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ANTINOCICEPTION INDUCED BY CENTRAL ADMINISTRATION OF HISTAMINE IN THE FORMALIN TEST IN RATS

ALI MOJTAHEDIN, ESMAEAL TAMADDONFARD* AND ALI ZANBOORI

Department of Basic Sciences,
Faculty of Veterinary Medicine, P.O. Box 1177,
Urmia University, Urmia – 57135, Iran

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Abstract : In the present study, effects of intracerebroventricular (icv) administration of histamine, mepyramine (H_1-receptor antagonist) and famotidine (H_2-receptor antagonist) have been investigated on the formalin test in rats. Subcutaneous injection of formalin (50 µl, 1%) into the ventral surface of the left hind paw produced a marked biphasic pain response (first phase: 0–5 min and second phase: 15–45 min). All the performed treatments did not significantly influence the first phase of pain. Histamine at the doses of 10 and 40 µg and mepyramine and famotidine at the same doses of 20 and 80 µg, significantly ($P<0.05$) decreased the late phase of formalin-induced pain. Pretreatments with mepyramine and famotidine at the same dose of 80 µg, significantly ($P<0.05$) prevented the histamine (40 µg)-induced antinociception. These results indicate that brain histamine produces antinociception, and both central H_1 and H_2 receptors may involve in the histamine-induced antinociception in the formalin test in rats.

Key words : brain, histamine, mepyramine, famotidine, formalin-induced pain, rats

INTRODUCTION

Several lines of evidence suggest that histamine, through its H_1, H_2, H_3 and H_4 receptors, participates in the mechanisms of pain (1, 2, 3, 4). Outside the brain and in the peripheral tissues such as skin, histamine stimulates nociceptive afferent fibers (5). At the spinal cord level, histamine trough its H_1 receptors, elicited a hyperalgesic response as assessed by the tail flick test in mice (6). Within the brain, the antinociceptive effects of centrally administered histamine and the involvement of its H_1, H_2 and H_3 receptors have been reported (7, 8, 9, 10, 11, 12, 13).

The formalin test is an important animal model in the study of acute long-lasting inflammatory pain (14). In this model, subcutaneous injection of diluted formalin into a hind paw elicits a biphasic pattern of pain-related behaviors, an early short-lasting neurogenic phase followed by a second and more sustained inflammatory phase (15). Histamine involves in the nociceptive and inflammatory responses induced by intraplantar injection of formalin in rats (16,

*Corresponding Author : Dr. Esmaeal Tamaddonfard, DVM, DVSc, Department of Basic Sciences, Faculty of Veterinary Medicine, P.O. Box 1177, Urmia University, Urmia 57135, Iran; Phone : +98-441-2770508; Fax : +98-441-2771926; Email : e_tamaddonfard@yahoo.com

17). There are few data regarding the direct effects of brain histamine and its central histamine H_1 and H_2 receptors involvement in the formalin-induced pain response in rats. In one study, centrally administered histamine attenuated nociception due to pedal edema induced by formalin in rats (10). The aim of the present study was to investigate the central effect of histamine, mepyramine (H_1-receptor antagonist) and famotidine (H_2-receptor antagonist) on the nociceptive response induced by formalin in rats.

MATERIAL 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 12:00 h to 16:00 h. The experimental protocol was approved by the Laboratory Animal Care and Use Center of the College of Veterinary Medicine of Urmia University.

Drugs

Drugs used in the present study included histamine dihydrochloride (Merck, Darmstadt, Germany) mepyramine (pyrilamine) and famotidine hydrochloride (Sigma-Aldrich Co., Steinheim, Germany). The drugs were dissolved in normal saline, expect of famotidine, which initially was dissolved in one drop of 1 M hydrochloric acid and then diluted with normal saline.

Surgery

After a 15-day adaptation period each rat was anaesthetized with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg) injected intraperitoneally, and then placed in a stereotaxic apparatus (Stoelting, Wood Lane, IL, USA). The scalp was incised and the skull was levelled off around the bregma. A 22 gauge, 12 mm stainless-steel guide cannula was inserted in the right lateral ventricle of the brain. The tip of the cannula was aimed at the following coordinates: 0.8 mm posterior to the bergma, 2 mm lateral to the midline and 4 mm below the top of the skull (12, 18). The cannula was then fixed to the skull using three screw and dental acrylic. A 12.5 mm stylet was inserted in the cannula to keep it patent prior to injection. Animals were allowed a 10-day recovery period before experiments were initiated.

