

The Effects of Histaminergic Agents in the Nucleus Accumbens of Rats in the Elevated Plus-Maze Test of Anxiety

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Objective: The nucleus accumbens (NAc) receive histaminergic neurons from tuberomammillary nuclei. There are reports indicating that central histamine systems are involved in many physiological behavioral processes, including anxiety. The aim of the present study was to assess whether the histaminergic system of the NAc is involved in anxiety-related behaviors.

Methods: Rats were anesthetized with intra-peritoneal injection of ketamine hydrochloride, plus xylazine and then were placed in a stereotaxic apparatus. In addition, two stainless-steel cannulae were placed 2 mm above the nucleus accumbens shell. Seven days after recovery from surgery, the behavioral testing was started. As a model of anxiety, the elevated plus maze which is a useful test to investigate the effects of anxiogenic or anxiolytic drugs in rodents, was used in male Wistar rats.

Results: Intra-NAc administration of histamine (0.01, 0.1 and 1 µg/rat) increased the percentage of open arm time (%OAT) and open arm entries (%OAE), but not locomotor activity, indicating an anxiolytic response. Furthermore, bilateral microinjections of different doses of the H₁ receptor antagonist pyrilamine (0.001, 0.01, 0.1 and 1 µg/rat) or the H₂ receptor antagonist ranitidine (0.001, 0.01, 0.1 and 1 µg/rat) into the NAc increased %OAT and %OAE, but not locomotor activity. However, both histamine and histamine receptor antagonists showed an anxiolytic-like effect; the antagonists (1 µg/rat) also decreased the histamine response.

Conclusion: The results may indicate a modulatory effect for the H₁ and H₂ histamine receptors of nucleus accumbens in the anxiety behavior of rats.

Keywords: Anxiety, Histamine, Maze learning, Pyrilamine, Ranitidine, Rats

Iran J Psychiatry 2010; 5:11-17

Histamine as a neurotransmitter may be involved in a wide range of physiological functions (for review see (1)), including learning and memory (2), morphine-state dependent learning (3) and novel environment motivated exploration (4, 5). Neuronal histamine increases emotional reactivity to aversive stimulation, which might increase learning motivation in avoidance and escape tasks (6). The involvement of the histaminergic system in the modulation of anxiety-related behaviors in animals has been suggested previously (7, 8). It has been shown that the histamine administration into the central amygdala may induce an anxiogenic response (9). On the other hand, anxiety-related stress may release histamine (10). Histamine may also be involved in the modulation of nucleus accumbens neurons (11).

Histamine acts through different receptor subtypes namely: H₁, H₂ and H₃ receptors. The H₁ receptors are a G-protein family of receptors whose activation leads to stimulation of phospholipase C and increase in

cAMP levels (12). The H₂ receptors are G-protein coupled receptors (13) whose activation leads to enhanced production of cAMP (14). The histamine H₃ receptor is an autoreceptor, regulating the release and synthesis of histamine (15). It has been shown that high densities of histamine H₁ receptors are present in the limbic system, including several hippocampal areas (1). Those mice lacking histamine H₁ receptors showed prolonged transfer latency in the light/dark box test indicating that the mutant mice were less fearful than the wild-type mice (16).

Although behavioral effects of histamine and related compounds have been evaluated in some brain regions [such as the dorsal and ventral hippocampus (17-19), the central amygdala (9), the nucleus accumbens (20), the inferior colliculus, the periaqueductal gray (21) and the nucleus basalis magnocellularis (7)], its influences on anxiety in the nucleus accumbens (NAc) have not been evaluated yet. The aim of the present study was to investigate the effects of histamine and histamine H₁

and H₂ receptor antagonists microinjected into the NAc and their possible roles in the modulation of anxiety-related behaviors using the elevated plus-maze test of anxiety in rats.

Materials and methods

Animals

Male Wistar rats bred in an animal house in the department of pharmacology (Tehran University of Medical Sciences, Iran), weighing 220-270 g at the time of surgery were used for this study. Four rats were housed per cage in a room with a 12 h light/dark cycle (lights on 07:00 h) and controlled temperature (22 ± 2 °C). They had access to food and water *ad libitum* and they were allowed to adapt to the laboratory conditions for at least 1 week prior to surgery. Each rat was handled about 3 min each day prior to behavioral testing. All experiments were performed between 9:00 h and 12:00 h and each rat was tested only once. Eight rats were used in each group of experiments. The study was approved by the Ethics Committee of the Tehran University of Medical Sciences which corresponds to the national guidelines for animal care and use.

