Role of Substance P on Histamine H₃ Antagonist-Induced Scratching Behavior in Mice

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Abstract. The purpose of the present study was to investigate the involvement of chemical mediators, other than histamine, in the scratching behavior induced by H₃ antagonists. Scratching behavior was induced by the histamine H₃ antagonists iodophenpropit and clobenpropit (10 nmol/site) when they were injected intradermally into the rostral part of the back of mast-cell-deficient (WBB6F1 W⁻/⁻) and wild-type (WBB6F1 +/+ ) mice. Subsequently, the effect of spantide, a tachykinin NK₁ antagonist, was measured for 60 min. The effects of the H₃ antagonists on in vitro histamine release from rat peritoneal mast cells were also investigated. When spantide was injected intradermally at a dose of 0.5 nmol/site, it significantly inhibited the response. Furthermore, iodophenpropit and clobenpropit (10⁻⁶ – 10⁻⁸ M) did not induce histamine release in isolated rat peritoneal mast cells. Our results indicate that substance P is involved in the skin responses elicited by the histamine H₃ antagonists. Moreover, the fact that these histamine H₃ antagonists did not induce significant increases in the histamine release from rat peritoneal mast cells suggests that the histamine H₃ receptor may not be present in the peripheral cells considered in this study.

Keywords: histamine, H₃ antagonist, scratching behavior, substance P, spantide

Introduction

The presence of histamine H₃ receptors was first confirmed in the brain by Arrang et al. in 1983 (1), and since then, several reports suggesting the probable effects of the histamine H₃ receptors on central and peripheral tissues have become available. Histamine inhibits its own synthesis and release by binding to the histamine H₁ receptors; hence, the blockage of the histamine H₃ receptors by H₃ antagonists induces histamine release, while stimulation with the agonists inhibits it. The histamine H₃ receptors in the central nervous system appear to be present primarily on nerve terminals, where they regulate not only histamine release but also the release of other neurotransmitters such as acetylcholine, dopamine, noradrenaline, and serotonin (2). It has been suggested that in the periphery, the receptors may exist in the perivascular nerve terminals (3) and that they could also control the release of various chemical mediators such as substance P (4).

It is well known that histamine plays a critical role in allergic and inflammatory reactions via the histamine H₁ receptors and in gastric secretion through the histamine H₂ receptors. Moreover, histamine has been reported to inhibit neurogenic sympathetic vasoconstriction via the histamine H₃ receptors in the porcine nasal mucosa (5). Rouleau et al. (6) and Meleod et al. (7) have also shown that the histamine H₁ agonists such as BP 2-94 and Sch 50971 inhibited inflammations in mice and guinea pigs, respectively. However, the details regarding the effect of the histamine H₃ antagonist remain unclear.

The itch, which is considered to be a prerequisite for scratching, is one of the main symptoms of allergic diseases, and it is widely accepted that histamine is directly involved in this response. Previously, we have...
reported that the H₃-receptor antagonists, namely, thiopropamide, AQ0145, iodophenpropit, and clobenpropit, induced scratching behavior in ICR and mast-cell-deficient mice. Such a response could not be completely blocked by H₁ antagonists such as chlorpheniramine and diphenhydramine, but could be blocked by the histamine H₃ agonist Rα-methylhistamine (8, 9). The evidence that histamine H₃ antagonists induced scratching behavior in mast-cell-deficient (W/W⁰) mice, which have been reported to lack skin mast cells by Kitamura et al. (10), led us to assume that non-mast cell histamine (11) or chemical mediators other than histamine were involved in the response. Therefore, the present study was conducted to clarify which chemical mediators are possibly involved in the scratching responses induced by the histamine H₃ antagonists.

Materials and Methods

Animals

The experiment was performed using 6–10-week-old genetically mast-cell-deficient (WBB6F1 W/W⁰) female mice and their normal littermates (WBB6F1 +/+ ) weighing 20 to 30 g (Japan SLC, Shizuoka), histamine-H₁-receptor-deficient female mice weighing 15 to 25 g (bred in our laboratory), female C57BL/6 mice weighing 15 to 25 g (Japan SLC), and male Wistar strain rats weighing 450 to 500 g (Japan SLC). The animals were kept in an air-conditioned room under controlled temperature (24 ± 2°C) and humidity (55 ± 15%) conditions. Food and water were given ad libitum. All the procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments at the Okayama University Advanced Science Research Center.

