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Cerebellar fastigial nucleus histamine and its H_2 but not H_1 receptors might inhibit acetic acid-induced visceral nociception and improve motor coordination in rats: role of opioid system

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Abstract

The cerebellum and its deep nuclei contribute to the regulation of important functions including motor coordination and pain. Histamine modulates some functions of the fastigial nucleus (FN) such as motor coordination. In this study, by application of histamine and activation of its H_1 and H_2 receptors, the FN processing of visceral pain, general locomotor activity and motor coordination were targeted. The possible mechanism of action was followed by the inhibition of opioid receptors. The right and left sides of the FN were surgically implanted with guide cannulas. Immediately after an intraperitoneal injection of acetic acid (1.00 mL, 1.00%), the first writhing onset latency and the writhing number over 60 min were recorded. Open-field and rotarod tests were applied for general locomotor and motor coordination assessment, respectively. Histamine and dimaprit (H_2 receptor agonist) increased first writhing onset latency, decreased the writhing number and increased falling time from the rod. These effects were prevented by ranitidine (H_2 receptor antagonist) pre-treatment. Significant alterations were not observed by histamine H_1 receptor agonist (2-pyridylethylamine) and antagonist (mepyramine). Naloxone, with no effect on falling time from the rod, inhibited the antinociceptive effects of histamine and dimaprit. Beam break number was not affected by the above-mentioned treatments. Based on the results, it can be suggested that histamine H_2, but not H_1 receptors at the FN might have had an inhibitory role on acetic acid-induced visceral pain and improved motor coordination. The antinociception, but not motor coordination might be mediated by FN opioid receptors.

Introduction

In addition to the very obvious roles in motor coordination, the cerebellum and its deep nuclei such as the fastigial nucleus (FN), the interpositus nucleus (IN) and the dentate nucleus (DN) regulate language, cognition and visuomotor adaptation.[1-3] Scholars have suggested important roles for FN in pain processing. For example, stimulation of cerebellar cortex enhanced, whereas stimulation of FN decreased the responses to colorectal distension.[4] Moreover, microinjection of calcitonin gene-related peptide (CGRP) into the FN caused tactile hypersensitivity and spontaneous pain.[5] In addition to CGRP, the FN neurons receive a variety of modulatory information from other inputs, including serotonergic, cholinergic, adrenergic, orexinergic, dopaminergic and histaminergic projections.[6,7]

Histaminergic axons exiting from tuberomammillary nucleus of hypothalamus affect endocrine, behavioral and visceral functions through H_1-H_4 receptors.[8] Brain histaminergic system is also involved in pain processing. In this regard, the roles of postsynaptic H_1 and H_2 receptors in thalamic submedius (Sm) nucleus and presynaptic H_3 receptor in agranular insular cortex in pain modulation have been reported.[9,10] The hypothalamocerebellar histaminergic projections to cerebellar deep nuclei modulates their functions. Reportedly, pre-administration of ranitidine (H_2 receptor antagonist), but not triprolidine (H_1 receptor antagonist) inhibited histamine-induced excitation of the cerebellar DN neurons.[11] Also, FN histamine through H_2 receptors improved motor coordination in rats.[12] However, there are no reports describing the involvement of cerebellar deep nuclei histamine and its receptors in modulation of visceral pain.

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It is well established that opioid receptors are involved in approximately all aspects of pain processing,[13,14] and are also employed in studies investigating the supra-spinal modulatory effects of histamine.[9,15] Naloxone (an opioid receptor antagonist) has been of interest to researchers who suggest possible reciprocal histamine-opioid system interactions in supra-spinal pain modulation.[16,17] More specifically, at the supra-spinal level, the antinociceptive effects of the activated H_1 and H_2 and the inhibited H_3 receptors have been shown to be sensitive to naloxone.[9,18]

Regarding the above-mentioned findings, the goal of this study was to investigate the function of histaminergic agents in the FN regulation of acetic acid-induced visceral pain. This purpose was performed by microinjection of histamine, mepyramine (H_1 receptor antagonist), 2-pyridylethylamine (2-PEA, H_1 receptor agonist), ranitidine (H_2 receptor antagonist) and dimaprit (H_2 receptor agonist) into the FN. Due to the main role of the cerebellum and its deep nuclei in motor coordination, rotarod test was used. General motor activity was assessed in an open-field test. To find out the mechanism of action, naloxone was applied in the FN with and without histaminergic agents.

Materials and Methods

Animals. All experiments were carried out on male Wistar rats (230 - 250 g with an average of 240 g) kept under standard conditions (22.00 ± 0.50 ˚C; 12:12 hr light–dark cycle) with unrestricted access to food and water. All experiments were conducted during the time between 10:00 AM - 14:00 PM. All experimental protocols were approved by Veterinary Ethics Committee of Urmia University Faculty of Veterinary Medicine (Ethical code: IR-UU-AEC-972-PD-3).

