

Histamine H₄ receptor antagonists are superior to traditional antihistamines in the attenuation of experimental pruritus

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Background: Histamine is a potent mediator of itch in humans, yet histamine H₁ receptor antagonists have been shown to be of limited use in the treatment of certain chronic pruritic diseases. The histamine H₄ receptor is a recently described histamine receptor, expressed on hematopoietic cells, linked to the pathology of allergy and asthma.

Objective: The contribution of the novel histamine H₄ receptor to histaminergic and allergic pruritus was investigated.

Results: Histamine and a selective histamine H₄ receptor agonist caused scratching responses in mice, which were almost completely attenuated in histamine H₄ receptor knockout mice or by pretreatment with the selective histamine H₄ receptor antagonist, JNJ 7777120. Pruritus induced by allergic mechanisms was also potently inhibited with histamine H₄ receptor antagonist treatment or in histamine H₄ receptor knockout mice. In all cases, the inhibitory effect of histamine H₄ receptor antagonist was greater than those observed with histamine H₁ receptor antagonists. The histamine H₄ receptor-mediated pruritus was shown to be independent of mast cells or other hematopoietic cells and may result from actions on peripheral neurons.

Conclusion: These results demonstrate that the histamine H₄ receptor is involved in pruritic responses in mice to a greater extent than the histamine H₁ receptor.

Clinical implications: Histamine H₄ receptor antagonists may have therapeutic utility for treating chronic pruritic diseases in humans where histamine H₁ receptor antagonists are not effective. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Histamine, histamine H₁ receptor, histamine H₄ receptor, pruritus, mast cells

Abbreviations used

H₁R: Histamine H₁ receptor
H₂R: Histamine H₂ receptor
H₃R: Histamine H₃ receptor
H₄R: Histamine H₄ receptor
H₄R^{-/-}: Histamine H₄ receptor knockout
H₄R^{+/+}: Histamine H₄ receptor wild-type

Pruritus associated with chronic diseases such as atopic dermatitis is poorly controlled in the clinic and has a major effect on the quality of life of patients. Histamine H₁ receptor (H₁R) antagonists are commonly prescribed despite any clear evidence of their effectiveness. Any minor effects are generally attributed to central sedative properties of first-generation drugs,¹ or to other anti-inflammatory properties associated with second-generation and third-generation antagonists.² In contrast, itch and other symptoms associated with acute dermal allergic responses and urticaria are well controlled both by sedating, centrally acting H₁R antagonists and by nonsedating, peripherally acting, second-generation and third-generation compounds.^{3,4} The clinical efficacy of histamine H₂ receptor (H₂R) antagonists in itch is minimal,⁵ whereas histamine activity at the histamine H₃ receptor (H₃R) potentially increases itch.⁶ The discovery of the histamine H₄ receptor (H₄R) opens the possibility of its involvement in pruritic diseases.

Numerous endogenous substances are regarded as mediators of itch, including neuropeptides, amines, cytokines, neurotrophins, proteases, cannabinoids, and opioids, as recently reviewed.⁷ These may act directly to sensitize or stimulate itch-sensing, afferent C fibers, or indirectly via skin mast cells to release other pruritogenic mediators. C fibers have been observed to be intimately associated with mast cells, the main source of pruritogenic mediators in the skin.^{8,9} Of these mediators, histamine has been extensively studied for its pruritogenic effects and has been shown to be a potent pruritogen when applied to both normal human skin¹⁰ and diseased skin.¹¹ Its association with itch in response to allergic stimuli has been well documented.^{12,13}

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The lack of efficacy of currently marketed antihistamines that target H₁ and H₂ receptors in some chronic pruritic conditions has led to the hypothesis that mast cell mediators other than histamine are the cause of itch in these conditions,^{14,15} but definitive evidence for this is lacking. Given the fact that histamine and its main cellular sources, mast cells and basophils, are known to be increased in the circulation and skin of patients with atopic dermatitis and chronic urticaria,¹⁶⁻¹⁸ the possibility exists that histamine receptors other than, or in addition to, those already clinically evaluated may be involved in these pruritic responses.

Recently, the novel H₄R has been shown to be important in mast cell and eosinophil function, as well as in allergic inflammation *in vivo*.^{19,20} The H₄R is expressed on many hematopoietic cells, including mast cells in human skin,²¹ that are known to be involved in pruritic responses. It is possible, therefore, that the H₄R may mediate itch that is not controlled by H₁R antagonists. In support of this, an initial investigation into the role of H₄R in itch using nonselective agonists and antagonists suggested that the receptor may be mediating histamine-induced itch in mice.²² Here we use the selective H₄R antagonist JNJ 777120, H₄R knockout mice, and a selective H₄R agonist to define the role of H₄R in pruritic responses to a variety of pruritogens and to investigate the pathways involved.

