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Role of histamine H_1 receptor in pain perception: a study of the receptor gene knockout mice

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Abstract

To study the participation of histamine H_1 receptors in pain perception, histamine H_1 receptor knockout mice were examined for pain threshold by means of three different kinds of nociceptive tasks. These included assays for thermal nociception (hot-plate, tail-flick, paw-withdrawal), mechanical nociception (tail-pressure), and chemical nociception (abdominal constriction, formalin test, capsaicin test) which evoked pain by the activation in nociceptive Aδ and C fibers. The mutant mice lacking histamine H_1 receptors showed significantly fewer nociceptive responses to the hot-plate, tail-flick, tail-pressure, paw-withdrawal, formalin, capsaicin, and abdominal constriction tests. Sensitivity to noxious stimuli in histamine H_1 receptor knockout mice significantly decreased when compared to wild-type mice. This data indicates that histamine plays an important role in both somatic and visceral pain perceptions through histamine H_1 receptors. The difference in the effect of histamine H_1 receptors antagonist, the active (D-) and inactive (L-) isomers of chlorpheniramine on ICR mice further substantiates the evidence of the role of histamine H_1 receptors on pain threshold.

Keywords: Histamine H_1 receptor; Pain perception; Histamine; Somatic pain; Visceral pain; Gene targeting

1. Introduction

The exact mechanism of pain perception has not been clearly elucidated. It is known that the excitatory neurotransmitter glutamate coexists with substance P in primary afferents that respond to painful stimulation. Glutamate and substance P on the dorsal horn mediate pain messages. There seem to be many different mechanisms for pain modulation including acetylcholine (Bartolini et al., 1992), γ-aminobutyric acid (GABA) (Malcangio et al., 1992), catecholamines (Jones and Gebhart, 1986) and 5-hydroxytryptamine (Samanin and Valzelli, 1971). Some are working in and around the peripheral receptors and others within the central nervous system. When tissues are damaged, a variety of chemical substances are released from the skin tissue itself, from blood cells, and from nerve endings. Histamine is also released from local cells and directly excites nociceptors. Several studies have demonstrated that histamine H_1 receptors play some role in physiological and pathological pain perception. The histaminergic neuron system arises from the tuberomammillary nucleus of the posterior hypothalamus receiving input mainly from the limbic system and projects efferent nerve fibers to almost all parts of the brain. There is also a minor descending pathway which comes from hypothalamic neurons (Watanabe et al., 1984). Its fibers can be found in the dorsal raphe nucleus and periaqueductal gray region, which are considered to be important for pain modulation (Basbaum and Fields, 1984). In the mammalian central nervous system, histamine is thought to modulate many functions of the hypothalamus such as circadian rhythms, locomotor

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activity, arousal, feeding, and drinking (Schwartz et al., 1991). These actions are mediated through three distinct receptors denoted as histamine H_1, H_2, and H_3 receptors. However, much remains to be learned about their role in the central nervous system.

The mutant mice lacking histamine H_1 receptor were generated by homologous recombination, using gene targeting (Inoue et al., 1996). In brains of homozygous mutant mice, no specific binding of [^{3}H]pyrilamine was seen. We previously studied the behavior of the mutant mice with several general tasks such as monitoring locomotor activity in an open vs. a home cage, administering a passive avoidance test, a resident–intruder aggression test and a formalin test to clarify the role of the histamine H_1 receptors (Yanai et al., 1998). Previous work published on pain perception was based mainly on the use of histamine H_1 receptor antagonists. This paper examines whether histamine H_1 knockout mice show decreased sensitivity to noxious stimuli by various behavioral tests. We used the following tests of nociception: hot-plate, tail-flick, tail-pressure, paw-withdrawal, formalin, capsaicin, and abdominal constriction. In order to compare the pain threshold, we also examined the effects of histamine H_1 receptors antagonist, the active (D-) and inactive (L-) isomers of chlorpheniramine on nociceptive responses in abdominal constriction, hot-plate and tail-pressure tests among ICR mice. These studies demonstrate that histamine has an important effect on the perception of nociceptive stimuli through histamine H_1 receptors.

2. Materials and methods

2.1. Animals

This study was carried out in accordance with the guidelines of the Ethics Committee of the International Association for the Study of pain (Zimmermann, 1983). Male mutant mice $(-/-)$, wild-type mice $(+/+)$, and heterozygote mice $(+/-)$ weighing 22–28 g were used in this study. These mice were bred in our laboratory and were produced using gene targeting methods. Approximately 10 mice were housed per cage, with a controlled temperature (22 ± 4°C), under an automatically controlled light cycle (light on 0600–1800 h) and with free access to food and water prior to the experiment. They were allowed to acclimatize to the examination room at least 48 h before the experiments. The experiments were performed between 1000 and 1700 h daily. In some experiments, male ICR mice of 5–7 weeks old were purchased from Japan SLC (Hamamatsu, Japan). The D- and L-chlorpheniramine was dissolved with distilled water in a volume of 10 ml/kg and injected intraperitoneally into ICR mice. In all of the experiments, no animals were used more than once. All experiments were performed with the permission of the institutional animal care and use committee.

