Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 522 (2005) 55–62
www.elsevier.com/locate/ejphar

Intrathecally-administered histamine facilitates nociception through tachykinin NK_1 and histamine H_1 receptors: A study in histidine decarboxylase gene knockout mice

Akiko Yoshida [a,b], Jalal Izadi Mobarakeh [a,c,*], Eiko Sakurai [a], Shinobu Sakurada [d], Tohru Orito [d], Atsuo Kuramasu [a], Masato Kato [b], Kazuhiko Yanai [a,*]

[a] Department of Pharmacology, Tohoku University School of Medicine, Seiryo-machi 2-1, Aoba-Ku, Sendai 980-8575, Japan
[b] Department of Anesthesiology, Tohoku University School of Medicine, Seiryo-machi1-1, Aoba-Ku, Sendai 980-8574, Japan
[c] Department of Pharmacology and Physiology, Pasteur Institute of Iran, 69 Pasteur Ave., Tehran 13164, Iran
[d] Department of Physiology and Anatomy, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-Ku, Sendai 981-8558, Japan

Received 8 August 2005; accepted 18 August 2005
Available online 5 October 2005

Abstract

Intrathecal injection of histamine elicited behavioral responses consisting of scratching, biting and licking in conscious mice. To study the participation of histamine in pain perception, histidine decarboxylase knockout mice were examined for pain threshold by means of three different kinds of noxious stimuli: thermal nociception (hot-plate, tail-flick, and paw-withdrawal), mechanical nociception (tail-pressure), and chemical nociception (formalin test and capsaicin test). Mutant mice lacking histidine decarboxylase showed significantly fewer nociceptive responses to the hot-plate, tail-flick, paw-withdrawal, tail-pressure, formalin and capsaicin tests. Sensitivity to noxious stimuli in the histidine decarboxylase knockout mice was significantly lower when compared to the wild-type mice. The intrathecally-administered histamine (400 pmol) significantly shortened the latency in the histidine decarboxylase knockout mice, but not in the wild-type mice in tail-flick tests. Pyrilamine, a histamine H_1 receptor antagonist, but not ranitidine, a histamine H_2 receptor antagonist, produced inhibition of the induced behavioral responses in the tail-flick test when co-administered with histamine. Sendide, a tachykinin NK_1 receptor antagonist, inhibited histamine-induced nociceptive behavior in the histidine decarboxylase knockout mice. In contrast, the treatment with D-(−)-2 amino-5-phosponovaleric acid (D-APV), an *N*-methyl-D-aspartate (NMDA) receptor antagonist, did not prevent the induction of the behavioral responses by histamine. These studies substantiate the evidence that nociceptive behavior induced by intrathecal injection of histamine is largely mediated through tachykinin NK_1 and histamine H_1 receptors in the spinal cord.

Keywords: Histamine; Histamine H_1 receptor; Tachykinin NK_1 receptor; NMDA receptor; Nociceptive response; Histidine decarboxylase gene knockout mouse

1. Introduction

Histamine, one of the inflammatory chemical mediators, is known to be involved both in peripheral and central nociceptive mechanisms. When tissues are injured, stimulated mast cells release histamine resulting in local vasodilation, plasma exudation, as well as depolarization of sensory nerve endings called nociceptors. These peripheral neurological events trigger subsequent processes of inflammatory pain including release of various neuropeptides and other pain-related molecules from primary afferent fibers, post-synaptic excitation of secondary neurons in the spinal dorsal horn, and signal transduction into the central nervous system (Parolaro et al., 1989; Baranauskas and Nistri, 1998; Scholz and Woolf, 2002). Participation of histamine as a neurotransmitter in central pain modulation is also well documented. Histamine releasing neurons are found exclusively in the tuberomammillary nucleus of the posterior hypothalamus. Efferent fibers raised from that region reach diverse areas in the central nervous system from the olfactory bulb to the spinal cord that is thought to be important in the nociceptive mechanism

* Corresponding authors. Department of Pharmacology, Tohoku University School of Medicine, Seiryo-machi 2-1, Aoba-Ku, Sendai 980-8575, Japan. Tel.: +81 22 717 8055; fax: +81 22 717 8060.
E-mail addresses: jalalizadi2002@yahoo.com (J.I. Mobarakeh), yanai@mail.tains.tohoku.ac.jp (K. Yanai).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.08.037

(Panula et al., 1984, 1989; Watanabe et al., 1984; Schwartz et al., 1991; Watanabe and Yanai, 2001; Brown et al., 2001; Haas and Panula, 2003). In addition, dense existence of the histaminergic fibers and the specific receptors in the midbrain periaqueductal gray matter suggests significant correlation between histamine and opioid receptor-mediated analgesia (Mason, 1999).

The role of histamine in pain processes has been previously investigated mostly using specific receptor-oriented approaches. Pharmacological approaches of histamine receptor subtypes have indicated significant involvement of histamine H_1 receptors in pain perception (Hough, 1988). The findings have been in part confirmed in recent studies using histamine H_1 receptor knockout mice, showing decreased sensitivities to noxious stimuli and enhancement of morphine-induced analgesia (Mobarakeh et al., 2000, 2002). Studies of α-fluoromethyl histidine, an L-histidine decarboxylase inhibitor, have also supported the concept of histamine-induced pain modulation (Watanabe et al., 1990). However, those previous methodologies include a limitation that the effects of endogenous histamine on in vivo physiological functions cannot be completely eliminated.

Recently, mutant mice lacking histidine decarboxylase have been generated by homologous recombination using a gene targeting technique (Ohtsu et al., 2001). The mice are apparently normal in macroscopic appearance, fertility, delivery and growth rates, but have no histidine decarboxylase activity resulting in a long lasting depletion of histamine in vivo. Previous studies using this model have revealed faster bacterial eradication (Hori et al., 2002) and stimulated eosinophil infiltration in the airway (Koarai et al., 2002) in the absence of tissue histamine. A significant advantage of this model is that endogenous histamine is almost completely absent, enabling the evaluation of roles of histamine in various physiological functions including nociceptive processes.