Stereotaxic surgery, microinjections and histology

Rats were anesthetized intraperitoneally with ketamine hydrochloride (50 mg/kg) and xylazine (4 mg/kg) and were placed in a stereotaxic frame (David Kopf Instruments) in a flat-skull position (incisor bar -3.3 mm). Bilateral stainless steel guide cannulae (22 gauge) were implanted 2 mm above the NAc shell according to stereotaxic coordinates AP, +1 mm forward of bregma; L, ± 1 mm from midline; V, -5.5 mm relative to dura (22) and were fixed to the skull with acrylic dental cement. The animals were allowed 7 days to recover before the test. Stainless steel stylets (27 gauge) were inserted into guide cannulae to maintain patency.

Intra-NAc injections were performed by means of an internal cannula (27-gauge, Supa; Iran), terminating 2 mm below the tip of the guides and were connected by polyethylene tubing to a 1- μ l Hamilton syringe. The rats received bilateral microinjections of 0.3 μ l on each side over a 60 sec period (0.6 μ l/rat). The inner cannula was left in place for an additional 60 sec to allow for the diffusion of the solution and to reduce the possibility of reflux. Based on the method used previously (23), 0.3 μ l of each drug with 5 min interval was used in the case of two injections. At the end of the study, injecting 0.6 μ l/rat of 1% methylene blue solution and determining the injected dye in the right and left NAc, identified and verified the injection site. The cannula placements were verified using the atlas of Paxinos and Watson (22). Data from the animals with injection sites located outside the NAc were not used in the analysis.

Behavioral test (Elevated plus-maze)

The method is the same as described previously (18). The elevated plus-maze was a wooden cross-shaped

maze, consisting of four arms arranged in the shape of a plus sign. Two of the arms had no side or end walls (open arms; 50 \times 10 cm). The other two arms had side walls and end walls, but were open on the top (closed arms; 50 \times 10 \times 40 cm). Where the four arms intersect, there was a square platform of 10 \times 10 cm. The maze was elevated to a height of 50 cm. In order to evaluate the total arm entries on the maze, rats were placed in a wooden test arena (50 \times 50 \times 35 cm) for five min prior to maze testing. Seven days after implantation, the effects of intra-NAc injections of the drugs were tested in the elevated plus-maze. At least 1 hour before testing, rats were placed in the room used for the experiments. The rats were randomly allocated to treatment conditions and tested in counterbalanced order. The rats were individually placed in the center of the maze facing a closed arm and were allowed 5 min of free exploration. The numbers of entries into the open arms, the number of entries into the closed arms, the total time spent in the open arms and the total time spent in the closed arms were measured. Entry was defined as all four paws in the arms. The percentage of open arm entries and open arm time as the standard anxiety indices (24) were calculated as follow: (a) %OAT (the ratio of times spent in the open arms to total times spent in any arms \times 100); (b) %OAE (the ratio of entries into open arms to total entries \times 100). (c) Total closed arm entries were measured as a relative pure index of locomotor activity (24).

Drugs

The drugs used in the present study were histamine dihydrochloride (Sigma Chemical Co., USA), pyrilamine maleate (Osve, Tehran, Iran) and ranitidine hydrochloride (Sigma Chemical Co., USA). All drugs were dissolved in sterile 0.9% saline. The drugs were injected in a volume of 0.3 μ l in each side of the NAc (0.6 μ l/rat). Total doses of the drugs used in both sides were expressed as μ g/rat.

Drug treatments

Experiment 1: Effects of histamine on anxiety-related behavior

Four groups of rats received saline (0.6 μ l/rat) or three different doses of histamine (0.01, 0.1 and 1 μ g/rat). The test session was performed 5 min after intra-NAc injections. %OAT, %OAE and locomotor activity were measured as described in the method section (Fig. 1).

