

The effect of intracerebro ventricular (ICV) injection of histamine and intraperitoneal injection of histidine on the acute trigeminal pain in rats

Hamed Ganjali^{1*} and Mahla Ganjali²

1- Phd student in department of basical sciences, Faculty of veterinary medicine, Ferdowsi University of Mashhad, Mashhad, Iran

2- M.Sc Student of Biotechnology. Bangalor university. Brindavan college of India

Corresponding author: Hamed Ganjali

ABSTRACT: The present study explored the interaction between histaminergic system. Orofacial pain was induced by subcutaneous (sc) injection of formalin (50 μ l, 1%) in the upper lip region and the time spent face rubbing was recorded in 3 min blocks for 45 min. Histamine and morphine suppressed both phases of pain. Naloxone alone non-significantly increased pain intensity and inhibited the antinociceptive effects of morphine and histamine. The results of the present study indicate that at the level of the hippocampus, histamine through its H₁ and H₂ receptors, mediates orofacial region pain.

Keywords: Histamine, histamine H₁ and H₂ receptors, morphine, naloxone, hippocampus, orofacial formalin test, rats.

INTRODUCTION

Histaminergic endings and its H₁, H₂ and H₃ receptors are distributed in various parts of limbic system (Schwartz et al., 1991), and mediate some of functions of the hippocampus such as anxiety, arousal state, hibernation, neurotransmission, learning and memory. Some interactions exist between histamine and opioid receptors in mediating the brain functions. The histamine precursor L-histidine potentiated, while zolantidine, but not pyrilamine (central histamine H₂-receptor antagonist) attenuated the discriminative stimulus effects of morphine. The present study was aimed to investigate the hippocampal interaction between histamine and morphine by intra-hippocampal microinjections of histamine, pyrilamine, ranitidine, morphine and naloxone in separate and combined treatments using orofacial formalin test in rats.

MATERIALS AND METHODS

Healthy adult male Wistar rats (280–330 g) were used throughout the study. Rats were maintained in groups of 6 per cage in a 12 h light-dark cycle (lights on at 07:00 h) at a controlled ambient temperature (22 \pm 0.5°C) with *ad libitum* food and water. Six rats were used in each experiment (Figure 1).



Figure 1. Rat used in the experiment

Experiments were performed between 12:00 h and 15:00 h. The experimental protocol was approved by the Veterinary Ethics Committee of the Faculty of Veterinary Medicine of Urmia University and was performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Drugs

Drugs used in the present study included histamine dihydrochloride, mepyramine maleate (pyrilamine), ranitidine hydrochloride, naloxone dihydrochloride (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) and morphine sulfate (Temad, Tehran, Iran). All drugs were dissolved in sterile normal saline 30 min before intra-hippocampal microinjection.

Surgery

After a 15-day adaptation period, each rat was anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg) injected intraperitoneally (ip), and then placed in a stereotaxic apparatus (Stoelting, Wood Lane, IL, USA). Two 23 gauge, 12 mm stainless-steel guide cannulas were bilaterally implanted into the right and left dorsal hippocampus at the following coordinates: 3.6 mm posterior to the bregma, 2.4 mm left and right sides of the midline and 2.7 mm below the top of the skull (Paxinos and Watson, 1997). The cannulas were then fixed to the skull using three screws and dental acrylic (Figure 2 and 3). A 29-gauge, 12 mm stylet was inserted to each cannula to keep them patent prior to injection. At least 14 days were allowed for recovery from the surgery.

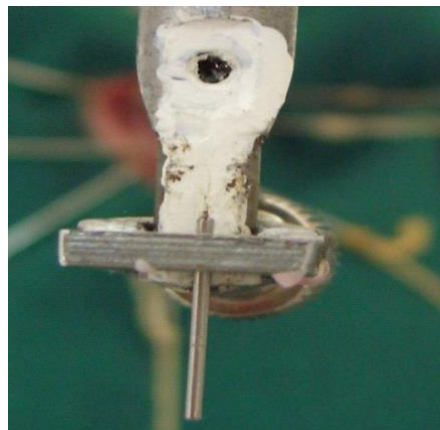


Figure 2. Stainless steel guide cannula



Fig 3. Stainless steel guide cannula was surgically implanted into the lateral ventricle of brain

Orofacial formalin test

Orofacial formalin test was performed according to the method described by Raboisson and Dallel (2004). Each rat was placed in Plexiglas observation chamber (30 cm × 30 cm × 30 cm) with a mirror mounted at 45° beneath the floor to allow an unobstructed view of the orofacial region. After a 30-min adaptation period, 50 µl of 1% diluted formalin solution was injected into the left side of upper lip sc just lateral to the nose using a 30-gauge

injection needle. Immediately following formalin injection, the rat was returned into the observation chamber (Figure 4).



Figure 4. Intracerebro ventricular injection of histamine in rats

The time each animal spent face rubbing with ipsilateral forepaw was recorded (using a stopwatch), in consecutive 3-min bins over a period of 45 min, and was considered as an index of nociception. Formalin injection induced a stereotyped response characterized by two well distinct phases (Clavelou et al., 1995). In the present study, data collected between 0 and 3 min post-formalin injection represented the first (early) phase and data collected between 15 and 33 min after injection of formalin represented the second (late) phase. All the observers were blinded to the protocol of the study (Figure 5).