METHODS

Animals

All experiments and procedures were performed according to on-site Institutional Animal Care and Use Committee standards and regulations. Female CD-1 mice (Charles River, Hollister, Calif) approximately 10 to 12 weeks old, H₄R knockout (–/–) and littermate, wild-type (+/+) mice (c57bl/6 sv129),²³ or mast cell-deficient (WBBF1-W/W^{*}) and sufficient control (WBB6F1-+/+) mice 10 to 12 weeks of age (Jackson Laboratories, Bar Harbor, Maine) were used. H₄R^{–/–}/+/+ bone marrow chimera mice were produced as described below. All were housed under controlled light (6:00-18:00 hours) and temperature (22°C) with food and water available ad libitum.

Reagents

The H₄ receptor antagonist JNJ 777120 was synthesized as previously described,²⁴ dissolved in a vehicle of 20% hydroxypropyl-β-cyclodextrin/water (HPβCD; wt/vol) at various concentrations, and administered per os (p.o.) at a volume of 10 mL/kg. Antagonists of other histamine receptors, H₁R, diphenhydramine, fexofenadine, desloratadine, loratadine, H₂R, ranitidine (Sigma Chemical Co, St Louis, Mo), and H₃R, JNJ 5207852²⁵ were formulated similarly for use in certain experiments. Histamine diphosphate and compound 48/80 (Sigma Chemical Co, St Louis, Mo) were dissolved in Dulbecco PBS (pH 7.4) at various concentrations and at 5 mg/mL, respectively, for intradermal administration. A selective H₄R agonist, 4-methylhistamine (synthesized at Johnson & Johnson Pharmaceutical Research & Development; LLC), was dissolved in PBS at various concentrations for intradermal administration.

For induction of IgE-mediated itch, antidinitrophenyl IgE (MP Biomedicals Inc, Aurora, Ohio) was made up at 1 μg/mL for intradermal administration of 20 ng in 20 μL PBS, and dinitrophenyl-

ovalbumin (Sigma Chemical Co, St Louis, Mo) at 1 mg/mL for intravenous injection of 100 μg in 100 μL PBS.

Production of H₄R bone marrow wild-type/knockout chimeras

Bone marrow chimeras were produced as previously described.²⁶ Briefly, H₄R^{–/–} mice were lethally irradiated with a dose of 400 Rad from a cesium 137-γ source. After 24 hours, mice received either 10⁶ bone marrow cells from H₄R^{+/+} mice or H₄R^{–/–} as control. Successful reconstitution of H₄R^{+/+} cells into H₄R^{–/–} was confirmed by the presence of H₄R RNA in the bone marrow, blood, and skin by RT-PCR. Experiments were then performed as described for other mice.

Induction of itch responses

In all experiments, the hair was clipped over the rostral part of the back 24 hours before intradermal injection of pruritogen. Where noted, animals received an oral dose of H₄R antagonist JNJ 777120 or other selective histamine receptor antagonists 20 minutes before injection of pruritogen. Animals were injected intradermally with 20 μL pruritogen (histamine, compound 48/80, H₄R agonist, 4-methylhistamine), or were given PBS as a vehicle control. Immediately after intradermal injection, the animals were returned to an acrylic cage (approximately 15 cm diameter × 30 cm high), to which they had been acclimated for at least 1 hour before the experiment, for observation of itch responses. Some animals were observed for passive cutaneous anaphylaxis-induced itch, where 20 ng anti-dinitrophenyl-IgE was injected intradermally 24 hours before 100-μg intravenous administration of dinitrophenyl-ovalbumin, at which point the described observation of itch was commenced. Antagonists were dosed 20 minutes before the intravenous injection.

Measurement of itch

Scratching behavior, which correlates with the sensation of itch in human beings,²⁷ was recorded with a video camera and the signal recorded on a DVD recorder. Itch was measured by blinded counting of the number of bouts in the 20-minute period immediately after the intradermal injection. A bout of scratching was defined as 3 or more individual rapid scratch movements with the hind paws around the injection site.

Measurement of histamine-induced paw edema

Female BALB/c mice (n = 5 per group) were used to test the efficacy of the various H₁R antagonists on histamine-induced paw edema. Diphenhydramine was administered at 20, 50, and 150 mg/kg and fexofenadine at 150 mg/kg p.o. in 20% HPβCD 20 minutes before intraplantar histamine injection. The assay was run as previously described²⁸ using the same dose of histamine as used in the pruritus model (300 nmol).

