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ORIGINAL ARTICLE

The role of histamine H_1 receptor in the anterior cingulate cortex on nociception level following acute restraint stress in male rats

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Abstract

Considering the importance of pain and stress, we decided to investigate the intra-anterior cingulate cortex (ACC) microinjection of histamine and mepyramine alone and concurrently on acute pain induced by hot plate following restraint stress in male rats. 24-gauge, 10 mm stainless steel guide cannula was implanted over the ACC in the incised scalp of 4 groups. Restraint stress in healthy rats produced a significant increase ($p < .05$) in the pain threshold. The simultaneous microinjection of 4 μg/side histamine and 8 μg/side mepyramine as a histaminergic system inverse agonist in healthy nonrestraint animals did not affect the pain threshold. Although Histamine decreased the threshold of pain meaningfully, mepyramine elevated it in a significant manner ($p < .05$). In the restrained animals, intra-ACC microinjection of histamine produced no significant impact on the pain threshold. However, intra-ACC microinjection of mepyramine before histamine, significantly ($p < .01$) altered the result and enhanced the threshold of pain. The results of our study demonstrated that histaminergic neurons have an important role in the processing of pain in the ACC following restraint stress.

KEYWORDS
ACC, acute pain, histaminergic system, mepyramine, restraint stress

1 | INTRODUCTION

The dynamic interactions between different forms of brain processes; containing sensory and contextual signals (i.e., motivational, emotional and cognitive) will end in the situation we call it pain.[1] The primary function of pain is to generate signals that promote appropriate behavior to protect the individual. Also, a subtle stimulus activates physiological processes that interpret the situation, best described as nociception. Therefore, pain is the subjective experience one feels as a result of the activation of these processes. It is worth noting that, nociceptive responses strongly affect the integrations between sensory and contextual signals.[2]

Stress is definitely another significant root that regulates physiological and behavioral responses of the body, just like pain. Although it has many negative impressions on mental and physical health, not every individual will be adversely affected and get hurt.[3] Stress has proven impacts on brain activity and also could be an effective trigger for changing multiple neural systems.[4] Consequently, the

Abbreviations: ACC, anterior cingulate cortex.

bilateral figure of stress on pain-related conditions can be expected. This bilateral figure is mainly considered by analgesia (insensibility to pain without loss of consciousness) or hyperalgesia (increased sensitivity responses to a painful stimulus)/ allodynia (pain triggered by harmless stimuli).[5]

Meanwhile, a decrease or increase in pain threshold (the amount of time elapsed before the participant reports the stimulus to be painful) is also dependent on a unique form of stress, mostly known as "acute restraint stress." It can produce a time-relative decrease in pain threshold and won't be affected by the administration of adrenergic blockers. Thus, the role of adrenergic pathways has remained unconfirmed.[5] Generally, the pain induced by acute restraint stress is an inescapable experience that leads to minor and major important biological-behavioral changes. Alterations in body temperature, heart rate, and mean arterial pressure are the most noteworthy biological aspects. Moreover, increased anxiety-like behaviors have been reported too.[6]

In addition to the above information, acute restraint stress itself is affected by the histaminergic system working in the brain.[7] Histamine plays a dual role as an inhibitory and excitatory neurotransmitter, which is frequently relevant to psychological stress.[8] The importance of histamine neurons in controlling many behavioral responses such as the state of being awake has been shown as well as stress. Hence, long-term block of the histaminergic system will have significant effects on behavioral and physiological challenges and of course, different body reaction to physical stress is one of them.[9] This system has a remarkable partnership with the pain induced by acute restraint stress. For attaining the mentioned point, it collaborates with agonists and antagonists of the H_1 and H_2 receptors. Actually, any impact of the histaminergic mechanism on pain enforced by restraint stress will be expected.[7] This effect can be dependent on the time of administration and also stress severity. As the H_2 receptor antagonists are capable of inhibiting morphine-induced antinociceptive activity, H_2 receptor agonists have the potential to enhance analgesic response 15 min before restraint stress.[10]

The anterior cingulate cortex (ACC), which is an important region in the brain's limbic system,[11] is activated in both acute and chronic pain.[12] Furthermore, ACC is a component of the medial prefrontal cortex and has proven effects on attention/ working memory.[13] This nucleus can also affect the psychological pain aspects since it has the potential to control endogenous pain and participate in the pleasant feeling after relieving pain. In general, because of the intricate role of the ACC in processing pain-related situations in the brain, this nucleus operates in three primary ways during the onset of acute pain triggers: It may be engaged in ascending assessment of nociceptive input, adjustment of cognitive, emotional and motivational factors to regulate the intellectual pain experience or the sensation of pleasure according to pain alleviation. In most studies of brain response to acute pain (harmful stimulus), ACC nucleus activation has been shown.[14] In fact, nociceptive sensory neurons collect and send signals to the different related nuclei in the brain, including ACC.[15]

The relationship between pain and stress has already been mentioned. Due to the various studies which have shown an increase in the activity of the ACC nucleus by emotional or psychological pain, the notable figure of this region on stress-related circumstances could be inferred too.[16] Since the ACC nucleus, as well as the histaminergic system, plays an important role in the processing of stress and also the effect of the histaminergic system on this nucleus has not been revealed yet, the authors have decided to investigate the effect of histamine H_1 receptor in the anterior cingulate cortex nucleus on pain level following acute restraint stress in male rats. Actually, through this experiment we were able to unlock the impact of histaminergic H_1 receptor on the ACC nucleus in case of exposure to the pain induced by acute restraint stress, and discover what effect agonists and inverse agonists of this receptor will have on the pain caused by the aforementioned type of stress.

2 | MATERIALS AND METHODS

2.1 | Animals

Male Wistar rats (weighing 200–240 g at the beginning of the experiment; purchased from the animal lab of Hamadan university of medical sciences), were used for the study. Every one of them was kept individually in standard polypropylene cages with free access to food and water under 12-h light, 12-h dark cycle and a temperature-controlled room (23 ± 1°C).[17] Rats were permitted to adapt to the testing conditions for 2 days prior to the behavioral tests. All procedures used in the present study were done under the ethical guidelines for the Care and Use of Laboratory Animals.