Drugs. Chemicals used in this research work were comprised of histamine dihydrochloride, 2-PEA, mepyramine maleate, dimaprit dihydrochloride, ranitidine hydrochloride, naloxone hydrochloride (Sigma-Aldrich Chemical Co., St. Louis, USA), and acetic acid (Merck, Darmstadt, Germany). Chemical solutions were prepared 30 min before use.

Study protocol. In the present study, animals were cannulated on day 1. Then, on the 10th, 13th and 20th days, acetic acid-induced visceral pain and open field and rotarod tests were applied, respectively. At the end of the experiments (day 21), the brains of the animals were removed to confirm the location of the cannulas. All experimenters were blinded to the study protocol. Between each test, the used apparatus was carefully cleaned and dried.

Animal grouping. In the present study, 132 rats were divided into 22 groups of six animals each as follow: Group 1 (Ns+Ns) received intra-FN microinjection of normal saline (250 nL) plus normal saline (250 nL). Groups 2 to7 received intra-FN microinjection of mepyramine (0.50 and 2.00 µg per 240 g), ranitidine (0.50 and 2.00 µg per 240 g) and naloxone (0.25 and 1.00 µg per 240 g) before 250 nL normal saline. Groups 8 to11 were treated by intra-FN microinjection of histamine (0.12, 0.25, 0.50 and 1.00 µg per 240 g) after 250 nL normal saline. Groups 12 to14 received intra-FN microinjection of mepyramine (2.00 µg per 240 g), ranitidine (2.00 µg per 240 g) and naloxone (1.00 µg per 240 g) before 1.00 µg per 240 g histamine. Groups 15 to18 were treated by intra-FN microinjection of 2-PEA (0.25 and 1.00 µg per 240 g) and prior micro-injection of mepyramine (2.00 µg per 240 g) and naloxone (1.00 µg per 240 g) before 2-PEA (1.00 µg per 240 g). Groups 19 to22 were treated by intra-FN microinjection of dimaprit (0.25 and 1.00 µg per 240 g) and prior micro-injection of ranitidine (2.00 µg per 240 g) and naloxone (1.00 µg per 240 g) before dimaprit (1.00 µg per 240 g).

Fastigial nucleus cannulation. Each rat was anesthetized by intraperitoneal (IP) injection of ketamine (80.00 mg kg^{-1}, Alfasan, Woerden, The Netherlands) and xylazine (8.00 mg kg^{-1}, Alfasan) combination and mounted on a stereotaxic surgery device (Stoelting, Wood Dale, USA). Guide cannulas were implanted bilaterally in the right and left sides of the FN, targeting the relative coordination 11.50 mm posterior to the bregma, 1.20 mm left and right to the midline, and 5.50 mm below the skull.[19] Following the stereotaxic surgery, a 10-day recovery period was considered.

Intra-FN microinjections. Normal saline and test drug solutions were microinjected into the FN in a fixed volume of 250 nL using a 1.00-µL Hamilton syringe connected to a 30-gauge injection needle. The micro-injection duration was 45 sec and injection needle were held for another 30 sec for more drug diffusion. The antagonists (mepyramine, ranitidine, and naloxone) and the agonists (histamine, 2-PEA, and dimaprit) were micro-injected 4 and 2 min before applying the pain and motor coordination tests, respectively. The drug doses used here were considered according to previous studies.[9,17]

Visceral nociceptive test. Visceral nociception was induced on day 11 after FN cannulation. To induce visceral nociception, each animal was placed in a clear Plexiglas box (30.00 × 30.00 × 30.00 cm) for a 30-min adaptation. Following microinjection protocol and IP injection of acetic acid (1.00 mL, 1.00%), first abdominal wall contraction (writhing) latency time was recorded and the writhing number were counted over 60 min. An abdominal constriction was described as a contraction wave of the abdominal wall followed by stretching of the hind limbs.[20]

Open-field test. On day 13, an electronic activity box (BorjSanat, Tehran, Iran) was used to assess the locomotion of animals in a Plexiglas box (40.00 × 40.00 × 40.00 cm). Following the microinjection protocol mentioned above, animals were carefully put in the center of the open field, and then the number of beam breaks

caused by animal movement were recorded in a 5-min session as a measure of general locomotor activity.[21]

Rotarod test. Rats were trained on days 17, 18 and 19 and tested on day 20 after cannulation surgery using a rotarod apparatus (RR410; Technic Azma, Tabriz, Iran). According to He *et al.,*[12] but with minor modification, on training days, animals were put on the rod at accelerating speeds of 0.00 (rotarod off), 5.00, 20.00, and 40.00 rpm for 45 sec in 5.00 trails with 10-min rest between them to reduce stress and fatigue. On testing day, after intra-FN microinjection, each rat was put on the rod which was rotating at the accelerating speed of 5.00 to 20.00 rpm for 45 sec to measure the latency time to the first fall from the rod. The maximum score of 45 sec was considered for the rats that did not fall from the cylinder.