Binding assays

Cell pellets from SK-N-MC cells transfected with mouse H₄ receptor or mouse H₁R were used. For the H₄R competition binding studies, [³H]histamine was used. The ligand used for the mouse H₁R binding was [³H]pyrilamine. The details of the assays are given by Thurmond et al.²⁸

Statistical analysis

Data were analyzed by using GraphPad Prism (v4.0) software (GraphPad Software, San Diego, Calif). Multiple test groups were compared with 1-way ANOVA with post hoc Bonferroni test. P values of < .05 are considered significant, and data are represented as the means ± SEMs.

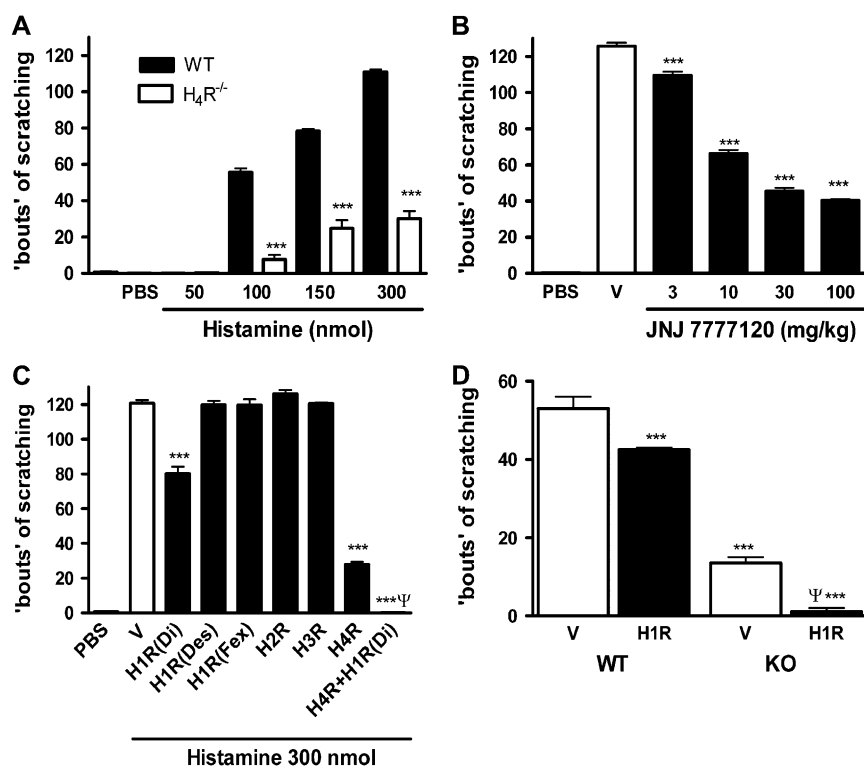


FIG 1. Effect of the H₄R on scratching induced by histamine. **A**, Histamine induced itch in a dose-dependent manner in wild-type (WT) mice, and this is attenuated in H₄R^{-/-} animals (n = 5). **B**, The H₄R antagonist JNJ 777120 (p.o.) blocks scratching to histamine (300 nmol) in CD-1 mice. **C**, Selective antagonists of the 4 known histamine receptors (H₁R(Di), diphenhydramine; H₁R(Des), desloratadine; H₁R(Fex), fexofenadine; H₂R, ranitidine; H₃R, JNJ 5207852; H₄R, JNJ 777120) were given at 20 mg/kg p.o. except Di (50 mg/kg) and Fex (150 mg/kg) before measuring scratching induced by intradermal injection of 300 nmol histamine into CD-1 mice (n = 5). **D**, The effects of the H₁R antagonist, diphenhydramine (10 mg/kg p.o.), on histamine-induced itch in H₄R^{-/-} (KO) or H₄R^{+/+} (WT) mice (n = 5). ***P < .001 vs vehicle or WT and ψP < 0.05 for H₁R vs vehicle in H₄R^{-/-}.

RESULTS

Histamine-mediated pruritus

Pruritic responses to histamine were examined in H₄R^{-/-} mice in comparison with wild-type controls. Histamine injected intradermally induced a pruritic response in a dose-dependent fashion in H₄R^{+/+} mice, whereas H₄R^{-/-} mice showed a significantly reduced ($P < .001$ vs +/+) response at all doses (Fig 1, A). To confirm the role of the H₄R in this response, intradermal injection of histamine also induced itch in a dose-dependent fashion in CD-1 mice (data not shown), with a dose of 300 nmol used for subsequent pharmacologic evaluation. In these studies, oral dosing of JNJ 777120, a selective H₄R antagonist,²⁸ at 3, 10, 30, 100 or mg/kg significantly reduced histamine-induced itch ($P < .001$ vs vehicle treated control) in a dose-dependent fashion. Maximal inhibition of 75% was observed at 30 mg/kg with a calculated ED₅₀ of around 5 mg/kg (Fig 1, B). Animals injected intradermally with PBS alone did not demonstrate an itch response.

