Pharmacological Reports
2008, 60, 219–224
ISSN 1734-1140

Short communication

Central effect of histamine in a rat model of acute trigeminal pain

Esmaeal Tamaddonfard, Emad Khalilzadeh, Nasrin Hamzeh-Gooshchi, Sona Seiednejhad-Yamchi

Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

Correspondence: Esmaeal Tamaddonfard, e-mail: e_tamaddonfard@yahoo.com

Abstract:
In conscious rats implanted with an intracerebroventricular (*icv*) cannula, effect of *icv* injections of histamine, chlorpheniramine (H_1-receptor antagonist) and ranitidine (H_2-receptor blocker) was investigated in a rat model of acute trigeminal pain. Acute trigeminal pain was induced by putting a drop of 5 M NaCl solution on the corneal surface of the eye and the numbers of eye wipes were counted during the first 30 s. Histamine (20, 40 μg) and chlorpheniramine (80 μg) significantly decreased the numbers of eye wipes. Ranitidine alone had no effect. Pretreatment with chlorpheniramine did not change the histamine-induced analgesia, whereas the histamine effect on pain was inhibited with ranitidine pretreatment. These results indicate that the brain histamine, through central H_2 receptors, may be involved in the modulation of the acute trigeminal pain in rats.

Key words:
brain, histamine, acute trigeminal pain, rats

Abbreviations: BTN – brainstem trigeminal nucleus, *icv* – intracerebroventricular, *ip* – intraperitoneal, MPGM – mesencephalic periventricular grey matter, MTN – mesencephalic trigeminal nucleus, PAG – preaquductal grey, ReN 1869 – R-1-(3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)--1-propyl)-3-piperidine carboxylic acid; RN – raphe nucleus, TMN – tuberomammillary nucleus,

Introduction

Evidences taken from various acute and chronic pain tests suggest that the brain histamine influences the central perception of pain. It has been reported that the intracerebroventricular (*icv*) injection of histamine produces antinociception in both hot plate and paw pressure nociceptive tests in rats [19], in the formalin test in mice [33] and in the neuropathic pain in rats [13]. It is recognized that the action of brain histamine on pain modulation is mediated through histamine H_1, H_2, H_3 and H_4 receptors [2, 11]. Co-administration of temelastine (H_1-receptor antagonist) and tiotidine (H_2-receptor blocker) with histamine into the preaquductal grey (PAG) has been shown to inhibit the histamine induced analgesia in the hot plate test in rats [34]. In addition, it was found that *icv* injections of ranitidine (H_2-receptor blocker) and thioperamide (H_3-receptor antagonist) but not pyrilamine (H_1-receptor antagonist) enhanced the nociceptive threshold assessed by the Von Frey test in a rat model of neuropathic pain [13]. Besides, antihyperalgesic effects of centrally administered 2-methylhistamine (H_1-receptor agonist) and dimaprit (H_2-receptor agonist) were reported in the carrageenan-induced hyperalgesia [26].

It was reported that imepip (H_3-receptor agonist) attenuated formalin-induced pain, and peripheral and central pretreatments with thioperamide (H_3-receptor antagonist) reversed the suppressive effect of imepip [3]. Histamine H_4-receptor antagonists such as JNJ7777120 and VUF6002 has been reported to reduce the hyperalgesia provoked by subplantar injection of carrageenan in rats [5].

The cornea is a densely innervated organ [24], and is used for the study of nociception in the trigeminal system, because corneal nociceptive receptors have a large representation in the trigeminal ganglion through the ophthalmic branch of trigeminal nerve [9]. Moreover, it has been shown that nociceptive neurons in trigeminal nucleus respond vigorously when capsaicin, nicotine or 5 M NaCl are applied on the corneal surface [4]. There are few behavioral methods in studying acute pain in trigeminal system. Noxious thermal and chemical stimuli have been used in these models [29, 35]. The wiping the eye with a forelimb, known as eye-wiping test, has been used for investigating the chemical pungency [17] or the presence of C-fiber activity [7]. Recently, the eye wiping induced by topical application of 5 M NaCl solution on the cornea surface, has been introduced as a sensitive animal model for the study of acute trigeminal pain mechanisms [8]. To date, the effect of brain histamine on the trigeminal region pain was not reported. Thus, the present study was designed to investigate the effect of *icv* injections of histamine, chlorpheniramine and ranitidine on the acute trigeminal pain induced by hypertonic saline applied topically on corneal surface in rats.

