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Ventrolateral periaqueductal gray exogenous and endogenous histamine attenuates sciatic nerve chronic constriction injury-induced neuropathic pain through opioid receptors

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Abstract

The aim of the present study was to investigate the effects of intra-ventrolateral periaqueductal gray (vlPAG) microinjection of histamine and thioperamide (a histamine H_3 receptor antagonist/inverse agonist) on neuropathic pain. To explore the possible mechanism, naloxone was microinjected alone or in combination with histamine and thioperamide. Neuropathic pain was induced by the left sciatic nerve chronic constriction injury. Both the right and left sides of vlPAG of the brain were surgically cannulated. Cold allodynia and mechanical hyperalgesia were recorded by acetone evaporation and von Frey filament tests. Areas under curve of allodynia and hyperalgesia were calculated. Histamine (0.50 and 2.00 μg per site), thioperamide (4.00 μg per site) and thioperamide (4.00 μg per site) before histamine (2.00 μg per site) suppressed cold allodynia and mechanical hyperalgesia after microinjection into the vlPAG. Microinjection of naloxone (0.25 and 1.00 μg per site) into the vlPAG had no effect on cold allodynia and mechanical hyperalgesia. The anti-allodynic and anti-hyperalgesic effects induced by microinjection of histamine (2.00 μg per site) and thioperamide (4.00 μg per site) into the vlPAG were inhibited by prior microinjection of naloxone (1.00 μg per site) into the same site. The above-mentioned agents did not alter locomotor activity. Based on our present results, it was concluded that exogenous (by histamine microinjection) and endogenous (by thioperamide microinjection) histamine of the vlPAG might contribute to the descending pain control mechanisms through a naloxone-sensitive mechanism.

Introduction

Neuropathic pain is a chronic pain state that is caused by the dysfunction of the somatosensory nervous system and may lead to a series of neurobiological events such as dysesthesia, hyperalgesia and allodynia.[1] Peripheral nerve injuries may result in cellular and molecular changes in supra-spinal structures such as anterior cingulated cortex (ACC) and periaqueductal gray (PAG) possibly involved in neuropathic pain maintenance.[2] The PAG, as an important axial structure of the midbrain, is comprised of four sub-regions including dorsomedial (dmPAG) dorsolateral (dlPAG) lateral (lPAG) and ventrolateral (vlPAG) sub-regions.[3] This midbrain structure receives descending projections from the higher brain areas such as medial prefrontal cortex (mPFC), ACC, amygdale and hypo thalamus as well as ascending nociceptive inputs from spinal dorsal horn.[4] In addition, the PAG through its reciprocal connections with the rostral ventromedial (RVM) of the medulla contributes to descending pain modulation.[5]

Brain histaminergic system originates from tubero-mammillary nucleus (TMN) of the hypothalamus and innervates almost all brain regions and through post-synaptic H_1, H_2 and H_4 and pre-synaptic H_3 receptors play a particular role in ascending and descending pain modulation.[6-8] For example, microinjections of histamine into the ACC, histamine H_1 and H_2 receptor agonists and antagonists into the thalamic submedius (Sm) and dentate gyrus (DG) have altered inflammatory pain intensity.[9-11] Microinjection of histamine H_3 receptor antagonist, thioperamide into the ACC and agranular insular cortex (AIC) suppressed formalin-induced inflammatory pain and

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spared nerve injury (SNI)-induced neuropathic pain, respectively, in rats.[9,12] Blood-brain barrier penetrating histamine H_1 (mepyramine) and H_2 (ranitidine) receptor antagonists suppressed cold and mechanical allodynia in tibial nerve transaction model of neuropathic pain in rats.[13]

Opioids and their mu-, delta- and kappa-opioid receptors are widely distributed in the peripheral and nervous systems and play a central role in processing of pain.[14,15] At the supra-spinal processing of pain, opioid receptors may modulate the antinociceptive effects of histamine. For example, microinjection of naloxone, an opioid receptor antagonist, into the ACC inhibited the antinociceptive effects induced by microinjection of histamine and thioperamide into the same site.[9]

The PAG receives a prominent projection from TMN histaminergic neuron system and expresses high densities of histamine H_3 and opioid receptors.[4,6,16] In addition, PAG mediates endogenous opioidergic signaling essential to opioid-induced analgesia.[17] Therefore, the present study was aimed to investigate the effects of exogenous, microinjection of histamine into the vlPAG, and endogenous, microinjection of thioperamide into the vlPAG, histamine on sciatic nerve chronic constriction injury (snCCI) model of neuropathic pain. In addition, the role of vlPAG histamine in descending pain control was also investigated after blockade of opioid receptor in this area of the brain with naloxone.

