Possible Participation of Histamine H3-Receptors in the Regulation of Anaphylactic Histamine Release from Isolated Rat Peritoneal Mast Cells

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Abstract—Anaphylactic histamine release from isolated rat peritoneal mast cells was concentration-dependently blocked by a 5-min treatment with exogenous histamine at 0.9 and 9 μM and enhanced by a 20- to 30-min treatment with thioperamide (H3-antagonist) at 3 μM with significance, but little affected by mepyramine (H1-antagonist) and cimetidine (H2-antagonist) at the cell concentration of 10^6 mast cells/ml. At a low concentration of mast cells (10^4 mast cells/ml), (R)-α-methylhistamine (α-MH), an H3-agonist, at 0.9–90 μM also inhibited the release in a concentration-dependent fashion. Thioperamide, but neither mepyramine nor cimetidine, significantly restored the decreased release by α-MH. However, the complete restoration by thioperamide could not be achieved because the drug itself slightly but concentration-dependently inhibited anaphylactic histamine release. On the other hand, not only betahistine and dimaprit but also α-MH did not suppress histamine release from the mast cells induced by compound 48/80. In rat plasma, considerable levels of histamine were detected. From these results, it is strongly suggested that histamine H3-like receptors are largely responsible for the negative feedback regulation of the anaphylactic histamine release from rat peritoneal mast cells.

Keywords: (R)-α-Methylhistamine, Thioperamide, Histamine, Mast cell, Compound 48/80

In the early 1980s, a third receptor of histamine was identified, which regulates the synthesis and release of histamine in the histaminergic nerve ends in the rat brain, and this was designated as the H3-receptor (1).

Since then, the development of specific and selective agonists and antagonists of histamine receptor subtypes has revealed that H3-receptors exist in other tissues including the ileum (2), airway (3, 4) and blood vessels (5); this was concluded from the observations that the neural stimulation-induced twitch responses of these tissues are inhibited by modulation of ACh, neuropeptides and other neurotransmitter release by H3-receptor stimulation, in a similar manner to that of the rat cerebral cortex. Thus, the majority of the investigations on H3-receptors were initially focused on the modulation of neurotransmitter release and/or histamine synthesis in the presynaptic ends of the brain or other tissues. In addition, there have recently been a number of reports on the roles of the receptor in effector organs: H3-receptor stimulation relaxed rabbit middle cerebral artery preconstricted with K+ by acceleration of nitric oxide and prostacyclin formation from the endothelium (6, 7); an H3-antagonist aggrated experimental allergic asthma in the guinea pig (8); an H3-agonist and H3-antagonist inhibited and enhanced, respectively, the histamine release from isolated gastric glands (9), and H3-receptor stimulation specifically down-regulated histamine synthesis in isolated fundic mucosal cells (10) in rabbits; an H3-receptor agonist reduced gastrin-induced vascular histamine release in the isolated rat stomach (11).

In the present study, we obtained evidence indicating that isolated rat peritoneal mast cells have surface H3-receptors, activation of which inhibits anaphylactic histamine release from these cells.

Materials and Methods

Reagents

Reagents used were histamine dihydrochloride and cimetidine (Nacalai Tesque, Kyoto), (R)-α-methylhistamine (α-MH) and thioperamide (donated from Green Cross, Osaka), mepyramine maleate, betahistine dihydrochloride, compound 48/80 and bovine serum albumin (BSA, Cohn fraction V; Sigma Chem., St. Louis, MO,
USA), dimaprit dihydrochloride (Research Biochem., Natick, MA, USA), gelatin (Merck, Darmstadt, Germany), heparin (Novo-Nordisk A/S, Gentofte, Denmark) and dimethyl sulfoxide (DMSO; Wako Pure Chem., Osaka). Other reagents used were the highest grade commercially available.

Histamine, betahistine, dimaprit, α-MH, mepyramine, cimetidine and compound 48/80 were dissolved in physiologic saline, and thioperamide was dissolved in 100% DMSO.