Using histidine decarboxylase knockout mice, therefore, this study aimed to clarify the roles of endogenous histamine in pain perception by evaluating various behavioral responses to noxious stimuli. The present results provide additional evidence regarding histamine-mediated pain modulation in the spinal cord.

2. Materials and methods

2.1. Animals

Histidine decarboxylase knockout mice of a 129Sv inbred strain were generated as previously described (Ohtsu et al., 2001). Male inbred strains of mutant (−/−) and wild-type 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 06:00–18:00) and with free access to food and water prior to the experiments. All mice were feed with a low histamine-containing diet (0.6 nmol/g). The ordinary diet contains 7280 nmol/g of histamine (Ohtsu et al., 2001). In our experimental conditions, the histamine contents in the histidine decarboxylase knockout and their wild-type mice were 0.018 and 0.059 nmol/g in the brain and 0.26 and 21.2 nmol/g in the skin, respectively. They were allowed to acclimatize to the examination room at least 48 h before the experiments. The experiments were performed between 10:00 and 17:00 daily. In all of the experiments, none of the animals were used more than once.

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). All experiments were performed with the permission of the institutional animal care and use committee. Experimental protocols were approved by respective Animal Care Committees of Tohoku University School of Medicine and Tohoku Pharmaceutical University.

2.2. PCR (polymerase chain reaction)

Mice were analyzed by PCR of genomic DNA from tail biopsies with slight modifications in order to verify whether the histidine decarboxylase was absent in mice.

The mutant allele was detected using 5′-AAA/CAT/CGC/ATC/GAG/CGA/GCA/CGT/ACT/CGG-3′ (5′ neo new) and 5′-ATG/TCC/TGA/YAG/CGG/TCC/GCC/ACA/CCC/AGC-3′ (3′ neo new) with the following PCR conditions: 40 cycles of 30 s at 95 °C, 1 min at 64 °C, 1 min at 72 °C (PCR band; approximately 250 bp).

The wild-type allele was also detected using 5′-AGT/GCG/GGA/CTG/TGG/CTC/CAC/GTC/GAT/GCT-3′ (Pr.3.30) and 5′-TAC/AGT/CAA/AGT/GTA/CCA/TCA/TCC/ACT/TGG-3′ (Pr.2.30) with the same PCR conditions (PCR band; approximately 144 bp) (Ohtsu et al., 2001).

2.3. Behavioral experiments

Hot-plate test: The responses to the heat stimulus were assessed using a hot-plate test at 47.5±0.1 °C. The weak heat stimulus was used in this study because the mutant mice were susceptible to the tissue damage. A positive response was noted when the mouse licked its hind paw 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 hind paw. Locomotion of the mouse on the plate was constrained by a Plexiglas wall (O' Callaghan and Holzman, 1975).

Tail-flick test: Pain thresholds were determined by using an automated tail-flick unit (Ugo Basile, Italy). Radiant heat as a tail-flick stimulus (D Amour and Smith, 1941) induces 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 tail-flick assay used different lamp intensities that typically yielded baseline latencies between 2–3 s and 11–12 s for detecting the antinociceptive and hypernociceptive changes, respectively (Mobarakeh et al., 2000; Sakurada et al., 2002).

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. (1998). 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.

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 hind paw 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.

Formalin test: In the formalin test, the mice were adapted in standard transparent cages (22.0×15.0×12.5 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 hind paw. The formalin test is based on the methods of Rosland et al. (1990), with a slight modification of the late-phase duration. Formalin (20 μl, 1.0% in saline) was subcutaneously injected under the skin of the dorsal surface of the right hind paw 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 was started immediately after the formalin injection and lasted for 5 min (0–5 min, Phase 1). The late response (Phase 2) was counted at 10 min after injection for 20 min (10–30 min). In both phases, only licking of the injected hind paw 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).

Capsaicin test: In the capsaicin test, the procedure was almost the same as in the formalin test. The mice were placed in a standard examination chamber. 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 hind paw, using a microsyringe with a 26-gauge needle (Sakurada et al., 1992).

Intrathecal injection procedure: The intrathecal (i.t.) injection procedure was adapted from the method of Hylden and Wilcox (1980). A 28-gauge stainless needle attached to a 50 μl Hamilton microsyringe was inserted between lumbar 5 and lumbar 6 in unanesthetized mice, and drugs were given slowly in a volume of 5 μl. In combined experiments, histamine was co-administered with various drugs in a total volume of 5 μl. A slight flick of the tail was used as an indication that the needle had penetrated the dura.

2.4. Chemicals

Sendide, [Tyr^6, D-Pfe^7, D-His^9]SP (6–10) and [D-Pfe^7, D-His^9]SP (6–11) were synthesized by solid-phase peptide methodology. The following drugs and chemicals were used: histamine dihydrochloride (Sigma, St. Louis, MO, USA), pyrilamine maleate salt (Sigma-RBI), ranitidine hydrochloride (Sigma-RBI), D-(−)-2 amino-5-phosponovaleric acid (D-APV) (Cambridge Research Biochemicals, Cambridge, UK). For i.t. injections, these compounds were dissolved in sterile artificial cerebrospinal fluid containing (mM): NaCl 126.6, KCl 2.5, $MgCl_2$ 2.0, and $CaCl_2$ 1.3.