2.2 | Drugs

In order to aim at the role of histamine H_1 receptor in the anterior cingulate cortex nucleus on nociception changes induced by acute restraint stress, one histamine H_1 agonist (Histamine dihydrochloride, Sigma Cat #H7250-5G) and one inverse agonist (Mepyramine maleate, Sigma Cat #P5514-5G) were used. Both drugs were obtained from an American pharmaceutical company; Sigma-Aldrich Corporation. They were dissolved in sterile normal saline 30 min before being administered intra-ACC via a cannula.

2.3 | Surgical procedure

Surgical procedure was carried out as explained before[18,19] with some modifications. Briefly, each rat, after being anesthetized by an intraperitoneal (i.p) injection of a mixture of Ketamine (80 mg/kg) and Xylazine (10 mg/kg), was placed in a stereotaxic apparatus (Steolting, Wood Dale, IL, USA). 24-gauge, 10 mm stainless steel guide cannula was implanted bilaterally over the ACC in the incised scalp according to the following coordinates: 2.4 mm anterior to the bregma, 0.6 mm left and right sides of the midline and 1.2–1.4 mm below the top of the skull. The process of fixing the

cannula was done using four screws and dental acrylic (Acropars, Tehran, Iran). All the animals were free for 10 days to recover from surgery.

2.4 | Experimental design

Wistar rats were randomly divided into 8 groups, each containing 6 animals. Restraint groups (4 out of 8) had their paw licking and jumping latencies measured and immediately restrained for 6 h. After microinjection into the ACC nucleus, paw licking and jumping latencies were measured again. Hot plate test was held for the first restraint group who had experienced surgery without intra-ACC microinjection. The second, third and fourth groups of restraint animals ($n = 6$) went through the same procedure except that they received drugs. The second one obtained 4 μg/side histamine, the third one 8 μg/side mepyramine and the fourth group simultaneously injected with 4 μg/side histamine plus 8 μg/side mepyramine. The remaining groups also consisted of a control group and three surgical groups without experiencing restraint stress. Histamine and mepyramine injectable doses were selected based on previous reports.[20–22] For this purpose, using previous research, a pilot study was designed, and doses were optimized. It is remarkable that mepyramine as an antihistamine is considered to be an inverse agonist, causing the opposite effect on the receptor compared to histamine. Therefore, it is more accurate to refer to this drug as "H_1-antihistamine" rather than "histamine antagonist".[23] Mepyramine, an inverse agonist, reduces inositol phosphate accumulation. It also lowers H_1R gene expression and decreases H_1R mRNA below its normal level. These findings indicate that inverse agonists can more effectively relieve allergy symptoms by not only preventing the increase in H_1R gene expression caused by stimuli, but also by reducing histamine baseline signaling through their inverse agonistic activity.[24]

2.5 | Restraint stress

Animals were restrained in a polyethylene glycol holder measuring 18 cm long with 5 cm radius for 6 h before the injection starts. Both ends of the holder were covered by plastic mesh to allow adequate ventilation.

2.6 | Intra-ACC microinjection

A 30-gauge injection needle in addition to a 30 cm polyethylene tube, was fitted to the 10 μL Hamilton syringe for intra-ACC microinjections. The volume of injected drug solution and injection period were 2 μL and 60 s, respectively.[25] It is worth mentioning that the injection needle was left in place for a few seconds to enhance the diffusion of the drug. Every group except control (2 μL normal saline) and scheme, fell into one of these treatments: 4 μg/side histamine, 8 μg/side mepyramine or 4 μg/side histamine plus 8 μg/side mepyramine. After each injection and before the hot plate test, the animal was given an opportunity to absorb the drug for 2 min.[26]

2.7 | Hot plate test

The original hot plate method was used to discuss the animal's pain threshold in this study. Every rat was individually placed on the hot plate with a temperature of 55 ± 1°C and 60 s cut-off time to avoid tissue damage. The latency time (the time between the instant of stimulation and the beginning of a response) for paw licking and jumping on the hot plate was considered as an animal's sensitivity to pain (characteristic of each animal that affects the way a painful stimulus is perceived). This test for all 8 groups ($n = 6$ each) was held for 2 times.

Analgesia was quantified as the percentage of the maximum possible effect (MPE%) to equalize the bases latency time in different animals, according to the following formula[27]:

$$\mathrm{MPE}\,\% = 100 \times \frac{(\mathrm{TL} - \mathrm{BL})}{(\mathrm{CT} - \mathrm{BL})}.$$

where MPE = Maximum Possible Effect, TL = Test Latency time, BL = Base Latency time (i.e. initial (0th) hotplate latency time), CT = Cut off Time.

2.8 | Cannula verification

As shown in Figure 1, the anterior cingulate cortex injection site was positioned according to the Paxinos and Watson rat brain atlas.[28] Prior to installing each cannula with the aid of guide cannulas, conformity of the ACC nucleus coordinates with the arms setting of stereotaxic apparatus was again verified based on the atlas data and correct positioning of cannulas have been ensured. Also, on the last day of the study, after euthanizing animals, brain samples were gathered and placed in 10% formaldehyde for 1 week. Finally, serial sections (3 μm) were prepared, and cannula placement in the ACC was microscopically controlled. Just the data from the rats with the correct location of injecting cannula were included in the analysis (Figure 1).

2.9 | Statistical analysis

The data were analyzed by Paired t-test or One-Way ANOVA with Tukey's post-hoc test, as appropriate, using SPSS software (version 16.0) and expressed as the Mean ± SEM. A value of $p < .05$ was regarded as significant. Graphs were drawn by plotting the MPE% for analgesia and latency time for the perception of pain as a function of time. The figures were prepared using GraphPad Prism software (version 6, GraphPad Software Inc., San Diego, CA).