Materials and Methods

Animals. In the present study, healthy adult male Wistar rats weighing 250 - 270 g were used. They were maintained in a laboratory under a 12.00:12.00 hr light-dark cycle (lights on at 07:00 hr) at a controlled ambient temperature (22.00 ± 0.50 ˚C) with free access to food and water. Experiments were performed between 11:00 hr and 15:00 hr. The Veterinary Ethics Committee of the Faculty of Veterinary Medicine of Urmia University (IR-UU-AEC-693/DA3/4/8/2019) approved all protocols used in the present study.

Chemical compounds. Histamine dihydrochloride, thioperamide maleate and naloxone dihydrochloride were purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA). All agents were freshly prepared before intra-vlPAG microinjection.

Study protocol. We used a 15-day time duration for each microinjection study. Rats underwent snCCI model of neuropathic pain on day 1. The vlPAG was surgically cannulated on day 7. The agent microinjection and acetone evaporation and von Frey filament tests were performed on day 14. Microinjection sites were verified on day 15.

Animal grouping. Sixty-six male Wistar rats (age: 12-15 months, weight: 250 - 270 g) were divided into 11 groups of six rats each.

Group 1 was sham group. This group had the exposure of the sciatic nerve and bregma of the skull without snCCI and brain cannulation; Group 2 underwent brain cannulation, snCCI and microinjection of normal saline; Groups 3 and 4 were microinjected with histamine (0.50 and 2.00 µg per site) after snCCI induction, respectively; Groups 5 and 6 were microinjected with thioperamide (1.00 and 4.00 µg per site) after snCCI induction, respectively; Group 7 was microinjected with thioperamide (4.00 µg per site) plus histamine (2.00 µg per site) after snCCI induction; Groups 8 and 9 were microinjected with naloxone (0.25 and 1.00 µg per site) after snCCI induction, respectively; and Groups 10 and 11 were microinjected with naloxone (1.00 µg per site) followed by histamine (2.00 µg per site) and thioperamide (4.00 µg per site) after snCCI induction, respectively.

Neuropathic pain model. Peripheral neuropathy was induced by snCCI model as described previously.[18] Rats were anesthetized with intraperitoneal (IP) injection of a mixture of 80.00 mg kg^{-1} ketamine (Alfasan, Woerden, The Netherlands) and 8.00 mg kg^{-1} xylazine (Alfasan). A small incision was made at the level of the left mid-thigh. The left sciatic nerve was exposed by blunt dissection through the biceps femoris. Connective tissue surrounding the nerve was carefully removed proximal to its trifurcation, and four 5-0 chromic catgut ligatures (Hur-Teb, Qazvin, Iran) were tied loosely around the nerve with about 1.00 mm spacing until the epineurium was slightly compressed. A typical minor twitching of the relevant muscles was observed when the nerve was constricted. Following surgery, the rats were received a single dose of penicillin G potassium (50,000 IU kg^{-1}; Jaber-Ebne-Hayyan Pharmaceutical Co., Tehran, Iran) and allowed to recover for one week before implantation of guide cannulas. In sham group, with no manipulation of sciatic nerve, surgery on skin and muscles were performed.

vlPAG cannulation. Among the PAG sub-regions, the vlPAG is a key component in opioid-dependent mechanism of descending pain modulation.[5] One week after snCCI induction, using the above mentioned protocol, anesthetized rats were placed in a stereotaxic apparatus (Stoelting, Wood Lane, USA) and the vlPAG were bilaterally implanted with two guide cannulas (24G, 13.00-mm) at the following coordinates: 7.80 mm posterior to the bregma, 0.80 mm left and right sides of the midline, and 5.50 mm below the top of the skull with 15.00° angle.[19] At the end of surgery, penicillin G potassium was IP injected at a dose of 50,000 IU kg^{-1}. Sham group underwent skull exposing but not cannulation.

vlPAG microinjection. Using a 30G, 13.00 mm needle attached to a 5.00 µL microsyringe (ILS, Innovative Systeme, GmbH, Stützerbach, Germany), intra-vlPAG microinjections of normal saline (control) and test agents were performed. Each vlPAG was microinjected with constant volumes of 0.25 µL of test agents over a period of

30 sec. The injection needle was left in place for a further 30 sec to facilitate diffusion of the drug.