**Harvest and purification of peritoneal mast cells from passively sensitized rats**

Eight-week-old, male Wistar rats (Japan SLC, Hamamatsu) were passively sensitized by i.p.-injection of 0.2 ml/animal of anti-dinitrophenylated Ascaris suum extract rat serum (48-hr passive cutaneous anaphylaxis titer: 256X). Forty-eight hours later, following anesthesia by inhalation of diethyl ether, the animals were killed by stunning and exsanguinated through an incision in the carotid artery. They were then injected i.p. with 100 ml/kg of Ca²⁺-free, 10 U/ml heparin-containing mast cell medium (Ca²⁺-free MCM, composition: 150 mM NaCl, 3.7 mM KC1, 3.0 mM Na₂HPO₄, 3.5 mM KH₂PO₄ and 6.0 mM glucose). After gentle abdominal massage, the peritoneal fluid, including the mast cells, was collected. The fluid was gently centrifuged (50 x g, 7 min, 4°C, 3 times) to obtain the cell pellet, which was suspended in Ca²⁺-free MCM at 10⁴ mast cells/ml and used as the partially purified rat mast cells. In some experiments, the partially purified mast cells were further purified according to the method of Sullivan et al. (12) by centrifugation through a 31.5% BSA layer. The mast cells obtained, designated as the purified rat mast cells, were suspended in Ca²⁺-free MCM at 10⁶ mast cells/ml. The mast cells had a purity of 6.0±0.5% (n=12) at the partially purified stage and a purity of 97.7±0.4% (n=10) at the purified stage.

**Conditions of anaphylactic histamine release**

First, the aliquots of suspended partially purified (10⁴ mast cells/ml) or purified rat mast cells (10⁶ mast cells/ml) were added with CaCl₂ at the final concentration of 0.9 mM and then preincubated at 37°C for 5 min or various times for the time-course experiments. Then the cells were treated with antagonists (for 21 min) and/or agonists (for 5 min, 20 min or various other times in the time-course experiments) of histamine, followed by a challenge with a specific antigen at the final concentration of 10 μg/ml at 37°C for 10 min. The reaction was stopped by cooling in ice-water followed by centrifugation at 1,700 x g for 15 min at 4°C. The supernatant was stored at −20°C until the histamine assay.

The agonists and antagonists at the concentrations used through the experiments did not substantially affect the spontaneous histamine release from the mast cells.

**Conditions of compound 48/80-induced histamine release**

After the preincubation with 0.9 mM CaCl₂ at 37°C for 5 min, partially purified mast cells (10⁴ mast cells/ml) were treated with betahistine, dimaprit or α-MH at various concentrations for 20 min and then stimulated with 0.05 μg/ml compound 48/80 for 10 min. Following procedures before the histamine assay were the same as those described above.

**Rat plasma for evaluation of histamine content**

To estimate the histamine levels in rat plasmas, the plasma was obtained by the following procedures: After anesthesia by inhalation of diethyl ether and i.v.-injection of 1,000 U/animal of heparin, 3.5 ml of blood was drawn from the abdominal aorta with a 20G needle and 5 ml plastic syringe; then within 20 sec, the sample was centrifuged at 12,000 x g for 3 min at room temperature. A 900-μl aliquot of the upper portion of the supernatant was removed and used for the histamine assay.

**Assay of histamine**

The supernatant samples were treated with 3% perchloric acid and centrifuged for 5 min to deproteinize them. Then their histamine contents were automatically assayed fluorometrically following purification by the reversed phase ion-paired method by a high performance liquid chromatography (HPLC; Toso, Tokyo) histamine analytical system, which employed a Chemcosorb 5-ODS-H column (4.0 φ x 150 mm; Chemco, Osaka). The conditions for HPLC were: solvent, 0.17 M KH₂PO₄ containing 0.1 mM 1-octanesulfonic acid sodium salt; flow rate, 0.6 ml/min at room temperature (13). For determination of the histamine content in mast cells, cell pellet (10⁴ or 10⁶ mast cells/specimen) was treated by the same procedures as described above. The degree of histamine release was expressed as a percentage of the mast cell histamine content or percent release of the control (stimulated with antigen or compound 48/80 and not treated with histamine agonists or antagonists).

**Assay of lactate dehydrogenase (LDH) from purified rat mast cells**

Purified rat mast cells (10⁵ mast cells/ml) were incubated with α-MH at 0.9–90 μM or thioperamide at 0.3–30 μM for 30 min at 37°C. LDH released from the purified rat mast cells into the medium was assayed by the decrease in ultraviolet absorption at 340 nm as described by Hill (14), with pyruvate as a substrate.
RESULTS

Effects of histamine and H₁, H₂ or H₃-antagonist on anaphylactic histamine release from the purified rat mast cell

When the purified mast cells (10⁶ mast cells/ml) were challenged with antigen, approximately 8% of their histamine contents were released. Treatment with 0.9 and 9 μM histamine, which was exogenously added, significantly influenced the release, inhibiting it by 20% and 25%, respectively, in a concentration-dependent fashion (Fig. 1). In the specimens that were not treated with antigen, an average of 0.22 μg/10⁶ mast cells/ml (2 μM) of histamine and less than 1/3 of that were spontaneously released into