To compare the relative contributions of the 4 known histamine receptors to histamine-induced itch, antagonists of these receptors were administered before intradermal

injection of histamine (Fig 1, C). Significant inhibition (~80%; $P < .001$) was again demonstrated for the H₄R antagonist JNJ 777120. Neither ranitidine nor JNJ 5207852, selective antagonists of H₂R and H₃R, respectively, demonstrated significant inhibition of histamine-mediated itch. Slight (~35%) but significant inhibition was observed with diphenhydramine, a H₁R antagonist with central nervous system activity. Fexofenadine and desloratadine had no effect. A dose response of fexofenadine showed no inhibition at 5, 50, or 150 mg/kg (see this article's Fig E1, A, in the Online Repository at www.jacionline.org). The dose response of diphenhydramine indicated a maximal effect on histamine-induced pruritus at 50 mg/kg (see this article's Fig E1, B, in the Online Repository at www.jacionline.org). The doses of fexofenadine and diphenhydramine used in subsequent experiments (150 mg/kg and 50 mg/kg, respectively) completely blocked histamine-induced paw edema (see this article's Fig E1, C, in the Online Repository at www.jacionline.org), indicating that those doses were sufficient to antagonize peripheral H₁R completely. JNJ 777120 has been shown to have no effect in this model.²⁸ Combination dosing of diphenhydramine and JNJ 777120 resulted in total ablation

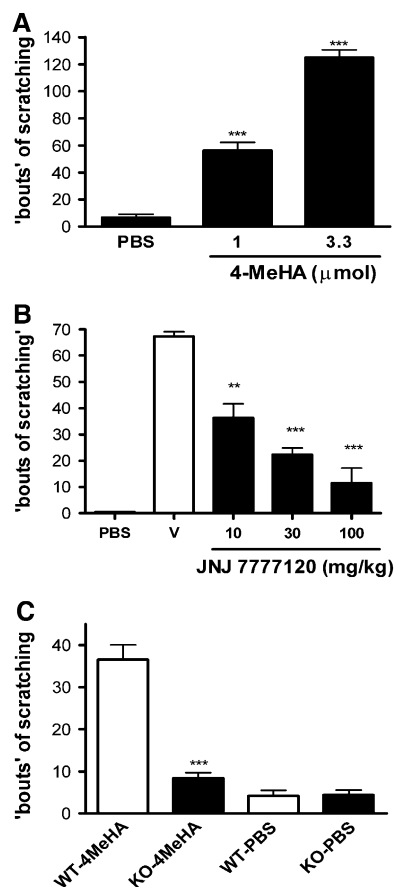


FIG 2. A selective H_4R agonist induces itch. **A**, Dose response of H_4R agonist, 4-methylhistamine (4-MeHA), induced scratching in mice ($n = 5$). **B**, Inhibition of itch induced by intradermal injection of 1 μ mol 4-methylhistamine in mice ($n = 5$) after p.o. dosing with various doses of the H_4R antagonist, JNJ 777120, or vehicle 20 minutes before. **C**, Itch induced by intradermal injection of 1 μ mol 4-methylhistamine or PBS in $H_4R^{-/-}$ (KO) mice versus wild-type (WT) controls ($n = 5$). ** $P < .01$; *** $P < .001$.

of the pruritic response ($P < .001$). In addition, the residual itching in knockout animals was completely abolished by dosing with diphenhydramine (Fig 1, D). The selectivity of these compounds for mouse H_4R and H_1R is given in this article's Table E1 in the Online Repository at www.jacionline.org. These results suggest that histamine-induced itch in mice is mediated by both H_1R and H_4R , with the H_4R having a more dominant role.

H_4R agonist-mediated pruritus

The contribution of H_4R to histamine-mediated itch was further evaluated by intradermal injection of a selective H_4R agonist, 4-methylhistamine.²⁹ This agonist caused a dose-dependent increase in scratching behavior similar to that seen with histamine (Fig 2, A), further supportive of a direct role for H_4R in pruritic responses. Significant dose-related inhibition of 4-methylhistamine-induced itch by oral administration of JNJ 777120 was also demonstrated (Fig 2, B). To verify further the role of the H_4R in this process, 4-methylhistamine was injected