Cold allodynia. The acetone evaporation test is a technique used to measure aversive behaviors triggered by evaporative cooling and is typically considered as a measure of cold allodynia.[20] Rats were placed in a clear plastic box (23.00 × 23.00 × 12.00 cm) with a wire mesh floor and allowed to habituate for 30 min prior to testing. After habituation, 100 µL acetone was sprayed on the plantar surface of the hind paws using a 1.00 mL syringe connected to a thin polyethylene tube. The time duration spent lifting and licking of the paw was recorded for two min after the start of the acetone spray. Paw lifting and licking time duration was expressed as an overall percentage response. Acetone spray was applied 20 min before and at 0, 20, 40 and 60 min after microinjection. Observers were blinded to the experimental conditions of the tested rats with the acetone spray test.

Mechanical hyperalgesia. Paw withdrawal frequency (PWF) was recorded in response to application of von Frey filaments (IITC-Life Science Instruments, Woodland Hill, USA) described previously.[12,21] Animals were placed on an elevated wire mesh floor enclosed in a transparent plastic box for a 30.00 min adaptation period. According to our previous study, the filament number 13.00 equivalent to 20.00 g was chosen for mechanical hyperalgesia assessment in experimental groups.[12] After a 30.00-min habituation period, positive (paw withdrawal) and negative responses were taken at 15 min before (–15 min, baseline values) and at 5, 25, 45 and 65 min after microinjection. Ten stimuli were applied with elapsed time 2 - 3 sec among them and PWF to filament application was recorded and expressed as PWF percentage [PWF (%): number of positive responses/number of trails × 100].

Microinjection sites verification. Initially, 0.25 µL methylene blue was microinjected into each side of vlPAG. Animals were deeply anaesthetized with a mixture of ketamine and xylazine, by the same protocol as mentioned above, and perfused intracardially with physiological saline followed by 10.00% formalin solution. The brains were removed and placed in a 10.00% formalin solution. Twenty-four hr later, we provided transverse sections (50.00 - 100 µm) and viewed under a loupe to localize the microinjection sites according to the atlas of Paxinos and Watson.[19]

Locomotor activity. Locomotor activities including line crossing and rearing numbers were recorded using an open-field test for a 5-min session after intra-vlPAG microinjection of test drugs.

Statistical analysis. The GraphPad Prism (version 5.00; Graph Pad Software Inc., San Diego, USA) was used to analyze the results. The data obtained from 20-min time points were analyzed using two-way repeated measure analysis of variance (ANOVA) and then Bonferroni post hoc test. Area under curve (AUC) was calculated by the trapezoidal method,[22] and analyzed by one-way ANOVA and post hoc Tukey's test. Values are expressed as mean ± SEM. A $p < 0.05$ was considered statistically significant.

Results

The locations of the cannula entrance into the brain were confirmed on the brain surface (Fig. 1A). The locations of the cannula tip placement (microinjection site) in the vlPAG (Fig. 1B) were confirmed in the brain section. The rat brain section of vlPAG (Fig. 1C) was adopted from the atlas of Paxinos and Watson.[19]

Figure 2A shows significant differences between treatments ($F_{(6,175)} = 410.89$, $p < 0.0001$), across times ($F_{(4,175)} = 125.48$, $p < 0.0001$), and between interactions ($F_{(24,175)} = 15.88$, $p < 0.001$) in the effects of histamine and thioperamide on snCCI-induced cold allodynia. In this context, histamine at doses of 0.50 and 2.00 µg per site and thioperamide at a dose of 4.00 µg per site significantly ($p < 0.0001$) decreased cold allodynia at all post-microinjection time points. In addition, prior microinjection of thioperamid (4.00 µg per site) increased the anti-allodynic effect of 2.00 µg per site histamine microinjected into the same site. The AUC analysis revealed significant ($F_{(6,27)} = 64.47$, $p < 0.0001$) differences among groups (Fig. 2B). Figure 2C shows significant differences between treatments ($F_{(6,175)} = 305.89$, $p < 0.0001$), across times ($F_{(4,175)} = 59.27$, $p < 0.0001$), and between interactions ($F_{(24,175)} = 10.65$, $p < 0.001$) in the effects of histamine and thioperamide on snCCI-induced mechanical hyperalgesia. In this context, cold allodynia at all post-microinjection time points was significantly ($p < 0.0001$) reduced by microinjection of histamine (0.50 and 2.00 µg per site) and thioperamide (4.00 µg per site).