![Fig. 1. Effects of exogenous histamine on the anaphylactic histamine release from purified rat mast cells. Histamine was added at the indicated final concentrations 5 min prior to the antigen challenge. Each column represents the mean±S.E. of 7 experiments. Amounts of spontaneous and anaphylactic histamine release were 0.22±0.05 and 1.25±0.14 μg/10⁶ mast cells/ml, respectively. Histamine content was 17.6±0.88 μg/10⁶ mast cells/ml. ** and ***: Statistical significance (paired t-test) of the difference from the control at P<0.01 and P<0.001, respectively. Spon: spontaneous histamine release, Cont: antigen-induced histamine release.]

![Fig. 2. Effects of (R)-α-methylhistamine (α-MH) on the anaphylactic histamine release from partially purified rat mast cells. α-MH was added 20 min before the antigen challenge at the indicated final concentrations. Each column represents the mean±S.E. of 6 experiments. Amounts of spontaneous and anaphylactic histamine release were 2.1 ± 0.27 and 28 ± 3.3 ng/10⁴ mast cells, respectively. Histamine content was 150±11.3 ng/10⁴ mast cells. ** and ***: Statistical significance (paired t-test) of the difference from the control at P<0.01 and P<0.001, respectively. Spon: spontaneous histamine release, Cont: antigen-induced histamine release.]

Table 1. Effects of mepyramine, cimetidine and thioperamide on the anaphylactic histamine release from purified rat mast cells

<table>
<thead>
<tr>
<th>Antagonists (μM)</th>
<th>% of histamine release</th>
<th>Treatment time (min) before antigen challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Spontaneous</td>
<td>2.0±0.18</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.8±0.76</td>
<td></td>
</tr>
<tr>
<td>Mepyramine (2.5)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cimetidine (4)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Thioperamide (3)</td>
<td>9.0±0.53</td>
<td>9.5±0.16</td>
</tr>
</tbody>
</table>

Each value represents the means±S.E. of 4 experiments. Histamine content was 23±0.6 μg/10⁶ mast cells/ml. * and **: statistically significant difference (paired t-test) from the control at P<0.05 and P<0.01, respectively. ND: not done.
Fig. 3. Time course effects of (R)-α-methylhistamine (α-MH) on the anaphylactic histamine release from partially purified rat mast cell. α-MH was added at the indicated time prior to the antigen challenge at final concentrations of 9 pM (-○-) and 90 pM (-●-). Each point represents the mean ± S.E. of 4 experiments. Amounts of spontaneous and anaphylactic histamine release were 1.8 ± 0.6 and 24 ± 2.3 ng/10^4 mast cells, respectively. Histamine content was 175 ± 38.3 ng/10^4 mast cells. □: Control (upper) and spontaneous (lower) levels. ** and ***: Statistical significance (paired t-test) of the difference from the control at P<0.01 and P<0.001, respectively.

Fig. 4. Effects of mepyramine, cimetidine and thioperamide on anaphylactic histamine release from partially purified rat mast cells. Drugs were added 21 min before the antigen challenge at the indicated concentrations. Each column represents the mean ± S.E. of 4 experiments. Amounts of spontaneous and anaphylactic histamine release were 2.0 ± 0.11 and 30 ± 2.3 ng/10^4 mast cells, respectively. Histamine content was 163 ± 1.21 ng/10^4 mast cells. *: Statistical significance (paired t-test) of the difference from the control at P<0.05 and P<0.01, respectively. Spon: spontaneous histamine release, Cont: antigen-induced histamine release.
the medium after the completion of the incubation and at the time of addition of exogenous histamine, respectively.

In addition, when the purified mast cells at the same concentration were treated with 3 μM thioperamide for 5 to 30 min and then challenged with the antigen, the anaphylactic histamine release was time-dependently enhanced, but neither mepyramine at 2.5 μM nor cimetidine at 4 μM stimulated the release (Table 1).

**Effects of α-MH on anaphylactic histamine release from partially purified rat mast cells**

Anaphylactic histamine release from the partially purified mast cells (10⁴ mast cells/ml) was only slightly decreased by the treatment with 0.09 or 0.9 μM α-MH. However, higher concentrations of the agonist significantly inhibited the release in a concentration-dependent manner (Fig. 2).