2.2. PCR and [^{3}H]pyrilamine binding

Mice were analyzed by PCR of genomic DNA from tail biopsies with slight modifications in order to verify whether the H_1 receptor subtype was absent in mice. The mutant allele was detected using 5′-TGAAGTATCTGGCTCT-GAGTGG-3′ (5′-primer, 5′-upstream of H_1 receptor gene) and 5′-TCTATCGCCTTCTTGACGAG-3′ (3′-primer complementary to neo^r gene sequence) with the following PCR conditions: 35 cycles of 1 min at 95°C, 1 min at 60°C, 2 min at 72°C (PCR band; approximately 0.98 kbp). The wild-type allele was also detected using 5′-TGAAG-TATCTGGCTCTGAGT GG-3′ (5′-primer as the same as mutant allele) and 5′-CCATCGATGGCTCCCTC CCTGGGAG-3′ (3′-primer complementary to H_1-receptor gene) (H_1-receptor PCR band; 1.2 kbp).

After the PCR analyses, [^{3}H] pyrilamine binding to brains were examined in the selected mice as described previously (Yanai et al., 1990). Tissues were homogenized in a Polytron (setting 5, 20 s) in approximately 20 volumes of ice-cold Na^+/K^+ phosphate buffer (50 mM, pH 7.5), and the homogenate was centrifuged twice at $50{,}000 \times g$ for 20 min. The final pellet was resuspended in 40 volumes of the ice-cold buffer. Incubation with [^{3}H]pyrilamine (Amersham, England) was carried out at 25°C for 30 min, and the reaction was terminated by addition of 5 ml of buffer and rapid filtration on a glass fiber filter (GF/B). Specific binding was defined as the radioactivity bound after subtraction of nonspecific binding, determined in the presence of 2 μM triprolidine. The amounts of H_1-receptor binding in the mutant $(-/-)$ and wild-type $(+/+)$ mice were 5.5 ± 4.6 and 247.9 ± 66.3 fmol/mg protein/nM, respectively.

2.3. Hot-plate test

Mice response to a heat stimulus was assessed using a hot-plate test at 50 ± 0.1°C. A positive response was noted when the mouse licked its hindpaw from the surface of the hot-plate test. A cut-off time of 60 s was used. A mirror was positioned behind the chamber and gave an unobstructed view of the animal's hindpaws. Locomotion of the mouse on the plate was constrained by a Plexiglas wall.

2.4. Tail-flick test

Radiant heat as a tail-flick stimulus (D Amour and Smith, 1941) depends on tail withdrawal at the radiant noxious heat endpoint and the response to thermal stimulation involves a spinal reflex. Times were determined individually for each mouse as the mean of two trials. The traditional tail-flick analgesia assay used a lamp intensity that typically yielded baseline latencies between 2 and 3 s with a maximum cut-off score of 10 s to minimize tissue damage.

2.5. Tail-pressure test

The pain or nociceptive threshold in mice was determined with an analgesia meter (tail-pressure) according to the method described by Leighton et al. (1988). The base of the tail was pressed and the level of pressure in mm Hg (10 mm Hg/s) that evoked biting or licking or struggling behavior was noted. Groups of mice ($n = 10$) were selected for each experiment.

2.6. Paw-withdrawal test

The paw-withdrawal responses were examined by the same method as the tail-flick using radiant heat. The animals were placed in a chamber and the right hindpaw was held on the radiant heat. The response to thermal stimulation was determined individually for each mouse as the mean of two trials (Calo et al., 1998). The withdrawal latency time depended on paw withdrawal at the radiant noxious heat endpoint. The traditional tail-flick test used a lamp intensity, which typically yielded baseline latencies between 2 and 3 s, with a maximum cut-off score of 10 s to minimize tissue damage.

2.7. Formalin test

In the formalin test, the mice were adapted in standard transparent cages ($22.0 \times 15.0 \times 12.5$ cm) approximately 1 h before injection of formalin. Transparent cages were also used as an observation chamber after injection of formalin. A mirror was positioned behind the chamber and gave an unobstructed view of the right hindpaw. The formalin test is based on that of Rosland et al., (1990), with a slight modification of the late-phase (phase 2) duration. Twenty microliters of formalin (2.0% in saline) was subcutaneously injected under the skin of the dorsal surface of the right hindpaw of the mouse using a microsyringe with a 26-gauge needle. Each mouse was immediately returned to the observation chamber after the injection. The recording of early response (phase 1) started immediately and lasted for 5 min (0–5 min). The recording of the late response (phase 2) started 10 min after the formalin injection and lasted for 20 min (10–30 min). In both phases, only licking of the injected hindpaw was defined as a nociceptive response and the total time of the response was measured with a hand-held stopwatch during the test period (Sakurada et al., 1995; Sato et al., 1999).