Drugs

The following drugs were obtained from the sources indicated in parentheses: iodophenpropit dihydrobromide (Tocris Cookson, Ltd., Bristol, UK), clobenpropit dihydrobromide (Tocris), spantide (Peptide Institute, Osaka), compound 48/80 (Sigma, St. Louis, MO, USA), and L-732,138 (Sigma). The drugs were dissolved in physiological saline.

Histamine release from mast cells

Peritoneal mast cells were harvested from the abdominal cavity of the rats and purified by Percoll density gradient centrifugation (12). The purified mast cells were then incubated for 10 min with a chilled physiological buffer solution containing 140 mM NaCl, 0.9 mM CaCl₂, 5.6 mM glucose, 5 mM HEPES, and 0.05% bovine serum albumin, adjusted to pH 7.4. Iodophenpropit and clobenpropit were added to each tube. The reaction was arrested 10 min later by cooling the tubes in ice water, followed by centrifugation at 200 x g for 15 min. The histamine content in the supernatant and precipitate was measured using an autoanalyzer (Bran Luebbe, Osaka) and a fluorometric detector (Model U-2000; Hitachi, Tokyo) (13).

Determination of histamine content in the skin

The mice were sacrificed under ether anesthesia and the rostral part of the back was excised, washed with ice-cold saline, and weighed. The samples were then minced and homogenized in 0.4 N perchloric acid using a Polytron homogenizer (Kinematica, Lucerne, Switzerland) on ice. After centrifugation at 1,500 x g for 10 min at 4°C, the histamine content of the supernatant was measured using an autoanalyzer and a fluorometric detector as described previously (13). One hour before starting the experiment, saline, iodophenpropit, or clobenpropit was administered intradermally into the rostral part of the back of the animals.

Procedure for evaluation of scratching behavior

To evaluate the scratching behavior, the animals were first placed in an observation cage for approximately 10 min for acclimatization. A microsyringe with a 27 gauge needle was used to inject 0.02 ml of iodophenpropit or clobenpropit intradermally into the rostral part of the back. Immediately after injecting the drugs, the animals were put back into the cage (1 animal/cage) and the scratching behavior was evaluated for 60 min according to the method described by Kuraishi et al. (14) using MicroAct. In this method, only the scratches made with the hind paw at the site of injection were counted, while those at the other areas such as the ears or head were disregarded. Spantide and L-732,138 were simultaneously injected with iodophenpropit and clobenpropit.

Statistical analyses

All the values are expressed as means ± standard errors of the mean (S.E.M.). Statistical evaluation of the results was performed by one-way analysis of variance (ANOVA) followed by the Dunnett’s test. A probability value of less than 0.05 was considered statistically significant.

Results

Effects of histamine H₃ antagonists on in vitro histamine release from rat peritoneal mast cells

The results are shown in Fig. 1. Compared with the
spontaneous histamine release values, iodophenpropit and clobenpropit did not induce in vitro histamine release from isolated rat peritoneal mast cells even at a concentration of $10^{-6}$ M. On the other hand, compound 48/80 (0.5 µg/ml) significantly increased the histamine release from mast cells.

**Changes in the histamine content in the dorsal skin induced by the histamine $H_3$ antagonists**

The administration of iodophenpropit and clobenpropit at a concentration of 10 nmol/site induced scratching behavior in both types of mice, but did not alter the histamine content in the skin of the mice (Table 1).

**Effects of tachykinin NK$_1$ antagonists on the scratching behavior induced by substance P and histamine $H_3$ antagonists**

As shown in Tables 2 and 3, when spantide was administered intradermally along with substance P and histamine $H_3$ antagonists at a dose of 0.5 nmol, it caused a significant decrease in the pruritus induced by substance P, iodophenpropit, and clobenpropit in both wild-type and mast-cell-deficient mice. Similarly, when L-732,138 was administered intradermally along with the histamine $H_3$ antagonists at a dose of 5 nmol, it also

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### Table 1. $H_3$ antagonists-induced changes in histamine contents of dorsal skin from wild-type and mast-cell-deficient mice

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (nmol)</th>
<th>Histamine contents ($\mu$g/g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wild-type</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>27.2 ± 1.7</td>
</tr>
<tr>
<td>Iodophenpropit</td>
<td>10</td>
<td>25.9 ± 1.5</td>
</tr>
<tr>
<td>Clobenpropit</td>
<td>10</td>
<td>28.9 ± 0.5</td>
</tr>
</tbody>
</table>

$H_3$ antagonists were injected into the rostral part of the back and 60 min later, mice were sacrificed and histamine contents were measured. Each value represents means ± S.E.M. (n = 5).