**Time course of the effects of α-MH on anaphylactic histamine release from partially purified rat mast cells**

Figure 3 shows the time course of the effects of α-MH at 9 and 90 μM on the anaphylactic histamine release from the partially purified mast cells (10⁴ mast cells/ml). Treatment for 1 min with 9 μM α-MH inhibited the release by only 7%, which was not significant. However, α-MH treatment for 5 to 20 min produced a significant inhibition, which reached a level of 20% to 32%. The inhibition was not increased by a 30-min incubation. Although the time course for the inhibition by 90 μM showed a similar pattern, the 1-min treatment of the agonist at this concentration significantly enhanced the release.

**Effects of H₁, H₂ and H₃-antagonists on the anaphylactic histamine release from partially purified rat mast cells**

Whether anaphylactic histamine release from 10⁴ mast cells/ml is influenced by the treatment of histamine recep-

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Fig. 5. Effects of mepyramine, cimetidine and thioperamide on (R)-α-methylhistamine (α-MH)-induced decreased release of anaphylactic histamine from partially purified rat mast cells. Mepyramine, cimetidine and thioperamide, and α-MH were added 21 and 20 min, respectively, before the antigen challenge. Each column represents the mean ± S.E. of 4 experiments. Amounts of spontaneous and anaphylactic histamine release were 1.9 ± 0.12 and 28 ± 1.2 ng/10⁴ mast cells, respectively. Histamine content was 155 ± 10.3 ng/10⁴ mast cells. ****: Statistical significance (paired t-test) of the difference from the group treated with α-MH at P < 0.001. Spon: spontaneous histamine release, Cont: antigen-induced histamine release.
Effects of H1-, H2 and H3-antagonists on the decreased anaphylactic histamine release by a-MH from partially purified rat mast cells

Whether H1, H2 and H3 antagonists influence the decreased release of anaphylactic histamine by a-MH was examined when the antagonists were administered prior to a-MH. As shown in Fig. 5, mepyramine at 0.25 or 2.5 μM or cimetidine at 0.4 or 4 μM showed minimal or no influence on the anaphylactic histamine release. Both 0.03 and 0.3 μM thioperamide slightly enhanced and inhibited the release, respectively, although not significantly, but higher concentrations of 3 and 30 μM concentration-dependently inhibited the release (Fig. 4).

Effects of H1-, H2 and H3-agonists on the histamine release induced by compound 48/80 from the partially purified rat mast cell

When partially purified mast cells (10⁴ mast cells/ml) were stimulated with compound 48/80 at 0.05 μg/ml, the amount of histamine released was similar to that induced by the antigen. The treatment with betahistine at 0.7 and 7 μM, dimaprit at 0.6 to 60 μM or a-MH at 0.08 to 80 μM did not inhibit the release (Fig. 6).

Histamine levels in rat plasma

In 8-week-old rats, which were used throughout the present experiments, a large amount of histamine, 48 ± 3.7 ng/ml (0.43 ± 0.03 μM, n = 12), was detected in the plasma.

Lactate dehydrogenase (LDH) release from the purified mast cells by a-MH and thioperamide

Table 2 shows the results of LDH release from purified rat mast cells (10⁴ mast cells/ml) induced by 0.9 to 90 μM a-MH or 0.3 to 30 μM thioperamide. The LDH release induced by either of these drugs was slight and no concentration dependencies were observed.

<table>
<thead>
<tr>
<th>a-MH or thioperamide concentration (μM)</th>
<th>% of LDH release</th>
<th>a-MH</th>
<th>Thioperamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-treated</td>
<td></td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>0.9</td>
<td>2.4</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2.6</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>2.6</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>—</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>—</td>
<td>2.5</td>
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</tr>
</tbody>
</table>

Each value represents the mean of 2 experiments.
DISCUSSION

In the present experiments, two concentrations of rat mast cell suspension were employed to determine if histamine receptors participate in the regulation of anaphylactic histamine release. A high concentration (10^6 mast cells/ml) of mast cells was used to study the possible influence of histamine on anaphylactic histamine release because exogenously added histamine at relatively high doses would make it nearly impossible to estimate the exact amount of anaphylactically released histamine if low cell concentrations were used. On the other hand, a low concentration (10^6 mast cells/ml) of mast cells was used to examine the effects of agonists and/or combination with antagonists of histamine on the anaphylactic histamine release, because under this condition, the spontaneously released histamine in such low concentrations would hardly interfere with the examination of the exact actions of these agonists and antagonists.