Figure 5. Steriotaxi for intracerebro ventricular injection of histamine

Cannula verification

At the end of each experiment, 0.25 μ l of methylene blue was injected into the each side of hippocampus. Animals were killed with the high dose diethyl ether, and perfused intracardially with physiological saline followed by 10% formalin solution. The brains were removed and placed in a formalin solution (10%) (Figure 6).

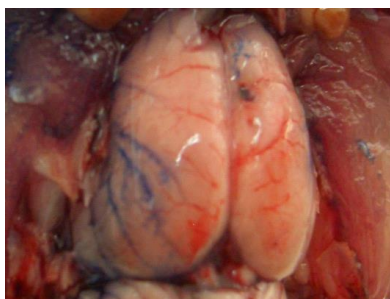


Figure 6. Brain of rat for doing experiment

After 24 h, the brains were sectioned coronally (50–100 μ m) and viewed under a loup to localize the injection site according to the atlas of Paxinos and Watson (Paxinos and Watson, 1997). The results obtained from rats with guide cannulas outside the hippocampus were eliminated from the data analysis.

Statistical analysis Data obtained from the subcutaneous injections of normal saline and formalin were analyzed using repeated measure ANOVA and Duncan test. To evaluate significant differences among intra-hippocampal microinjection treated groups, one-way analysis of variance (ANOVA) and Duncan's test were applied.

RESULTS AND DISCUSSION

Results

The rat brain section was modified from the atlas of Paxinos and Watson (Paxinos and Watson, 1997). The locations of the cannula tip placements in the hippocampus were confirmed with intra-hippocampal injection of methylene blue.

Subcutaneous injection of normal saline into the rat upper lip produced a negligible nociceptive response only in the first 3-min block. Diluted formalin, when subcutaneously injected into the upper lip, produced a typical pattern of face rubbing behavior.

Repeated measure ANOVA revealed a significant difference in face rubbing between first. Therefore, the formalin-induced nociceptive behavior showed a biphasic time course: the first phase began immediately after formalin injection and declined in approximately 10 min, while the second phase began about 15 min after formalin injection and lasted about 18 min and declined to the end of recording period (45 min). This shows the intra-hippocampal microinjections of histamine, mepyramine and ranitidine on the formalin-induced orofacial pain. Intra-hippocampal microinjection of histamine at a dose of 1 and 2 μg , but not at a dose of 0.5 μg , significantly decreased the intensity of nociceptive response in the first phase of formalin-induced nociception. The second phase of formalin-induced pain was significantly suppressed by intra-hippocampal microinjection of histamine at doses of 0.5, 1 and 2 μg . Intra-hippocampal microinjection of pyrilamine at doses of 1 and 4 μg alone did not change the intensity of first and second phases of pain. Pretreatment with pyrilamine (4 μg) significantly inhibited the antinociceptive effects of histamine (1 μg) in the first and second phases of formalin induced orofacial pain. Microinjection of ranitidine into the dorsal hippocampus alone at doses of 1 and 4 μg alone did not change the intensity of first and second phases of pain. Pretreatment with ranitidine (4 μg) significantly inhibited the antinociceptive effects of histamine (1 μg) in the first and second phases of formalin-induced orofacial pain. Microinjection of morphine into the dorsal hippocampus at a dose of 0.5 μg had no effect on pain response, whereas morphine at doses of 1 and 2 μg significantly suppressed both first and second phases of pain. Naloxone (4 μg) alone non-significantly increased pain response, and reversed the antinociceptive effects of morphine (2 μg) in the first phases of pain. No antinociceptive effect was observed in the first phase of formalin-induced pain, when intra-hippocampal microinjection of histamine (0.5 μg) was used with morphine (0.5 μg). Intra-hippocampal microinjections of histamine at doses of 1 and 2 μg with morphine.

(0.5 μg) produced antinociceptive effects when compared with normal saline and morphine (0.5 μg) treated groups. In the second phase of formalin-induced pain, intra-hippocampal microinjection of histamine at doses of 0.5, 1 and 2 μg with morphine (0.5 μg) produced antinociceptive effects when compared with normal saline and morphine (0.5 μg) treated groups. Intra-hippocampal microinjection of naloxone (4 μg) significantly prevented antinociceptive effects of histamine in both first and second phases of formalin-induced orofacial pain. Intra-hippocampal microinjections of pyrilamine and ranitidine at the same dose of 4 μg significantly inhibited morphine-induced antinociceptive effects in the first and second (phases of formalin-induced orofacial pain).