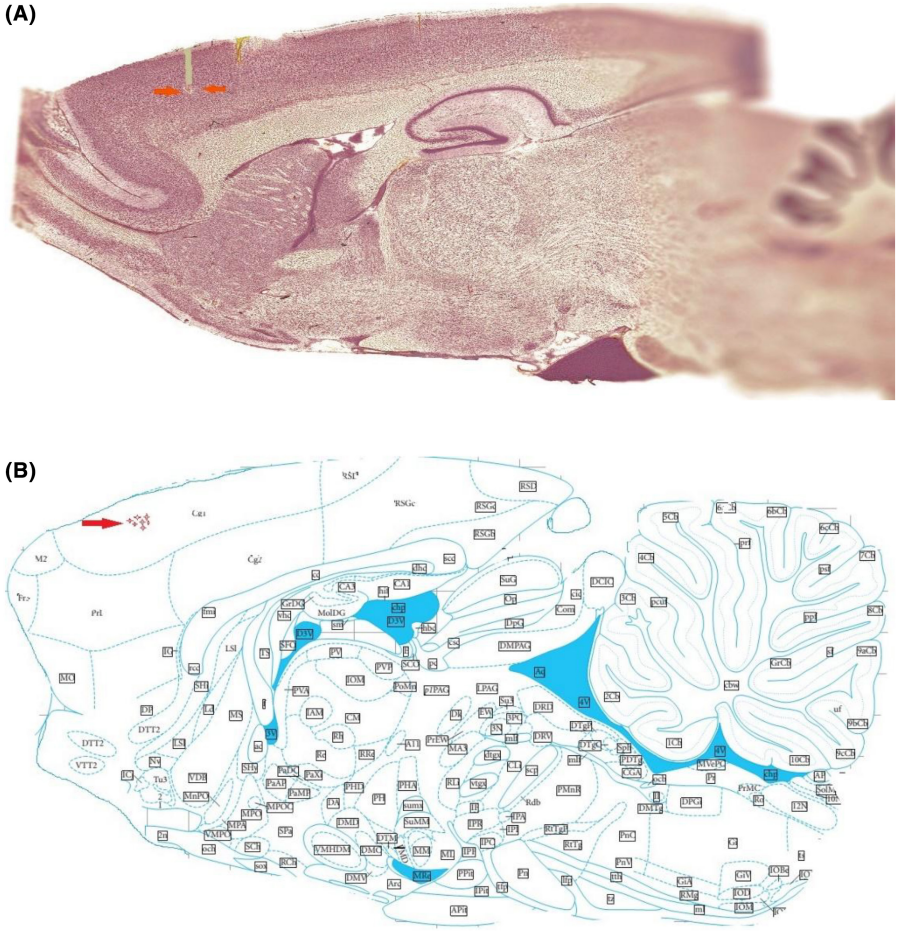

FIGURE 1 Longitudinal section of the rat brain illustrating the location of the ACC nuclei in the rat brain. (A) Placement of the tip of cannulas in the ACC (red arrow) of all rats included in the data analysis. (B) ACC nuclei region in rat according to Paxinos & Watson atlas in stereotaxic coordinates.[28]

2.10 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,[29] and are permanently archived in the Concise Guide to PHARMACOLOGY 2023/24: G protein-coupled receptors.[30]

3 | RESULTS

3.1 | The effect of restraint stress on acute pain induced by the hot plate in intact animals

The first group consisted of 6 intact rats which have not been surgically treated and just restrained for 6 h in a polyethylene glycol holder. The result showed that restraint stress in healthy rats significantly increased the pain threshold and consequently reduced the perception of pain ($t = 3.585$, df = 5, $p < .05$, Figure 2A).

3.2 | The effect of intra-ACC microinjection of histamine on acute pain induced by the hot plate in nonrestraint animals

Intra-ACC microinjection of histamine at the dose of 4 μg/side, significantly decreased the pain threshold. Actually, its administration elicited an increase in nociceptive behaviors on the hot plate including paw licking and jumping. As demonstrated, occurred changes are notable ($t = 4.365$, df = 5, $p < .05$, Figure 2B).

3.3 | The effect of intra-ACC microinjection of mepyramine on acute pain induced by the hot plate in nonrestraint animals

Unlike histamine, intra-ACC microinjection of mepyramine at the dose of 8 μg/side amplified the threshold of pain with a significant effect. Therefore, it can be stated that, it took longer for jumping and paw-licking behaviors to be seen ($t = 2.943$, df = 5, $p < .05$, Figure 2C).

