

Pruritus-associated response mediated by cutaneous histamine H₃ receptors

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Summary

Background Histamine is one of the most common chemical mediators causing pruritus, and H₁ receptor antagonists have been used as a first choice in its treatment. On the other hand, although the presence of H₃ receptors has been identified in the skin, few studies have investigated the involvement of H₃ receptors on pruritus.

Objective The purpose of this study was to examine whether H₃ receptor agonist or antagonist influences the incidence of scratching behaviour in ICR or mast cell-deficient WBB6F₁-W/W^V mice.

Methods The mice were given an intradermal injection of H₃ receptor agonist or antagonist into the rostral part of the back, and the occurrence of scratching behaviour at the injected site by the hind paws was counted over 60 min.

Results H₃ receptor antagonists, thioperamide and AQ0145 significantly increased the incidence of scratching behaviour in ICR mice. H₃ receptor agonist, (R)-alpha-methylhistamine, had no effect. On the other hand, (R)-alpha-methylhistamine significantly inhibited thioperamide or AQ0145-induced scratching behaviour. In addition, both thioperamide and AQ0145 elicited scratching behaviour in mast cell-deficient WBB6F₁-W/W^V mice.

Conclusion From these results, it may be concluded that H₃ receptors are involved in the modulation of pruritus in the skin, and mast cells are not essential in this response. In addition, H₃ receptor agonists can be useful as a novel therapeutic approach against pruritus.

Keywords AQ0145, H₃ agonist, H₃ antagonist, H₃ receptor, (R)-alpha-methylhistamine, scratching behaviour, thioperamide, WBB6F₁-W/W^V mice

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Introduction

Histamine is considered to be a potent chemical mediator of pruritus, an unpleasant sensation provoking a strong desire to scratch [1–3]. In humans, it has been reported that intradermal injection of histamine and compound 48/80, an agent that induces histamine release from the skin mast cells, causes a weal and flare response, which is accompanied by the sensation of pruritus [4–6]. On the other hand, H₁ receptor antagonists inhibit both histamine and compound 48/80-induced pruritus [5, 6]; therefore, they are widely used in the treatment of allergic diseases of the skin [7]. However, the pruritus of some allergic diseases is often resistant to H₁ receptor antagonists. Given these findings, it is suggested that other mechanisms or mediators of pruritus are involved in allergic disease.

In 1983, the H₃ receptor was identified as a presynaptic autoreceptor on histamine-containing neurons in the brain, regulating the release and synthesis of histamine by a feedback mechanism [8]. Moreover, Arrang et al. [9] studied the *in vivo* effects of the H₃ receptor agonist, (R)-alpha-

methylhistamine, and the H₃ receptor antagonist, thioperamide, in the rat lung, abdominal skin and spleen, and demonstrated that (R)-alpha-methylhistamine modified the release and synthesis of histamine. Recently, the H₃ receptor has been found to have a widespread distribution in animal and human peripheral tissues [10–15]. However, there is as yet very little information as to whether the H₃ receptor is involved in pruritus in the skin.

In this study, we studied whether H₃ receptor agonist or antagonist influences the incidence of scratching behaviour, which is considered to be a pruritus-associated response in mice. The participation of skin mast cells in scratching behaviour induced by a H₃ receptor-related compound was also elucidated.

Materials and methods

Animals

The experiment was performed using 6–10-week-old female ICR mice, mast cell-deficient (WBB6F₁-W/W^V) mice and their normal littermates (WBB6F₁-+/+) weighing 25–30 g (Japan SLC, Inc., Shizuoka, Japan). The animals were housed in an air-conditioned room maintained at 22–26 °C with a humidity of 40–70%. The mice were given standard laboratory rodent chow and water *ad libitum*. All procedures

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involving animals were conducted in accordance with the guidelines of the Animal Care and Use Committee, Faculty of Pharmaceutical Sciences, Okayama University.

Drugs

(R)-Alpha-methylhistamine and thioperamide were purchased from Sigma Chemical Co. (St Louis, MO, USA). AQ0145 was kindly provided by The Green Cross Corporation (Osaka, Japan). (R)-Alpha-methylhistamine, thioperamide and AQ0145 were dissolved in physiological saline.

Scratching behaviour

To evaluate scratching behaviour, the animals were put into an observation cage ($31 \times 18 \times 24.5 \text{ cm}^3$) for about 10 min to acclimatize. The mice were given an intradermal injection of 50 μL of saline with or without drugs into the rostral part of the back. Immediately after the injection of the drugs, the animals were put back into the observation cage (one animal/cage) and the occurrence of scratching behaviour at the injected site by the hind paws was counted over 60 min. Scratching behaviour was observed in accordance with the method of Kuraishi et al. [16]. (R)-Alpha-methylhistamine was administered intraperitoneally 30 min before the injection of the drugs.

