Dimaprit, a Histamine H2-Agonist, Inhibits Anaphylactic Histamine Release from Mast Cells and the Decreased Release Is Restored by Thioperamide (H3-Antagonist), but Not by Cimetidine (H2-Antagonist)

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ABSTRACT—Whether anaphylactic histamine release from rat peritoneal mast cells is influenced by betahistine, a histamine H1-receptor agonist/H3-antagonist, and dimaprit, an H2-agonist, was examined. Treatment with dimaprit at 6 and 60 μM for 20 min significantly inhibited the anaphylactic histamine release, whereas betahistine at up to 80 μM under the same conditions did not affect it. Treatment with dimaprit at 6 and 60 μM for 1 to 20 min and for 5 to 20 min, respectively, caused a time-dependent inhibition of the release, but up to 30 min treatment with 8 and 80 μM betahistine had no effect. The decreased histamine release induced by dimaprit was recovered by neither mepyramine nor cimetidine. However, thioperamide, an H3-selective antagonist, dose-dependently restored the diminished release. From these results, the inhibition of anaphylactic histamine release by dimaprit is not produced by the stimulation of H2 receptors, but involves the stimulation of H3-like receptors or H3-subtype receptors, which are distinct from the H3-receptors located in brain, and suggests that the receptor plays an important role in the negative feedback regulation of histamine release.

Keywords: Mast cell, Histamine, Dimaprit, Thioperamide, (R)-α-Methylhistamine

Histamine is one of the important mediators in both in vitro and in vivo anaphylaxis. Rat peritoneal mast cells have been frequently used as an in vitro model of anaphylaxis for research on subjects such as mechanisms of histamine release and evaluation of antiallergic drugs, because they are easily harvested from the peritoneal cavity and can be highly purified by density gradient centrifugation, and contain a large amount of histamine, a considerable portion of which is released in response to immunological and non-immunological stimuli.

Meanwhile, it is well known that histamine released during anaphylaxis or administered exogenously manifests a variety of pharmacological phenomena, especially enhancement of capillary venule permeability, stimulation of secretory glands and airway smooth muscle contraction, through the stimulation of H1- or H2-receptors. Whereas investigations of the pharmacological response of tissues or cells to histamine have extensively been conducted, it is still obscure how anaphylactic histamine release from mast cells is modulated by histamine, although indirect lines of evidence of the possible involvement of H2-receptors in the regulation of anaphylactic histamine release from the lung tissue in vitro or passive cutaneous anaphylaxis in vivo have been reported (1–3). This can be due to the difficulties in distinguishing anaphylactic histamine release from exogenously added histamine or the lack of selective agonists and antagonists for subtypes of histamine receptors until recently.

In this paper, we report that anaphylactic histamine release from the rat peritoneal mast cells is suppressed by dimaprit, an H2-agonist (4), and the reduced release is sufficiently restored by the coexistence of thioperamide, a highly selective H3-antagonist (5, 6), but not cimetidine, a selective H2-antagonist (7).

MATERIALS AND METHODS

Reagents

Reagents and their sources used were as follows: Mepyramine maleate and betahistine dihydrochloride
(Sigma Chem., St. Louis, MO, USA), histamine dihydrochloride and cimetidine (Nacalai Tesque, Kyoto), thioperamide (donated by Green Cross, Osaka), dimaprit dihydrochloride (Research Biochem., Natick, MA, USA), gelatin (Merck, Darmstadt, FRG), heparin (Kodama, Tokyo) and bovine serum albumin (BSA, Cohn fraction V; Sigma Chem.).

Histamine, betahistine, dimaprit, mepyramine and cimetidine were dissolved in physiologic saline and thioperamide was dissolved in 10% dimethyl sulfoxide before use.

**Physiologic solution**

Mast cell medium (MCM) was used for harvesting, washing and suspension of mast cells as the physiologic solution throughout the experiments. The composition of Ca\(^{2+}\)-free MCM was: 8.76 g/l NaCl, 0.28 g/l KCl, 1.07 g/l Na\(_2\)HPO\(_4\)·12H\(_2\)O, 0.48 g/l KH\(_2\)PO\(_4\), 1.08 g/l glucose and 1.0 g/l BSA, with or without 10 U/ml heparin.

**Animals**

Seven-week-old, male Wistar rats weighing 150–170 g were purchased from Japan SLC (Hamamatsu).