Experiment 2: Effects of histamine receptor antagonists on anxiety-related behavior

In this experiment, five groups of rats received saline (0.6 μ l/rat) or different doses of histamine H₁ receptor antagonist, pyrilamine (0.001, 0.01, 0.1 and 1 μ g/rat). The other five groups of rats received saline (0.6 μ l/rat) or different doses of histamine H₂ receptor antagonist, ranitidine (0.001, 0.01, 0.1 and 1 μ g/rat). The test session was performed 5 min after intra-NAc injections of the drugs. %OAT, %OAE and locomotor activity were measured (Fig. 2 and 3).

Experiment 3: Effects of intra-NAc administration of histamine receptor antagonists on histamine-induced anxiolytic response

In this experiment, four groups of rats received saline (0.6 µl/rat) or three different doses of histamine (0.01, 0.1 and 1 µg/rat) as control groups. The test session was performed 5 min after intra-NAc injections. %OAT, %OAE and locomotor activity were measured (Fig. 4, Left panel). Another four groups of rats received saline (0.6 µl/rat) or the same doses of intra-NAc histamine plus pyrilamine (1 µg/rat) with a 5 min interval (Fig. 4, Middle panel). Another four groups of rats received ranitidine (1 µg/rat) 5 min before intra-NAc injection of saline (0.6 µl/rat) or the different doses of histamine (Fig. 4, Right panel). The test session was performed 5 min after intra-NAc injection of histamine. %OAT, %OAE and locomotor activity were measured (Fig. 4).

Statistical analysis

One-way ANOVA was used to compare the effects of different doses of histamine with its control. Two-way ANOVA was used for evaluation of interactions between drugs. Following a significant *F*-value, post-hoc analysis (Tukey-test) was performed for assessing specific group comparisons. Differences with *P* < 0.05 between experimental groups at each point were considered statistically significant.

Results

Effects of histamine on anxiety-related behavior

Fig. 1 shows the effects of histamine on anxiety-related parameters in the elevated plus-maze. A one-way ANOVA revealed that histamine increased %OAT [$F(3,28)=16.47, p<0.001$] and %OAE [$F(3,28)=4.78, p<0.05$] indicating an anxiogenic response. No significant change in the locomotor activity was observed following the administration of histamine [$F(3,28)=1.4, P>0.05$]. The data indicates that histamine administration into NAc is able to induce an anxiolytic effect.

The effects of histamine receptor antagonists on anxiety-related behavior

Fig. 2 and 3 show the effects of histamine H1 receptor antagonist pyrilamine or histamine H2 receptor antagonist ranitidine alone on anxiety-related behavior. One -way ANOVA using post hoc analysis revealed that pyrilamine at the doses of 0.01 and 0.1 µg/rat increased %OAT [$F(4,35)=5.78, P<0.01$] and at the dose of 0.1 increased %OAE [$F(4,35)=5.7, p<0.01$], but not locomotor activity [$F(3,35)=1.89, P>0.05$](Fig 2).

Moreover, one -way ANOVA using post hoc analysis revealed that ranitidine at the doses of 0.001, 0.01 and 0.1 µg/rat increased %OAT [$F(4,35) = 10.9, p<0.001$] and at the dose of 0.01 and 0.1 µg/rat increased %OAE [$F(4,35)=11.6, p<0.001$], but not locomotor activity [$F(3,35)=1.48, p>0.05$](Fig 3).