Materials and Methods

Animals

Healthy adult male Wistar rats, weighing 220–250 g were used in this study. Rats were maintained in polyethylene cages with food and water available *ad libitum*, in a laboratory with controlled ambient temperature (23 ± 0.5°C) and under a 12 h light-dark cycle (lights on from 07:00). Experiments were carried out between 14:00 h and 17:00 h. The experimental protocol was approved by the Laboratory Animal Care and Use Center of the College of Veterinary Medicine of Urmia University.

Experimental protocol

After a 15-day adaptation period, each rat was anesthetized with a mixture of ketamine (80 mg/kg, *ip*) and xylazine (10 mg/kg, *ip*) and then was placed in a stereotaxic apparatus (Stoelting, Wood Lane, IL, USA). A 22-gauge, 12 mm stainless steel guide cannula was introduced *icv* at the following coordinates: 0.8 mm posterior to the bregma, 2 mm lateral to the midline and 4 mm below the top of the skull [28]. The cannula was fixed to the skull using three screws and dental acrylic (Acropars, Tehran, Iran). A 14-day recovery period was allowed before the experiments were initiated.

On the days of experiments, every dose of histamine dihydrochloride (Merck, Darmstadt, Germany), chlorpheniramine maleate and ranitidine hydrochloride (Sigma-Aldrich Co., Steinheim, Germany) dissolved in a constant volume of 1 μl of 0.9% NaCl was injected *icv* using a 5 μl Hamilton microsyringe over a period of 30 s. One specific group of rats was assigned to one specific drug treatment condition and each group comprised eight rats. Thus, each rat received 2, 3 or 4 different treatments and four days was allowed between *icv* injections.

For induction of trigeminal pain, each rat was placed on a 50 × 50 cm wooden table. After a 15 min habituation period, one drop (40 μl) of NaCl 5 M solution was topically applied on the surface of the cornea using a fine dropper and then the number of eye wipes performed with ipsilateral forepaw were counted for a period of 30 s [8]. In control group, one drop of NaCl 0.15 M solution was topically applied.

At the end of the experiments, the rats were icv injected with 10 μl of methylene blue and then were deeply anesthetized with the high dose of ether and decapitated. The brains were removed and placed in formaldehyde (10%) solution. After 24 h, the brains were sliced into 1 mm slices and the placement of the tip of the cannula was visually controlled. Data from rats with an incorrect placement of the cannula were excluded from analysis.

All the values are expressed as the means ± SEM. The data were analyzed by using analysis of variance (ANOVA) followed by Duncan's test. Statistical significance was set up at $p < 0.05$.

Results

None of tested animals reacted to topically applied NaCl 0.15 M solution, thus the obtained results (0 ± 0) are not shown in the Figures.

Histamine injected *icv* at the doses of 5 and 10 μg was without effect, whereas at the doses of 20, 40 μg, the amine significantly ($p < 0.05$) decreased the numbers of eye wipes. No significant change was observed between the effects of histamine at 20 and 40 μg (Fig. 1).

Icv injection of chlorpheniramine at the dose of 40 μg produced no effect on the pain response, but the numbers of eye wipes were reduced by *icv* injection of chlorpheniramine at the dose of 80 μg. Ranitidine at the doses of 40 and 80 μg, used *icv*, produced no significant effect on pain response (Fig. 2).

The pain suppressive effect induced by *icv* injected histamine (40 μg) was not affected with chlorpheniramine (80 μg), but pretreatment with *icv* injection of ranitidine (80 μg) before histamine (40 μg) prevented the histamine-induced antinociception (Fig. 3).