2.8. Capsaicin test

In the capsaicin test, the procedure was almost the same as in the formalin test. The mice were placed in standard transparent cages ($22.0 \times 15.0 \times 12.5$ cm) 1 h before the examination. A mirror was positioned behind the chamber to allow clear observation of the paws. After this period of adaptation, 20 μl of capsaicin (5.2 nmol) was injected under the skin of the dorsal surface of the right hindpaw, using a microsyringe with a 26-gauge needle. Intrathecal (i.t.) injections were made in unanesthetized mice at the L5 and L6 intervertebral space by the Hylden and Wilcox (1980) technique. Briefly, a volume of 5 μl (0.2 nmol capsaicin) was injected intrathecally with a 28-gauge needle connected to a 50-μl Hamilton microsyringe, the animal being lightly restrained to maintain the position of the needle. The mouse was immediately returned to the observation chamber after injection and the period of observation started. The amount of time the animals spent licking the injected paw was timed with a stopwatch. The animal was observed individually for 15 min immediately after the subcutaneous injection of capsaicin (Sakurada et al., 1992).

2.9. Abdominal stretch (visceral pain)

For these assays of chemical nociception, mice were habituated for 1 h to the testing room, and after injecting a noxious substance into the peritoneal cavity, the mice were placed in individual Plexiglas observation chambers. The number of stretching movements or abdominal constrictions or writhes-lengthwise stretches of the torso with a concomitant concave arching of the back were counted and totaled for 15 min after the intraperitoneal (i.p.) injection of either 0.6% acetic acid in a volume of 5 ml/kg or magnesium sulfate ($MgSO_4$). In some experiments, D- and L-chlorpheniramine was injected intraperitoneally to ICR mice at a dose of 10 mg/kg. Forty-five minutes after the treatment, acetic acid for one group and magnesium sulfate for the other, was injected in a volume of 5 ml/kg on ICR mice.

2.10. Chemicals

Drugs were obtained from the following sources: Histamine H_1 receptors antagonist, the active (D-) and inactive (L-) isomers of chlorpheniramine were gifts from Yoshitomi Pharmaceutical (Japan) and Essex Japan Pharmaceutical, respectively. Other chemicals used in this study were commercial products of reagent grade (Wako, Japan; Sigma, St. Louis, MO, USA). All solutions were made with distilled water on the same day of utilization.

2.11. Statistical analysis of data

Statistical analysis of the results was performed using the Dunnett's test for multiple comparison, after analysis of variance (ANOVA). Differences were considered to be significant if $P < 0.05$. All values are expressed as the mean ± S.E.M. for n determination.

3. Results

3.1. Hot-plate, tail-flick, tail-pressure and paw-withdrawal tests in histamine H_1 receptor knockout mice

Histamine H_1 receptor knockout (−/−), heterozygote (+/−) and wild-type (+/+) mice were examined for pain threshold using a hot-plate test, a tail-flick test, a tail-pressure test and a paw-withdrawal test. As shown in Fig. 1, the responses to behavioral tasks were quite different between the three kinds of mice. The latency to respond to several thermal painful stimuli significantly differed in the wild-type and mutant mice. In the hot-plate and tail-flick tests, the latency to respond to thermal stimuli was significantly prolonged in the mutant and heterozygous mice when compared to the wild-type mice (Fig. 1A and B). Because the hot-plate evokes behavior that is integrated at the spinal and supraspinal levels, it is difficult to specify the locus of the contribution. Similar results, however, were observed in the paw-withdrawal test that monitored reflex withdrawal to noxious heating of the hindpaw (Fig. 1D). Taken together, these results suggest that primary afferent fibers play an important role in the modulation of normal pain responsiveness, although the participation of central nervous system cannot be ruled out. The latency for evoking a withdrawal reflex of the tail to a mechanical stimulus differed significantly among the three groups (Fig. 1C). The latency to respond to the mechanical stimulus of tail pressure was significantly prolonged in the mutant mice. The behavior of the heterozygote in response to the mechanical stimulus differed significantly from the wild-type and mutant mice. The magnitude of the behavior observed in the heterozygote was between that of the wild-type and mutant mice.