Statistical analysis

All data are presented as means $\pm$ SEM. Statistical analysis was performed using one-way ANOVA and the Dunnett's test. A probability value of less than 0.05 was considered significant.

Results

H_3 receptor agonist-induced scratching behaviour in ICR mice

As shown in Fig. 1, the intradermal injection of (R)-alpha-methylhistamine (10 $\mu\text{g}/\text{site}$) into the rostral back caused no apparent scratching behaviour on the skin around the injected site by the hind paws (similar to saline). In addition,

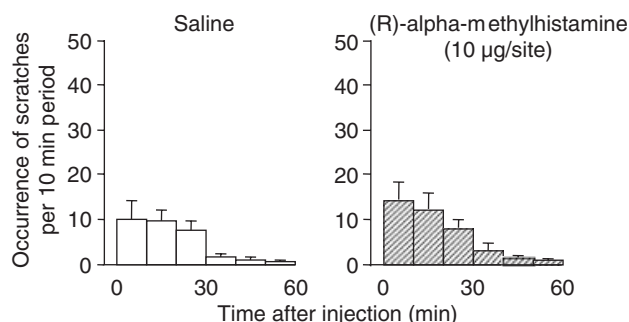


Fig. 1. Time course of scratching behaviour induced by an intradermal injection of (R)-alpha-methylhistamine (10 $\mu\text{g}/\text{site}$) in ICR mice. The ICR mice were given an intradermal injection of saline or (R)-alpha-methylhistamine at a dose of 10 $\mu\text{g}/\text{site}$ into the rostral back and the occurrence of scratching behaviour of the skin around the injected site was counted. Each column and bar represents the mean $\pm$ SEM of ten experiments.

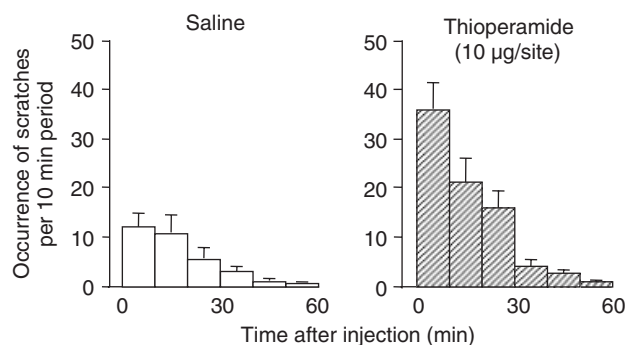


Fig. 2. Time course of scratching behaviour induced by an intradermal injection of thioperamide (10 $\mu\text{g}/\text{site}$) in ICR mice. The ICR mice were given an intradermal injection of saline or thioperamide at a dose of 10 $\mu\text{g}/\text{site}$ into the rostral back and the occurrence of scratching behaviour of the skin around the injected site was counted. Each column and bar represents the mean $\pm$ SEM of ten experiments.

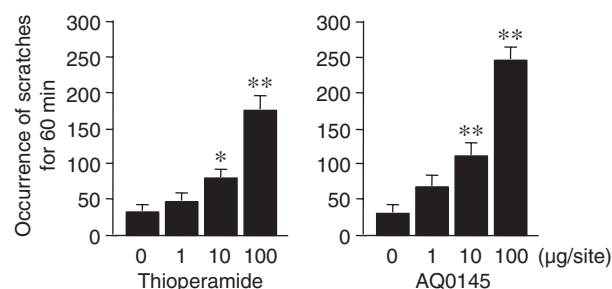


Fig. 3. Dose-response effect of H_3 receptor antagonist-induced scratching behaviour in ICR mice. The ICR mice were given an intradermal injection of saline, thioperamide (1, 10 and 100 $\mu\text{g}/\text{site}$) or AQ0145 (1, 10 and 100 $\mu\text{g}/\text{site}$) into the rostral back and the occurrence of scratching behaviour of the skin around the injected site was counted over 60 min. Each column and bar represents the mean $\pm$ SEM of ten experiments. *, **: Significantly different from saline-treated mice by ANOVA and the Dunnett's test ($P < 0.05$ and $P < 0.01$, respectively).

intradermal injection of (R)-alpha-methylhistamine at a dose of 100 $\mu\text{g}/\text{site}$ also caused no significant scratching behaviour compared with that of saline-treated mice (data not shown).