2.5. Statistical analysis of data

Results are given as the mean±standard error of mean (S.E.M.). Student's two-tailed *t*-test was used to verify significance between two groups; the other tests were performed using Dunnett's test for multiple comparison after analysis of variance (ANOVA). *P* value <0.05 was considered significant. We used a computer-associated curve-fitting program (GraphPad Prism, GraphPad Software, Inc., San Diego, CA) for the statistical significance of differences between groups.

3. Results

3.1. Hot-plate test, tail-flick test, paw-withdrawal test, and tail-pressure test, in histidine decarboxylase knockout mice

Histidine decarboxylase knockout (−/−) and wild-type (+/+) mice were examined for pain threshold using a hot-plate test, a tail-flick test, a paw-withdrawal test and a tail-pressure test. The latency to respond to several thermal painful stimuli significantly differed in the wild-type and mutant mice. In the hot-plate test, tail-flick test and paw-withdrawal test, the latency to respond to thermal stimuli was significantly prolonged in the mutant mice compared to the wild-type mice (Fig. 1A–C). The latency to respond to the mechanical stimulus of tail pressure was also significantly longer among the mutant mice (Fig. 1D).

3.2. Pain responses in formalin and capsaicin tests

Histidine decarboxylase knockout mice showed decreased pain sensitivity in the first phase (Phase 1) of the formalin test when compared to the wild-type mice (Fig. 2A). The licking time of Phase 1 is thought to provide a different measure of the acute pain produced mainly by direct chemical activation of C-fibers. We also observed a significant decrease in pain sensitivity in the second phase (Phase 2), which has been proposed to be associated with the release of inflammatory mediators including the central sensitization process in addition with on-going nociceptor activation in the peripheral system (Fig. 2B). The subcutaneous injection of capsaicin into the dorsal surface of a hind paw increased licking responses toward the injected paw in a dose-dependent manner. This characteristic behavior of licking evoked by a hind paw injection of capsaicin, an intensely noxious chemical stimulus that directly activates C-fibers, was also significantly less in the mutant mice when compared to the wild-type mice (Fig. 2C).

3.3. Tail-flick response induced by i.t.-administered histamine

The i.t. administered histamine (400 pmol) significantly shortened the latency of the histidine decarboxylase knockout mice, but not the wild-type mice in tail-flick tests (Fig. 3A and B).

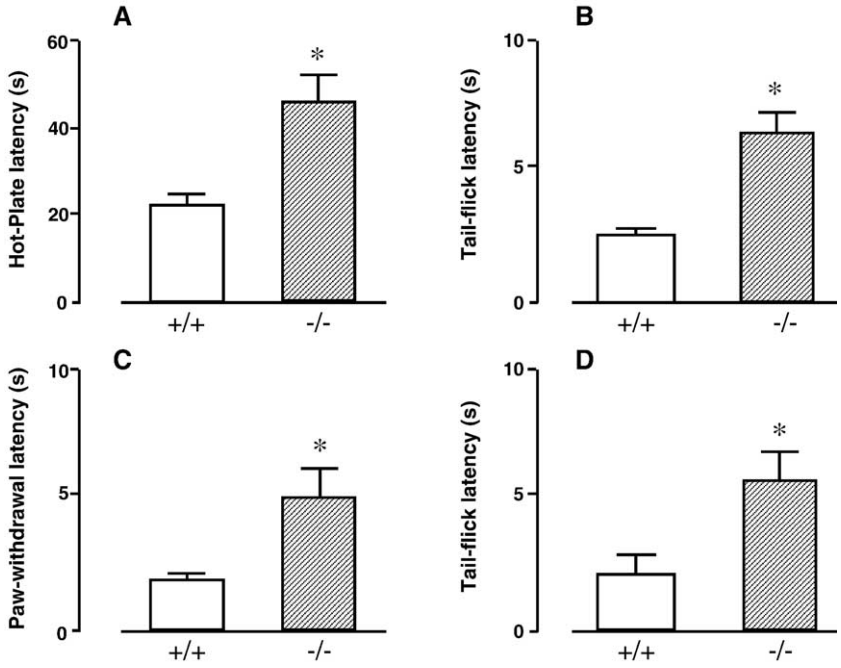

Fig. 1. Pain responses to thermal and mechanical stimuli in the HDC knockout mice. (A) Hot-plate latency at 47.5±0.1 °C. (B) Tail-flick test latency. (C) Paw-withdrawal latency. (D) Tail-pressure test. The nociceptive responses were compared between the wild-type (+/+) and mutant (−/−) mice. Each bar represents the mean±S.E.M. of 10 mice. Asterisks indicate significant differences from those of the wild-type mice (control); *$P<0.05$. In all tests, the mutant mice showed significantly decreased responses.