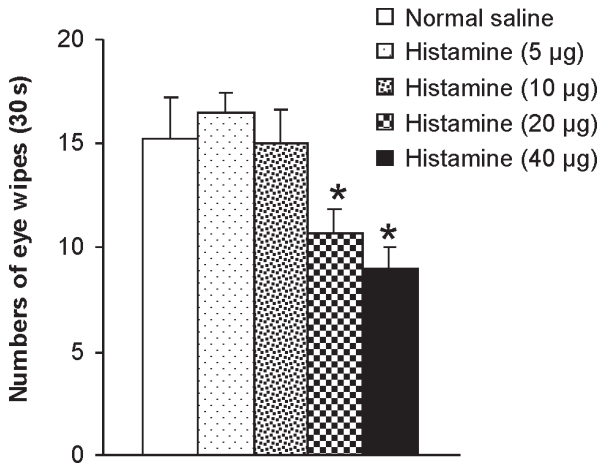

Fig. 1. Effect of *icv* injection of histamine on the numbers of eye wipes induced by 5 M NaCl solution applied to corneal surface. Values are the means ± SEM (n = 8 for normal saline and histamine 5 and 10 μg and n = 8 for histamine 20 and 40 μg).* $p < 0.05$ as compared to normal saline and histamine 5 and 10 μg treatments

Discussion

The results presented here indicate that *icv* injection of histamine produced antinociception in the acute trigeminal pain in rats. The cell bodies of the histaminergic neuronal system are concentrated in the tuberomammillary nucleus (TMN) of the hypothalamus and send axons to innervate the entire central nervous system [12]. The areas such as external layers of the dorsal horn of the spinal cord, mesencephalic periventricular grey matter (MPGM), raphe nucleus (RN) and

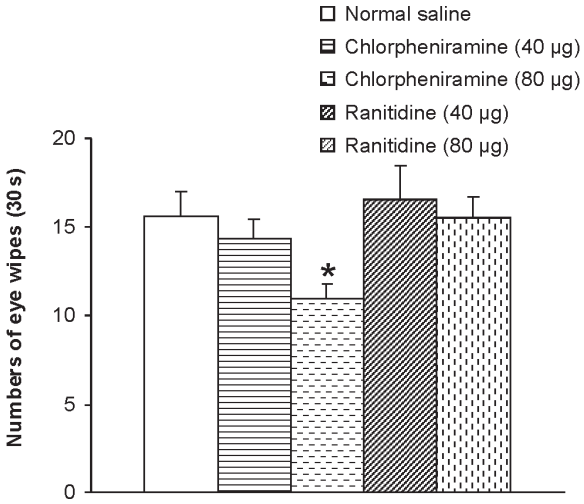

Fig. 2. Effect of *icv* injections of chlorpheniramine and ranitidine on the numbers of eye wipes induced by 5 M NaCl solution applied to corneal surface. Values are the means ± SEM (n = 8 for normal saline and chlorpheniramine 40 and 80 μg and n = 8 for ranitidine 40 and 80 μg).* $p < 0.05$ as compared to normal saline and other treatments

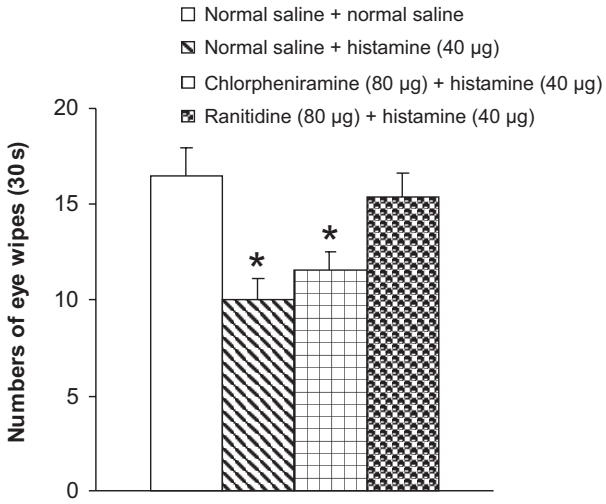

Fig. 3. Effect of *icv* pretreatments with chlorpheniramine and ranitidine before *icv* injection of histamine on the numbers of eye wipes induced by 5 M NaCl solution applied to corneal surface. Values are the means ± SEM (n = 8).* $p < 0.05$ as compared to normal saline plus normal saline and ranitidine plus histamine treatments

mesencephalic trigeminal nucleus (MTN), known to be involved in the nociceptive control [6], are also innervated by the hypothalamic histamine system [16, 30].