### Table 2. Effect of spantide on scratching behavior induced by histamine $H_3$ antagonists in wild-type and mast-cell-deficient mice

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (nmol)</th>
<th>Scratches per 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wild-type</td>
</tr>
<tr>
<td>Spontaneous</td>
<td>—</td>
<td>6.0 ± 1.7</td>
</tr>
<tr>
<td>Control (Substance P, 100 nmol/site) + Spantide</td>
<td>0.05</td>
<td>44.8 ± 5.6</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>35.8 ± 5.8**</td>
</tr>
<tr>
<td>Control (Iodophenpropit, 10 nmol/site) + Spantide</td>
<td>0.05</td>
<td>35.5 ± 5.1</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>17.8 ± 1.9**</td>
</tr>
<tr>
<td>Control (Clobenpropit, 10 nmol/site) + Spantide</td>
<td>0.05</td>
<td>33.4 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>17.1 ± 2.6**</td>
</tr>
</tbody>
</table>

Mice were injected with spantide intradermally and simultaneously with $H_3$ antagonists into the rostral part of the back and the scratching behavior was measured for 60 min. Each value represents means ± S.E.M. (n = 8). *: **: Significantly different from the control group at $P<0.05$ and $P<0.01$, respectively.
caused a significant decrease in the scratching behavior.

**Histamine H₃ antagonist-induced scratching behavior in C57BL/6 and histamine-H₁-receptor-deficient mice**

An intradermal injection of iodophenpropit and clobenpropit at a dose of 100 nmol/site into the skin of histamine-H₁-receptor-deficient mice induced significant scratching behavior. This response was significantly different from that of the control group; however, when compared with the scratching behavior induced by the same drugs in other strains of mice such as C57BL/6 mice, the response was found to be approximately 3 times weaker (Fig. 2).

### Discussion

During the past few years, several reports have been published providing evidence that histamine H₃ receptors can function as heteroreceptors to modulate the release of different chemical mediators such as noradrenaline, 5-hydroxytryptamine, dopamine, acetylcholine, and substance P from the central nervous system and peripheral tissues (2, 15, 16). Furthermore, some of our previous experiments clearly demonstrated that when administered locally into the skin of mice, the H₃ antagonists induced allergy symptoms such as pruritus or vessel extravasation (9, 17). However, although we assumed that these reactions occurred due to the release of histamine and substance P, which are highly compromised during an allergy, the extent to which these chemical mediators participated in the reactions remains obscure. When iodophenpropit and clobenpropit were tested to evaluate their ability to

### Table 3. Effect of L-732,138 on scratching behavior induced by histamine H₃ antagonists in wild-type and mast-cell-deficient mice

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (nmol)</th>
<th>Spontaneous</th>
<th>Control (Substance P, 100 nmol/site) + L-732,138</th>
<th>Control (Iodophenpropit, 10 nmol/site) + L-732,138</th>
<th>Control (Clobenpropit, 10 nmol/site) + L-732,138</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wild-type</td>
<td>Mast-cell-deficient</td>
<td>Wild-type</td>
<td>Mast-cell-deficient</td>
</tr>
<tr>
<td>Spontaneous</td>
<td>—</td>
<td>4.6 ± 1.6</td>
<td>6.8 ± 1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (Substance P, 100 nmol/site) + L-732,138</td>
<td>—</td>
<td>63.8 ± 6.5</td>
<td>77.0 ± 8.0</td>
<td>40.5 ± 6.1</td>
<td>40.5 ± 6.1</td>
</tr>
<tr>
<td>Control (Iodophenpropit, 10 nmol/site) + L-732,138</td>
<td>0.5</td>
<td>47.6 ± 8.3</td>
<td>63.1 ± 10.0</td>
<td>17.5 ± 3.0</td>
<td>17.5 ± 3.0</td>
</tr>
<tr>
<td>Control (Clobenpropit, 10 nmol/site) + L-732,138</td>
<td>0.5</td>
<td>35.5 ± 4.7**</td>
<td>40.5 ± 6.1**</td>
<td>14.9 ± 2.8**</td>
<td>14.9 ± 2.8**</td>
</tr>
<tr>
<td>Control (Clobenpropit, 10 nmol/site) + L-732,138</td>
<td>5</td>
<td>29.6 ± 5.4</td>
<td>32.6 ± 2.4</td>
<td>14.9 ± 2.8**</td>
<td>14.9 ± 2.8**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23.6 ± 4.5</td>
<td>28.6 ± 3.4</td>
<td>14.9 ± 2.8**</td>
<td>14.9 ± 2.8**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.9 ± 2.8**</td>
<td>17.5 ± 3.0**</td>
<td>14.9 ± 2.8**</td>
<td>14.9 ± 2.8**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>42.4 ± 5.2</td>
<td>38.4 ± 5.3</td>
<td>17.5 ± 3.0**</td>
<td>17.5 ± 3.0**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33.6 ± 4.2</td>
<td>30.6 ± 4.9</td>
<td>17.5 ± 3.0**</td>
<td>17.5 ± 3.0**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.4 ± 4.0**</td>
<td>12.9 ± 2.2**</td>
<td>17.5 ± 3.0**</td>
<td>17.5 ± 3.0**</td>
</tr>
</tbody>
</table>