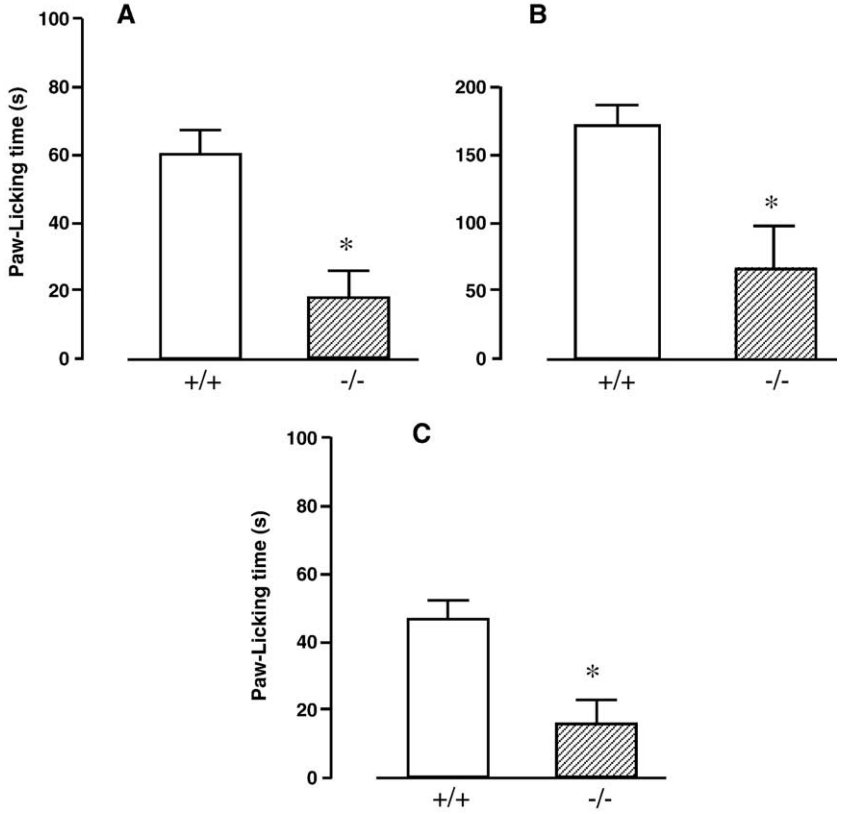

Fig. 2. Pain responses to noxious chemical stimuli in the HDC knockout mice. (A) Formalin test (Phase 1). Duration of licking the injected paw during 0–10 min was counted. (B) Formalin test (Phase 2). Duration of licking the injected paw during 10–30 min was counted. (C) Capsaicin test. Duration of licking the injected paw was counted. Each bar represents the mean±S.E.M. of 10 mice. Asterisks indicate significant differences from those of the wild-type mice (control); *$P<0.05$. In all tests, responses to intraplantar pain stimuli were significantly reduced in mutant mice compared to wild-type mice.

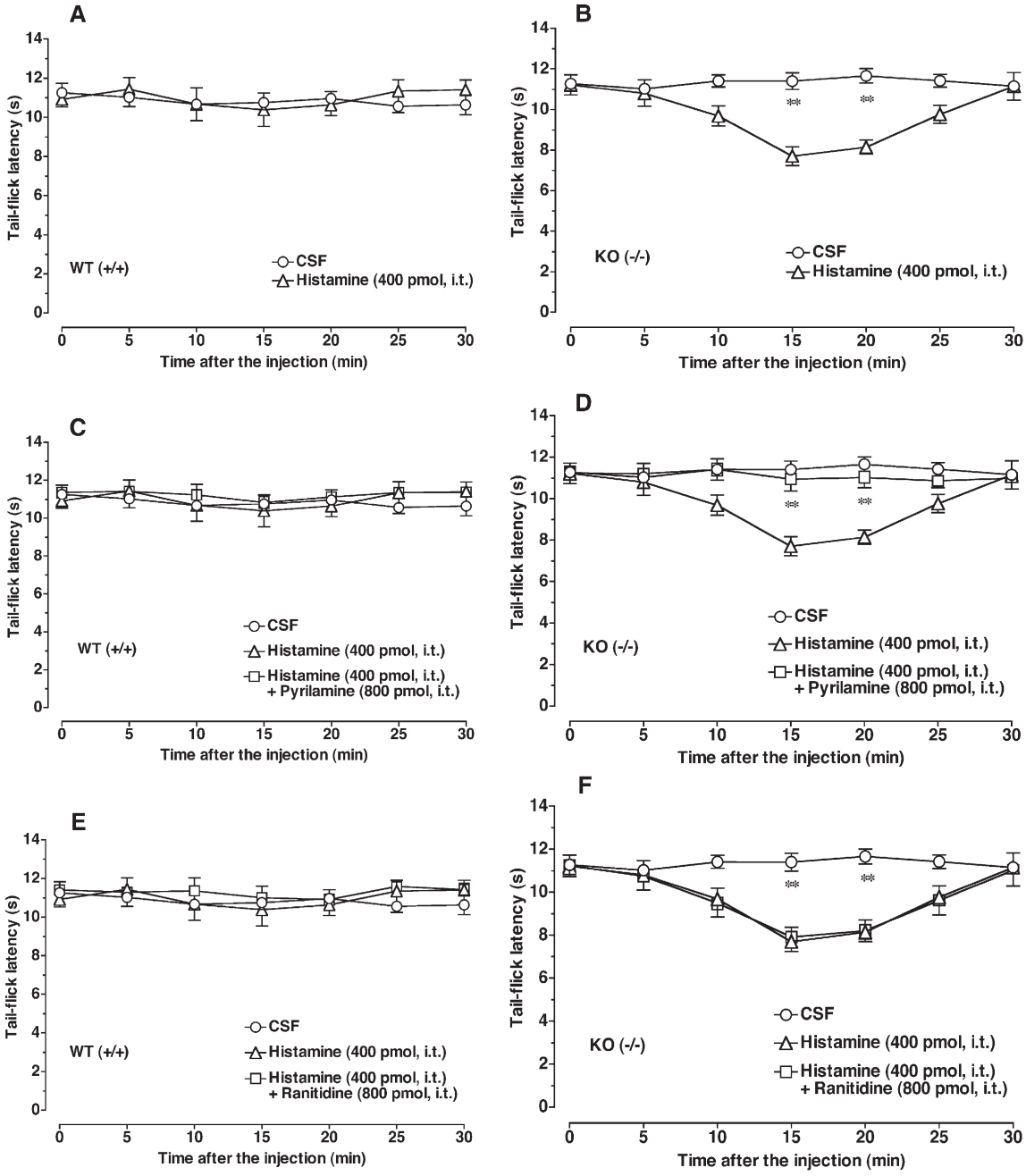

Fig. 3. Effects of intrathecally-administered histamine on the tail-flick test in the histidine decarboxylase knockout mice. (A) Histamine (400 pmol) or CSF was administered intrathecally to the wild-type mice. (B) Histamine (400 pmol) or CSF was administered intrathecally to the mutant mice. (C) Histamine (400 pmol) and pyrilamine (800 pmol) were co-administered intrathecally to the wild-type mice. (D) Histamine (400 pmol) and pyrilamine (800 pmol) were co-administered intrathecally to the mutant mice. (E) Histamine (400 pmol) and ranitidine (3200 pmol) were co-administered intrathecally to the wild-type mice. (F) Histamine (400 pmol) and ranitidine (3200 pmol) were co-administered intrathecally to the mutant mice. The pretreatment control values were determined by two consecutive measurements for 10 min intervals. Each value represents mean±S.E.M. of 10 mice in each group. Asterisks indicate significant differences from those of pretreatment; $P<0.05$, $^{**}P<0.01$.