3.2. The effects of D-, and L-chlorpheniramine on the response to the thermal or mechanical stimulus

The latency to the thermal or mechanical stimulus was examined in ICR mice after the treatment of the active (D-) and inactive (L-) isomers of chlorpheniramine as shown Fig. 2. After the administration of D-chlorpheniramine, ICR mice showed less responsiveness to the thermal stimulus of the hot-plate test (Fig. 2A). The effect of the active isomer was only significant at 60 min after the administration. On the other hand, we could not observe any changes of response after the treatment of L-chlorpheniramine or saline. Although the threshold for evoking a withdrawal reflex of the tail to a thermal stimulus did not differ in the D-chlorpheniramine-treated mice, the latency to respond to a mechanical stimulus was significantly prolonged by the treatment of D-chlorpheniramine (Fig. 2B).

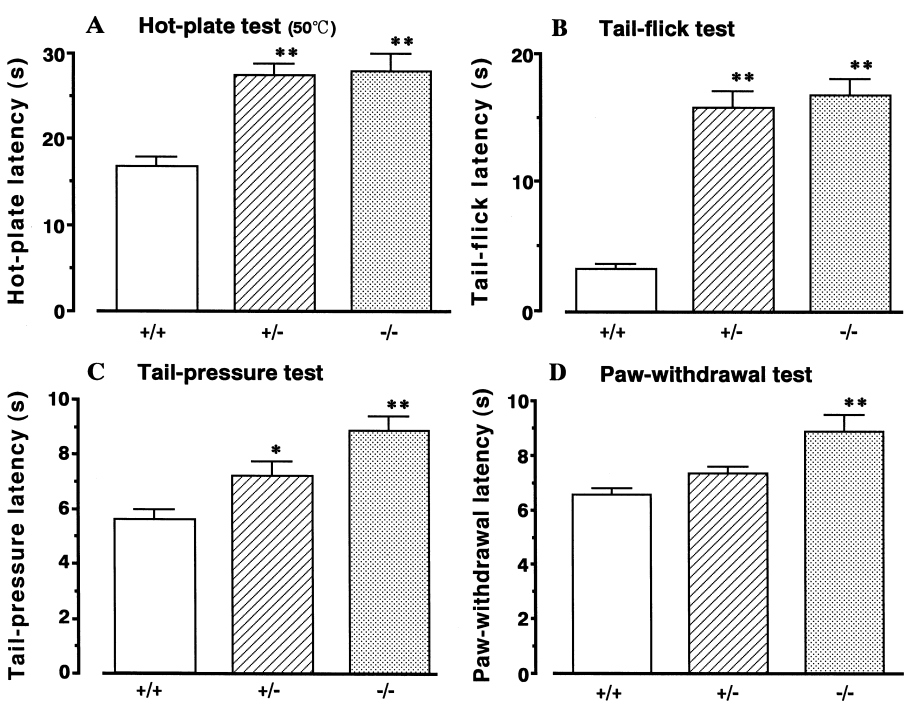

Fig. 1. Pain responses to thermal or mechanical stimuli in histamine H_1 receptor knockout mice. (A) Hot-plate latency at 50 ± 0.1°C between wild-type (+/+), heterozygous (+/−), and the mutant (−/−) mice. (B) Tail-flick latency. (C) Tail-pressure test. (D) Paw-withdrawal latency. The nociceptive responses were examined between the wild-type, heterozygous and mutant mice. Each bar represents the mean ± S.E.M. in 10 mice. Asterisks indicate significant differences from those of the wild-type mice (control); $^{*}P < 0.05$, $^{**}P < 0.01$.