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

Rats were passively sensitized by intraperitoneal injection of 0.2 ml/animal of rat antiserum (48-hour passive cutaneous anaphylaxis titer: 256 X) against dinitrophenylated *Ascaris suum* extract. Forty-eight hours later, 10 ml/100 g body weight of Ca\(^{2+}\)-free MCM containing 10 U/ml heparin was injected i.p.; the abdomen was gently massaged for 1 min; and then the peritoneal fluid, including the mast cells was collected. The fluid was gently centrifuged (50 × g, 7 min, 4°C, 3 times), and the resultant cell pellet was suspended in Ca\(^{2+}\)-free MCM at 10⁴ mast cells/ml and used for the experiments. The mast cells had a purity of 6.3 ± 0.40% (n = 18).

**Conditions of anaphylactic histamine release**

After the addition of CaCl\(_2\) at final concentrations of 0.9 mM and preincubation at 37°C for 5 min, the aliquots of the suspended mast cells were treated with histamine receptor agonists and/or antagonists for 20 min or various other times and challenged with a specific antigen (final concentrations of 10⁻⁵ g/ml) at 37°C for 10 min. The reaction was halted by cooling the mixture in ice-water, followed by centrifugation at 1,700 × g for 15 min at 4°C. The supernatant was stored at −20°C until 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 a histamine analytical system of high performance liquid chromatography (HPLC, Toso, Tokyo) with a cation ex-

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**Fig. 1.** Effect of betahistine and dimaprit on in vitro anaphylactic histamine release from rat peritoneal mast cells. Drugs were added 20 min before the antigen challenge at the final concentrations indicated. Each column represents the mean ± S.E. of 6 experiments. Amounts of spontaneous and anaphylactic histamine release were 2.4 ± 0.30 and 52.9 ± 9.78 ng/10⁴ mast cells, respectively. Histamine content was 205 ± 10.8 ng/10⁴ mast cells. ** and ***: Statistically significant difference (paired t-test) from the control at P < 0.01 and 0.001, respectively.
change column (TSK-gel SP-2SW, 4.6 φ × 100 mm, Tosoh). The conditions for HPLC were: solvent, 0.28 M KH₂PO₄; flow rate, 0.6 ml/min, at room temperature (8, 9).

RESULTS

Effects of betahistine and dimaprit

The mast cells were treated with betahistine, an H₁-agonist (10)/H₃-antagonist (11, 12), at 0.07–70 µM and dimaprit, an H₂-agonist (4), at 0.06–60 µM for 20 min at 37°C, and their effects on anaphylactic histamine release were examined. Betahistine at up to 7 µM did not affect the release, although a higher concentration (70 µM) tended to slightly inhibit it. Dimaprit at 0.06 µM did not suppress the release either. However, at the concentrations ranging from 0.6–60 µM, it produced a dose-dependent decrease in the release, which reached approximately 25% at the maximum (Fig. 1).

Time course of the effects of betahistine and dimaprit

The time course of the effects of betahistine and dimaprit on the anaphylactic histamine release was assessed by incubating the mast cells with the drugs for 1–30 min prior to the antigen challenge. Treatment with 7 µM betahistine for 1 min exhibited a modest inhibition of the release. Further treatment did not generate a conspicuous influence on the release at all. Treatment with betahistine at 70 µM for 1–10 min demonstrated similar effects on the histamine release to those at the concentration of 7 µM. However, 20- and 30-min incubation slightly decreased the release. On the other hand, although a 1-min treatment with dimaprit at 6 and 60 µM only affected the release with slight suppression and enhancement, respectively, further treatment with either concentration for 5–20 min exerted a concentration-dependent, obvious inhibition of the release. It was not observed that longer incubation (30 min) increases the suppression at either concentration (Fig. 2).

Effect of H₁-, H₂- and H₃-antagonists on the decreased release induced by dimaprit

Whether mepyramine (H₁-antagonist) (13), cimetidine (H₂-antagonist) (7) or thioperamide (H₃-antagonist) (5, 6) influences the decreased anaphylactic release of histamine induced by dimaprit was examined by treating the mast cells with the respective antagonists for 21 min and adding the agonist 1 min later. As shown in Fig. 3, neither mepyramine nor cimetidine affected the decreased release (60% of the control). Contrary to expectation, 0.3–30 µM thioperamide, however, concentration-dependently restored the decreased release, which reached approximately 90% of the control level at the concentration of 30 µM.