Verification of cannulas placement. At the end of each experiment (day 20), the FNs were microinjected with 250 nL of Methylene Blue. After euthanizing Thereafter, the rats were euthanized by IP injection of 100 mg kg^{-1} ketamine and 10.00 mg kg^{-1} xylazine followed by intra-cardiac injection of 1.00 mL xylazine. The brains were carefully removed and the surface of the brains was photographed to record the cannula entrance points. The brains were then placed in 10.00% formalin solution. Seven days later, coronal and longitudinal brain sections were prepared (100 - 200-µm thick) and observed under a loupe to be compared and verified according to the atlas of Paxinos and Watson.[19] Data from four rats with guide cannulas outside the FN were eliminated from data analysis.

Statistical analysis. Data were statistically analyzed using Graph Pad Prism (version 8.2.1; GraphPad Software Inc., San Diego, USA). First writhing onset latency, writhing number, beam break number and falling latency from the rod obtained from experimental groups were analyzed by one-way ANOVA followed by Tukey's post hoc test. Data were presented as mean ± SEM. The statistical significance of $p < 0.05$ was considered for all results.

Results

Figure 1 shows the cannulas point of entrance on the surface of the brain right over the FN (A) and also the correct placement of the tip of the cannulas in the longitudinal (B) and coronal (C) sections of the FN. The longitudinal (D) and coronal (E) sections were adopted from the atlas of Paxinos and Watson.[19]

First writhing onset latency in intra-FN normal saline-treated group was 6.66 ± 0.52 min after IP injection of acetic acid. Mepyramine (0.50 and 2.00 µg per 240 g), ranitidine (0.50 and 2.00 µg 240 g^{-1}) and naloxone (0.25 and 1.00 µg per 240 g) did not significantly ($p > 0.05$, Fig. 2A) alter the first writhing onset latency. One-way ANOVA revealed significant differences among groups regarding the effects of histamine alone and after mepyramine, ranitidine and naloxone ($F(7,40) = 54.34$, $p < 0.0001$, Fig. 2B). Tukey's post hoc test analysis indicated that histamine at doses of 0.25, 0.50 and 1.00 µg per 240 g, but not at a dose of 0.12 µg per 240 g, significantly increased the first writhing onset latency (Fig. 2B). Moreover, prior administration of ranitidine (2.00 µg per 240 g) and naloxone (1.00 µg per 240 g), but not mepyramine (2.00 µg per 240 g) reversed the increasing effect of 1.00 µg per 240 g histamine (Fig. 2B). There were no significant ($p > 0.05$, Fig. 2C) differences among groups receiving 2-PEA alone (0.25 and 1.00 µg per 240 g) and after mepyramine (2.00 µg per 240 g) and naloxone (1.00 µg per 240 g). Considering dimaprit alone and ranitidine and naloxone before dimaprit, one-way ANOVA revealed significant ($F(4,25) = 33.04$, $p < 0.0001$, Fig. 2D) differences. Follow-up analysis with Tukey's test indicated that dimaprit at doses of 0.25 and 1.00 µg per 240 g increased the first writhing onset latency (Fig. 2D). Pre-treatment with ranitidine (2.00 µg per 240 g) and naloxone (1.00 µg per 240 g) reversed the increasing effects of dimaprit (Fig. 2D).

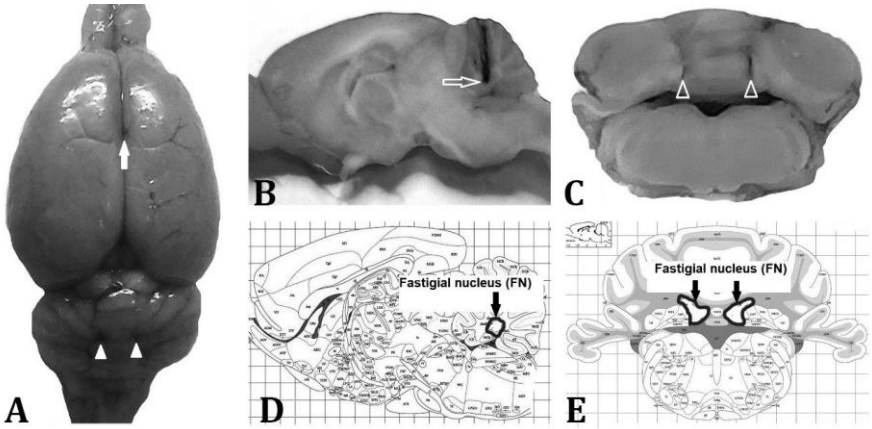

Fig. 1. Location of the fastigial nucleus (FN) cannula entrance points on the brain surface (solid white arrow heads, **A**), cannula tips in the brain lateral (hollow white arrow, **B),** and cross-section (hollow white arrow heads, **C)** views in the FN of the rats included in the data analysis**. D,** and **E)** Respective atlas plates adopted from Paxinos and Watson[19] in which FN has been shown with bold line and black solid arrows. The bregma (solid white arrow, in A) was used as stereotaxic reference point.