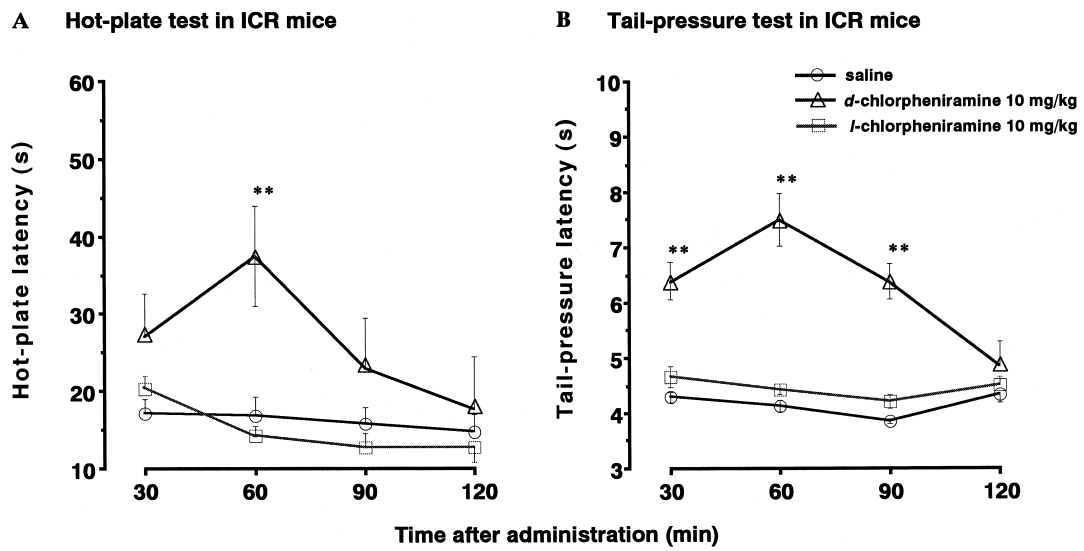

Fig. 2. Effects of chlorpheniramine (D- and L-isomer) in the pain responses to different thermal or mechanical stimuli. (A) Hot-plate test in ICR mice. (B) Tail-pressure test in ICR mice. The nociceptive responses were examined at 30, 60, 90 and 120 min after the administration of saline (○), D-chlorpheniramine (△) and L-chlorpheniramine (□). The statistical significance was examined among the three groups treated with saline and D- and L-chlorpheniramine. $^{**}P < 0.01$.

3.3. Pain responses to noxious chemical stimulation of 2.0% formalin in cutaneous tissue of histamine H_1 receptor knockout mice

Histamine H_1 receptor knockout mice showed decreased pain sensitivity in the first phase (Phase 1) of the formalin test when compared to the wild-type mice (Fig. 3A). The licking time of Phase 1 is thought to provide a different measure of the acute pain produced by direct chemical activation of C-fibers. We also observed a significant decrease in pain sensitivity in the second phase (Phase 2), which is proposed to result from a central sensitization process set up by activity during the first phase (Fig. 3B). The behavior of the heterozygote in response to 2.0% formalin differed significantly from the wild-type and mutant mice in both Phase 1 and Phase 2. The magnitude of the behavior observed in the heterozygote was between that of the wild-type and mutant mice.

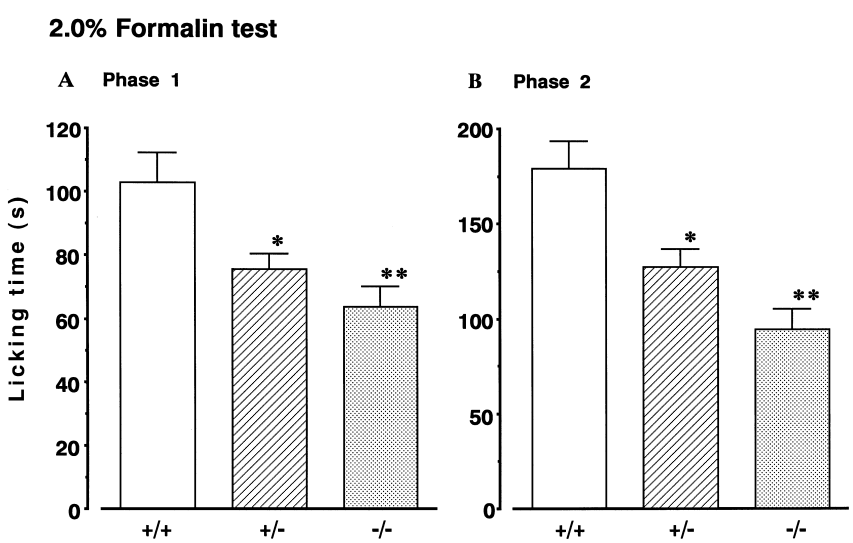

Fig. 3. Pain responses to the injection of formalin into the paws of histamine H_1 receptor knockout mice. Data represent the amount of time that the animal spent licking the injected paw in the early (A) (0–10 min, Phase I) and late phase (B) (10–30 min, Phase II). The data are expressed as the mean ± S.E.M. of 10 mice. The nociceptive responses were examined among the wild-type (+/+), heterozygous (+/−) and mutant (−/−) mice. $^{*}P < 0.05$; $^{**}P < 0.01$.