The orofacial region is one of the most densely innervated (by the trigeminal nerve) areas of the body, which focuses some of the most common pains. It is also the site of frequent chronic and referred pains [31]. It is believed that pain and injury in organs such as mouth, nose and eyes activate the brainstem trigeminal nucleus (BTN) [18]. It has been reported that the afferents from cornea especially enter into two spatially distinct parts of the BTN [21]. Furthermore, an electrical activity evoked by CO_2 pulses applied to the cornea was reported in the MTN neurons [1]. On the other hand, most studies have concluded that the brain histamine is involved in the modulation of proprioception through the action on the MTN [10, 15, 16]. Therefore, it seems that the antinociceptive effect of centrally injected histamine, observed in the present study, may be related to the action of histamine on the brainstem mesencephalic structures.

According to the present results, it is clear that the histamine H_1 receptor blocker, chlorpheniramine, produced antinociception in the absence of histamine, but in the presence of histamine, chlorpheniramine did not change the histamine-induced antinociception. Histamine H_1 receptors play an important role in both somatic and visceral pain perception since mutant mice lacking the histamine H_1 receptors, showed fewer nociceptive responses in various pain tests [22]. It has been reported that *icv* injection of 2-(3-trifluoromethylphenyl)histamine dihydrogenmaleate, 2-thiazolylethylamine (H_1-receptor agonists) and pyrilamine (H_1-receptor antagonist) produce hypernociception and antinociception, respectively, which suggests that H_1 receptor activation increases sensitivity to noxious stimuli [20]. In another study, it was found that intracerebral microinjection of temelastine into the PAG or into the RN prevented the histamine-induced antinociception, but in the absence of histamine, nociceptive response was not changed by temelastine [34]. Moreover, using tail flick and paw pressure tests of nociception, it was reported that *icv* injection of chlorpheniramine alone produced no antinociceptive effect, whereas, the antinociception induced by SKF 91488, an inhibitor of histamine catabolism, was prevented by chlorpheniramine pretreatment [14]. *Icv* injection of pyrilamine, a non-sedating H_1-receptor blocker, was reported to produce no effect on the pain threshold in the neuropathic pain of rats [13]. In addition, the tricyclic compound, ReN 1869, a novel histamine H_1 receptor antagonist that penetrates the blood-brain barrier, has been found to induce antinociception in chemical (formalin, capsaicin and phenylquinone writhing) but not in thermal (hot plate and tail flick) tests of nociception [27]. The differences between the findings would be related to the nature and sensitivity of the experimentally induced pain models applied and to the kind of H_1-receptor blocker used. The antinociception induced by the high dose of chlorpheniramine (80 μg), observed in the present study, may be related to its side effects, because chlorpheniramine belongs to the first class of H_1 antihistamines, and sedation, drowsiness and poor motor coordination are the side effects of the first class antihistamines [36, 37]. In the present study, a synergistic effect was not observed in the chlorpheniramine pretreatment. This may be related to the side effects produced by chlorpheniramine that may inversely affect the histamine-induced antinociception. However, the results of this study are not able to reveal a clear role for the involvement of histamine H_1 receptors in the histamine-induced antinociception, at least in the pain test used here.

The results of our study also show that the antinociceptive effect of centrally injected histamine is mediated through central H_2 receptors. The involvement of central histamine H_2 receptors in antinociception has been studied in thermal, mechanical and chemical nociceptive tests. It was reported that the thresholds for pain perception in histamine H_2 receptor gene knockout mice were higher than those of wild-type mice [23]. In the hot plate test in rats, the pain threshold enhancement was reported after *icv* injections of cimetidine and ranitidine [25]. Moreover, microinjection of H_2 receptor antagonist, cimetidine, into the PAG has been found to inhibit the histamine-induced antinociception [34]. On the other hand, in the tail flick nociceptive test in rats, intrathecal injection of ranitidine was not able to produce antinociception [32]. However, in the present study, ranitidine did not produce analgesia in the absence of histamine, but in the presence of histamine, ranitidine blocked the histamine-induced antinociception.