The writhing number was obtained 54.68 ± 3.63 in 60 min after IP injection of acetic acid in intra-FN normal saline-treated group. The writhing numbers were not changed by mepyramine (0.50 and 2.00 µg per 240 g), ranitidine (0.50 and 2.00 µg per 240 g) and 0.25 and 1.00 µg per 240 g naloxone (p > 0.05, Fig. 3A). Considering the effects of histamine alone and after mepyramine, ranitidine and naloxone on the writhing number, one-way ANOVA expressed significant differences ($F(7,40) = 43.29$, $p < 0.0001$, Fig. 3B). Tukey's post hoc test analysis indicated that histamine at doses of 0.25, 0.50 and 1.00 µg per 240 g, but not at a dose of 0.12 µg 240 g^{-1}, significantly decreased writhing number (Fig. 3B). Moreover, prior administration of ranitidine (2.00 µg per 240 g) and naloxone (1.00 µg per 240 g), but not mepyramine (2.00 µg per 240 g) reversed the reducing effect of 1.00 µg per 240 g histamine (Fig. 3B). In 2-PEA alone (0.25 and 1.00 µg per 240 g) and after mepyramine (2.00 µg per 240 g) and naloxone (1.00 µg per 240 g) receiving groups, no significant (p > 0.05, Fig. 3C) differences were observed. Significant ($F(4,25) = 16.31$, $p < 0.0001$, Fig. 3D) differences in the writhing number were found in dimaprit alone and ranitidine and naloxone before dimaprit treated groups. Further analysis with Tukey's test indicated that dimaprit at doses of 0.25 and 1.00 µg per 240 g decreased writhing number (Fig. 3D). The reducing effect of dimaprit was reversed by ranitidine (2.00 µg per 240 g) and naloxone (1.00 µg per 240 g) pre-treatment (Fig. 3D).

Fall latency from the rod after intra-FN microinjection of normal saline was 20.85±1.78 sec. This value was not significantly (p > 0.05, Fig. 4A) altered after intra-FN microinjection of mepyramine (0.50 and 2.00 µg per 240