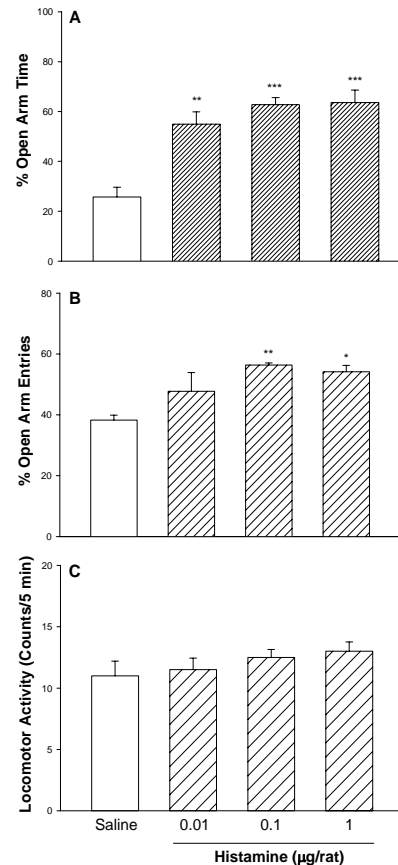


Figure 1. The effects of intra-NAc injection of histamine on anxiety. Rats were injected with saline (0.6 µl/rat) or histamine (0.01, 0.1 and 1 µg/rat). The test was performed 5 min after intra-NAc injections. Each bar is mean±S.E.M. %OAT (A),%OAE (B) or locomotor activity (C). N=8, *P<0.05, ** P< 0.01, ***P< 0.001 when compared to the saline treated rats.

The effects of histamine receptor antagonists on histamine response

Fig 4 indicates the effects of histamine receptor antagonists on histamine-induced anxiolytic-related behavior. A two-way ANOVA showed that pyrilamine (1 µg/rat) could significantly alter the influence of histamine on %OAT [$F(3,56)=22.08, p<0.001$] and locomotor activity [$F(3,56)=7.02, p<0.001$], but not %OAE [$F(3,56)=1.17, p>0.5$].

Furthermore, two-way ANOVA also revealed a significant difference between the response of histamine in the presence or absence of ranitidine on %OAT [$F(3,56)=9.35, p<0.001$], %OAE [$F(3,56)=3.3, p<0.05$], but not locomotor activity [$F(3,56)=2.4, p>0.05$]. Post hoc analysis indicated that pyrilamine and ranitidine significantly reversed the

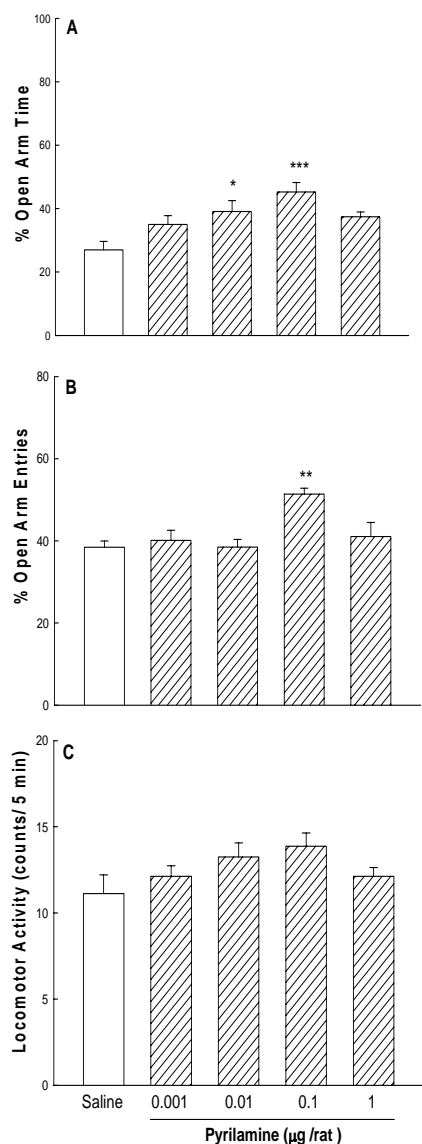


Fig. 2

Figure 2. The effects of intra-NAc injection of pyrilamine on anxiety. Rats were injected with saline (0.6 µl/rat) or pyrilamine (0.001, 0.01, 0.1 and 1 µg/rat). The tests were performed 5 min after intra-NAc injections. Each bar is mean±S.E.M. %OAT (A), %OAE (B) or locomotor activity (C).

N=8, * P<0.05, ** P<0.01, ***P<0.001 when compared to the saline treated rats.

increase in the %OAT and %OAE induced by histamine. However, co-administration of a higher dose histamine plus pyrilamine decreased the locomotor activity.