In conclusion, the present results suggest that the activation of brain histamine produces an antinociceptive effect in the trigeminal region pain in rats. The antinociception induced by histamine is mediated through histamine H_2 receptors.

References:

1. Bereiter DA, Bereiter DF, Hirata H: Topical cannabinoid agonist, WIN55,212-2, reduces cornea-evoked trigeminal brainstem activity in the rat. Pain, 2002, 99, 547–556.
2. Brown RE, Stevens DR, Haas HL: The physiology of brain histamine. Prog Neurobiol, 2001, 63, 637–672.
3. Cannon KF, Leurs R, Hough LB: Activation of peripheral and spinal histamine H_3 receptors inhibits formalin-induced inflammation and nociception, respectively. Pharmacol Biochem Behav, 2007, 88, 122–129.
4. Carstens E, Kuenzier N, Handwerker HO: Activation of neurons in rat trigeminal subnucleus caudalis by different irritant chemicals applied to the oral and ocular mucosa. J Neurophysiol, 1998, 80, 465–492.
5. Coruzzi G, Adami M, Guaita E, de Esch IJ, Leurs R: Antiinflammatory and antinociceptive effects of the selective histamine H_4-receptor antagonists JNJ7777120 and VUF6002 in a rat model of carrageenan-induced acute inflammation. Eur J Pharmacol, 2007, 563, 240–244.
6. Dubner R, Bennet GJ: Spinal and trigeminal mechanisms of nociception. Annu Rev Neurosci, 1983, 6, 384–418.
7. Farazifard R, Kiani R, Noorbakhsh M, Esteky H: Effects of neonatal C-fiber depletion on the integration of paired-whisker inputs in rat barrel cortex. Exp Brain Res, 2005, 162, 115–121.
8. Farazifard R, Safapour F, Sheibani V, Javan M: Eye-wiping test: a sensitive animal model for acute trigeminal pain studies. Brain Res Brain Res Protoc, 2005, 16, 44–49.
9. Felipe CD, Gonzalez GG, Gallar G, Belmonte C: Quantification and immunocytochemical characteristics of trigeminal ganglion neurons projecting to the cornea: effect of corneal wounding. Eur J Pain, 1999, 3, 31–39.
10. Fujise T, Yoshimatsu H, Kurokawa M, Oohara A, Kang M, Nakata M, Sakata T: Satiation and masticatory function modulated by brain histamine in rats. Proc Soc Exp Biol Med, 1998, 217, 228–234.
11. Haas HL, Pannula P: The role of histamine and the tuberomammillary nucleus in the nervous system. Nat Rev Neurosci, 2003, 4, 121–130.
12. Hough LB: Cellular localization and possible functions for brain histamine: recent progress. Prog Neurobiol, 1988, 30, 469–505.
13. Huang L, Adachi N, Nagaro T, Liu K, Arai T: Histaminergic involvement in neuropathic pain produced by partial ligation of the sciatic nerve in rats. Reg Anesth Pain Med, 2007, 32, 124–129.
14. Jochem J, Rybus-Kalinowska B, Żwirska-Korczała K: Studies on the role of H_1 receptors in endogenous central histamine-induced antinociceptive effect in rats. Acta Med Mart, 2002, 2, 14–19.
15. Inagaki N, Yamatodani A, Shinoda K, Shiotani Y, Tohyama M, Watanabe T, Wada H: The histaminergic innervation of the mesencephalic nucleus of the trigeminal nerve in rat brain: a light and electron microscopical study. Brain Res, 1987, 418, 388–391.
16. Lazarov NE, Gratzl M: Selective expression of histamine receptors in the mesencephalic trigeminal neurons. Neurosci Lett, 2006, 404, 67–71.
17. Lee J, Lee J, Kim J, Kim SY, Chun MW, Cho H, Hwang SW et al.: N-(3-acyloxy-2-benzylpropyl)-N'-(4-hydroxy-3-methoxybenzyl) thiourea derivatives as potent vanilloid receptor agonists and analgesics. Bioorg Med Chem, 2001, 9, 19–32.