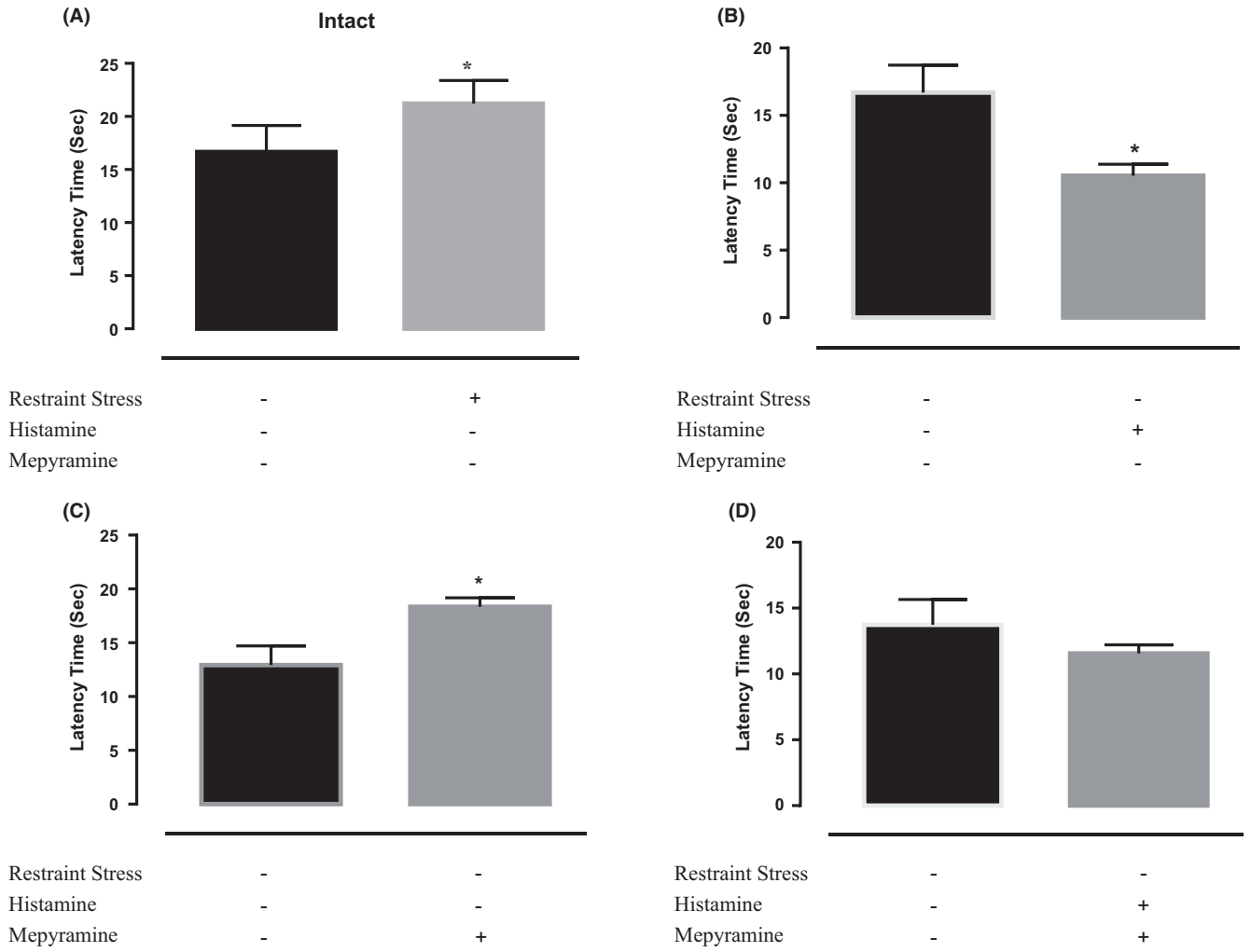

FIGURE 2 (A) The effect of acute restraint stress on acute pain induced by the hot plate in intact animals. Paw licking and jumping were measured as the main criteria for pain threshold. Both foresaid factors were evaluated immediately after 6 h of restraint stress and the hot plate temperature was 55 ± 1°C. Data shown as Mean ± SEM of six rats per group and analyzed by paired *t*-test using SPSS software. $^{*}p < .05$ compared with the base values. RS, restraint stress. The effect of intra-ACC microinjection of (B) histamine (4 μg/side), (C) mepyramine (8 μg/side), and (D) histamine and mepyramine combination on acute pain induced by the hot plate in non-restraint animals. Paw licking and jumping were measured as the main criteria for pain threshold. Both aforementioned factors were evaluated 2 min after the intra-ACC microinjection of histamine or mepyramine. In combination therapy, histamine was microinjected 10 min after mepyramine. About 2 min later paw licking and jumping behaviors were examined. The hot plate temperature was 55 ± 1°C. Data shown as Mean ± SEM of six rats per group and analyzed by paired *t*-test using SPSS software. $^{*}p < .05$ compared with the base values.

3.4 | The effect of simultaneous intra-ACC microinjection of histamine and mepyramine on acute pain induced by the hot plate in nonrestraint animals

At first, the intra-ACC microinjection was held with 8 μg/side of mepyramine. After 10 min histamine was injected at the dose of 4 μg/side. About 2 min later paw licking and jumping behaviors were examined. The aforementioned procedure did not contribute to any meaningful change in the threshold of pain. The result means that inhibiting histamine receptors by mepyramine (Histamine H_1 receptor inverse agonist) in nonrestraint animals does not lead to the distinctive perception of pain in the ACC nucleus compared to control. (Figure 2D).

3.5 | The effect of restraint stress on acute pain induced by the hot plate in the sham group

The fifth group ($N = 6$) underwent surgery but received no medication. The animals were restrained for 6 h in polyethylene glycol holders. Acute restraint stress significantly increased the threshold of pain in sham-operated animals. It is worth noting that, the results of this group and the control one were similar. Restraint stress resulted

in a significant increase in latency time in both groups ($t = 3.897$ df = 5, $p < .05$, Figure 3A).

3.6 | The effect of intra-ACC microinjection of histamine on acute pain induced by the hot plate following restraint stress

Intra-ACC microinjection of histamine as an H_1 and H_2 receptors agonist at the dose of 4 μg/side to restrained animals (6 h; $N = 6$), slightly increased pain threshold. As a result, activating histamine receptors in the ACC nucleus following restraint stress does not exhibit any significant change or a vigorous analgesic effect (Figure 3B).

3.7 | The effect of intra-ACC microinjection of mepyramine on acute pain induced by the hot plate following restraint stress

Microinjection of mepyramine into the ACC nucleus at the dose of 8 μg/side on restrained animals for 6 h, meaningfully suppressed the feeling of pain induced by hot plate following restraint stress. To conclude, the observed changes in the threshold of pain were significant ($t = 0.2974$, df = 5, $p < .01$, Figure 3C).

3.8 | The effect of simultaneous intra-ACC microinjection of histamine and mepyramine on acute pain induced by the hot plate following acute restraint stress

At first, the intra-ACC microinjection was held with 8 μg/side of mepyramine and after 10 min histamine was injected at the dose of 4 μg/side. Both injections were performed immediately after 6 h of restraint stress. About 2 min later paw licking and jumping behaviors were examined. This approach clearly changed the severity of the animal's jumping and paw-licking behaviors on the hot plate. Results demonstrate a significant increase in the threshold of pain which indicates that blocking histamine H_1 receptors with mepyramine as a histaminergic system inverse agonist, curbs the perception of pain ($t = 2.94$, df = 5, $p < .05$, Figure 3D).

