentration (μM)				
	0	0.01	0.1	1	5
Mean	28.81	23.35	20.62	16.95	13.68
SEM	2.41	2.81	2.05	1.75	1.36
<i>P</i>		< 0.001	< 0.0001	< 0.00001	< 0.0001

Inhibitory effect of exogenous histamine on anti-IgE-induced histamine release from human basophils. Leucocyte stimulation was carried out in two stages and the effects of exogenous histamine on the first stage evaluated. Leucocytes were divided in aliquots and suspended in Tyrode's buffer without Ca^{2+} , containing different histamine concentrations (0, 0.01, 0.1, 1 and 5 μM). After incubation for 10 min at 37°C in the presence of an optimal concentration of anti-IgE, the cells were washed twice in Ca^{2+} -free Tyrode's buffer, and incubated for 30 min at 37°C in Ca^{2+} -containing Tyrode's buffer. Histamine release in the supernatant was measured by an automated fluorometric method. Results are expressed as percent histamine release (mean $\pm$ SEM; $n = 8$).

dependent, more pronounced with the highest dose employed (100 μM), and related to the stimulus used to activate human basophils. The different behaviour can probably be explained by the different amounts of histamine released by the two agents. F-met peptide is usually a more potent histamine releaser than anti-IgE, and this has been confirmed also in our experiments. The enhancing effect of cimetidine is in fact prominent at low levels of histamine release, whereas little or no enhancement can be found when basophil leucocytes release high percentages of histamine. This is in keeping with the observations of other authors (4, 9, 18), who observed that H_2 antagonists have a more pronounced enhancing effect when antigen-induced histamine release is low (i.e. < 20 % of total histamine content). Alternatively, the reason for the different effect of cimetidine should be searched for in the different mechanisms of action of the two stimuli. Anti-IgE induces histamine release through cross-linking of membrane-bound IgE (7), whereas f-met peptide interacts with a membrane receptor, which differs from IgE and C5a receptors (16). Also, anti-IgE- and f-met peptide-induced histamine release are differently affected by a hypertonic environment (17). Therefore, it cannot be excluded that the different effect of cimetidine is related to the different mechanisms of action of the two stimuli.

Released histamine was demonstrated to behave as a feed-back inhibitor of histamine release

from human basophil leucocytes, as well as from rat and human brain tissue and peripheral nerve endings (1-3, 6, 12, 13). Several experimental data indicate that H_2 , but not H_1 or H_3 , receptors are involved in the regulation of histamine release from human basophil leucocytes: firstly, H_2 , but not H_1 or H_3 , antihistamines enhance histamine release from human basophil leucocytes (12, 13, 19), and, secondly, dimaprit, a selective H_2 agonist, can mimic the inhibitory effect of extracellular histamine on antigen-induced histamine release from human basophils (19). Our results are in keeping with the previous findings indicating that H_2 receptors mediate the feed-back inhibition of histamine release from human basophils, and demonstrate that thioperamide, a selective H_3 antagonist, and (R) α methyl-histamine, a selective H_3 agonist, are devoid of any effect on IgE-mediated and IgE-independent histamine release from these cells. In contrast, it is demonstrated that H_3 receptors regulate histamine synthesis and release from histaminergic nerve endings (1, 2, 6); therefore, it can be suggested that the histamine receptors controlling histamine release vary from cell type to cell type.

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