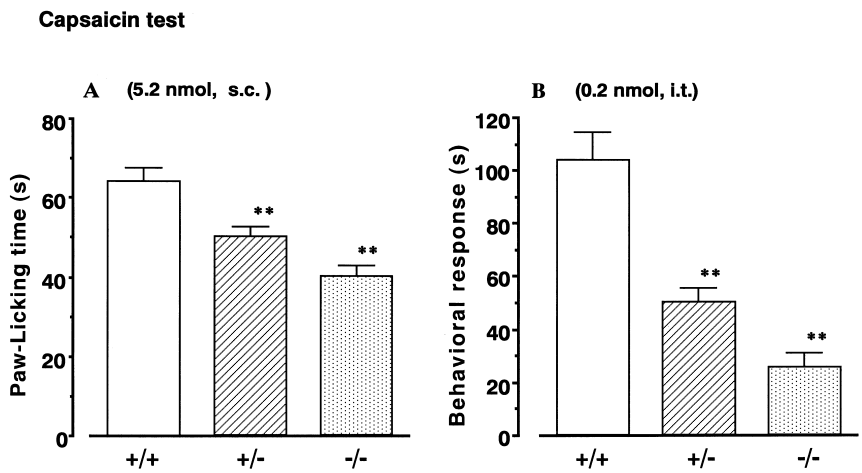

Fig. 4. Pain responses to the subcutaneous and i.t. injection of capsaicin in histamine H_1 receptor knockout mice. (A) Time spent on paw licking after the injection of capsaicin (5.2 nmol) into the dorsal surface of the hind paw of each mouse. Values represent the amount of behavioral response as the mean ± S.E.M. of 10 mice. The data are expressed as the mean ± S.E.M. of 10 mice. The nociceptive responses were examined between the wild-type (+/+), heterozygous (+/−) and mutant (−/−) mice. $^{**}P < 0.001$. (B) Effects of i.t. injection of capsaicin (0.2 nmol) on the behavioral response. The nociceptive responses were examined among the wild-type (+/+), heterozygous (+/−) and mutant (−/−) mice. $^{**}P < 0.01$.

3.4. Capsaicin test in histamine H_1 receptor knockout mice

The subcutaneous injection of capsaicin into the dorsal surface of a hindpaw produced a dose-dependent licking response toward the injected paw. This characteristic behavior appeared immediately, peaked at 0–5 min and decayed 5 min after the capsaicin injection. The pain behavior of licking evoked by a hindpaw injection of capsaicin, an intensely noxious chemical stimulus that directly activates C-fibers, was also significantly less in the heterozygote and mutant mice when compared to wild-type mice (Fig. 4A). We also injected capsaicin (0.2 nmol) intrathecally into the wild-type, heterozygous and mutant mice in order to reveal the site of action (Fig. 4B). Capsaicin is known to cause the release of substance P from the spinal cord. Histamine H_1 receptor knockout mice showed a marked decrease in the behavioral responses induced by the i.t. injection of capsaicin when compared to the wild-type mice. The behavior of the heterozygote also differed significantly from the wild-type.

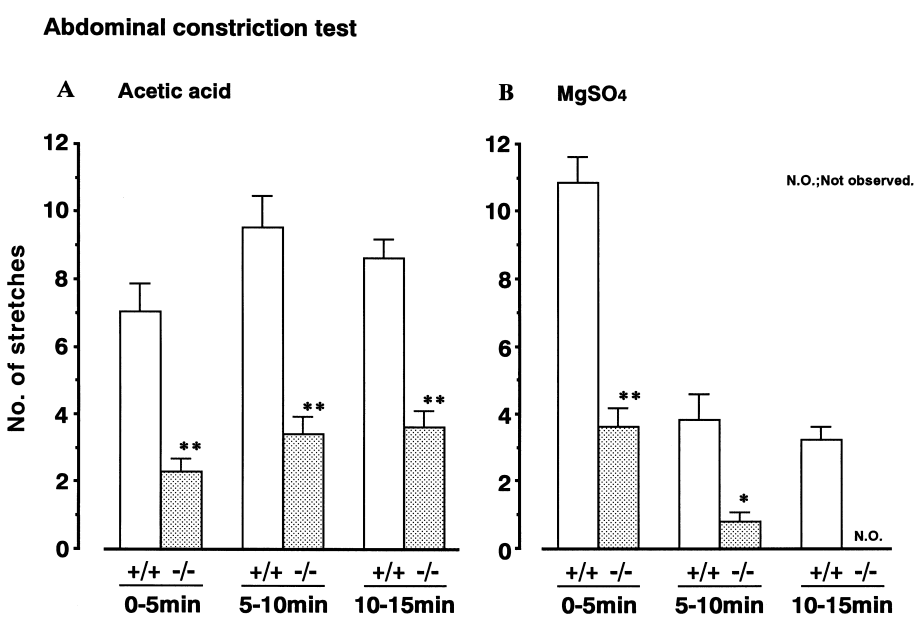

Fig. 5. Pain responses to noxious chemical stimulation of visceral tissue in histamine H_1 receptor knockout mice. Visceral pain response (abdominal stretching) produced by i.p. injection of dilute acetic acid (A), a stimulus associated with inflammation ($n = 30$) or i.p. injection of $MgSO_4$ (B), a stimulus that produces pain without inflammation ($n = 30$). Data represented the number of abdominal stretching during 5 min at 0–5, 5–10, and 10–15 min after injection of acetic acid or $MgSO_4$. Data are expressed as the mean ± S.E.M. from 30 wild-type and mutant mice. $^{*}P < 0.01$; $^{**}P < 0.001$.