18. Malick A, Burstein R: Cells of the trigeminohypothalamic tract in the rat. J Comp Neurol, 1998, 400, 125–144.
19. Malmberg-Aiello P, Lamberti C, Ghelardini C, Giotti A, Bartolini A: Role of histamine in rodent antinociception. Br J Pharmacol, 1994, 111, 1269–1279.
20. Malmberg-Aiello P, Lamberti C, Ipponi A, Bartolini A, Schunak W: Evidence for hypernociception induction following histamine H_1 receptor activation in rodents. Life Sci, 1998, 63, 463–467.
21. Marfurt CF, Del Toro DR: Corneal sensory pathway in the rat: a horseradish peroxidase tracing study. J Comp Neurol, 1987, 267, 450–459.
22. Mobarakeh JI, Sakurada S, Katsuyama S, Kutsuwa M, Kuramasu A, Lin ZY et al.: Role of histamine H_1 receptor in pain perception: a study of the receptor gene knockout mice. Eur J Pharmacol, 2000, 391, 81–89.
23. Mobarake JI, Takahashi K, Sakurada S, Kuramasu A, Yanai K: Enhanced antinociceptive effects of morphine in histamine H_2 receptor gene knockout mice. Neuropharmacol, 2006, 51, 612–622.
24. Muller IJ, Marfurt CF, Kruse F, Tervo TMT: Corneal nerves: structure, contents and functions. Exp Eye Res, 2003, 76, 521–542.
25. Netti C, Guidobono F, Sibilia V, Villa I, Cazzamalli E, Pecile A: Central effects of the histamine H_2 receptor agonist and antagonist on nociception in rats. Agents Actions, 1988, 23, 247–249.
26. Netti C, Sibilia V, Guidobono F, Villani P, Pecile A, Braga PC: Evidences for an inhibitory role of central histamine on carrageenin-induced hyperalgesia. Neuropharmacology, 1994, 33, 205–210.
27. Olsen UB, Eltrop Ct, Ingvardsen BK, Jorgensen TK, Lundbaek JA, Thamsen C, Hansen AJ: Ren 1869, a novel tricyclic antihistamine, is active against neurogenic pain and inflammation. Eur J Pharmacol, 2002, 435, 43–57.
28. Paxinos G, Watson C: The rat brain in stereotaxic coordinates. Compact Third Edition, Academic Press, San Diego, USA, 1997.
29. Pelissier T, Pajot T, Dallel R: The orofacial capsaicin test in rats: effects of different capsaicin concentrations and morphine. Pain, 2002, 96, 81–87.
30. Schwartz JC, Arrang JM, Garbarg M, Pollard H, Ruat M: Histaminergic transmission in the mammalian brain. Physiol Rev, 1991, 71, 1–51.
31. Sessle BJ: Acute and chronic orofacial pain: brainstem mechanisms of nociceptive transmission and neuroplasticity, and their clinical correlates. Crit Rev Oral Biol Med, 2000, 11, 57–91.
32. Shu HW, Chung KM, Kim HY, Huh SO, Song DK: Effects of histamine receptor antagonists injected intrathecally on antinonociception induced by opioid

administered intracerebroventricularly in the mouse. Neuropeptides, 1994, 33, 121–129.
33. Tamaddonfard E, Rahimi S: Central effect of histamine and peripheral effect of histidine on the formalin-induced pain response in mice. Clin Exp Pharmacol Physiol, 2004, 31, 518–522.
34. Thoburn KK, Hough LB, Nalwalk JW, Mischler SA: Histamine-induced modulation of nociceptive responses. Pain, 1994, 58, 29–37.
35. Tzabazis A, Klyukinov M, Manering M, Nemenov ML, Shafer SL, Yeomans DC: Differential activation of trigeminal C or A δ nociceptors by infrared diode laser in rats: behavioral evidence. Brain Res, 2005, 1037, 148–156.
36. Welch MJ, Meltzer EO, Simons FE: H_1-antihistamines and the central nervous system. Clin Allergy Immunol, 2002, 17, 337–388.
37. Woodward JK: Pharmacology of antihistamines. J Allergy Clin Immunol, 1995, 86, 606–612.

Received:
August 30, 2007; in revised form: November 28, 2007.