Discussion

In the present study, the possible involvement of the nucleus accumbens (NAc) H₁ and H₂ histamine sensitive sites in anxiety-related behavior has been

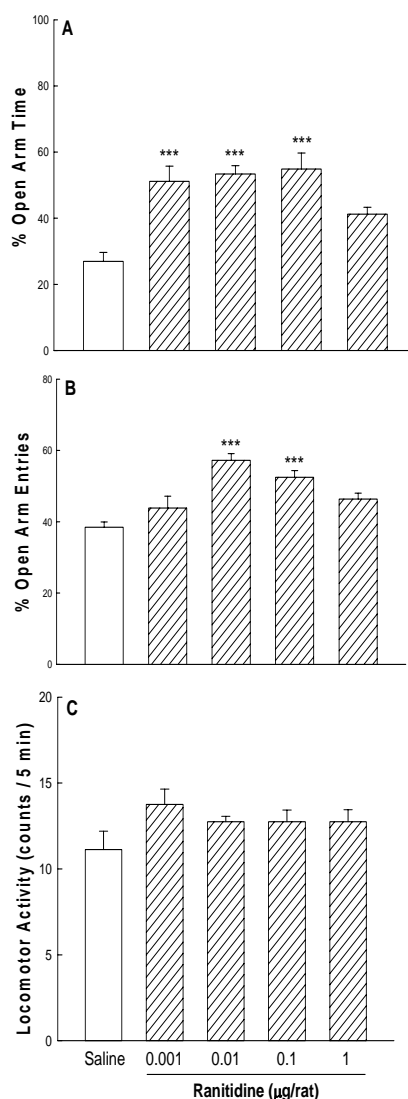


Fig. 3

Figure 3. The effects of intra-NAc injection of ranitidine on anxiety. Rats were injected with saline (0.6 µl/rat) or ranitidine (0.001, 0.01, 0.1 and 1 µg/rat). The tests were performed 5 min after intra-NAc injections. Each bar is mean±S.E.M. %OAT (A), %OAE (B) or locomotor activity (C).

N=8, ***P<0.001, when compared to the saline treated rats.

investigated using the elevated plus-maze as a model of anxiety for the selective identification of anxiolytic and anxiogenic drug effects in rodents (25). The present results show that intra-NAc microinjection of histamine increased %OAT (% Open Arm Times) and %OAE (% Open Arm Entries), the parameters of anxiety related behaviors without locomotor impairment in the elevated plus-maze. This may indicate that histamine exerts its anxiolytic effect when injected into the NAc. Histamine synthesizing neurons

from posterior hypothalamus of the rat brain project fibers to different structures such as the hippocampus and the NAc (26, 27). Moreover, several studies have suggested that the distributions of histamine receptors may mediate the actions of histamine in certain brain areas such as cerebral cortex or hippocampus and also in the NAc (26, 28, 29).

The histamine receptors may modulate behavioral functions of NAc cells (30). Our present data are in agreement with other reports that microinjection of histamine into the rat ventral hippocampus decreased fear-like behaviors in the elevated asymmetric plus-maze (31). In contrast, an anxiogenic effect following intra-ventral hippocampal administration of histamine has also been reported in rats (17). Our data is opposed to that obtained in our previous study in the central amygdala (9) and that by Malmberg-Aiello et al. (8) in which it has been reported that activation of histamine H₁ receptors could reduce the time spent in the lighted compartment of light/dark box, indicating a probable anxiogenic effect for histamine H₁ receptors. Furthermore, intra-NAc microinjection of histamine has been shown to produce a dual effect in the asymmetric plus-maze (32). Thus histamine may cause opposite effects in different regions of brain and may induce a modulatory influence on anxiety-related behavior. Histaminergic systems in the modulation of anxiety may be strongly dependent on the site of drug administration (33).

When histamine H₁ receptor antagonist pyrilamine and histamine H₂ receptor antagonist ranitidine were challenged against histamine in the present study, the anxiolytic responses of histamine on %OAT and %OAE were reduced, which may support involvement of histamine H₁ and H₂ receptors in the histamine response. The results are in agreement with previous reports indicating that anxiolytic effects of histamine in the nucleus accumbens were blocked by both H₁ and H₂ histamine receptor antagonists (32). These findings may indicate that anxiolytic effects of histamine may be mediated either through post-synaptic H₁ or H₂ histamine receptors. Histamine functions through three different histamine receptor subtypes: postsynaptic histamine H₁ and H₂ receptors in addition to presynaptic histamine H₃ receptors which control the release of neuronal histamine (34) and many other neurotransmitters (35). There may be the possibility that the dose of histamine used in the present study acts on pre-synaptic H₃ receptors and induces an anxiolytic response. On the other hand, an important interaction between neuronal histamine and acetylcholine levels and also the change in the 5-HT turnover have been suggested (6). The H₁ receptor activation by histamine may inhibit 5-HT release (36). However, in the present experiments, administration of pyrilamine and ranitidine by themselves also induced anxiolytic response. Therefore, the possibility of modulatory effect of histamine H₁ and H₂ receptor mechanism(s) in the NAc in the anxiety related behaviors seems likely. It may be possible that pyrilamine acts indirectly