H_3 receptor antagonist-induced scratching behaviour in ICR mice

Figure 2 shows the incidence of scratching behaviour over 60 min after the intradermal injection of thioperamide. Thioperamide at a dose of 10 $\mu\text{g}/\text{site}$ elicited an apparent scratching behaviour compared with saline-treated control. The effect of thioperamide peaked in the initial 10-min period and almost subsided 30 min after the injection. The effects of H_3 receptor antagonists (thioperamide and AQ0145) on the incidence of scratching behaviour for 60 min are shown in Fig. 3. Both thioperamide and AQ0145 caused dose-related increases in the incidence of scratching behaviour in ICR mice. Significant increases were observed at doses of 10 and 100 $\mu\text{g}/\text{site}$ for both thioperamide and AQ0145.

Inhibition by (R)-alpha-methylhistamine on H_3 receptor antagonist-induced scratching behaviour in ICR mice

(R)-Alpha-methylhistamine showed a dose-related inhibition of the scratching behaviour induced by thioperamide (10

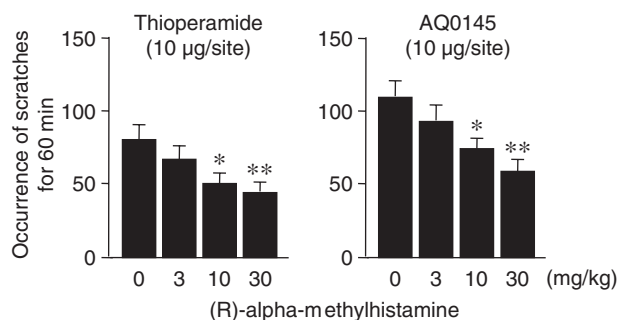


Fig. 4. Inhibition by (R)-alpha-methylhistamine on H_3 receptor antagonist-induced scratching behaviour in ICR mice. The ICR mice were given an intraperitoneal injection of saline or (R)-alpha-methylhistamine (3, 10 and 30 mg/kg) and 30 min later an intradermal injection of thioperamide (10 µg/site) or AQ0145 (10 µg/site) was made into the rostral back. The occurrence of scratching behaviour of the skin around the injected site was counted over 60 min. Each column and bar represents the mean $\pm$ SEM of ten experiments. *, **: Significantly different from vehicle-treated mice by ANOVA and the Dunnett's test ($P < 0.05$ and $P < 0.01$, respectively).

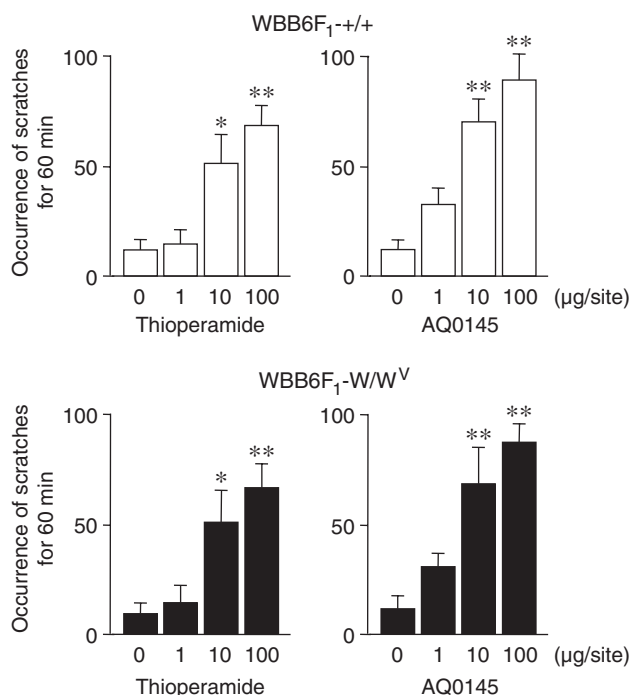


Fig. 5. Dose-response effect of H_3 receptor antagonist-induced scratching behaviour in WBB6F1-+/+ and WBB6F1-W/W^V mice. The WBB6F1-+/+ or WBB6F1-W/W^V mice were given an intradermal injection of saline, thioperamide (1, 10 and 100 µg/site) or AQ0145 (1, 10 and 100 µg/site) into the rostral back and the occurrence of scratching behaviour of the skin around the injected site was counted over 60 min. Each column and bar represents the mean $\pm$ SEM of ten experiments. *, **: Significantly different from saline-treated mice by ANOVA and the Dunnett's test ($P < 0.05$ and $P < 0.01$, respectively).