Discussion

The present study shows that the subcutaneous injection of formalin into the upper lip produced a distinct biphasic pattern in the face rubbing performed by ipsilateral forepaw. Subcutaneous injection of formalin at the concentrations of 0.2–10% into the upper lip region induced a biphasic pattern in the face rubbing in rats [8]. During the orofacial formalin test, two distinct phases due to different mechanisms of nociception are produced, the first phase is associated to direct stimulation of C-nociceptors, whereas the second phase reflects integration between nociceptors and spinal and brainstem signaling (Dallel et al., 1995). Face rubbing with the ipsilateral forepaw due to formalin injection into the upper lip, has been mentioned as a specific nociceptive response (Raboisson and Dallel, 2004). Some researchers have reported vocalization, grooming and scratching due to electrical, mechanical, thermal and chemical (capsaicin, formalin) stimulation of the orofacial region in rats (Ahn et al., 2005). However, nociceptive behavior obtained from the present study is in agreement with other investigations (Capuano et al., 2009).

In this study intrahippocampal microinjection of histamine produced antinociception and histamine H₁ and H₂ antagonists, pyrilamine and ranitidine, respectively, prevented histamine-induced antinociception. The cell bodies of the histaminergic neuron system are concentrated in the tuberomammillary nucleus (TMN) of the hypothalamus, and send out axons to innervate the entire central nervous system (Brown et al., 2001).

Hippocampal formation receives a weak to moderate histaminergic innervation, and the distribution of histamine H₁ and H₂ receptors in various parts of limbic system have been well documented (Brown et al., 2001). There is no report regarding the hippocampal changes of histamine and its H₁ and H₂ receptors in acute and chronic pain states. In other brain nuclei such as striatum, Huang et al. (Huang et al., 2007) reported an increase of histamine concentrations in a rat model of neuropathic pain. Moreover, an increase of histamine concentrations in the PAG was reported after intraplantar injection of paraformaldehyde solution in rats (Murotani et al., 2010). The involvement of brain histamine as a modulator of pain has been investigated by injection of the amine into the ventricles of brain or by microinjection of the amine into the brain nuclei. Central histamine H₁ and H₂ receptors involvement in the histamine-induced antinociception was reported in formalin test in mice and rats (Mojtahedin et al., 2008), and in acute trigeminal pain in rats. Microinjection of histamine into the PAG or into the dorsal raphe nucleus (DRN) produced antinociception in the rat hot plate test. Histamine-induced antinociception was inhibited by central pretreatment with temelastine (an antagonist of histamine H₁ receptors) and tiotidine (an antagonist of histamine H₂ receptors). In addition, microinjections of histamine into the dentate gyrus, a part of hippocampal formation, decreased licking/biting and shaking of the formalin injected paw, and prior microinjections of chlorpheniramine (an antagonist of histamine H₁ receptors) and ranitidine into the same site inhibited the suppressive effects of histamine. In conclusion, the results of the present study suggest that at the level of the hippocampus the histaminergic and opioidergic systems interact with each other in mediating the pain originating from orofacial structures.

Hippocampal opioid and histamine H₁ and H₂ receptors are involved in the interaction between histamine and morphine.

REFERENCES

- Ahn DK, Lee KR, Lee HJ, Kim SK Choi HS, Lim EJ, Park JS. 2005. Intracisternal administration of chemokines facilitated formalin-induced behavioral response in the orofacial area of freely moving rats. *Brain Res Bull*, 66, 50–58.
- Brown RE, Stevens DR, Haas HL. 2001. The physiology of brain histamine. *Prog Neurobiol*, 63, 637–672.
- Capuano A, De Corato A, Treglia M, Tringali G, Dello Ruso C, Navarra P. 2009. Antinociceptive activity of buprenorphine and lumiracoxib in the rat orofacial formalin test: a combination analysis study. *Eur J Pharmacol*, 605, 57–62.
- Clavelou P, Dallel R, Orliquest T, Woda A, Raboisson P. 1995. The orofacial formalin test in rats: effects of different formalin concentrations. *Pain*, 62, 295–301.
- Dallel R, Raboisson P, Clavelou P, Saade M, Woda A. 1995. Evidence for a peripheral origin of the tonic nociceptive response to subcutaneous formalin. *Pain*, 61, 11–16.
- Huang L, Adachi N, Nagaro T, Liu K, Arai T. 2007. Histaminergic involvement in neuropathic pain produced by partial ligation of the sciatic nerve in rats. *Reg Anesth Pain Med*, 32, 124–129.
- Mojtahedin A, Tamaddonfard E, Zambouri A. 2008. Antinociception induced by central administration of histamine in the formalin test in rats. *Indian J Physiol Pharmacol*, 52, 249–254.
- Murotani T, Ishizuka T, Nakazawa H, Wang X, Mori K, Sasaki K, Ishida T, Yamatodani A. 2010. Possible involvement of histamine, dopamine, and noradrenalin in the periaqueductal gray in electroacupuncture pain relief. *Brain Res*, 1306, 62–68.
- Paxinos G, Watson C. 1997. The rat brain in stereotaxic coordinates. Compact third edn., Academic Press, San Diego.
- Raboisson P, Dallel R. 2004. The orofacial formalin test. *Neurosci Biobehav Rev*, 28, 219–226.
- Schwartz JC, Arrang JM, Garbarg M, Pollard H, Rouat M. 1991. Histaminergic transmission in the mammalian brain. *Physiol Rev*, 71, 1–51.