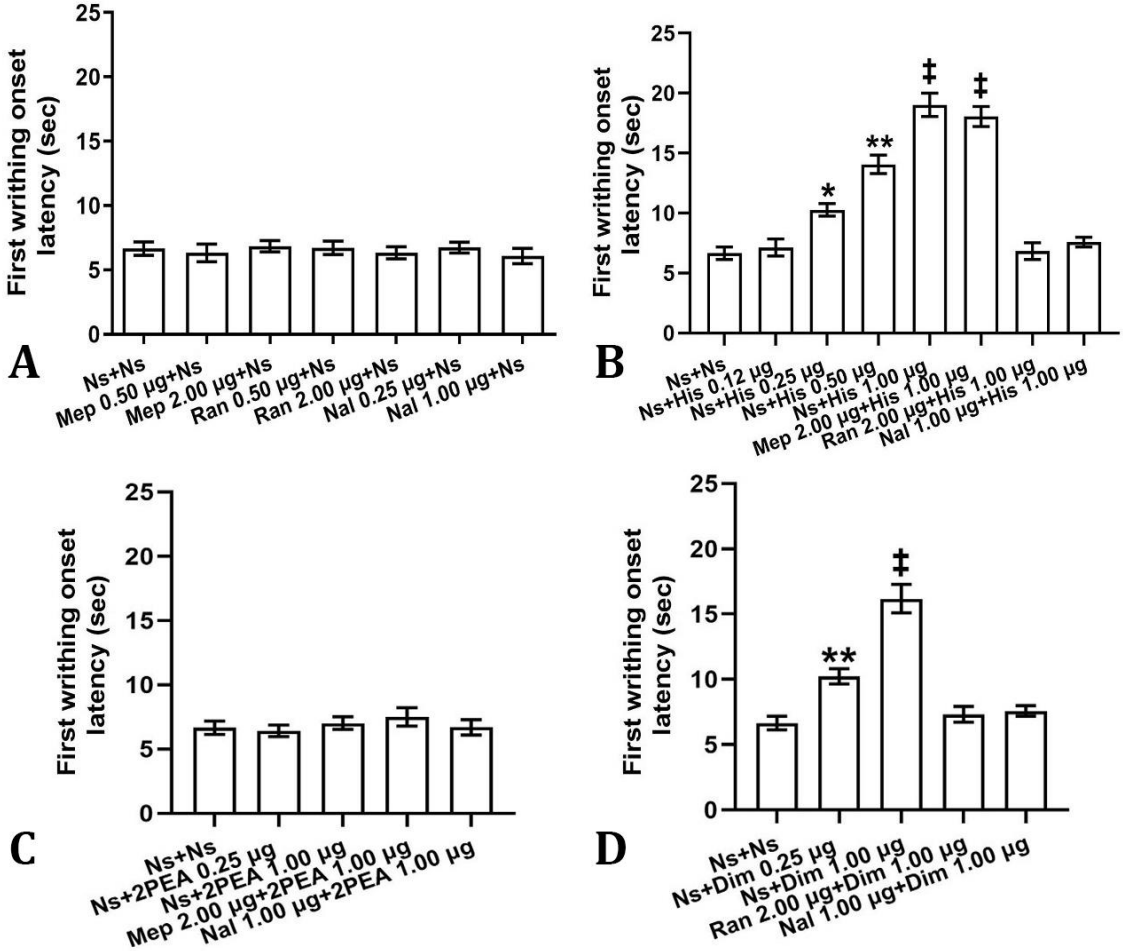

Fig. 2. Nociceptive response (first writhing latency time) induced by intraperitoneal injection of acetic acid after intra-fastigial nucleus (FN) microinjection of **A)** mepyramine (Mep), ranitidine (Ran) and naloxone (Nal), **B)** histamine (His), mepyramine plus histamine, ranitidine plus histamine and naloxone plus histamine, **C)** 2-pyridylethylamine (2-PEA), mepyramine plus 2-PEA and naloxone plus 2-PEA and **D)** dimaprit (Dim), ranitidine plus dimaprit and naloxone plus dimaprit. In all the double microinjections into the FN, time intervals of 4.00 and 2.00 minutes were observed before induction of visceral pain. All drugs were administered as µg per 240 g^{-1}. Ns: normal saline. Values from each group are the mean ± SEM (n = 6). * $p < 0.05$, ** $p < 0.01$, † $p < 0.001$ and ‡ $p < 0.0001$ compared to Ns+Ns, respectively.

g), ranitidine (0.50 and 2.00 µg per 240 g) and naloxone (0.25 and 1.00 µg per 240 g). Considering the effects of histamine alone and after mepyramine, ranitidine and naloxone on fall latency from the rod, one-way ANOVA revealed significant differences ($F(7,40) = 13.39$, $p < 0.0001$, Fig. 4B).

Tukey's post hoc test analysis indicated that histamine doses of 0.25, 0.50 and 1.00 µg per 240 g, but not at the dose of 0.12 µg per 240 g, significantly increased fall latency from the rod (Fig. 4B). Also, prior administration of ranitidine (2.00 µg per 240 g), but not naloxone (1.00 µg per rat) and mepyramine (2.00 µg per rat) reversed the enhancing effect of 1.00 µg per 240 g histamine (Fig. 4B). No significant differences were observed in fall latency from the rod among groups receiving 2-PEA alone (0.25 and 1.00 µg per 240 g) and after mepyramine (2.00 µg per 240 g) and naloxone (1.00 µg per 240 g), ($p > 0.05$, Fig. 4C).

Regarding dimaprit alone and ranitidine and naloxone before dimaprit, one-way ANOVA revealed significant ($F(4,25) = 8.538$, $p < 0.001$, Fig. 4D) differences. Further analysis with Tukey's test indicated that dimaprit at a dose of 1.00 µg per 240 g, but not at a dose of 0.25 µg per 240 g increased time spent on rotarod (Fig. 4D). The increasing effect of dimaprit was reversed by ranitidine (2.00 µg per 240 g), but not naloxone (1.00 µg per 240 g) pre-treatment (Fig. 4D).

Beam break number after intra-FN microinjection of normal saline was 92.17 ± 5.04 in 5 min. All the above-mentioned treatments did not significantly alter the beam break number (data not shown).