In the experiment with the high concentration of purified rat mast cells, exogenously added histamine concentration-dependently inhibited the anaphylactic histamine release. At the end of the anaphylactic reaction, the control sample (no exogenously added histamine) contained spontaneously released histamine at levels high enough to suggest that even the anaphylactic histamine release of the control should be substantially inhibited, although the concentration of histamine released spontaneously in the medium was obviously lower than the final concentrations of exogenously added histamine when it was assayed at the time of histamine addition. It is unlikely that exogenously added histamine influences the histamine content of the mast cells because only less than 0.35% of the radioactivity was incorporated into the cells when the purified mast cells (10^6 mast cells/ml/tube) were incubated with radiolabeled [3H]-histamine at 50 nM final concentration (185 kBq) at 37°C for 3 hr.

In accordance with the above suggestion, subsequent experiments at the same concentration of mast cells in the presence of histamine-receptor antagonists revealed that H2-receptor antagonists, but not H1- and H2-receptor antagonists, enhanced the anaphylactic histamine release.

Similar to the effect of histamine, a-MH concentration-dependently decreased the anaphylactic histamine release from rat peritoneal mast cells (10^6 mast cells/ml) at micromolar order of concentrations. In the time course experiments, 1-min treatment with the drug at a high concentration of 90 pM significantly enhanced the anaphylactic histamine release, the mechanisms of which are not known yet. A treatment of more than 10 min with this concentration, however, significantly inhibited the release. It has been reported that nanomolar concentrations of a-MH induce biochemical or pharmacological activities, and similar concentrations of thioperamide inhibit them or biological events induced by stimulators other than a-MH (2-5, 15, 16). Compared with the concentrations of a-MH and/or thioperamide described in these papers, 2 or 3 orders of magnitude higher concentrations were needed for the respective inhibition and recovery of the anaphylactic histamine release from the rat peritoneal mast cells. The reason for this difference can not yet be explained. However, one possible answer may lie in the following fact: fairly high histamine concentrations were detected in the plasma of this species, suggesting that the peritoneal mast cells are always exposed to histamine at high concentrations in vivo. The levels of histamine in the rat plasma in this paper were approximately 5 times higher than those reported previously (17). Therefore, the assay of histamine in the respective plasmas from 3 groups consisting of 10 to 15 rats/group was further carried out, but the histamine concentrations of each group were similar to the mean ± S.E. value obtained in the present results.

Human basophils have been proven to have H2-receptors, stimulation of which elevates the intracellular levels of cyclic 3',5'-adenosine monophosphate to inhibit anaphylactic histamine release (18). In addition, it has been reported that cimetidine enhances the release by a blockade of the receptor (19). Furthermore, in human lung mast cells, anaphylactic histamine release is also demonstrated to be blocked by stimulation of their H2-receptor (20). On the other hand, anaphylactic histamine release from rat peritoneal mast cells has been demonstrated to be only slightly influenced by cimetidine treatment (21).

Besides the present results that a-MH decreased the anaphylactic histamine release from isolated rat mast cells, we have recently found that dimaprit (H2-agonist) (22) at relatively high concentrations of 0.6 to 60 pM also concentration-dependently decreased the release, which was restored by the coexistence of thioperamide, but not by mepyramine and cimetidine, while betahistine (H1-agonist (23)/H3-antagonist (24, 25)) does not influence the anaphylactic histamine release (26). On the other hand, as shown in the present results, dimaprit and a-MH as well as betahistine trivially influenced compound 48/80-induced histamine release. Moreover, a-MH did not change the Ca ionophore A 23187-induced histamine release (S. Kohno, unpublished data). From these results, it seems likely that the regulation of the histamine release by dimaprit and a-MH is specific for that induced by anaphylaxis.

Even at a high concentration, a-MH (90 pM) or thioperamide (30 pM) could only induce the release of a very small quantity of LDH from the purified rat mast cells, indicating that these drugs have little cytotoxic action on
the cell at such concentrations. However, thioperamide at high concentrations, at which restoration of the decreased release induced by α-MH is observed, caused a substantial reduction of the anaphylactic histamine release from mast cells, the mechanism of which is not yet known. Therefore, this explains why thioperamide can not completely recover the decreased release.

Taken together, these results strongly suggested that H3-receptors, but neither H1- nor H2-receptors, are solely responsible for the negative feedback regulation of anaphylactic histamine release from rat peritoneal mast cells by histamine.

It has been reported that dimaprit has no H3-receptor-stimulating activity in the rat brain (1). Taking this report into consideration together with our previous report (26) on the inhibition of anaphylactic histamine release by dimaprit described above and the relatively high concentrations of α-MH necessary to inhibit the release in the present results, it is suggested that H3-receptors on the rat peritoneal mast cell are a subtype distinct from those in the rat brain.

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