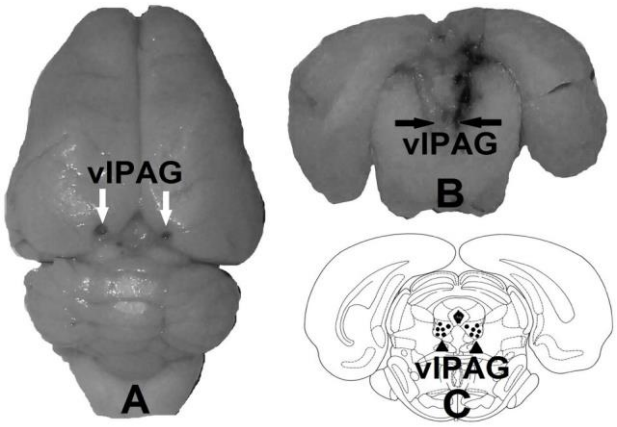

Fig. 1. Schematic illustrations of the rat brain showing the cannula entrance location (white arrows) of the vlPAG on the brain surface (A), cannula tip placement in the vlPAG (black arrows, B) of rats included in the present study. Atlas plate of vlPAG with microinjection sites (arrowheads, C) was adopted from Paxinos and Watson.[19] vlPAG: ventrolateral periaqueductal grey.