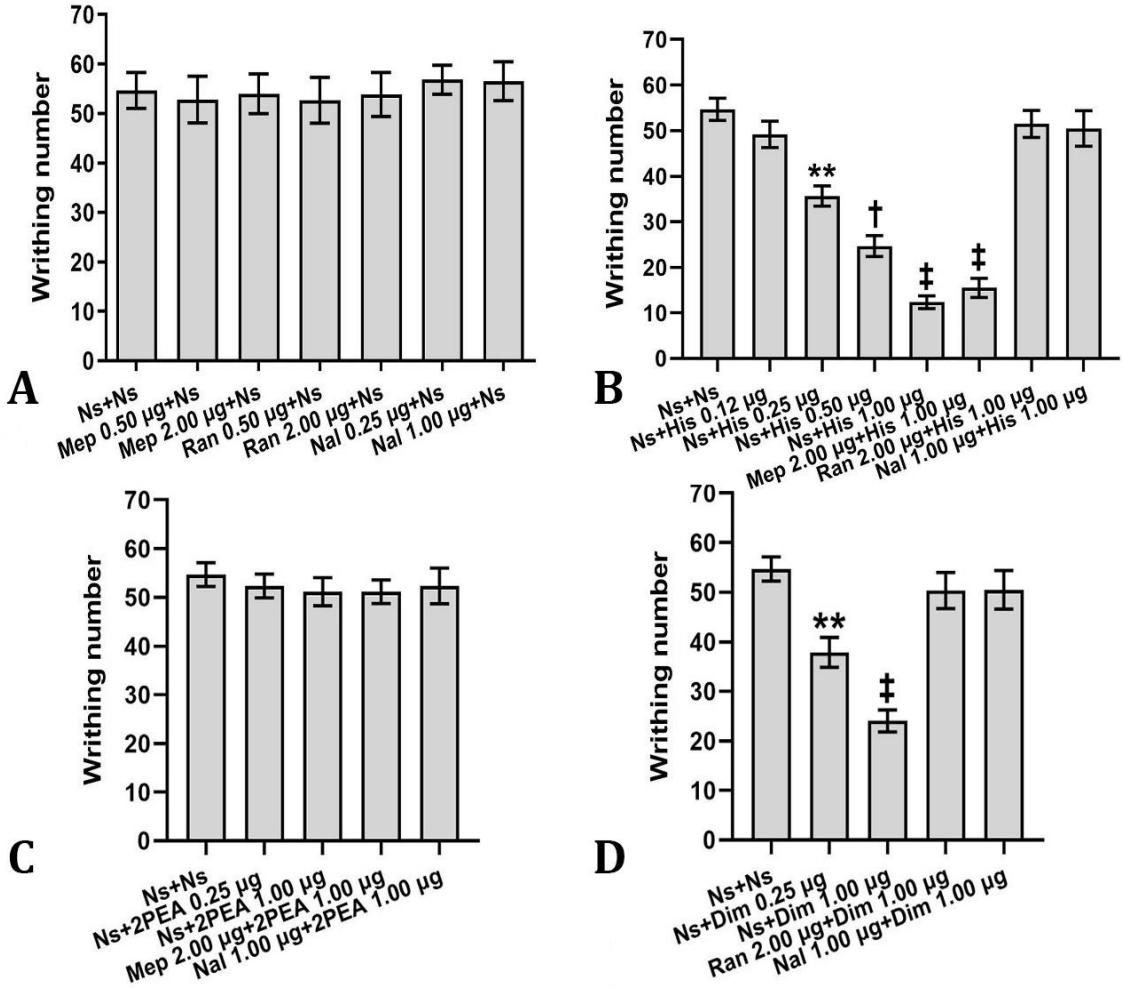

Fig. 3. Nociceptive response (writhing number over 60 min) induced by intraperitoneal injection of acetic acid after intra-fastigial nucleus (FN) microinjection of **A)** mepyramine (Mep), ranitidine (Ran) and naloxone (Nal), **B)** histamine (His), mepyramine plus histamine, ranitidine plus histamine and naloxone plus histamine, **C)** 2-pyridylethylamine (2-PEA), mepyramine plus 2-PEA and naloxone plus 2-PEA and **D)** dimaprit (Dim), ranitidine plus dimaprit and naloxone plus dimaprit. In all the double microinjections into the FN, time intervals of 4.00 and 2.00 minutes were observed before induction of visceral pain. All drugs were administered as µg per 240 g^{-1}. Ns: normal saline. Values from each group are the mean ± SEM (n = 6). ** $p < 0.01$, † $p < 0.001$ and ‡ $p < 0.0001$ compared to Ns+Ns, respectively.