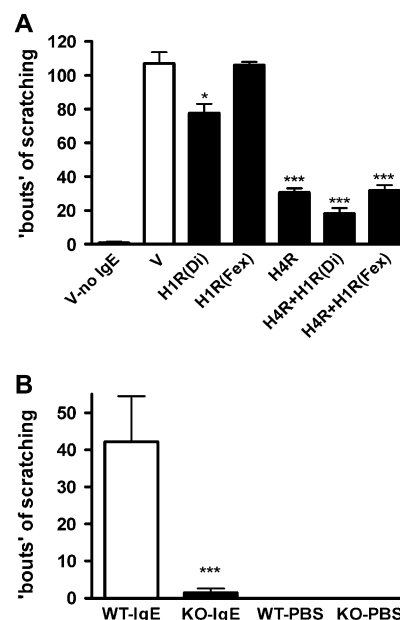


FIG 3. The role of the H_4R in antigen-specific IgE-mediated scratching. **A**, Comparative activity of H_1R and H_4R antagonists versus scratching evoked by antigen-specific IgE in mice ($n = 5$). H_4R antagonist, JNJ 777120, dosed p.o. at 30 mg/kg, or H_1R antagonists, diphenhydramine ($H1R(Di)$, 50 mg/kg) and fexofenadine ($H1R(Fex)$, 150 mg/kg) p.o. or in combination ($H4R + H1R$) given 20 minutes before dinitrophenyl is injected intravenously. **B**, Antigen-specific IgE-induced scratching in $H_4R^{-/-}$ (KO) mice versus wild-type (WT) controls ($n = 5$). * $P < .05$; *** $P < .001$ vs vehicle or WT-IgE.

into $H_4R^{-/-}$ mice, and no pruritic response was observed in comparison with wild-type controls (Fig 2, C).

Passive cutaneous anaphylaxis-mediated pruritus

Histamine-induced scratching is a well characterized model for pruritic responses in both mice and humans. However, the relative importance of histamine to allergic pruritus is uncertain because mast cells, which are thought to contribute to this symptom, can release a number of different pruritogens on activation, in addition to histamine. We therefore investigated the role of the H_4R in a model of IgE-dependent mast cell-mediated itch, which is perhaps more representative of allergic-type itch responses. In this case, antigen-specific IgE was injected intradermally, and when antigen was given systemically 24 hours later, scratching was observed at the site of IgE injection. This is a result of specific degranulation of mast cells at this site. Dosing allergic mice with JNJ 777120 at 30 mg/kg was effective in significantly attenuating itch in response to antigen challenge ($P < .001$ vs vehicle), with a 72% reduction in the observed effect (Fig 3, A). The centrally acting H_1R antagonist, diphenhydramine, caused a moderate (27%) but significant ($P < .001$) reduction in pruritus, whereas the noncentrally acting agent, fexofenadine, showed no reduction in IgE-evoked scratching. These data are in agreement with the limited perturbation of IgE-mediated pruritus by H_1R antagonists or in H_1R

knockouts.^{30,31} When JNJ 777120 was dosed in conjunction with each of the H₁R antagonists, significant inhibition ($P < .001$) was again observed that was similar to JNJ 777120 alone. The slight reduction observed in combination with diphenhydramine was not statically significant. Further confirmation for the role of the H₄R in this response was obtained in H₄R^{-/-} mice. Although allergen provoked a robust scratch response in H₄R^{+/+} mice, the scratch response to IgE was virtually abolished in H₄R^{-/-} mice ($P < .001$ vs $+/+$; Fig 3, B). These results clearly indicate that the H₄R, with some minor contribution of H₁R, is involved in the pruritic response seen with mast cell degranulation even in this system where multiple potential pruritogens are released.

Role of hematopoietic cells in H₄R-mediated pruritus

The expression of the H₄R is thought to be mainly restricted to hematopoietic cells. Mast cells are the most obvious source of histamine in many pruritic diseases and certainly are key players in the previously discussed IgE model in which no scratching is observed in mast cell-deficient mice (data not shown). In addition to producing histamine, mast cells also express the H₄R,^{21,23} and therefore histamine might be argued to have some autocrine effects on mast cells through the H₄R that could be important in driving the observed pruritic responses. Although it is known that the H₄R does not affect IgE-mediated degranulation of mast cells,²³ we addressed this possibility by examining whether JNJ 777120 could block histamine-induced itch in mast cell-deficient mice. Mast cell-deficient mice showed no significant difference in itch response to histamine compared with mast cell-sufficient mice (Fig 4, A). Additionally, mast cell-sufficient and mast cell-deficient animals treated with JNJ 777120 showed comparable reduction in pruritic responses to histamine ($P < .001$ and $.01$, respectively). Finally, intradermal application of the H₄R specific agonist, 4-methylhistamine, caused scratching comparable to that observed in mast cell-sufficient mice (Fig 4, B). These results suggest that H₄R expression on mast cells is not required for inducing pruritic responses.