Drug injection

For intracerebroventricular administrations of normal saline (control), histamine (2.5, 10 and 40 µg), mepyramine and famotidine at the same doses of 5, 20 and 80 µg, a 28 gauge, 12.5 mm injection needle was attached to a 30 cm polyethylene tube fitted to a 5 µL Hamilton syringe. Then, the rat was restrained by hand, the stylet was withdrawn, and the injection needle was inserted into the guide cannula. The volume of the solutions to be injected into lateral ventricle was 1 µL and the injection was made over a period of 60s. One specific group of rats was assigned to one specific drug treatment condition and each group comprised six rats. Thus, each rat was received 2 or 3 treatments and five days were allowed between intracerebroventricular injections.

Formalin test

Formalin test was used for induction of nociception. Before rats were pain tested, they were placed in a plexiglass observation chamber (30 × 30 × 25 cm) for 30 min on

three successive days to minimize stress-activated pain suppressive mechanisms (19). The formalin test was applied as follows. Fifty microlitres of 1% formalin was injected subcutaneously into the ventral surface of right hind paw using a 29-gauge injection needle (20). The rat was then placed in the observation chamber with a mirror mounted at 45° beneath the floor to allow an unobstructed view of the paw. The time spent licking and biting the injected paw was taken as a measure of nociceptive response and was recorded in five min intervals for 1 h. In the present study, data collected between 0 to 5 min post-formalin injection represented phase one (early phase) and data collected between 15 to 45 min after injection of formalin represented phase two (late phase). In control rats the intraplantar injection of appropriate amount of normal saline was performed. All the observers were blinded to the protocol of the study.

Cannula verification

During the surgery and before icv injections, the rising of the cerebrospinal fluid through the cannula was observed. For additional confirmation of the placement of the cannula in the lateral ventricle of the brain, at the end of experiments, the rats were intracerebroventrcularly injected with 10 µl methylene blue and then were deeply anaesthetized 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 place of the tip of the cannula and distribution of the dye in the lateral ventricle were visually controlled. Data from rats with an incorrect placement of the cannula were excluded from analysis.

Statistical analysis

Data were expressed as mean ± SEM. Differences among treated groups were statistically evaluated using the factorial analysis of variance (ANOVA) followed by Duncan's test. Differences were considered significant at $P<0.05$.

RESULTS

Intraplantar injection of normal saline produced no considerable pain response. Therefore the results (first phase: 3.0±0.9 s and second phase: 1.2±0.5 s) obtained from the normal saline are not presented in the figures. Intracerebroventricular injections of histamine, mepyramine and famotidine produced no significant effects on the first phase of formalin-induced pain (Figs. 1, 2, 3 and 4).

Intracerebroventricular injection of histamine at the doses of 10 and 40 µg, but not at the 2.5 µg, significantly ($P<0.05$) decreased the second phase of the time spent licking and biting of the injected paw-induced by intraplantar injection of formalin (Fig. 1).

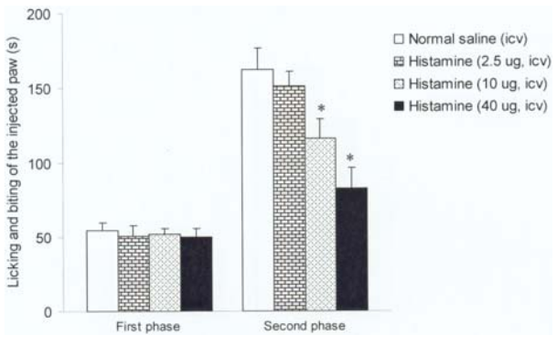

Fig. 1: Effect of intracerebroventricular injection of histamine on the formalin-induced nociception in rats. (*$P<0.05$ as compared with normal saline and histamine 2.5 µg), n=six rats for normal saline and histamine (2.5 µg) and six rats for histamine (10 and 40 µg) treatments.

The second phase of formalin-induced nociceptive response was significantly ($P<0.05$) lowered by intracerebroventricular

injection of mepyramine at the doses of 20 and 80 µg. Mepyramine at the dose of 5 µg produced no significant effect (Fig. 2).

Intracerebroventricular injection of famotidine at the doses of 20 and 80 µg, but not at the dose of 5 µg, significantly (P<0.05) decreased the second phase of nociception induced by formalin (Fig. 3).

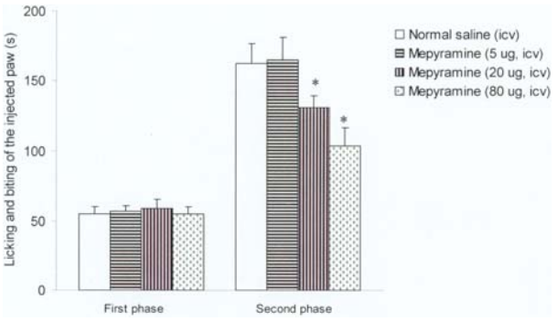

Fig. 2: Effect of intracerebroventricular injection of mepyramine on the formalin-induced nociception in rats. (*P<0.05 as compared with normal saline and mepyramine 5 µg), n=6 rats for mepyramine treatments.