µg/site) and a significant effect was observed at doses higher than 10 mg/kg (Fig. 4). Similar to thioperamide, AQ0145 (10 µg/site)-induced scratching behaviour was also inhibited by (R)-alpha methylhistamine; a significant effect was observed at doses of 10 and 30 mg/kg (Fig. 4).

H_3 receptor antagonist-induced scratching behaviour in WBB6F1-+/+ and WBB6F1-W/W^V mice

The effects of H_3 receptor antagonists (thioperamide and AQ0145) on the incidence of scratching behaviour after intradermal injection in WBB6F1-+/+ and WBB6F1-W/W^V mice are shown in Fig. 5. Thioperamide and AQ0145 caused dose-related increases in the incidence of scratching behaviour both in WBB6F1-+/+ and WBB6F1-W/W^V mice. Significant increases were observed at doses of 10 and 100 µg/site for both drugs.

Discussion

Recently, numerous studies have shown that H_3 receptors are also present in various peripheral tissues other than the brain [9–15]. In the periphery, it has been suggested that the H_3 receptors exist in perivascular nerve terminals, and regulate the release of histamine [9], acetylcholine [15] or noradrenaline [17]. Furthermore, it is well-known that histamine regulates substance P release from peripheral ending sensory nerves via H_3 receptors [13, 18]. Previous studies demonstrated the existence of H_3 receptors on the C-fibres of peripheral endings of the sensory nerves [9, 13]. These findings suggest the possibility that H_3 receptors are involved in pruritus of the skin.

(R)-Alpha-methylhistamine and thioperamide, the first H_3 receptor agonist and antagonist, respectively, to be recognized, were used to characterize the H_3 receptors definitively [9], in addition to also being used in various experiments to investigate the physiological and pharmacological roles of the H_3 receptors. In the present study, we found that the intradermal injection of the H_3 receptor antagonist, thioperamide, significantly induced scratching behaviour in ICR mice. On the other hand, no apparent scratching behaviour was observed after intradermal injection of the H_3 receptor agonist, (R)-alpha-methylhistamine. As shown before, H_3 receptor antagonists stimulate the synthesis and release of histamine, and agonists show opposite direction. This is the reason why H_3 agonists caused no scratching behaviour. Recently, AQ0145 has been synthesized, and it showed both potent H_3 receptor affinity and antagonistic activity [19]. In addition, AQ0145 has been reported to decrease seizure susceptibility from electrically induced convulsion in mice, suggesting the efficacy of this compound *in vivo* [20]. As shown in the present study, an intradermal injection of AQ0145 increased the incidence of scratching behaviour in mice. The relative potency of AQ0145 was higher than that of thioperamide. Similar findings also demonstrated that the effect of AQ0145 against H_3 receptors was five times higher than thioperamide in binding assay *in vitro* [19]. On the other hand, H_3 receptor antagonist-induced scratching behaviour was found to be antagonized by pretreatment with the H_3 receptor agonist in the present study. These results suggest that the effect of the H_3 receptor antagonist is mediated by H_3 receptors in the skin. It is generally considered that (R)-alpha-methylhistamine show a sedative effect. However, we already demonstrated that inhibition of scratching behaviour cannot be ascribed to the sedative action of drugs such as H_1 receptor antagonists and diazepam [21].

From previous observations, it can be assumed that H₃ receptors exist in the skin [11, 18, 22]. However, no evidence has been found for the presence of H₃ receptors on cutaneous endothelial or mast cells [12]. Kohno et al. [23] reported that H₃ receptors may be present on rat peritoneal mast cells. On the other hand, Rozniecki et al. [24] indicated that neither H₃ receptor agonist nor H₃ receptor antagonist had any effect on rat peritoneal mast cells. It is well-known that mast cell-deficient WBB6F₁-W/W^V mice have been extensively used as an important model for studying the role of mast cells. These mice have been shown to have less than 1% of the mast cells found in the skin of their controls or other normal mice [25]. As shown in the present study, significant increases in the incidence of scratching behaviour were also observed in mast cell-deficient mice after intradermal injection of H₃ receptor antagonists. These observations strongly suggest that mast cells in the skin are not essential in the H₃ receptor antagonist-induced scratching behaviour.

In conclusion, H₃ receptors are involved in the modulation of pruritus in the skin, and mast cells are not essential in this response. Therefore, H₃ agonists may be useful as novel therapeutic agents against pruritus.

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