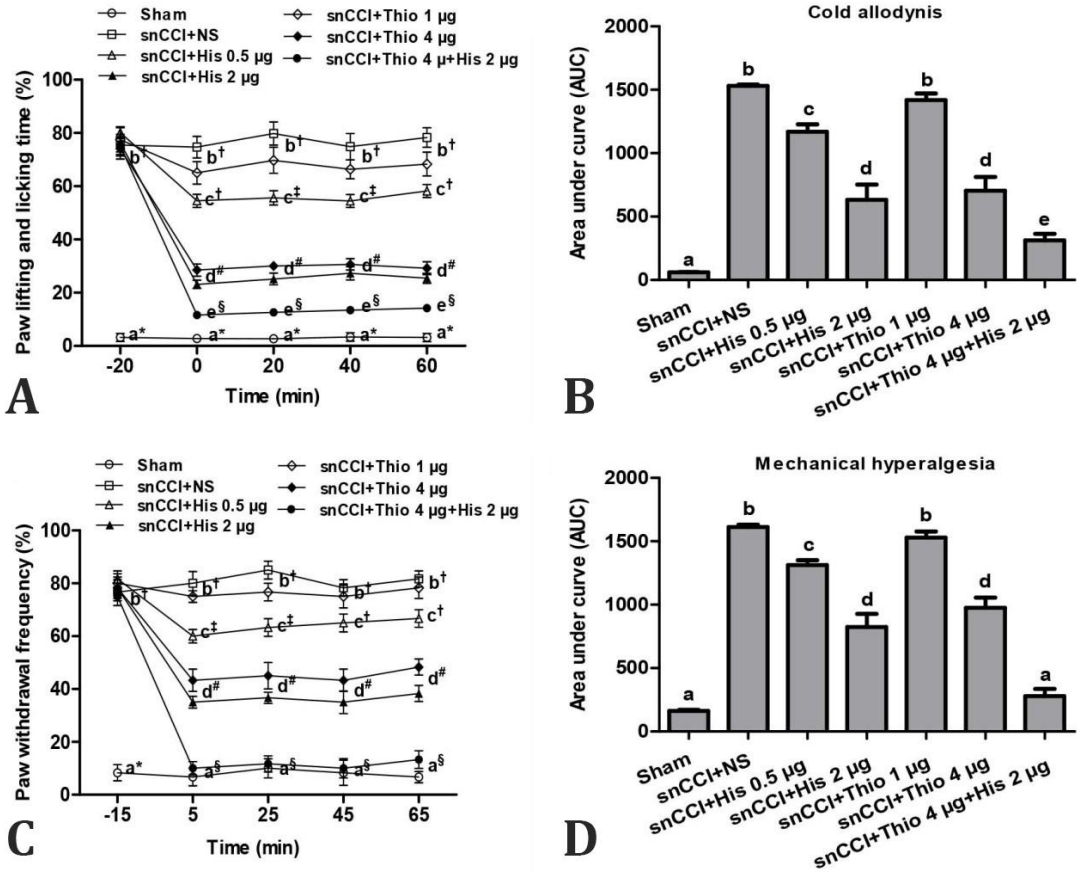

Fig. 2. Effects of intra-vlPAG microinjection of histamine, thioperamide and thioperamide before histamine on cold allodynia (A), related area under curve (AUC, B), mechanical hyperalgesia (C) and related area under curve (AUC, D) in snCCI-induced neuropathic pain. Thioperamide and histamine were microinjected four and two min before neuropathic pain symptoms recording, respectively. These test drugs were used in µg per site. Values are the mean ± SEM of six rats per group. Similar letters (a *vs* a, b *vs* b, c *vs* c, d *vs* d and e *vs* e: $p > 0.05$) and symbols (* *vs* *, † *vs* †, ‡ *vs* ‡, # *vs* # and § *vs* §: $p > 0.05$) indicate no significant differences among time points and among treated groups, respectively. Non-similar letters (a *vs* b: $p < 0.0001$, a *vs* c and b *vs* e: $p < 0.001$, a *vs* d, b *vs* d and c *vs* e: $p < 0.01$ and a *vs* e, b *vs* c, c *vs* d and d *vs* e: $p < 0.05$) and symbols (* *vs* †: $p < 0.0001$, * *vs* ‡ and † *vs* §: $P < 0.001$, * *vs* #, † *vs* #, ‡ *vs* §: $p < 0.01$ and * *vs* §, † *vs* ‡, ‡ *vs* # and # *vs* § $p < 0.05$) indicate significant differences among time points and among treated groups, respectively. vlPAG: Ventrolateral periaqueductal grey, snCCI: Sciatic nerve chronic constriction injury, NS: Normal saline, His: Histamine, and Thio: Thioperamide.

The anti-hyperalgesic effect of 2.00 µg per site histamine was significantly ($p < 0.0001$) increased by prior microinjection of thioperamid (4.00 µg per site) into the same site. The AUC analysis revealed significant ($F_{(6,27)} = 104.11$, $p < 0.0001$) differences among groups (Fig. 2D).

Figures 3A and 3B show the effects of intra-vlPAG microinjection of naloxone on cold allodynia and mechanical hyperalgesia, respectively, induced by snCCI. Naloxone at doses of 0.25 and 1.00 µg per site produced no significant effects on cold allodynia ($F_{(4,100)} = 0.38$, $p > 0.05$, Fig. 3A) and mechanical hyperalgesia ($F_{(4,100)} = 0.39$, $p > 0.05$, Fig. 3B) intensities at all post-microinjection time points. The AUC analysis also revealed no significant ($F_{(3,15)} = 0.99$, $p > 0.05$, Fig. 3C, cold allodynia) and ($F_{(3,15)} = 0.85$, $p > 0.05$, Fig. 3C, mechanical hyperalgesia) differences among snCCI plus normal saline and snCCI plus naloxone 0.25 and 1.00 µg per site treated groups. In contrast, AUC analysis revealed significant differences on cold allodynia ($F_{(3,15)} = 340.18$, $p < 0.0001$, Fig. 3C) and mechanical hyperalgesia ($F_{(3,15)} = 296.45$, $p < 0.0001$, Fig. 3C) between intact group with snCCI plus normal saline and snCCI plus naloxone (0.25 and 1.00 µg per site) treated groups.

Figure 4A-D shows the effects of prior intra-vlPAG microinjection of naloxone on histamine- and thioperamide-induced anti-allodynic and anti-hyperalgesic effects. Prior microinjection of naloxone (1.00 µg per site) significantly ($p < 0.001$) prevented histamine (2.00 µg per site)-, and thioperamide (4.00 µg per site)-induced anti-allodynia and anti-hyperalgesia at all post-microinjection time points (Figs. 4A and 4C). The AUC analysis revealed significant differences in cold allodynia ($F_{(6,27)} = 131.05$, $p < 0.0001$, Fig. 4B) and mechanical hyperalgesia ($F_{(6,27)} = 96.15$, $p < 0.0001$, Fig. 4D) among groups.