3.9 | The effect of Intra-ACC microinjection of histamine, mepyramine, and their combination on analgesia in nonrestraint animals in the hot plate test

One-way ANOVA analysis of data revealed that intra-ACC microinjection of histamine in nonrestraint animals decreased analgesia in a significant manner, in contrast to intra-ACC microinjection of mepyramine, which exhibited a significant increase ($p < .001$). Furthermore, the combination of mepyramine with histamine in nonrestraint rats did not show any meaningful difference when compared with sham-operated animals. Accordingly, MPE% of analgesia for intra-ACC microinjections of histamine and mepyramine dropped significantly for the former and elevated meaningfully for the latter (Figure 4A).

3.10 | The effect of intra-ACC microinjection of histamine, mepyramine, and their combination on analgesia following acute restraint stress in the hot plate test

As illustrated in Figure 4B, the results of one-way ANOVA analysis of data revealed that intra-ACC microinjection of histamine following acute restraint stress in animals did not increased analgesia in a significant manner when compared with sham-operated or intact rats. However, intra-ACC microinjection of mepyramine not only induced a meaningful change in analgesia, but its combination with histamine also intensified analgesia with a significant result as compared with histamine ($p < .001$, $p < .05$; respectively), sham and intact groups, so as MPE% of analgesia. The result means that the obstruction of histamine receptors with mepyramine will increase analgesia and prevent pain perception in the ACC nucleus (Figure 4B).

3.11 | Comparison of the effect of intra-ACC microinjection of histamine, mepyramine, and histamine and mepyramine combination on restraint and nonrestraint animals

The effect of intra-ACC microinjection of histamine was compared in restraint and nonrestraint animals. The results showed that restraint stress significantly increases the histamine's impact. Consequently, analgesia or latency time is enhanced meaningfully ($t = 12.81$, df = 10, $p < .001$, Figure 5A).

The effect of intra-ACC microinjection of mepyramine on restraint and nonrestraint animals, was same the as histamine. Precisely, restraint stress increased the analgesic function of mepyramine. Therefore, in the presence of restraint stress, mepyramine can have a greater analgesic effect ($t = 9.953$, df = 10, $p < .001$, Figure 5B).

Just like the two other groups, comparison of the combination injection of histamine and mepyramine yielded the same result on restraint and nonrestraint animals. In general, restraint stress in all three cases strengthened the analgesic effect, compared to the nonrestarint groups ($t = 14.49$, df = 10, $p < .001$, Figure 5C).

4 | DISCUSSION

In the present study, we investigated the role of histamine H_1 receptors in the anterior cingulate cortex on acute pain induced by

