

Central Effect of Exogenous Histamine on Pain Induced by Sub-Plantar Injection of Formalin in Rabbits

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Received 27 October 2009, Accepted 21 February 2010

Abstract

In the present study, the effects of intracerebroventricular (ICV) administration of normal saline (control), histamine, mepyramine (a histamine H₁-receptor antagonist) and ranitidine (a histamine H₂-receptor antagonist) were investigated on the formalin-induced pain in rabbits. Subcutaneous (SC) injection of a formalin (100 µl, 5%) solution into the ventral surface of the right hind paw was performed, and the time durations spent licking and biting the injected paw were measured in 10 min blocks for 1 h. The SC injection of formalin produced a short-lasting (10 min) pain response. The ICV injection of histamine at doses of 25, 50 and 100 µg significantly ($P < 0.05$) decreased the time duration spent licking and biting the injected paw. Mepyramine and ranitidine, used alone produced no effects. The ICV pretreatments with mepyramine and ranitidine at the same dose of 200 µg significantly ($P < 0.05$) prevented histamine (100 µg, ICV)-induced antinociception. These results indicate that activation of brain histamine with ICV injection of exogenous histamine produces antinociception. Central histamine H₁ and H₂ receptors may be involved in the centrally administered histamine-induced antinociception in the formalin-induced pain in rabbits.

Key words: Brain, Histamine, Formalin-induced pain, Rabbits.

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Introduction

Several lines of evidence suggest that brain histamine may be involved in central perception of pain. The intracerebroventricular (ICV) injection of histamine elicited antinociceptive effects in hot plate, paw pressure and abdominal wall constriction tests of nociception in rats and mice.¹ In formalin test in mice, both acute and tonic phases of pain were attenuated by centrally administered histamine.² It was found that ICV injection of histamine enhanced nociceptive threshold assessed by the Von Frey test in a rat model of neuropathic pain.³ The ICV administration of histamine decreased the number of eye wipes induced by putting a drop of hypertonic saline solution on corneal surface in rats.⁴ It is recognized that the action of brain histamine on pain modulation is mediated through histamine central H₁, H₂ and H₃ receptors.⁵ The ICV injection of H₁ agonist (2-TEA: 2-thiazolylethylamine) and antagonist (pyrilamine) produced hypernociception and antinociception, respectively.⁶ Both H₂ agonist (4-methylhistamine) and antagonist (ranitidine) enhanced the threshold of nociception in rats.⁷ Imepip, a histamine H₃ receptor agonist, attenuated formalin-induced pain, and peripheral and central pretreatments with thioperamide (H₃-receptor antagonist) reversed the suppressive effect of imepip.⁸

Formalin, as a nociceptive stimulus, has been frequently used to study of pain mechanisms in rats and mice, and according to these studies, a marked biphasic nociceptive behavior was produced by small amounts (20-100 µl) of dilute solutions (0.1-10%) of formalin applied in various parts of body.^{9,10} However, Aloisi *et al.* (1993) reported a brief period of leg lifting caused by formalin (100 µl, 5%) injection into the hind paw in rabbits.¹¹ In addition, a short (10 min) period of head movements was reported after subcutaneous injection of

formalin (100 µl, 5%) in the ear skin of rabbits.¹²

The present study was designed to investigate the effects of ICV injection of histamine and its H₁ and H₂ antagonists, mepyramine and ranitidine, respectively, on pain response induced by sub-plantar injection of formalin in rabbits.

Materials and Methods

Animals. Thirty six healthy adult male New Zealand white rabbits weighing between 2.5-3 kg, bred in the animal house of the Urmia Faculty of Veterinary Medicine, were individually maintained in standard aluminium cages (50 × 50 × 40 cm) under controlled temperature (21-23 °C) on a 12 hrs light/dark cycle. They were fed with a commercial diet and water was available *ad libitum*. Six rabbits were used in each drug treatment. The Laboratory Animal's Care and Use Center of Faculty of Veterinary Medicine of Urmia University approved all procedures performed in this study.

Drugs. Histamine dihydrochloride (Merck, Darmstadt, Germany) at doses of 12.5, 25, 50 and 100 µg and mepyramine maleate and ranitidine hydrochloride (Sigma-Aldrich Co., Steinheim, Germany) at the same doses of 50, 100 and 200 µg were used in the present study. The ICV injections of mepyramine and ranitidine at the same dose of 200 µg before ICV injection of histamine (100 µg) were also performed. All drugs were dissolved in normal saline (0.9% NaCl solution, control) 30 min before ICV injections.

Lateral cerebral ventricle cannulation. For cannulation of the lateral ventricle of the brain, a stainless steel guide cannula was surgically implanted into the left lateral cerebral ventricle as described previously.¹³ In brief, each rabbit was anaesthetized with a mixture of 50 mg kg⁻¹ ketamine (alfasan, Woerden-Holland) and 5 mg kg⁻¹ xylazine (alfasan, Woerden-Holland) injected intramuscularly (IM).