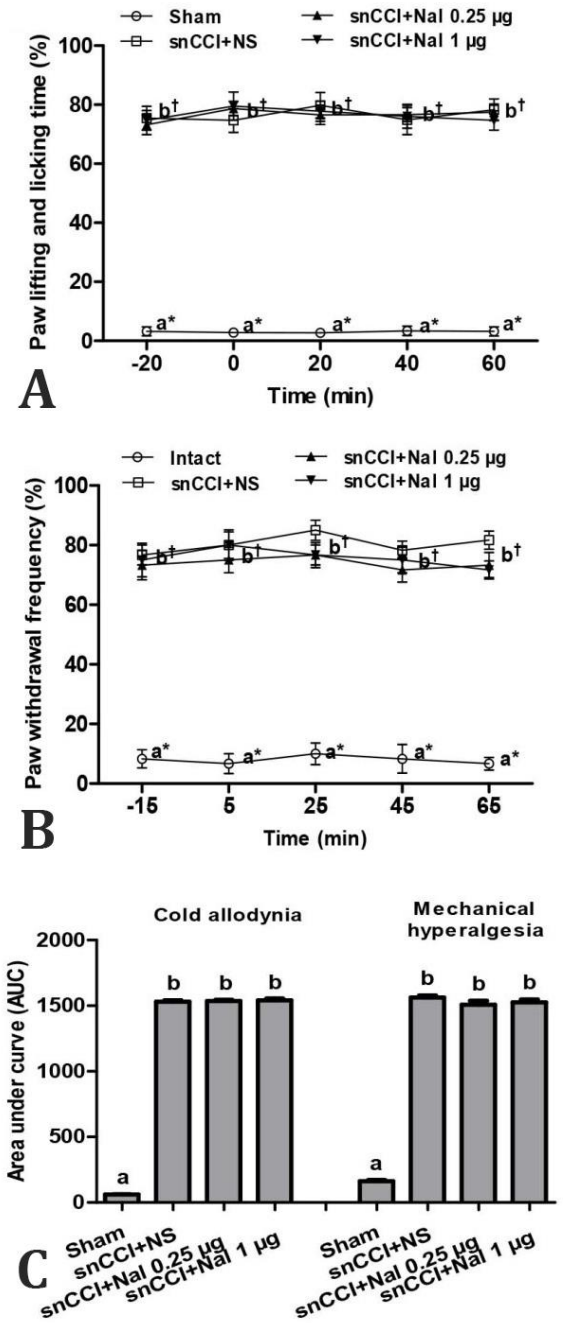

Fig. 3. Effects of intra-vlPAG microinjection of naloxone on cold allodynia (A), mechanical hyperalgesia (B) and related area under curves (AUC, C) in snCCI-induced neuropathic pain. Naloxone was microinjected six min before neuropathic pain symptoms recording. These test drugs were used in µg per site. Values are the mean ± SEM of six rats per group. Similar letters (a *vs* a, b *vs* b: $p > 0.05$) and symbols (* *vs* *, † *vs* †: $p > 0.05$) indicate no significant differences among time points and among treated groups, respectively. Different letters (a *vs* b: $p < 0.0001$) and symbols (* *vs* †: $p < 0.0001$) indicate significant differences among time points and among treated groups, respectively. vlPAG: Ventrolateral periaqueductal grey, NS: Normal saline, snCCI: Sciatic nerve constriction injury, and Nal: Naloxone.

Line crossing and rearing numbers were 27.33 ± 2.58 and 14.5 ± 1.23, respectively, in sham group. With no significant differences with sham group, these values reached to 23.83 ± 2.02 (line crossing number) and 13.67 ± 1.20 (rearing number) in snCCI rats receiving intra-vlPAG microinjection of normal saline. Intra-vlPAG microinjection of histamine, thioperamide and naloxone did not alter line crossing and rearing numbers (data not shown).

Discussion

The results of the present study showed that intra-vlPAG microinjection of histamine suppressed both the cold allodynia and mechanical hyperalgesia in snCCI model of neuropathic pain. Due to a weak penetration of histamine from the blood-brain barrier, scholars have been frequently used intracerebroventricular and intra-brain nuclei administration of the amine for investigating its integrative effects.[23,24] Although there are no reports showing the effects of intra-vlPAG histamine on neuropathic pain, in hot-plate model of acute pain it was found that microinjection of histamine into the periaqueductal gray or the nearby dorsal raphe (PAG/DR) suppressed nociceptive response in rats.[25] The PAG/DR-microinjected histamine induced antinociception was inhibited by prior microinjection of temelastine and tiotidine, histamine H_1 and H_2 receptor antagonists, respectively, into the same site.[25] In another pain processing areas of the brain such as striatum,[26] sciatic nerve partial ligation-induced neuropathic pain increased the level of histamine in this area.[27] In this context, microinjection of histamine into the AIC, an important brain area in perception, modulation and chronification of pain,[28] suppressed mechanical hyperalgesia and pain aversion induced by SNI model of neuropathic pain in rats.[12] All of these findings clearly indicated that exogenous histamine might have a potent role in supra-spinal processing of pain.