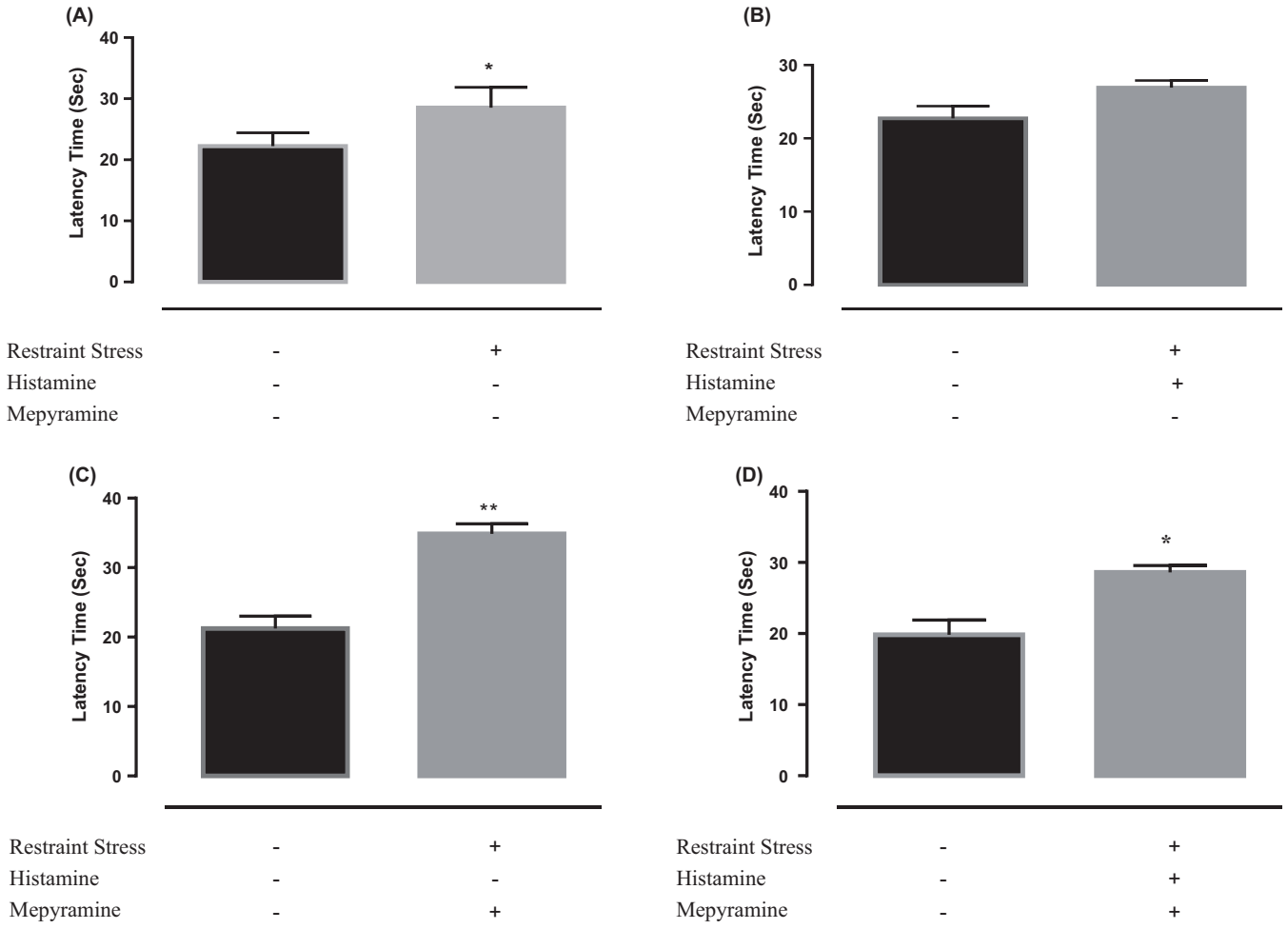

FIGURE 3 (A) The effect of restraint stress on acute pain induced by the hot plate in an operated sham group of animals. The effect of intra-ACC microinjection of (B) histamine, (C) mepyramine and (D) histamine and mepyramine combination, on acute pain induced by the hot plate in restraint animals. Animals were restrained for 6 h in polyethylene glycol holders. Histamine (4 μg/side) or mepyramine (8 μg/side) intra-ACC microinjection was performed immediately after the removal of rats from holders. In combination therapy, histamine was microinjected 10 min after mepyramine and immediately after 6 h of restraint stress. About 2 min later, paw-licking and jumping behaviors were measured. Both mentioned factors had been measured as the main criteria for pain threshold. The hot plate temperature was 55 ± 1°C. Data is shown as Mean ± SEM of six rats per group and analyzed by paired *t*-test using SPSS software. $^{*}p < .05$, $^{**}p < .01$ compared with the base values. RS, restraint stress.

hot plate following acute restraint stress. Above mentioned pieces of evidence pointed out that acute restraint stress results in significant analgesia. According to previous research, acute restraint stress serves as a significant stressor in various animal species, leading to a complex interplay of hormonal responses and functional alterations in the central nervous system. Altered behavioral and hormonal responses, as well as transient changes in neurotransmitters, aminobutyric acid, and glutamate systems due to acute restraint stress, result in pain attenuation and analgesia. Moreover, it has been revealed that the pain-relieving impact of some opiate compounds is enhanced as a result of exposure to this kind of stress.[31,32]

The results of the current study demonstrated that the histamine H_1 receptor in the anterior cingulate cortex regulates spinal and supraspinal flows of pain. The paw licking and jumping behaviors in the hot plate test equal rat forebrain neuronal activities, indicating that supraspinal mechanisms are responsible for painful stimuli.[17,33] ACC nucleus possesses many different neurons, including histaminergic ones. The existence of histamine H_1 (but not H_2 and H_3) receptors in the cingulate cortex has been revealed by PET imaging.[34] It is interesting to know that ACC activation in response to anxiety and also painful stimulus has been attributed. Since the ACC is a part of the limbic system, it will be activated in both acute and chronic pain and plays a crucial role in controlling stress.[35] The affection of this nucleus from psychological pain aspects leads to an important regulatory figure.[14] This would also indicate the effective role of the histaminergic system in actuating the ACC nucleus relative to stress and pain. Furthermore, it has been reported that these histamine receptors contribute to the increased antinociceptive behaviors following restraint stress.[12]

In the current study, histamine intra-ACC microinjection as an H_1, H_2 and H_3 receptors agonist to nonrestraint animals decreased pain threshold and the changes are significant. It is worth noting that,