To investigate whether H₄R expression on other hematopoietic cells could be involved in H₄R-mediated pruritus, we reconstituted H₄R^{-/-} mice with H₄R^{+/+} bone marrow, so that any H₄R expression in these mice could only be on cell types derived from bone marrow, and measured itch responses to 4-methylhistamine. The presence of H₄R^{+/+} cells in these mice was confirmed by RT-PCR of bone marrow, blood, and skin (data not shown). Consistent with the results in Fig 2, H₄R^{+/+} mice scratched in response to the H₄R agonist, and this was completely attenuated in H₄R^{-/-} mice or in H₄R^{-/-} mice reconstituted with knockout bone marrow (Fig 4, C). Reconstitution of the H₄R^{-/-} mice with wild-type bone marrow was insufficient to restore scratching in response to agonist (Fig 4, C). Therefore, it can be concluded that H₄R expression on nonhematopoietic cells is responsible for mediating the pruritic responses.

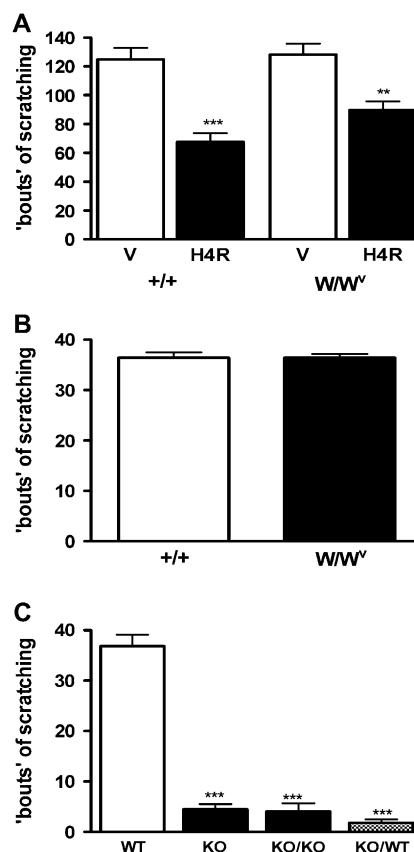


FIG 4. H₄R-mediated scratching does not require mast cells or other hematopoietic cells. **A**, Bouts of scratching were measured after intradermal injection of 300 nmol histamine in mast cell-sufficient (WBB6F1-+/+) and mast cell-deficient (WBB6F1-W/W^V) mice ($n = 10$). The inhibition by H₄R antagonists was analyzed in both strains by p.o. dosing of JNJ 777120 (30 mg/kg) or PBS (V) 20 minutes before histamine injection. **B**, Bouts of scratching were measured after intradermal injection of 1 μ mol 4-methylhistamine in mast cell-sufficient (WBB6F1-+/+) and mast cell-deficient (WBB6F1-W/W^V) mice ($n = 5$). **C**, Bouts of scratching were measured after intradermal injection of 1 μ mol 4-methylhistamine into wild-type (WT), H₄R^{-/-} mice (KO) or H₄R^{-/-} mice reconstituted with bone marrow from H₄R^{-/-} (KO/KO) or wild-type (KO/WT) mice ($n = 5$). ** $P < .01$ vs vehicle; *** $P < .001$ vs vehicle or wild-type.

Compound 48/80-mediated pruritus

Compound 48/80 is best known as a potent mast cell degranulator, but it also has the ability to activate sensory nerve fibers directly.³² Intradermal injection of compound 48/80 produced a robust itch, but this response was similar in both mast cell-deficient mice and mast cell-sufficient control mice when dosed with vehicle, consistent with previous reports.³³ Treatment with JNJ 777120 caused a highly significant ($P < .001$) and marked inhibition of scratching behavior in both strains (Fig 5, A). In CD-1 mice, pretreatment with JNJ 777120 (3, 10, 30, 100 mg/kg) led to a dose-dependent inhibition of 48/80-mediated itch, which was significantly reduced at all doses ($P < .001$ vs vehicle). Maximal inhibition of around 62% was observed at 30 mg/kg, which did not differ significantly from similar inhibition at higher doses (Fig 5, B).