Mice were injected with L-732,138 intradermally and simultaneously with H₃ antagonists into the rostral part of the back and the scratching behavior was measured for 60 min. Each value represents means ± S.E.M. (n = 8). **: Significantly different from the control group at P<0.01.
induce histamine release from rat peritoneal mast cells in vitro, it was found that at a dose that was sufficient to induce scratching behavior in vivo, neither of the drugs had increased the histamine content in the supernatant obtained after the reaction was completed. According to Bent et al. (18), the histamine H3 antagonist thioperoxamide enhanced the histamine release from human adenoidal mast cells; these results differ from those obtained in our experiments. This discrepancy could probably be due to the higher dose of the drugs used. On the other hand, an intradermal injection of iodophenpropit and clobenpropit at a dose of 10 nmol/site into the skin of mice did not decrease the histamine content in the skin significantly. Moreover, the change in the histamine content in the skin after the intradermal injection of iodophenpropit and clobenpropit was lower than the threshold level of histamine that was necessary to induce scratching behavior as reported by Inagaki et al. (19). Lippert et al. (20) have also recently demonstrated that the presence of the histamine H3 receptors in connective tissue mast cells is doubtful. Based on these findings, it is reasonable to presume that the scratching behavior induced by histamine H3 antagonists is not mediated by histamine.

The modulation of substance P release by the histamine H3 receptors has been postulated in several studies (21). Nemmar et al. (22) reported that the nerve terminals in the afferent neurons contain certain neuropeptides such as substance P and that pretreatment with the histamine-H3-receptor agonist imetit prevented a capsaicin-induced substance P release from the C-fibers. Moreover, as previously demonstrated, exogenous substance P at a dose of 100 nmol could induce significant scratching behavior in mast-cell-deficient as well as wild-type mice (9). These findings contributed to the fact that substance P is highly compromised during itching and allergic reactions. We also provided evidence for the possible role of substance P in the scratching behavior induced by histamine H3 antagonists in mast-cell-deficient and wild-type mice, which is a hypothesis that was elucidated through experiments involving tachykinin NK1 antagonists. In both types of mice, spantide and L-732,138 clearly and significantly inhibited the scratching behavior induced by iodophenpropit and clobenpropit when administered simultaneously with these drugs, and this data provides considerable support to confirm the participation of substance P in H3 antagonist-induced itch reactions.

The authenticity of these results was strengthened by the data obtained from experiments using histamine-H3-receptor-deficient mice. Mice lacking the histamine H3 receptors have become important models to elucidate the physiological roles of histamine, and evidence suggests that intranasal instillation of histamine did not induce allergic symptoms in these mice (23). Similarly, when histamine was injected intraderrmally into the skin of these mice, it did not induce scratching behavior even at a dose of 100 nmol, whereas substance P at the same concentration significantly increased the response (data not shown). Intradermal administration of iodophenpropit and clobenpropit significantly increased the scratching behavior in histamine-H3-receptor-deficient mice, although the response was weak compared with that observed in wild-type mice. Impaired locomotor activity and exploratory behavior have been reported in mice that lack the histamine H1 receptor (24), which is probably the reason for the weak response to histamine H1 antagonist administration in this type of mice.

Based on the present results, we have provided data to support our previous hypothesis that substance P is the main mediator of the itch induced by histamine H3 antagonists. Moreover, it seems likely that histamine H3 antagonists regulate substance P release via prejunctional histamine H3 receptors that are located on peripheral nerve endings of sensory nerves. Since substance P has been demonstrated to be involved in allergic reactions and scratching behavior, the determination of the effectiveness of the histamine H3 agonists in blocking substance P derived pruritic responses is an interesting aspect of research to be pursued.

References