The difference in the responses between the two strains is probably due to the supersensitivity of histamine receptors in histidine decarboxylase knockout mice. We also examined the effects of histamine H_1 and H_2 receptor antagonists on histamine-evoked hyperalgesia. Pyrilamine, a histamine H_1 receptor antagonist, but not ranitidine, a histamine H_2 receptor antagonist, inhibited the histamine-evoked hyperalgesia in the tail-flick test (Fig. 3D and F). The i.t. co-administration of histamine H_1 or H_2 receptor antagonists did not elicit any observable behavioral responses in the wild-type mice (Fig. 3C and E).

3.4. Effect of tachykinin NK1 receptor and NMDA receptor antagonists on histamine-induced behavioral responses

Sendide, a tachykinin NK_1 receptor antagonist, significantly inhibited histamine-elicited nociceptive behaviors in

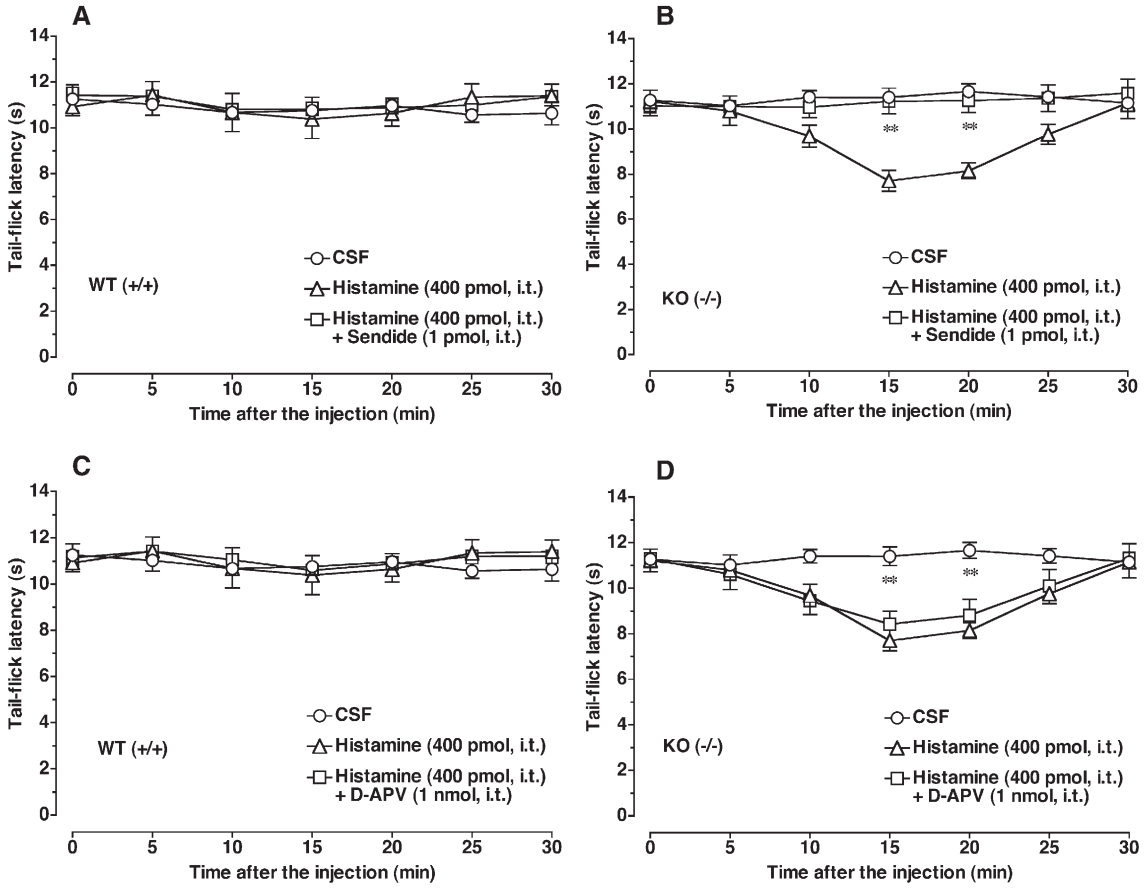

Fig. 4. Effects of intrathecally-administered sendide and D-APV on histamine-elicited hyperalgesia in the tail-flick test. (A) Histamine (400 pmol) and sendide (1 nmol) were co-administered intrathecally to the wild-type mice. (B) Histamine (400 pmol) and sendide (1 nmol) were co-administered intrathecally to the mutant mice. (C) Histamine (400 pmol) and D-APV (1 nmol) were co-administered intrathecally to the wild-type mice. (D) Histamine (400 pmol) and D-APV (1 nmol) were co-administered intrathecally to the mutant mice. The pretreatment control values were determined by two consecutive measurements for 10 min intervals. Each value represents mean±S.E.M. of 10 mice in each group. Asterisks indicate significant differences from those of pretreatment; $P<0.05$, $^{**}P<0.01$.

the histidine decarboxylase knockout mice in the tail-flick test (Fig. 4B). In contrast, the treatment with D-APV, an NMDA receptor antagonist, did not prevent the induction of the behavioral responses by histamine (Fig. 4D). The i.t. injection of sendide or D-APV did not elicit any observable behavioral responses in the wild-type mice (Fig. 4A and C).