Thereafter, the head of rabbit was fixed in a stereotaxic apparatus (Stoelting, Wood Lane, IL, USA). The scalp was incised and the skull was leveled off around the bregma. A 1 mm diameter hole was drilled through the skull 1 mm anterior to the bregma and 2.5-3 mm lateral to the midline and a 22-gauge, 18 mm length stainless steel guide cannula was vertically inserted until cerebrospinal fluid rose in the cannula. The cannula was then anchored with dental acrylic cement (Acropars, Tehran, Iran) to three preplaced stainless steel screws in the skull. After surgery, penicillin G procaine was injected IM at a dose of 60000 IU kg⁻¹, and the rabbit was returned to its cage.

Drug injection. An interval of at least 10 days was allowed between cannula implantation and ICV injection of drugs. Drugs were injected using a 25 µl Hamilton's microsyringe. The volume of the drug solution to be injected into the lateral ventricle was 5 µl and the injection was made over a period of 30 s. Each rabbit received two or three treatments, and seven days was allowed between treatments. The ICV injection of histamine was performed 5 min before sub-plantar injection of formalin. Injection of formalin into the ventral surface of the hind paw was performed 10 min after ICV injection of mepyramine and ranitidine.

Induction of pain. The formalin test was used for the induction of pain. The rabbits were placed in the formalin test cages (50×50×45 cm) for 45 minutes. Thereafter, 100 µl of formalin (5%) solution was injected SC into the ventral surface of the right hind paw. Control animals received 100 µl normal saline. Licking and biting of injected paw was taken as a measure of the pain response. The time durations spent the licking and biting of the injected paw were measured in 10 min blocks for a period of 1 hr after formalin injection.

Verification of the cannula. During the surgery and before ICV injections, an efflux 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 rabbits were ICV injected with 50 µl of methylene blue and then were deeply anaesthetized with a high dose of ether and decapitated. The brains were removed and placed in a formaldehyde (10%) solution. After 24 h, the brains were sliced into 1mm slices and the placement of the tip of the cannula and distribution of the dye in the lateral ventricle were visually controlled. Data from rabbits with an incorrect placement of the cannula were excluded from the analysis.

Statistical analysis. Data obtained from the subcutaneous injections of normal saline and formalin were analyzed using repeated measure ANOVA followed by Duncan's test. To evaluate significance differences among drug-treated groups, one-way analysis of variance (ANOVA) and Duncan's test were applied. In figures, all values are expressed as the mean ± S.E.M. A value of $P < 0.05$ was considered statistically significant.

Results

Sub-plantar injection of normal saline produced a weak response of licking and biting of the injected paw. After sub-plantar injection of formalin, a significant ($P < 0.05$) difference was observed between first 10 min block with other 10 min blocks (Fig. 1).

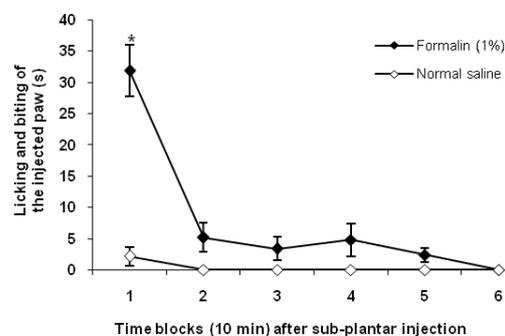


Fig 1. Time (seconds) spent of licking and biting of the injected paw after sub-plantar injection of normal saline and formalin in rabbits, * $P < 0.05$ as compared with normal saline and other 10 min blocks. n = 6 rabbits.

The ICV injection of histamine at a dose of 12.5 µg produced no significant ($P > 0.05$) effect, whereas at doses of 25, 50 and 100 µg, histamine significantly ($P < 0.05$) reduced formalin-induced pain response (Fig. 2).

The ICV injection of mepyramine and ranitidine at doses of 50, 100 and 200 µg, produced no significant ($P > 0.05$) effect on the durations of licking and biting of the injected paw induced by formalin (Fig. 3).

Pretreatments with mepyramine and ranitidine at the same dose of 200 µg significantly ($P < 0.05$) prevented histamine (100µg)-induced antinociception (Fig. 4).

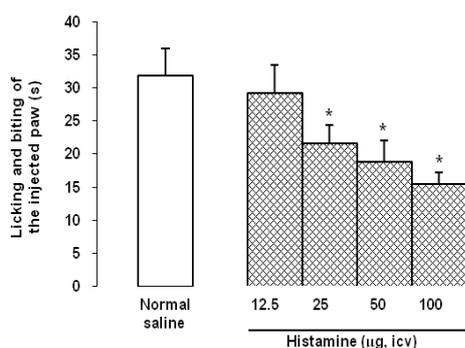


Fig 2. Effect of ICV injection of histamine on the formalin-induced pain response in rabbits, $*P < 0.05$ as compared with normal saline (control), $n = 6$ rabbits for normal saline and histamine (12.5 and 25 µg) and 6 rabbits for histamine (50 and 100 µg).