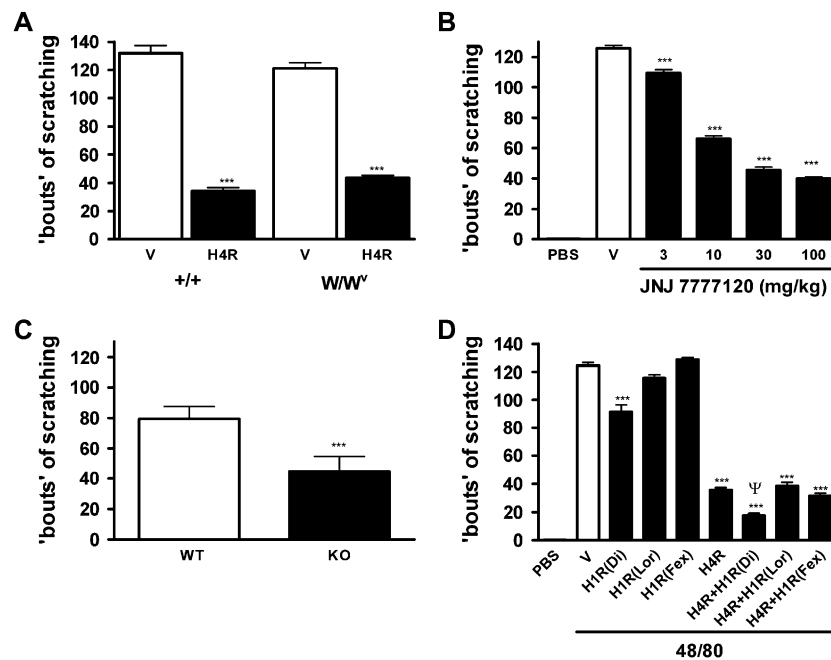


FIG 5. Effect the H₄R on scratching induced by compound 48/80. **A**, Bouts of scratching were measured after intradermal injection of 100 μ g compound 48/80 in mast cell-sufficient (WBB6F1-+/+) and mast cell-deficient (WBB6F1-W/W^V) mice (n = 5). The inhibition of 48/80 by the H₄R antagonist JNJ 777120 (30 mg/kg p.o.) was analyzed. **B**, Inhibition by itch induced by compound 48/80 in CD-1 mice (n = 5) after p.o. dosing with JNJ 777120 twenty minutes before. **C**, Itch induced by compound 48/80 in H₄R^{-/-} (KO) mice versus wild-type controls (n = 5). **D**, Comparative activity of H₁R and H₄R antagonists versus scratching evoked by compound 48/80 CD-1 mice (n = 5). H₄R antagonist, JNJ 777120 (30 mg/kg p.o.), or H₁R antagonists, diphenhydramine (H1R(Di), 50 mg/kg p.o.), fexofenadine (H1R(Fex), 150 mg/kg p.o.) and loratadine (H1R(Lor), 10 mg/kg p.o.) or in combination (H4R+H1R). ***P < .001 vs vehicle or wild-type control; Ψ P < .05 for H₁R/H₄R vs H₄R alone. V, Vehicle.

Similarly, compound 48/80 injected intradermally induced a pruritic response in H₄R^{+/+} mice, whereas H₄R^{-/-} mice showed a significantly reduced ($P < .001$ vs +/+) response (Fig 5, C). Therefore, the mast cell degranulating capacity of 48/80 is not responsible for itch responses in our model, but the itch it produces is still mediated by the H₄R.

To examine the potential complimentary effects of H₄R and H₁R antagonism, JNJ 777120 was compared with centrally acting diphenhydramine and noncentrally active H₁R antagonists, fexofenadine and loratadine, against scratching evoked by compound 48/80 (Fig 5, D). Diphenhydramine was minimally but significantly efficacious ($P < .001$), whereas neither fexofenadine nor loratadine demonstrated any activity. The inhibition in scratching observed after JNJ 777120 was, as before, highly significant ($P < .001$). Combining diphenhydramine and JNJ 777120 afforded additive protection, whereas the addition of fexofenadine or loratadine to JNJ 777120 provided no benefit.

DISCUSSION

The ability of histamine to produce pruritic responses in humans is incontrovertible. However, its involvement in various chronic pruritic diseases has been debated, largely because of the ineffectiveness of existing H₁R and H₂R

antagonists in the clinic. Here we demonstrate the definitive role of the histamine H₄ receptor in murine itch and investigate the possible mechanisms involved in H₄R-mediated pruritus using clinically relevant pruritogens and allergic mechanisms.