4. Discussion

In the present study, we clearly demonstrated that the depletion of endogenous histamine decreased the behavioral responses to several noxious stimuli using histidine decarboxylase knockout mice. Our studies using histamine-related gene knockout mice further substantiate the histamine-mediated pain modulation, confirming that endogenous histamine plays an excitatory role in the perception of pain. In addition, the intrathecal administration of histamine (400 pmol) evokes hyperalgesia in histidine decarboxylase knockout mice as assayed by the tail-flick test, but hyperalgesia was not observed in the wild-type mice at a low dose of histamine. Another interesting finding of the present study is that hyperalgesia evoked by intrathecally-administered histamine is blocked by the intrathecal treatment of histamine H_1 and tachykinin NK_1 receptor antagonists.

The latencies to respond to all of the thermal tests applied (hot-plate, tail-flick and paw-withdrawal) and the mechanical stimuli test (tail pressure) were significantly prolonged in the mutant mice when compared to the wild-type mice. These findings suggest that endogenous histamine stimulates afferent pain pathways evoked by nociceptor and that the level of histamine is an important determinant to affect the threshold of pain stimuli. However, these results do not distinguish predominant histamine involvement between their peripheral and central actions because the present tests can monitor nociceptive reflexes both at spinal and supraspinal levels except for the tail-flick test (D Amour and Smith, 1941). The chemical stimuli using formalin and capsaicin in this study provided further insights for this issue. Rodents show typical biphasic responses of biting and licking when formalin is injected into the hind paw. It is considered that the early response (Phase 1) is evoked by direct chemical activation of peripheral C-fibers and the late response (Phase 2) is mediated by the subsequent sensitization of the nociceptive neurons in the spinal dorsal horn with concomitant activation of nociceptor as occurred in Phase

1 (Rosland et al., 1990). Capsaicin injection also induces central sensitization through direct C-fiber activation resulting in similar behavioral responses (Sakurada et al., 1992). Our data demonstrates that histidine decarboxylase knockout mice are less responsive to these stimuli, suggesting the involvement of endogenous histamine both in the peripheral and central mechanisms.

These results seem to conflict with previous findings reported by Thoburn et al. (1994). The authors described the antinociception produced by intracerebrally-administered histamine in mice, suggesting that histamine directly stimulates the periaqueductal gray matter neurons resulting in the activation of the descending pain suppression system. In addition, pharmacological experiments using histamine H_3 receptor ligands, L-histidine, and histamine *N*-methyltransferase or histidine decarboxylase inhibitors suggested that substances enhancing brain histamine levels induced antinociception (Malmberg-Aiello et al., 1994, 1997). Such ambiguities exist in the role of the histaminergic neuron system for nociception from classical pharmacological studies. The discrepancy between their results and ours may be explained, in part, by the difference in the histamine doses and the involved neuronal pathways. Malmberg-Aiello et al., 1994, 1997, reported biphasic effects of intracerebrally-administered histamine on pain responses. In that paper, very low doses of histamine produced hyperalgesia, while high doses inversely produced analgesia.

We previously reported that the intrathecally-administered histamine evoked hyperalgesia at doses of 600–1600 pmol in mice, while hyperalgesia was not observed at higher doses (Sakurada et al., 2002, 2003). These findings combined with our present results suggest that histamine is predominantly involved in facilitation of the ascending pain transmission at physiological tissue concentration. On the other hand, the descending pain suppression system could be activated when a higher range of histamine is administered. There are cumulating data verifying critical involvement of histamine H_1 receptors in physiological and pathological pain perception from the studies of histamine-related gene knockout mice (Mobarakeh et al., 2000, 2002). These data further substantiate the hypothesis that the activation of histamine H_1 receptors by endogenous histamine is functioning as a stimulatory factor of pain transmission (Watanabe and Yanai, 2001). Since the transcripts of histamine H_1 receptor genes are expressed in the dorsal root ganglions, histamine plays an important role for physiological and pathological pain perception at the ascending C-fibers and spinal cord (Kashiba et al., 1999).

Possible changes in dynamics of the nociceptive neuropeptides are an alternative explanation for the observed insensitivity to pain in the histidine decarboxylase knockout mice. Since histamine is known to mediate the release of substance P and glutamate in inflammatory conditions, an assumption might be justified that the almost complete elimination of tissue histamine alters the neuronal environment including these peptides resulting in the inhibition of central pain transmission (Carlton et al., 1998; McHugh and McHugh, 2000; Riedel and Neeck, 2001). In fact, tachykinin NK_1 receptor antagonists and substance P antiserum were reported to attenuate hyperalgesic responses induced by intrathecally-administered histamine (Sakurada et al., 2003). In addition, the depletion of tissue histamine presumably would up-regulate or down-regulate the histamine receptors in specific areas although the apparent receptor bindings did not change in the brain of mutant mice (data not shown).