In the present study, alone intra-vlPAG microinjection of thioperamide attenuated cold allodynia and mechanical hyperalgesia and prior microinjection of this drug increased the suppressive effects of histamine in snCCI-model of neuropathic pain. These results were consistent with our pervious results in which microinjection of thioperamide not only produced antinociception but also increased histamine-induced analgesia in inflammatory pain.[9] Our recent study on SNI-induced neuropathic pain reported that microinjection of thioperamide and immepip (a histamine H_3 receptor agonist) into the AIC produced anti-hyperalgesic and hyperalgesic effects, respectively, and prior microinjection of thioperamide inhibited immepip-induced hyperalgesia.[12] These findings clearly indicated that thioperamide acted as an antagonist/inverse agonist. Histamine H_3 receptors have

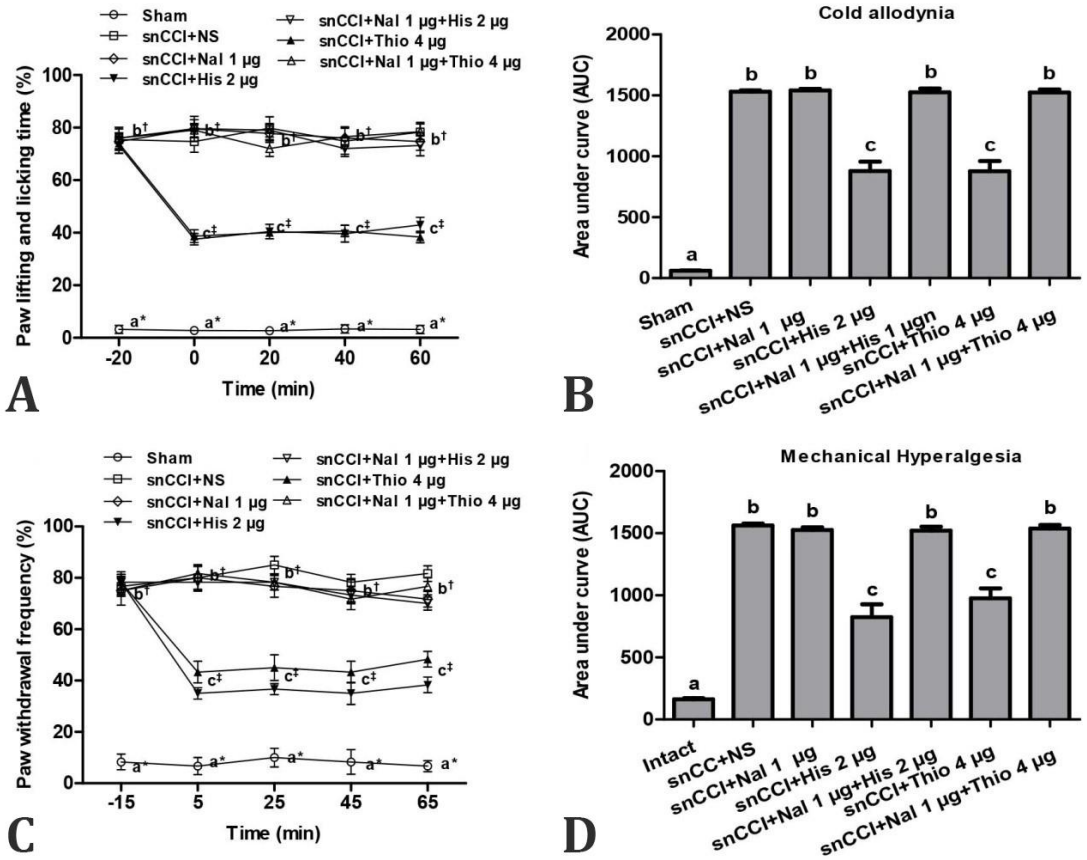

Fig. 4. Effects of prior intra-vlPAG microinjection of naloxone on the same site microinjected histamine- and thioperamide-induced anti-cold allodynia (A), its related area under curve (AUC, B), anti-mechanical hyperalgesia (C) and its related area under curve (AUC, D) in snCCI model of neuropathic pain. Naloxone, thioperamide and histamine were microinjected six, four and two min before neuropathic pain symptoms recording, respectively. These test drugs were used in µg per site. Values are the mean ± SEM of six rats per group. Similar letters (a *vs* a, b *vs* b, c *vs* c: $p > 0.05$) and symbols (* *vs* *, † *vs* † and ‡ *vs* ‡: $p > 0.05$) indicate no significant differences among time points and among treated groups, respectively. Non-similar letters (a *vs* b: $p < 0.0001$ and a *vs* c and b *vs* c: $p < 0.01$) and symbols (* *vs* †: $p < 0.0001$ and * *vs* ‡ and † *vs* ‡: $p < 0.01$) indicate significant differences among time points and among treated groups, respectively. vlPAG: Ventrolateral periaqueductal grey, snCCI: Sciatic nerve chronic constriction injury, NS: Normal saline, Nal: Naloxone, His: Histamine, and Thio: Thioperamide.

an auto-receptor role at the histaminergic synapses. Histamine H_3 receptor antagonists/inverse agonists such as clobenpropit, ciproxifan and thioperamide by blockade of these receptors increase the release of histamine, whereas activation of them with R-α-methylhistamine, immepip and imetit (histamine H_3 receptor agonists/inverse antagonists) results histamine release inhibition.[7] Histamine H_3 receptors are also involved in descending modulation of neuropathic pain. It has been reported that blocking the auto-inhibitory histamine H_3 receptor by microinjection of thioperamide into the locus coelureus (LC) facilitates release of histamine and increases descending noradrenergic pain inhibition.[29] Therefore it seems that activation of endogenous histamine by its H_3 receptor antagonist/inverse agonist reduced neuropathic pain symptoms at the vlPAG level.