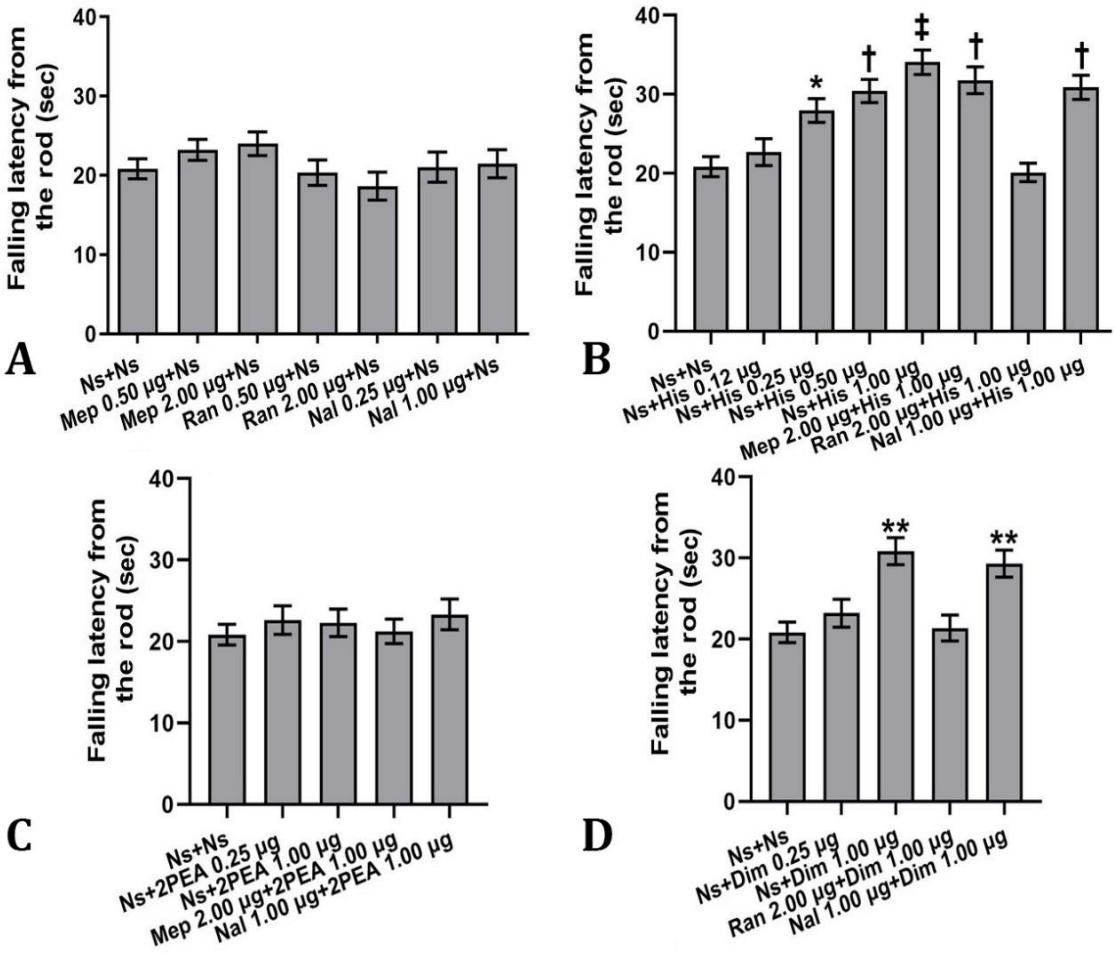

Fig. 4. Fall latency from the rod after intra-fastigial nucleus (FN) microinjection of **A)** mepyramine (Mep), ranitidine (Ran) and naloxone (Nal), **B)** histamine (His), mepyramine plus histamine, ranitidine plus histamine and naloxone plus histamine, **C)** 2-pyridylethylamine (2-PEA), mepyramine plus 2-PEA and naloxone plus 2-PEA and **D)** dimaprit (Dim), ranitidine plus dimaprit and naloxone plus dimaprit. In all the double microinjections into the FN, time intervals of 4.00 and 2.00 minutes were observed before induction of visceral pain. All drugs were administered as µg per 240 g. Ns: normal saline. Values from each group are the mean ± SEM (n = 6). ** $p < 0.01$, † $p < 0.001$ and ‡ $p < 0.0001$ compared to Ns+Ns, respectively.

Discussion

In this study, microinjection of histamine H_1 and H_2 receptor antagonists, mepyramine and ranitidine, respectively, did not cause any significant alterations in acetic acid-induced visceral nociceptive responses. In addition, intra-FN microinjection of histamine increased first writhing latency time and decreased writhing number which might indicate a supraspinal inhibiting effect of histamine. This antinociceptive effect of histamine was inhibited by prior microinjection of ranitidine but not mepyramine into the same area. These findings did not accurately express the mediating role of H_1 and H_2 receptors. To cover this issue, H_1 and H_2 receptor selective agonists were used in the continuation of the research. In this regard, following microinjection of 2-PEA alone and mepyramine before 2-PEA into the FN, there were no significant effects on visceral pain responses. On the other hand, dimaprit attenuated visceral pain responses and ranitidine prevented these effects of dimaprit. All of these findings indicated that at the FN level, histamine might process visceral nociception through H_2, but not H_1 receptors. Recent studies have suggested important roles for the cerebellum in visceral pain modulation. Chemical stimulation of the rat cerebellar cortex increased the colorectal distension-induced abdominal reflex associated with perceiving visceral pain, whereas chemical stimulation of the FN produced an opposite effect.[4] In addition, intra-FN microinjection of L-glutamate has been shown to attenuate hyperalgesia in a chronic visceral hypersensitivity model, suggesting FN's possible involvement in visceral pain modulation.[22] Moreover, it has been reported that downregulation of Pellino-1 (an E3 ubiquitin ligase) in the FN acting through