The pruritic response to histamine and a selective H₄R agonist was dramatically attenuated in H₄R^{-/-} mice and by treatment with JNJ 777120, a selective, potent, and orally active H₄R antagonist.²⁸ The histamine-induced pruritic response in mice is thought to be similar to the pruritic response induced by histamine in human beings²⁷; however, the role of H₄R in a more physiologically relevant model of itch was additionally evaluated using an IgE and mast cell-dependent model in which surface-bound IgE on mast cells is cross-linked by antigen to cause degranulation and activation.³⁴ Using this model, scratching induced by mast cell degranulation was again significantly inhibited in H₄R^{-/-} mice or by treatment with JNJ 777120. Indeed, the inhibition was almost complete, which is an interesting observation, given that mast cell activation and degranulation can produce many putative pruritogens, such as tryptase and leukotriene B₄, in addition to histamine. This could suggest that the H₄R is also acting downstream of other mediators at a more central level to transmit pruritic signals. In support of this, we have demonstrated that pruritic responses to compound 48/80 administration could be inhibited by H₄R

perturbation. This potent pruritogen has been demonstrated by us, and by others, to exert direct effects on sensory nerves and mediate itch independent of its effects on mast cells.^{32,33} Although controversial, there are reports of H₄R expression in the spinal cord and brain.³⁵ This could be in parallel with its expression in the periphery, much like H₁R and H₃R. Together, these results clearly indicate that the H₄R can mediate both histaminergic and allergic pruritus in mice, and may modulate conduction of other pruritic signals.

Given that several histamine receptors could be involved in pruritus, we investigated the relative contribution of the 4 known histamine receptors using selective antagonists. In all of our investigations, H₄R antagonism was by far the most effective inhibitor of itch, with only the H₁R antagonist among the other drugs tested, diphenhydramine, demonstrating significant activity. This was true for histamine, IgE, and compound 48/80-induced itch. Notably, the comparative clinical efficacy of the different histamine receptor antagonists in chronic conditions is consistent with the data presented here in the acute mouse models. Centrally acting H₁R antagonists, such as diphenhydramine, have limited effectiveness in these models and in chronic human disease, whereas H₁R antagonists that have limited brain penetration, such as fexofenadine and desloratadine, are not effective in mice or human beings. It is interesting to speculate, therefore, that the acute mouse models presented herein could be more representative of chronic pruritus in human beings. Despite the fact that the exact clinic relevance of these acute animal models to chronic human pruritus is still unclear, our observation that H₄R antagonism appears to be more effective in these models opens the possibility that histamine may indeed be involved in chronic pruritic diseases, but its actions are mediated mainly through the H₄R, which is not antagonized by current drugs.

The efficacy of first-generation H₁R antagonists in some pruritic disease has frequently been attributed to sedative effects,^{1,36} and the sedative effects of diphenhydramine in mice have been described previously.³⁷ It is important to specify, therefore, that although we have shown that the H₄R antagonist JNJ 777120 crosses the blood–brain barrier, it does not affect measures of sedation in rodents (data not shown), so the described inhibitory effects of H₄R antagonism on pruritus are not secondary to sedation.

The pruritogenic activity of intradermally administered H₄R agonist was suggestive of a peripheral location for histamine H₄R mediating pruritus, but the exact site of its involvement required investigation. H₄R has been shown to be expressed on human skin mast cells,²¹ and we have previously demonstrated its expression on murine mast cells and its involvement in the modulation of mast cell–dependent inflammation.²⁸ Although we have shown H₄R is not involved in the control of IgE-mediated mast cell degranulation,²³ we could not be sure that the effects in mast cell–mediated itch models were not a result of modulation of other mast cell–related functions or mediators. The observations that histamine-induced itch and the

inhibitory effects of the H₄R antagonist were unaltered in mast cell–deficient mice (WBB6F1 W/W^V), which have few or no mast cells in skin,³⁸ indicated that H₄R on mast cells was not required for initiation of H₄R-mediated pruritus. In addition, the findings that bone marrow from H₄R^{+/+} mice was unable to reconstitute H₄R-mediated pruritic responses in H₄R^{-/-} mice argues against the involvement of mast cells or other hematopoietic cells in these events. These results, combined with the direct pruritic effect of H₄R agonist injected locally, are suggestive of a peripheral, nonmast cell–associated H₄R mediating the pruritic responses. Although we cannot rule out a role for H₄R on other cells in the skin, such as keratinocytes, it seems probable that histamine is acting directly on the peripheral terminals of primary sensory neurons through activation of H₄ receptors. There is 1 report of H₄R expression in human nasal mucosal neurons, which may support this hypothesis.³⁹ We are currently investigating the potential expression and function of H₄R on cutaneous afferent nerve fibers, where receptor expression has been demonstrated for other mediators of itch such as tryptase and substance P.^{40,41} As discussed, this could parallel expression and activity of the receptor in the central nervous system.

In conclusion, we have demonstrated, using potent and selective ligands of the H₄R, that this novel receptor is involved in pruritic responses in mice, independent of its function in hematopoietic cells. Although more work is necessary, the current results show that H₄R antagonists are more efficacious than other antihistamines in mouse models of itch. This and their clear anti-inflammatory effects^{20,28} hold promise that these novel pharmacologic agents may provide alternative and effective therapies for the treatment of inflammatory pruritic disease.

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