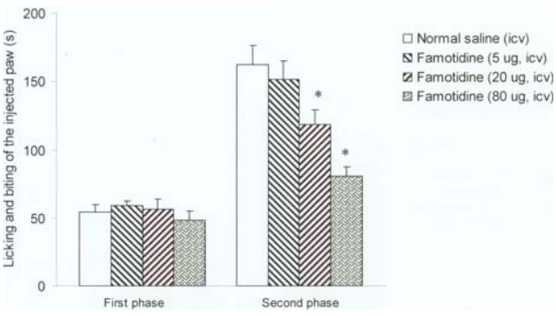

Fig. 3: Effect of intracerebroventricular injection of famotidine on the formalin-induced nociception in rats. (*P<0.05 as compared with normal saline and famotidine 5 µg), n=6 rats for famotidine treatments.

Intracerebroventricular pretreatments with mepyramine and famotidine at the same dose of 80 µg significantly (P<0.05) inhibited the suppressive effect of intracerebroventricular histamine (40 µg) on the formalin-induced nociception (Fig. 4).

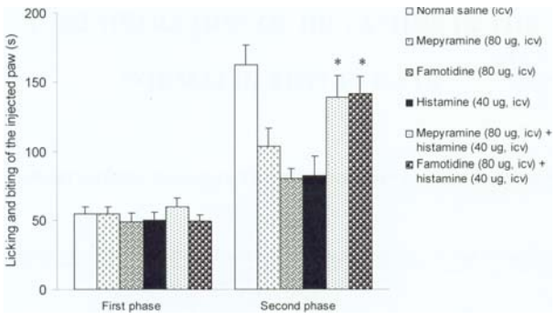

Fig. 4: Pretreatment effect of mepyramine and famotidine on the histamine-induced antinociception in rats. (*P<0.05 as compared with mepyramine, famotidine and histamine), n=6 rats for mepyramine plus histamine and 6 rats for famotidine plus histamine treatments.

DISCUSSION

In the present study, it was found that intracerebroventricular administration of histamine without any effect on the first phase, suppressed the second phase of formalin-induced pain in rats. The first phase in turn can be attributed to a direct algogenic effect of formalin on the nociceptors, and the second phase to release of local inflammatory mediators responsible for sensitization of primary and spinal sensory neurons and subsequent signal transduction into the brain (15). Histamine, one of the local inflammatory mediators involves both in the first and second phases of formalin-induced pain (16, 17). On the other hand, in the brain, the areas such as external layers of the dorsal horn of the spinal cord, mesencephalic periventricular grey matter, raphe nucleus (RN) and mesencephalic trigeminal nucleus (MTN), known to be involved in the nociceptive control (21), are innervated by the hypothalamic histamine system (22). Tamaddonfard and Rahimi (11) reported an analgesic effect of centrally administered histamine in the formalin test in mice.

Moreover, centrally administered histamine attenuated nociception due to pedal edema induced by formalin in rats (10). It seems that central histamine-induced antinociception may be at the supraspinal level, because Sakurada et al. (6) reported a hyperalgesic effect of intrathecally administered histamine in mice. However, taken together with aforementioned findings, the results of the present study suggest that the brain histaminergic system can provide modulation of inflammatory pain.

In the present study, histamine H_1 antagonist, mepyramine, itself produced antinociception, but inhibited 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 (23). Moreover, the analgesic effects of H_1 antihistaminics in the most types of nociceptive tests have been reported (24). Mepyramine belongs to the first generation of H_1 antihistaminics and easily penetrates the brain after oral administration (25). In the formalin test in mice, subcutaneous injection of pyrilamine did not produce any antinociceptive effect (26), but in the tail flick and hot plate tests of nociception, intraperitoneal injection of pyrilamine inhibited the histamine-induced antinociception (27). In one study, chronic administration of mepyramine via the drinking water attenuated both phases of formalin-induced pain response in rats (28).

In the present study, histamine H_2 antagonist, famotidine, itself produced antinociception, but inhibited the histamine-induced antinociception. The involvement of histamine central 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_1 receptor gene knockout mice were higher than those of wild-type mice (29). There is not any report regarding the central effect of famotidine in the formalin test in rats. In the hot plate test in rats, the pain threshold enhancement was reported after intracerebroventricular injections of cimetidine and ranitidine (30). Moreover, intracerebroventricular injection of cimetidine induced antinociceptive response in the heat tail flick nociceptive test in rats (8).

In conclusion, the present results suggest that the activation of brain histamine produces an antinociceptive effect in the formalin test in rats. Both mepyramine and famotidine show antinociceptive effect. Central histamine H_1 and H_2 receptors may involve in the central histamine-induced antinociception.

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