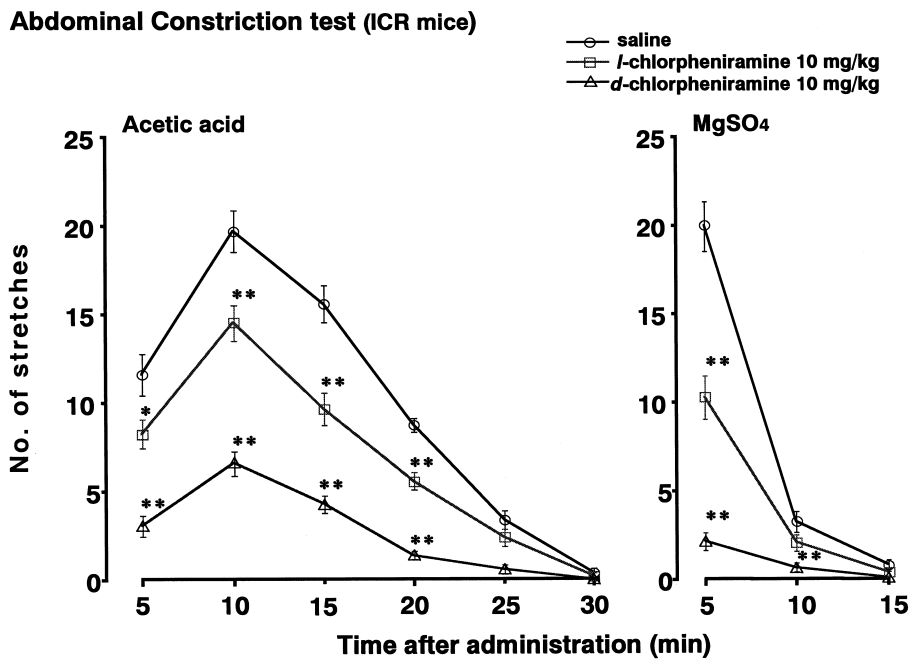

Fig. 6. The effects of D- and L-chlorpheniramine on visceral pain response (abdominal stretching) produced by i.p. injection of dilute acetic acid or $MgSO_4$. The abdominal stretching was induced by acetic acid or $MgSO_4$ at 45 min after administration of saline, D- and L-chlorpheniramine. Each points with bar represents the mean ± S.E.M. of 10 mice. $^{*}P < 0.05$; $^{**}P < 0.01$.

3.5. Abdominal stretching behavior in histamine H_1 receptor knockout mice

Finally, we tested the wild-type and the mutant mice in two models of acute visceral pain. One was caused by an i.p. injection of $MgSO_4$ that induces an immediate pain response independent of inflammation (Gyires and Torma, 1984). The other was caused by acetic acid that is secondary to a delayed inflammatory response. In both cases, we found reduced pain sensitivity in the mutant mice as shown in Fig. 5A and B.

3.6. The effects of D-, and L-chlorpheniramine on the response to visceral pain

The abdominal stretching behavior caused by an i.p. injection of acetic acid and $MgSO_4$ was examined in ICR mice after the treatment of the active (D-) and inactive (L-) isomers of chlorpheniramine as shown Fig. 6. After the administration of D-chlorpheniramine, ICR mice showed less responsiveness to visceral pain. The effect of the active isomer was significant at all time points of counting. On the other hand, the effect of L-chlorpheniramine was smaller than that of D-chlorpheniramine.

4. Discussion

A possible role of histaminergic neurotransmission in the control of pain has been postulated (for review, see Hough, 1988; Schwartz et al., 1991; Onodera et al., 1994). Intracerebroventricular administration of histamine into rodents impairs several nociceptive responses. Most of the papers report antinoceptive effects due to activation of histamine H_2 receptors (Malmberg-Aiello et al., 1994). As for the histamine H_1 receptors, the papers published so far are based mainly on the use of histamine H_1 receptor antagonists. Prevention of histamine-induced antinociception by histamine H_1 antagonists has been reported (Parolaro et al., 1989). Even though histamine H_1 antagonists inhibit the analgesia evoked by histamine in doses that are inactive alone, histamine H_1 receptor antagonist also produce analgesic activity when given either alone or with opiates (Suzuki et al., 1994). Such ambiguities exist in the role of the histaminergic neuron system for nociception.

Although drugs exert their primary actions by interacting with the specific target molecules, they also have other actions. At higher concentrations, most drugs can interact with a wide variety of biological molecules, often resulting in functional alterations. The data obtained by pharmacological experiments should be re-evaluated from a more specific point of view. In the current study, the pain threshold of histamine H_1 receptor knockout mice was studied in several tests including three different kinds of noxious stimuli: thermal, chemical and mechanical. Histamine H_1 receptor knockout mice showed less response to pain for the three different noxious stimuli when compared to the wild-type mice. From our studies, it is suggested

that activation of histamine H_1 receptors can increase sensitivity to noxious stimuli.