DISCUSSION

In 1973, Lichtenstein and coworkers reported that

![Fig. 2. Time course effect of betahistine and dimaprit on in vitro anaphylactic histamine release from rat peritoneal mast cells. Drugs were added at the time indicated before the antigen challenge. Each point represents the mean±S.E. of 6 experiments. Amounts of spontaneous and anaphylactic histamine release were 2.1±0.30 and 38.6±4.66 ng/10⁴ mast cells, respectively. Histamine content was 209±22.9 ng/10⁴ mast cells. * *, ** and ***: Statistically significant difference (paired t-test) from the control at P<0.05, 0.01 and 0.001, respectively.](image-url)
anaphylactic histamine release from human basophils is regulated by H₂-receptors, the stimulation of which results in the inhibition of the release by elevation of the cellular levels of cyclic adenosine monophosphate (14, 15). On the other hand, anaphylactic histamine release from rat peritoneal mast cells has been reported not to be influenced by an H₂-antagonist, cimetidine, suggesting that H₂-receptors are not involved in the regulation of anaphylactic histamine release in this cell (16, 17).

To further clarify this point, we used not only H₁- and H₂-agonists but also H₁-, H₂, and H₃-antagonists and investigated whether histamine receptors are responsible for regulating the anaphylactic histamine release from the rat peritoneal mast cells in vitro. Because the rat peritoneal mast cells spontaneously release a considerable amount of histamine (spontaneous histamine release: 0.48±0.07 μM at 10⁵ mast cells/ml by incubation at 37°C for 30 min, n = 5), 10⁴ mast cells/ml, a concentration that is as low as 1/10 of the usual one used, was employed to eliminate as much as possible any interference by endogenously released histamine with the action of exogenously added histamine receptor agonists and antagonists. Betahistine, a drug with H₁- and weak H₂-agonistic activities (10) and H₃-antagonistic activities (11, 12), showed a very weak inhibition of anaphylactic histamine release even at high concentrations and with considerably long term incubation. From this result, it can be concluded that the H₁-receptor is not involved in the modulation of histamine release.

On the contrary, dimaprit, which is a potent H₂-agonist in terms of accelerating the atrium rate, stimulating the gastric acid secretion and contracting the uterus (18) and a weak H₃-antagonist in terms of inhibiting the brain histamine release (19), produced 25-40% inhibition of the release, although relatively high concentrations were required for the inhibition. The decreased release was not restored by the pretreatment with either an H₁- or H₂-antagonist, suggesting that the inhibition of the release by the agonist is mediated by neither H₁-receptors nor H₂-receptors.

Surprisingly, thioperamide, a highly selective H₃-antagonist (5, 6), induced restoration from the decreased release concentration-dependently, although fairly high concentrations were needed for that and complete recovery by the antagonist was not observed.

Recent experiments in our laboratory revealed that thioperamide has no cytotoxic effect on purified rat peritoneal mast cells at 30 μM and shows no potentiation of the anaphylactic histamine release in itself in similar conditions to the present experiments. Furthermore, (R)-α-methylhistamine, a highly specific H₂-agonist (5), at similar concentrations to those of dimaprit used in the
REFERENCES are distinct from autoreceptors of H3 located in brain.

It has been reported that dimaprit and betahistine prevent the H3-agonist-induced inhibition of histamine release from depolarized slices of rat cerebral cortex (Kₐ = 3 and 7 µM, respectively) (18, 19). We do not have any rational explanations for why betahistine only slightly inhibited the histamine release at the high concentration (70 µM) at only the 20- and 30-min treatment. It is also not known so far whether dimaprit partially antagonizes the dimaprit itself-induced inhibition of the histamine release, via blockade of H₃-receptors.

Compared with concentrations of thioperamide for antagonizing the H₃-agonist-induced inhibition of either histamine release from or histamine synthesis in rat brain slices depolarized by K⁺ (6), much higher concentrations were needed to recover the decreased anaphylactic histamine release from the rat mast cells. In rat plasma, considerable concentrations of histamine (approximately 0.4 µM) were detected (S. Kohno et al., manuscript in preparation). This is the probable reason why relatively high concentrations of histamine (approximately 0.4 µM) were detected (S. Kohno et al., manuscript in preparation).

Concentrations of thioperamide (70 pM) were detected (S. Kohno et al., manuscript in preparation).

Taken these results together, it is strongly suggested that dimaprit exerts the inhibition of anaphylactic histamine release from the rat peritoneal mast cells via stimulation of H₁-like receptors or H₃-subtype receptors, which are distinct from autoreceptors of H₃ located in brain.

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