toll-like receptor 4/nuclear factor kappa B pathway produces a protective effect against chronic visceral hypersensitivity in a colorectal distension rat model.[23] Based on the fact that the cerebellum and its deep nuclei receive a high density of histaminergic axons,[24,25] and knowing that histamine H_2 receptors are distributed in cerebellar cortex and deep nuclei including the FN, IN, Purkinje, and molecular layer cells,[25] it is believed that histamine through H_2 receptors regulates some functions of the cerebellum. In this context, intra-cerebellar nuclei (FN and IN) microinjection of histamine and dimaprit improved motor performances in rats, conversely, ranitidine (H_2 receptor antagonist) declined motor performances, whereas, H_1 receptor antagonist and agonist (triprolidine and 2-PEA, respectively) had no effect on motor performances.[12] There are no report describing the roles of cerebellum and its deep nuclei histamine in the regulation of pain responses, but in other brain areas involved in pain processing, the histaminergic system through H_1, H_2 and H_3 receptors has created regulatory effects in different aspects of pain. For example, microinjection of ranitidine into the thalamic ventral posteriolateral nucleus prevented dimaprit-induced antinociception in the formalin-induced muscle pain.[17] In addition, at the thalamic ventral posteromedial nucleus level, the involvement of histamine H_1, H_2 and H_3 receptors in modulation of acute trigeminal pain have been reported.[18] Moreover, applying exogenous histamine (by microinjection of histamine) and increasing endogenous histamine (by microinjection of thioperamide, an inhibitor of histamine H3 receptors) in the ventrolateral periaqueductal gray (vlPAG), an important area in pain processing, pain-alleviating effects have been reported in neuropathic rats.[10] By putting these findings together, the significant role of the histaminergic system and its H_2 receptors in the FN processing of visceral pain is revealed to some extent.

In the present study, prior administration of naloxone into the FN inhibited the reduction of visceral pain caused by microinjection of histamine and dimaprit into the same site. These findings indicated the existence of a functional interaction between histamine H_2 and opioid receptors within the FN. In the areas involved in pain processing, functional interaction between histamine and opioid systems has been reported. For example, pre-micro-injection of naloxone as well as naloxanazine (a specific antagonist of mu-opioid receptors) into the thalamic Sm nucleus inhibited dimaprit's attenuating effect on formalin-induced orofacial pain.[9] Prior administration of naloxone into the vlPAG inhibited histamine, and thioperamide-induced anti-hypersensitivity in chronic constriction injury model of neuropathic pain in rats.[26] Considering the finding that there is a high density of opioid receptors in different parts of the cerebellum, including Purkinje cells and deep nuclei,[27,28] and also that the FN has a high distribution of H_2 histamine receptors,[25] the possibility of the existence of a functional interaction between histamine and opioids in the FN does not seem unlikely.

In the present study, microinjection of histamine and dimaprit, but not 2-PEA into the FN increased fall latency from the rod. The enhancing effects of histamine and dimaprit were inhibited by ranitidine, but not naloxone. These results indicated that histamine through H_2 receptors in the FN might improve locomotor performance on rotarod. Microinjection of histamine and dimaprit into the FN lengthened the endurance time of rats on the rotating rod and ranitidine produced an opposite effect.[12] In this context, intra-FN microinjection of 2-PEA and triprolidine (selective histamine H1 receptor antagonist) did not change motor performance on rotarod.[12] Moreover, microinjection of histamine into another deep nucleus of the cerebellum, the IN, increased the time spent in the rotarod test and decreased the time spent in the beam balance test.[29] In addition to improving effects on motor balance, microinjection of histamine into the FN through H_2 receptors produced a protective effect in the stress-induced gastric mucosal damage model.[30] Our current results also showed that intra-FN microinjection of naloxone did not inhibit the improving effects of histamine and dimaprit, suggesting that opioid receptors might not be involved. Research on the role of opioid receptors in the function of the FN in motor coordination and balance requires the use of agonists and antagonists of the receptors.

Based on the results of our current study, no significant changes were observed in the number of beam breaks after the microinjection of histaminergic agents into the FN. This result confirmed the findings of He *et al.* that showed microinjection of histaminergic agents into the FN could not change crossing and rearing numbers in an open field test.[12] Most, but not all, antinociceptive drugs may decrease nociceptive behaviors partially due to reducing the animal's locomotor activity rather than merely due to their analgesic effect. Therefore, special care needs to be taken to ensure that the recorded antinociceptive effect is solely due to a decrease in pain perception and not by a certain motor malfunction which blocks the occurrence of the nociceptive behavior.[31]

These results demonstrated that histamine through H_2 receptors produced visceral antinociceptive effects without changing locomotor activity and enhances rat motor coordination in the cerebellar FN. It is suggested that the hypothalamocerebellar histaminergic projections might play a modulatory role not only in pain processing but also in motor coordination.

In conclusion, according to the results of this study, microinjection of histamine and dimaprit into the FN produced antinociceptive effects in acetic acid-induced visceral pain and improved locomotor performance in

rotarod test. These effects were prevented by intra-FN microinjection of ranitidine. Histamine H_1 receptor in the FN did not seem to have any modulatory role. The antinociceptive, but not motor improving effects induced by histamine and dimaprit were inhibited by naloxone microinjection into the FN. These results strongly suggested that at the level of the cerebellar FN, histamine H_2 receptors might interact with opioid receptors in producing visceral antinociceptive effect, whereas such interaction might not be employed in motor function.

Acknowledgments

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Conflict of interest

No financial or other conflicts of interest are declared by the authors.

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