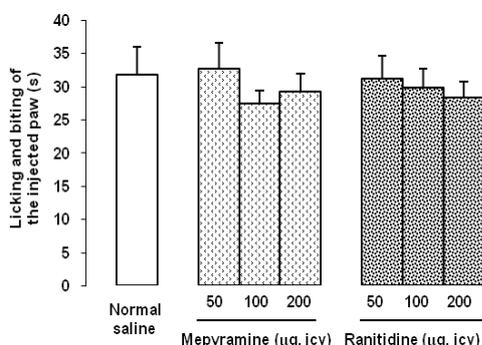


Fig 3. Effect of ICV injections of mepyramine and ranitidine on the formalin-induced pain response in rabbits, $n = 6$ rabbits for mepyramine and 6 rabbits for ranitidine.

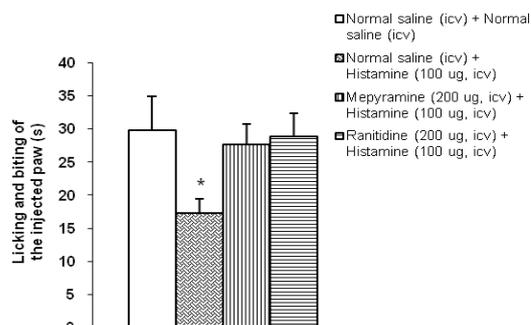


Fig 4. Effect of ICV pretreatments with mepyramine and ranitidine on the histamine-induced antinociception in the formalin test in rabbits, $*P < 0.05$ as compared with normal saline plus histamine (100 µg), $n = 6$ rabbits for normal saline plus normal saline and normal saline plus histamine and 6 rabbits for mepyramine plus histamine and ranitidine plus histamine

Discussion

In the present study, sub-plantar injection of formalin produced a monophasic pain response, which lasted for 10 min. This finding is in agreement with those studies which performed for the study of pain in rabbits.^{11, 12}

The present results indicated that the activation of brain histamine with ICV injection of exogenous histamine produced an antinociceptive effect in the rabbit model of formalin test. The cell bodies of histaminergic neuronal system are found only in the tuberomammillary nucleus of the hypothalamus, and their fibers and terminals innervate the entire central nervous system.¹⁴ The areas such as the external layers of dorsal horn of the spinal cord, preaqueuductal gray and raphe nucleus, known to be involved in nociceptive control¹⁵ are also innervated by histaminergic system of hypothalamus.¹⁴ Antinociceptive effects of brain histamine and the involvement of its receptors in various inflammatory models of pain in rodents have been investigated by injection of the amine into the lateral ventricle or into the specific nuclei of brain. The ICV injection of histamine attenuated formalin-induced nociception, paw edema as well as protein

concentration in edema fluid.¹⁶ In another study, centrally administered histamine reduced hyperalgesia induced by intraplantar injection of carrageenan.¹⁷ The ICV injection of histamine suppressed the late phase of formalin-induced pain in rats.¹⁸ Microinjection of histamine into the dorsal hippocampus suppressed the formalin-induced orofacial pain in rats.¹⁹

In the present study, histamine H₁ and H₂ antagonists -when used alone-, did not change formalin-induced pain response. The ICV injection of chlorpheniramine alone did not affect the nociceptive response in the hot plate test in rats²⁰ whereas in the acute corneal pain in rats, an antinociceptive effect induced by ICV injection of chlorpheniramine was reported.⁴ The ICV injections of mepyramine and famotidine alone suppressed the second phase of formalin-induced pain in rats.¹⁸ The tricyclic compound, ReN 1869, a novel histamine H₁ 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.²¹ Microinjection of mepyramine and ranitidine into the hippocampus did not influence both phases of formalin-induced orofacial pain in rats.¹⁹

In this study, both H₁ and H₂ antagonists prevented histamine-induced antinociception. This indicates that both histamine H₁ and H₂ receptors may be involved in the antinociceptive effect induced by histamine. The histamine H₁ and H₂ receptors play important roles in both somatic and visceral nociception because histamine H₁ and H₂ receptors knockout mice showed fewer nociceptive responses in various pain tests.^{22, 23} It was found that intracerebral microinjection of temelastine (H₁-receptor antagonist) and cimetidine into the periaqueductal gray or into the raphe nucleus prevented the histamine-induced antinociception.²⁴ Moreover, using tail flick and paw

pressure tests, the antinociceptive effect induced by SKF 91488, an inhibitor of histamine catabolism, was prevented by chlorpheniramine pretreatment.²⁰ In a rat model of acute trigeminal pain, the preventive effect of ranitidine, but not chlorpheniramine on the histamine-induced antinociception was reported.⁴ The ICV pretreatments with chlorpheniramine and ranitidine prevented histamine-induced antinociception in rats.¹⁸ In the rat model of orofacial formalin-induced pain, microinjection of mepyramine and ranitidine into the dorsal hippocampus prevented histamine-induced antinociception.¹⁹ The differences between the findings may be associated with the nature and sensitivity of the experimentally induced pain models the kind of histamine H₁ and H₂ receptor blockers used applied.

In conclusion, the present results suggest that the activation of brain histamine with ICV injection of exogenous histamine produces an antinociceptive effect in the formalin-induced pain in rabbits. Moreover, central histamine H₁ and H₂ receptors may be involved in the antinociception induced by centrally administered histamine.

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