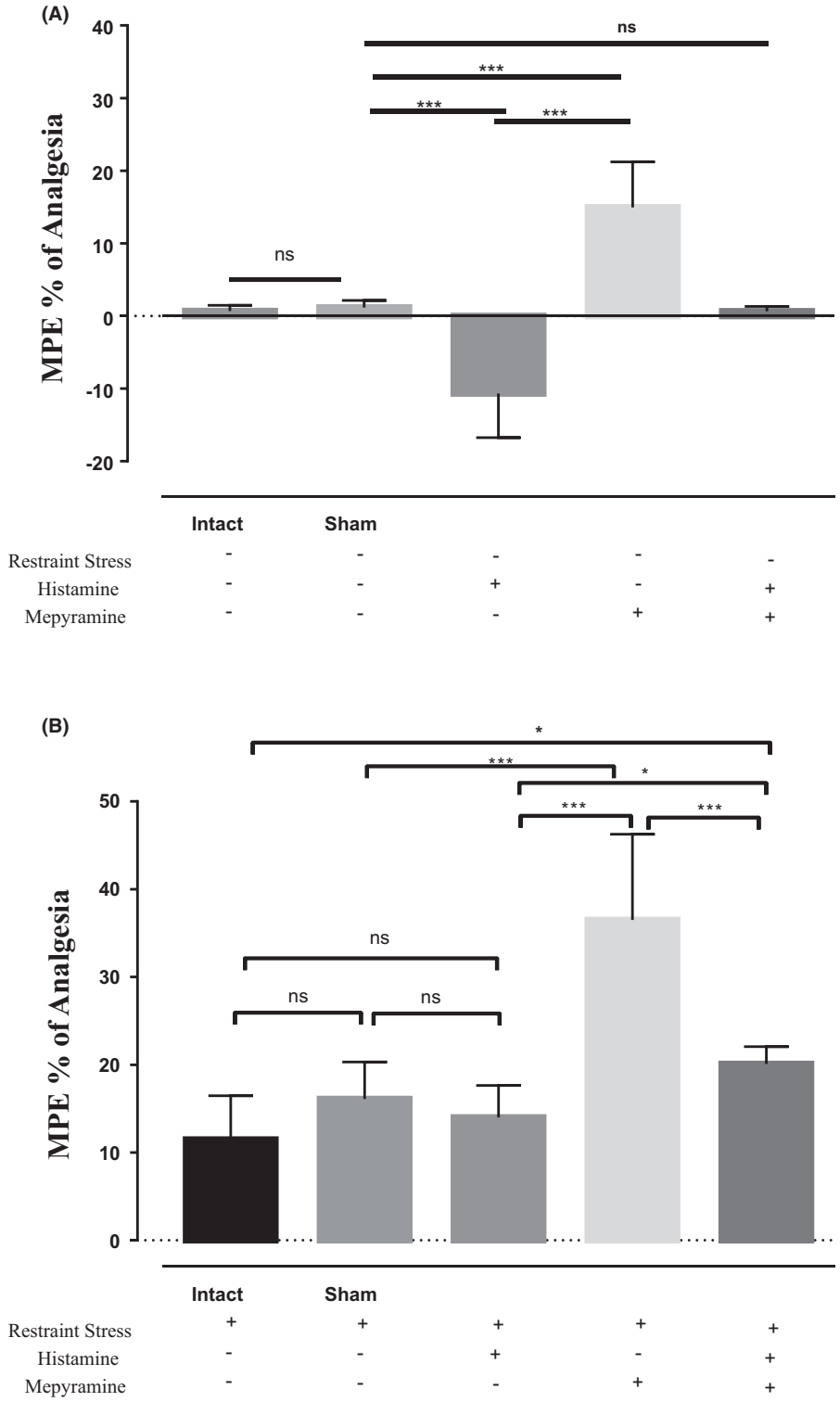

FIGURE 4 The analgesic effect of intra-ACC microinjection of histamine, mepyramine and histamine-mepyramine combination, on acute pain induced by the hot plate in (A) rats without restraint stress and (B) rats with acute restraint stress. In the restraint group, animals were restrained for 6 h in polyethylene glycol holders. Histamine (4 μg/side) or mepyramine (8 μg/side) intra-ACC microinjection was performed immediately after the removal of rats from holders. In combination therapy, histamine was microinjected 10 min after mepyramine and immediately after 6 h of restraint stress. About 2 min later paw licking and jumping behaviors were measured. The hot plate temperature was 55 ± 1°C. Data shown as Mean ± SEM of the percentage of maximum possible effect (MPE%) of analgesia for six rats per group and analyzed by One-Way ANOVA followed by Tukey's test using SPSS software. $^{*}p < .05$, $^{***}p < .001$.

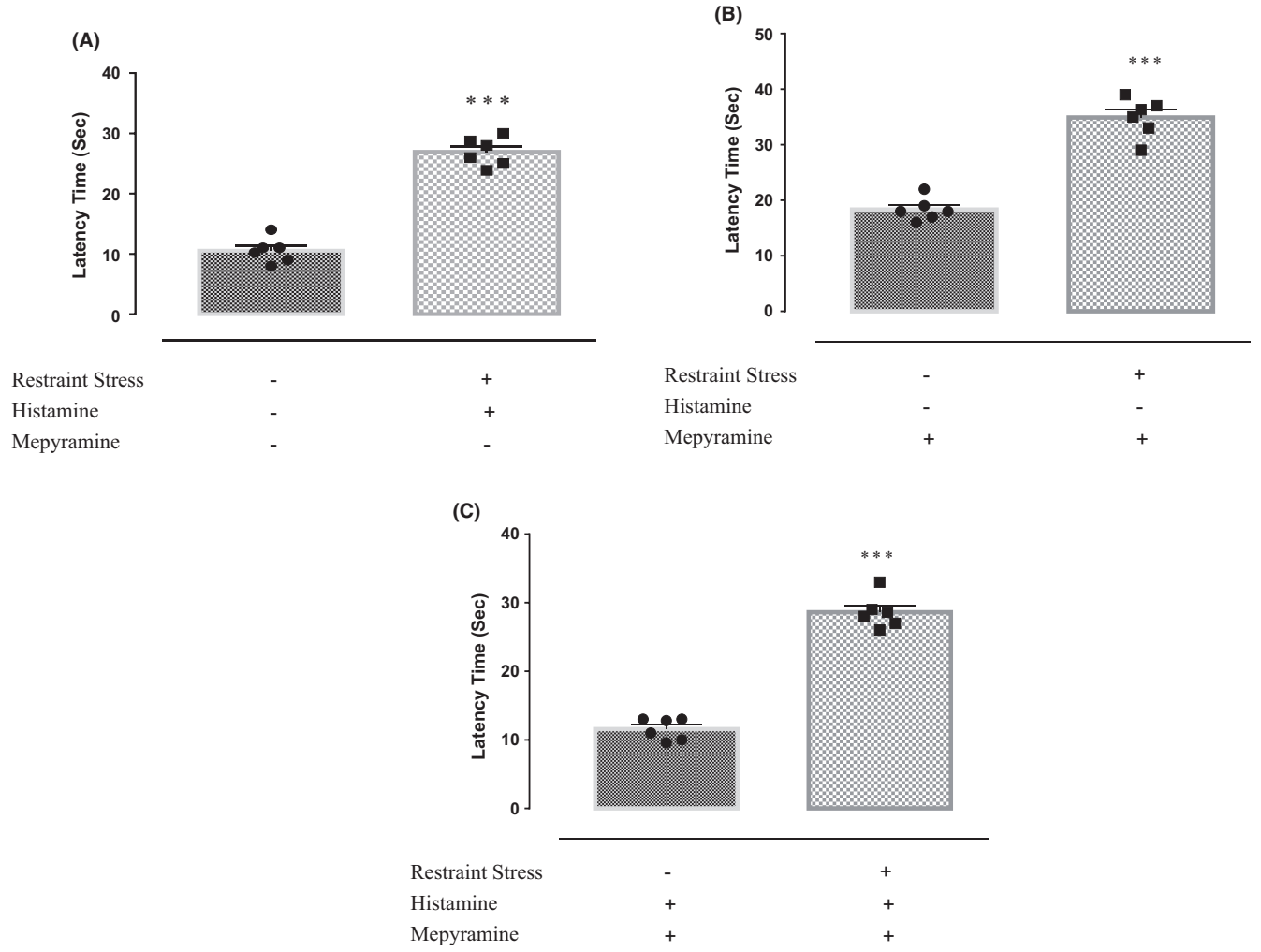

FIGURE 5 Comparison of the effect of intra-ACC microinjection of (A) histamine, (B) mepyramine and (C) histamine and mepyramine combination on restraint and nonrestraint animals. In nonrestraint groups, paw licking and jumping were measured as the main criteria for pain threshold. Both aforementioned factors were evaluated 2 min after the intra-ACC microinjection of histamine or mepyramine. In combination therapy, histamine was microinjected 10 min after mepyramine. About 2 min later paw licking and jumping behaviors were examined. In restraint groups nimals were restrained for 6 h in polyethylene glycol holders. histamine (4 μg/side) or mepyramine (8 μg/side) intra-ACC microinjection was performed immediately after the removal of rats from holders. In combination therapy, histamine was microinjected 10 min after mepyramine and immediately after 6 h of restraint stress. About 2 min later paw licking and jumping behaviors were measured. Both mentioned factors had been measured as the main criteria for pain threshold. The hot plate temperature was 55 ± 1°C. At the end, the effect of each injection on restraint and nonrestraint animals was compared. Data shown as Mean ± SEM of six rats per group and analyzed by paired *t*-test using SPSS software. ***$p < .001$ compared with the base values.