In addition, intrathecally-administered histamine (400 pmol) induced hyperalgesia in the histidine decarboxylase knockout mice, while the wild-type mice did not show any changes at this dose. The difference in responses is probably due to the functional supersensitivity induced by the depletion of histamine. It is well known that the nociceptive pathway has receptors for excitatory amino acid, glutamate and substance P resulting in transmission of the nociceptive information to the spinal dorsal horn. Histamine is known to be one of the pain mediators, and it directly or indirectly activates nociceptors. Lacking histamine can induce hyperalgesia to histamine at a dose of 400 pmol in histidine decarboxylase knockout mice, while the intrathecally-administered histamine evoked hyperalgesia at doses of 600–1600 pmol in normal ddY mice (Sakurada et al., 2002, 2003). We also demonstrated that the co-administration of pyrilamine inhibited hyperalgesia induced by intrathecally-administered histamine, and that ranitidine did not inhibit the histamine-elicited hyperalgesia. This result suggests that hyperalgesic responses to histamine in histidine decarboxylase knockout mice may be mediated through H_1 receptors in the spinal cord. Histamine H_1 receptor gene knockout mice exhibited hypoalgesic properties, demonstrating that histamine can facilitate pain perception through histamine H_1 receptors (Mobarakeh et al., 2000, 2002). The present study is essentially consistent with our previous reports using histamine H_1 receptor knockout mice. Both knockout mice studies clearly demonstrate that histamine and its receptors are vital for facilitating pain transmission peripherally and centrally.

The transcripts of histamine H_1 receptor genes were detected in many substance P and calcitonin gene-related peptide (cGRP) immunoreactive dorsal root ganglion neurons induced by nerve injuries in the peripheral tissues (Kashiba et al., 1999; Kashiba and Senba, 2001). We also showed that the co-administration of sendide, a tachykinin NK_1 receptor antagonist, inhibited the histamine-elicited hyperalgesic responses, but D-APV, an NMDA receptor antagonist did not inhibit hyperalgesia. This result suggests that histamine-induced hyperalgesic responses are mediated through tachykinin NK_1 receptors in the spinal cord. These results are also supported by our previous behavioral experimental studies (Sakurada et al., 2002, 2003).

This report using histidine decarboxylase knockout mice demonstrates that endogenous histamine may be involved in the facilitation of ascending pain transmission and that the hyperalgesic responses evoked by intrathecally-administered histamine might be mediated through tachykinin NK_1 and histamine H_1 receptors in the spinal cord. Although the role of the histamine system in pain perception is not simple, the long-lasting depletion of endogenous histamine in histidine decarboxylase knockout mice will enable greater understanding of these issues. Further studies using histidine decarboxylase

knockout mice are needed to clarify the role of the histamine system in physiological and pathological pain perception.

Acknowledgements

This work was supported in part by Grants-in-Aid for scientific research from the Japan Society for the Promotion of Science (JSPS), Goho Life Science Foundation and a 21st Century COE program (Bio-nano-technology) from the Ministry of Education, Culture, Sports, Science and Technology, Japan. We thank Professor Hiroshi Ohtsu for kindly providing the histidine decarboxylase knockout mice.

References

Baranauskas, G., Nistri, A., 1998. Sensitization of pain pathways in the spinal cord: cellular mechanisms. Prog. Neurobiol. 54, 349–365.

Brown, R.E., Stevens, D.R., Haas, H.L., 2001. The physiology of brain histamine. Prog. Neurobiol. 63, 637–672.

Calo, G., Rizzi, A., Marzola, G., Guerrini, R., Salvadori, S., Beani, L., Regoli, D., Bianchi, C., 1998. Pharmacological characterization of the nociception receptor mediating hyperalgesia in the mouse tail withdrawal assay. Br. J. Pharmacol. 125, 373–388.

Carlton, S.M., Zhou, S., Coggeshall, R.E., 1998. Evidence for the interaction of glutamate and NK_1 receptors in the periphery. Brain Res. 790, 160–169.

D Amour, F.E., Smith, B.L., 1941. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72, 74–76.

Haas, H., Panula, P., 2003. The role of histamine and the tuberomammillary nucleus in the nervous system. Nat. Rev., Neurosci. 4, 121–130.

Hori, Y., Nihei, Y., Kurokawa, Y., Kuramasu, A., Makabe-Kobayashi, Y., Terui, T., Doi, H., Satomi, S., Sakurai, E., Nagy, A., Watanabe, T., Ohtsu, H., 2002. Accelerated clearance of *Escherichia coli* in experimental peritonitis of histamine-deficient mice. J. Immunol. 169, 1978–1983.

Hough, L.B., 1988. Cellular localization and possible functions for brain histamine. Recent Prog. Prog. Neurobiol. 30, 469–505.

Kashiba, H., Senba, E., 2001. Primary sensory neurons expressing histamine H_1-receptor mRNA. Nippon Yakurigaku Zasshi 118, 43–49.

Kashiba, H., Fukui, H., Morikawa, Y., Senba, E., 1999. Gene expression of histamine H_1 receptor in guinea pig primary sensory neurons: a relationship between H_1 receptor mRNA-expressing neurons and peptidergic neurons. Mol. Brain Res. 66, 24–34.

Koarai, A., Ichinose, M., Ishigaki-Suzuki, S., Yamagata, S., Sugiura, H., Sakurai, E., Makabe-Kobayashi, Y., Kuramasu, A., Watanabe, T., Shirato, K., Hattori, T., Ohtsu, H., 2002. Disruption of L-histidine decarboxylase reduces airway eosinophilia but not hyperresponsiveness. Am. J. Respir. Crit. Care Med. 167, 758–763.

Leighton, G.E., Rodriguez, R.E., Hill, R.G., Hughes, J., 1998. Kappa-opioid agonist produce antinociception after i.v. and i.c.v. but not intrathecal administration in the rat. Br. J. Pharmacol. 93, 553–560.

Malmberg-Aiello, P., Lamberti, C., Ghelardini, C., Giotti, A., Barlolini, A., 1994. Role of histamine in rodent antinociception. Br. J. Pharmacol. 111, 1269–1279.

Malmberg-Aiello, P., Lamberti, C., Ipponi, A., Hanninen, J., Ghelardini, C., Bartolini, A., 1997. Effects of two histamine-*N*-methyltransferase inhibitors, SKF 91488 and BW 301U, in rodent antinociception. Naunyn-Schmiedeberg's Arch. Pharmacol. 355, 354–360.