Recently, Malmberg-Aiello et al. (1998) reported clear evidence for hypernociception following histamine H_1 receptor activation using a new, highly selective histamine H_1 receptor agonist. In their report, 2-(3-trifluoromethylphenyl) histamine dihydrogenmaleate (FMPH) was used to characterize the hypernociception caused by histamine H_1 receptor activation. The effects of FMPH on the pain threshold in the hot plate test were biphasic. In low concentrations, FMPH lowered the reaction latency in the hot-plate test while FMPH increased the reaction latency in higher doses. The effects at lower doses are probably due to the activation of histamine H_1 receptors. Their data are in accordance with our data of histamine H_1 receptor knockout mice. In addition, our results are consistent with other previous observations (Glick and Crane, 1978; Chung et al., 1984; Bhattacharya and Paramar, 1985; Parolaro et al., 1989; Oluyomi and Hart, 1991; Braga et al., 1992; Mogil and Grisel, 1998).

Antinoceptive effects of histamine H_1 receptor antagonists are well documented both in laboratory animals and in humans (Oluyomi and Hart, 1991). Histamine H_1 antagonists (antihistamines) are known to block not only histamine H_1 receptors, but also antagonize muscarinic, serotonergic, and dopaminergic actions. In our experiments, two stereoisomers of chlorpheniramine (D- and L-isomer) were used to examine whether the pure histamine H_1 receptor antagonism can cause antinoception effects. Stereoselectivity of the binding has been shown previously by inhibition of [^{3}H] pyrilamine binding in vivo in guinea pig brain, the D-isomer being about 100 times more potent than the L-isomer (Yanai et al., 1990). The active isomer was more potent for the antinociceptive effects than the L-isomer in both somatic and visceral pain.

Histamine mediates a variety of physiological reactions in peripheral tissues, as well as in the central nervous system through three types receptors (Schwartz et al., 1991). Histamine itself is also present in peripheral nerves (Mac Donald et al., 1981). About 15% of the trigeminal and lumber dorsal root ganglion were intensely labeled with a probe of the histamine H_1 receptor gene (Kashiba et al., 1999). It was also reported that the mRNA of histamine H_1 receptor genes was detected in many substance P and calcitonin gene-related peptide immunoreactive neurons following the peripheral nerve injuries. This data suggests that histamine H_1 receptors may be transported peripherally and centrally and are present on sensory endings in the skin and dorsal horn. Histamine H_1 bindings within the superficial layer of the dorsal horn and in the peripheral are probably lost in histamine H_1 receptor knockout mice. The differential roles of histamine H_1 receptors on afferent fibers and the spinal cord in both dorsal and ventral horns remain unclear. The measured endpoints of the tail-pressure, hot-plate, formalin and i.t. capsaicin tests in this paper are the duration of licking, biting or scratching times. Licking and scratching behaviors are thought to be mediated in higher centers. Our data suggests that both peripheral perception and central sensitization could be attenuated in histamine H_1 receptor knockout mice.

A variety of brain structures have been implicated in pain suppression, one is a zone of neurons in the midbrain such as the periventricular and periaqueductal gray. The stimulation of the periaqueductal gray can cause a profound analgesia or an absence of pain, thus affecting the behavioral state. Also the periaqueductal gray can influence the raphe nuclei of the medulla which in turn can modulate the flow of nociceptive information through the dorsal horns of the spinal cord. It is not ruled out that neuronal histamine can modulate the activity of periaqueductal gray and raphe nuclei.

This study suggests that histamine could modulate nociception at the cutaneous tissue level, the dorsal horn in the spinal cord and supraspinal including dorsal raphe nucleus in the medulla and periaqueductal gray in the midbrain. The dorsal raphe nucleus and the periaqueductal gray region are considered to participate in pain modulation as descending pain-control pathways. As observed in all tests, histamine is probably working for hypernociception in the peripheral and neuronal sites. From the present results, we conclude that histamine can modulate analgesia through histamine H_1 receptors. Gene targeting has developed rapidly over the past several years, so that large numbers of mice with different gene deficiencies have been generated to study the functional roles of the gene. The study using knockout mice is a promising approach to confirm the data obtained by classical pharmacological tools.

In the present report, we clearly show for the first time using histamine H_1 receptor knockout mice that it is possible to induce antinociception by removing histamine H_1 receptors. This was illustrated by the observation that mutant mice lacking histamine H_1 receptors show a lower pain response with respect to normal mice. This indicates that it is also possible to increase sensitivity to noxious stimuli by activating histamine H_1 receptors. It thus seems likely that the histaminergic system, like many other neuronal systems, plays an important role in the modulation of central perception of nociceptive stimuli. The increase in the pain threshold resulting from blocking histamine H_1 receptors provides a theoretical support for the use of histamine H_1 antagonists as mild analgesics in the clinic.

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