the intra-ACC microinjection of mepyramine as an H_1 antagonist did not yield a similar outcome in such animals and return increased pain threshold. Moreover, the microinjection of both drugs concurrently in nonrestraint animals caused a slight decrease in the threshold of pain with no remarkable effect. Previously, it has been reported that microinjection of histamine into ACC can have a mentionable impress on acute pain. However, mepyramine inhibits the reported histamine effect and causes antinociceptive behaviors.[21] The present study marked that microinjection of mepyramine in nonrestraint rats could decrease the perception of acute pain induced by hot plate and increase analgesia, which is in agreement with the previous report.[21]

Additionally, in the present study, a significant increase in the pain threshold for animals that were restrained and underwent surgery without medication (operated sham group) was attained. It is notable that, after restraint stress with no medication, the animal realization of pain was decreased and it seems that acute restraint stress results in analgesia in normal animals. In both groups of animals (sham and intact), the results were similar and restraint stress significantly enhaced the the pain threshold, resulting in an increase in analgesia, compared to nonrestraint animals.

In this case, histamine intra-ACC microinjection in a group of animals with acute restraint stress did not cause a notable elevation in the pain threshold, so that, histamine was not able to demonstrate antinociceptive effect in these animals by decreasing the hot plate induced pain at the supraspinal level. On the other hand, intra-ACC microinjection of mepyramine as a histamine H_1 receptors inverse

agonist produced a significant increase in the pain threshold following acute restraint stress. Furthermore, blocking the H_1 receptors with mepyramine repeated the same effect of mepyramine intra-ACC microinjection and significantly increased the pain threshold. It is necessary to mention that the inhibition of histamine H_1 receptors with mepyramine in restraint animals could elevate the pain threshold following acute pain induced by the hot plate to a great extent. Furthermore, following comparing the effect of intra-ACC microinjection of histamine, mepyramine, and both drugs concurrently on restraint and nonrestraint animals, it was found that restraint stress significantly increases the analgesic effect in each of these injections, Generally, it demonstrated a profound analgesic effect, compared to the group of animals that did not experience restraint stress. In agreement with the results of the current study, the histamine blockage mechanism of mepyramine resulted in an alleviated pain perception.[21] ACC suppresses incoming signals at the dorsal horn of the spinal cord via activating the periaqueductal gray.[36] In this case, microinjection of histamine into the periaqueductal gray and raphe magnus of restraint animals slightly enhanced the threshold of the pain in the hotplate test,[37] which is totally in association with the findings of the present study. Also, in this context, it has been reported that the intracerebral injection of histamine elevated the threshold of the nociception in neuropathic pain.[38]

The previous investigation describes that acute restraint stress leads to a time-dependent increase in pain threshold via nonadrenergic mechanisms in rats. Prazosin, a selective α_1 antagonist and propranolol, a β-adrenergic blocking agent had no effect on the pain threshold which confirms the lack of adrenergic mechanisms in the pain induced by acute restraint stress.[5] The results of the current study demonstrated that the histaminergic system, specially H_1 receptor in the ACC nucleus has an important role in the regulation of nociception following acute restraint stress; So, the role of histamine was confirmed by the blocking effect of mepyramine.

5 | CONCLUSION

In the present study, we investigated the role of histamine H_1 receptors on acute pain following restraint stress. The results of the current study described an important impression of the histaminergic system on decreasing the acute pain induced by hot plate following restraint stress in the ACC core. Acute restraint stress had significantly increased the threshold of pain. Also, it affects central nervous system through various pathways, including hormonal, behavioral, and psychological responses, functional alterations, and neurotransmitter changes which will lead to an enhancement in the pain threshold. Furthermore, acute restraint stress itself is influenced by the histaminergic system in the brain and its agonist/antagonists, as shown by our study too. As a result, this form of stress has the ability to induce analgesia via different roots of which histaminergic system is one of them. Although the intra-ACC microinjection of histamine significantly decreased the pain threshold in nonrestraint animals, it left no remarkable impact on restraint animals. In return, mepyramine antagonized the effect of histamine in nonrestraint animals. In the restraint group mepyramine meaningfuly increased pain threshold, and confirmed the remarkable role of these receptors on the threshold of pain due to restraint stress. Finally, the present results showed that restraint stress strengthened the effects of histamine and mepyramine and developed analgesia. The authors are recommended to examine other histamine receptors including H_2 and H_3 because they also appear to be involved in pain perception following restraint stress.

AUTHOR CONTRIBUTIONS

RD was involved in doing behavioral study and drafting. FZ, the advisor of the study, carried out the statistical analyzes and drafting. MM, the advisor of the study, involved in drafting and final checking of the manuscript. RH, the supervisor of the study, was involved in concept, design, support of study, drafting and final checking of the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflict of interests.

DATA AVAILABILITY STATEMENT

Data will be provided upon request.

ETHICS STATEMENT

All methods employed in the current study were conducted in adherence to the National Institutes of Health ethical guidelines for the Care and Use of Laboratory Animals and approved by the University of Medical Sciences of Hamadan (UMSHA) Ethical Committee, Hamadan, Iran (ID: IR.UMSHA.REC.1396.294).

LIST OF HYPERLINKS

Histamine: https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=1204.

Mepyramine: https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=1227.

H_1 receptor: https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=262.

H_2 receptor: https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=263.

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