Mason, P., 1999. Central mechanisms of pain modulation. Curr. Opin. Neurobiol. 9, 436–441.

McHugh, J.M., McHugh, W.B., 2000. Pain: neuroanatomy, chemical mediators, and clinical implications. AACN Clin. Issues 11 (2), 168–178.

Mobarakeh, J.I., Sakurada, S., Katsuyama, S., Kutsuwa, M., Kuramasu, A., Lin, Z.Y., Watanabe, T., Hashimoto, Y., Watanabe, T., Yanai, K., 2000. Role of histamine H_1 receptor in pain perception: a study of the receptor gene knockout mice. Eur. J. Pharmacol. 391, 81–89.

Mobarakeh, J.I., Sakurada, S., Katsuyama, S., Hayashi, T., Orito, T., Okuyama, K., Sakurada, T., Kuramasu, A., Watanabe, T., Watanabe, T., Yanai, K., 2002. Enhanced antinociception by intrathecally-administered morphine in histamine H_1 receptor gene knockout mice. Neuropharmacology 42, 1079–1088.

O' Callaghan, J.P., Holzman, S.G., 1975. Quantification of the analgesic activity of narcotic antagonists by a modified hot-plate procedure. J. Pharmacol. Exp. Ther. 192, 497–505.

Ohtsu, H., Tanaka, S., Terui, T., Hori, Y., Makabe-Kobayashi, Y., Pejler, G., Tchougounova, E., Hellman, L., Gertsenstein, M., Hirasawa, N., Sakurai, E., Buzas, E., Kovacs, P., Csaba, G., Kittel, A., Okuda, M., Hara, M., Mar, L., Numayama-Tsuruta, K., Ohuchi, K., Ichikawa, A., Falus, A., Watanabe, T., Nagy, A., 2001. Mice lacking histidine decarboxylase exhibit abnormal mast cells. FEBS Lett. 502 (1–2), 53–56.

Panula, P., Yang, H.Y., Costa, E., 1984. Histamine-containing neurons in the rat hypothalamus. Proc. Natl. Acad. Sci. U. S. A. 81, 2572–2576.

Panula, P., Pirvola, U., Auvinen, S., Airaksinen, M.S., 1989. Histamine-immunoreactive nerve fibers in the rat brain. Neurosience 28, 585–610.

Parolaro, D., Patrini, G., Massi, P., Mainardi, P., Giagnoni, G., Sala, M., Gori, E., 1989. Histamine as a central modulator of rat intestinal transit. J. Pharmacol. Exp. Ther. 249, 324–328.

Riedel, W., Neeck, G., 2001. Nociception, pain, and antinociception: current concepts. Z. Rheumatol. 60, 404–415.

Rosland, J.H., Tjolsen, A., Maehle, B., Hole, K., 1990. The formalin test in mice: effect of formalin concentration. Pain 42, 235–242.

Sakurada, T., Katusmata, K., Tan-No, K., Sakurada, S., Kisara, K., 1992. Capsaicin test in mice for evaluating tachykinin antagonists in the spinal cord. Neuropharmacology 12, 1279–1285.

Sakurada, T., Katusmata, K., Yogo, H., Tan-No, K., Sakurada, S., Ohba, M., Kisara, K., 1995. The neurokinin-1 receptor antagonist, sendide, exhibits antinociceptive activity in the formalin test. Pain 60, 175–180.

Sakurada, S., Orito, T., Sakurada, C., Sato, T., Hayashi, T., Mobarakeh, J.I., Yanai, K., Onodera, K., Watanabe, T., Sakurada, T., 2002. Possible involvement of tachykinin NK_1 and NMDA receptors in histamine-induced hyperalgesia in mice. Eur. J. Pharmacol. 434, 29–34.

Sakurada, S., Orito, T., Furuta, S., Watanabe, H., Mobarakeh, J.I, Yanai, K., Watanabe, T., Sato, T., Onodera, K., Sakurada, C., Sakurada, T., 2003. Intrathecal histamine induced spinally mediated behavioral responses through tachykinin NK_1 receptors. Pharmacol. Biochem. Behav. 74, 487–493.

Sato, T., Sakurada, S., Takahashi, N., Sakurada, T., Tan-no, K., Wako, K., Kisara, K., 1999. Contribution of spinal mu-opioid receptors to morphine-induced antinociception. Eur. J. Pharmacol. 369, 183–187.

Scholz, J., Woolf, C.J., 2002. Can we conquer pain? Nat. Neurosci. 5, 1062–1067 (suppl).

Schwartz, J.C., Arrang, J.M., Garbarg, M., Pollard, H., Ruat, M., 1991. Histaminergic transmission in the mammalian brain. Physiol. Rev. 71, 1–51.

Thoburn, K.K., Hough, L.B., Nalwalk, J.W., Mischler, S.A., 1994. Histamine-induced modulation of nociceptive responses. Pain 58, 29–39.

Watanabe, T., Yanai, K., 2001. Studies on functional roles of the histaminergic neuron system by using pharmacological agents, knockout mice and positron emission tomography. Tohoku J. Exp. Med. 195, 197–217.

Watanabe, T., Taguchi, Y., Shiosaka, S., Tanaka, J., Kubota, H., Terano, Y., Tohyama, M., Wada, H., 1984. Distribution of the histaminergic neuron system in the central nervous system of rats; a fluorescent immunohistochemical analysis with histidine decarboxylase as a marker. Brain Res. 295, 13–25.

Watanabe, T., Yamatodani, A., Maeyama, K., Wada, H., 1990. Pharmacology of α-fluoromethylhistidine, a specific inhibitor of histidine decarboxylase (a histamine-forming enzyme). Trends Pharmacol. Sci. 11, 363–367.

Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109–110.