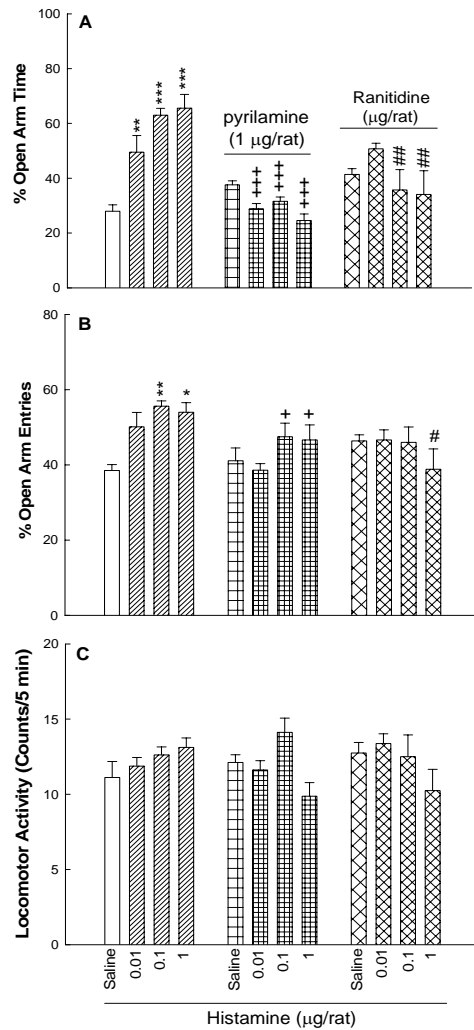


Fig. 4

Fig. 4. The effects of intra-NAc injection of histamine receptor antagonists on histamine-induced anxiolytic response. Rats were injected with saline (0.6 µl/rat), pyrilamine (1 µg/rat) or ranitidine (1 µg/rat) 5 min before injection of saline or histamine (0.01, 0.1 and 1 µg/rat). The tests were performed 5 min after intra-NAc injections. Each bar is mean±S.E.M. %OAT (A), %OAE (B) or locomotor activity (C).

N=8, *P<0.05, **P< 0.01, ***P< 0.001, when compared to the saline treated rats. +P< 0.05, +++P< 0.001, when compared to respective control group. #P< 0.05, ##P<0.01, when compared to respective control group.

through acetylcholine or 5-HT receptor mechanisms and alters anxiety-related behaviors. This issue of the antagonist response needs more experiments to be clarified. Furthermore, there are reports showing either anxiogenic or anxiolytic effects following the administration of H₁ and H₂ blockers applied to different brain regions. It has been reported that intra-nucleus basalis magnocellularis administration of both H₁ receptor antagonist, chlorpheniramine and the H₂ receptor antagonist ranitidine reduce anxiety in rats (7).

In contrast, Santos et al. (21) showed that administration of ranitidine into the periaqueductal gray and inferior colliculus induced fear-like behaviors. Thus, the authors reported a significant difference between the effects of H₂ receptor blockade in the periaqueductal gray and inferior colliculus. Such controversial results have also been reported for histamine. Our data show that histamine did not impair the locomotor activity. However, only a higher dose of pyrilamine in combination with histamine decreased locomotion. Therefore, the influence of locomotion in the response induced by the drugs cannot be involved. In conclusion, if modulation of anxiety by histamine is the case as suggested by other investigators (32), one may suggest that the antagonists are also involved in the modulation mechanism by histamine.

Acknowledgments

The authors would like to thank Dr. Touraj Nayer-Nouri for his valuable assistance

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