Our present results showed that alone microinjection of naloxone into the vlPAG did not alter neuropathic pain intensity, whereas anti-allodynia and anti-hyperalgesia induced by intra-vlPAG microinjection of histamine and thioperamide were prevented by prior microinjection of naloxone into the same site. These results indicated that antinociception induced by exogenous and endogenous histamine was dependent on naloxone-sensitive opioid receptors at the vlPAG. There are not any reports showing the effects of naloxone on antinociception induced by histamine and thioperamide at the vlPAG level. Meanwhile, in other areas of brain such as ACC, a major area of brain involving in sensory and affective dimensions processing of pain,[30] microinjection of naloxone prevent histamine-, and thioperamide-induced antinociceptive effects.[9] Naloxone is a competitive antagonist of mu-, kappa- and sigma-opioid receptors with higher affinity for the mu-

opioid receptors,[31] and has been frequently used to explore the role of endogenous opioid system in pain mechanisms at the PAG level. For example, analgesic effect induced by intra-PAG microinjection of 26Fα, an endogenous ligand of the glutamine RF-amide peptide receptor (QRFP receptor) were inhibited by systemic injection of naloxone in rat formalin test.[32] Naloxone reversed the antinociceptive effects induced by intra-vPAG microinjection of DAMGO ([D-Ala2,N-MePhe4,Gly(ol)5]-enkephalin, a mu-opioi receptor agonist) and DPDPE ([D-Pen,D-Pen5]-enkephalin, a delta-opioid receptor agonist) in SNI model of neuropathic pain.[33] In contrast, the anti-allodynic effects induced by intra-PAG microinjection of neuropeptide FF was not inhibited by naloxone in two spinal nerve ligation model of neuropathic pain.[34] All the above mentioned findings and the results of our present study clearly indicated that histamine and thioperamide induced anti-allodynic and anti-hyperalgesic effects were mediated through a naloxone-sensitive mechanism at vlPAG level.

The present results showed that intra-vlPAG microinjection of histamine, thioperamide and naloxone did not alter locomotor activities in an open-field test. In other brain areas such as ACC and DG, microinjection of histamine and its H3 receptor antagonist (thioperamide) and naloxone did not change locomotor activity in an open-field test.[9,11]

In conclusion, the results of the present study showed that intra-vlPAG microinjection of histamine, exogenous histamine, and thioperamide, an endogenous histamine releaser, suppressed neuropathic symptoms such as cold allodynia and mechanical hyperalgesia. Naloxone-sensitive opioid receptors might have been involved in these suppressive effects of histamine and thioperamide. Supraspinal histamine and its H3 receptors might contribute to descending pain modulation through PAG opioid-dependent mechanism.

Acknowledgments

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Conflict of interest

The authors declare that there are no conflicts of interest.

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