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# Histaminergic system of the lateral septum in the modulation of anxiety-like behaviour in rats

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**Abstract**

The central histaminergic system is known to have modulatory influence on anxiety-related behaviour both in animals and humans through histamine $H_1$ and/or $H_2$ receptors. In the present study, the effects of intra-lateral septal microinjections of histaminergic agents on anxiety-related behaviours in male Wistar rats have been investigated. 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. Intra-lateral septal administration of histamine (0.5 and 1 μg/rat) decreased the percentage of open arm entries and open arm time but not locomotor activity, showing an anxiogenic response. The intra-lateral septal injections of different doses of the histamine H1 receptor antagonist, pyrilamine (5, 10 and 20 μg/rat) or the histamine H2 receptor antagonist, ranitidine (5, 10 and 20 μg/rat) could not significantly alter the anxiety-like parameters in the plus-maze test. However, intra-lateral septal injections of different doses of pyrilamine (10 and 20 μg/rat) or ranitidine (10 and 20 μg/rat) significantly reversed histamine (1 μg/rat)-induced anxiogenic effect. The results may indicate that the histaminergic system of lateral septum modulate anxiety-like behaviour through histamine H1 and H2 receptors.


*Keywords:* Histamine; Pyrilamine; Ranitidine; Anxiety; Elevated plus-maze; (Rat)

## 1. Introduction

Our previous studies indicate that histaminergic system in the central amygdala (Zarrindast et al., 2005b), the dorsal (Zarrindast et al., 2006) and ventral hippocampus (Rostami et al., 2006) are involved in mediating of anxiety-like behaviour. Moreover, several lines of evidence suggested that histaminergic system of the nucleus accumbens (Orofino et al., 1999), inferior colliculus, periaqueductal gray (Santos et al., 2003) and nucleus basalis magnocellularis (Privou et al., 1998) may have an important role in the modulation of anxiety. On the other hand, anxiety-related stress may release histamine (Yoshiotomi et al., 1985). Histamine acts through the interaction with presynaptic ($H_3$) and post-synaptic ($H_1$, $H_2$, and $H_4$) receptors (For review see Brown et al., 2001). From the existing studies it may be concluded that different histamine receptors ($H_1$, $H_2$ and $H_3$) may be involved in mediating of anxiety-like behaviour. The impetus for the suggestion that the histamine $H_1$ receptors are involved in anxiogenic effects came from observations of Yanai and coworkers (1998). They have shown that mice lacking histamine $H_1$ receptors are significantly less anxious than wild-type mice in the elevated plus-maze. It has also been suggested that the stimulation of histamine $H_1$ receptors has an anxiogenic effect (Yuzurihara et al., 2000b). Furthermore, the anxiolytic effects have been reported by microinjection of chlorphenir-amine, the histamine $H_1$ receptor antagonist or ranitidine, the histamine $H_2$ receptor antagonist into the nucleus basalis magnocellularis region (Privou et al., 1998). In contrast, intra-nucleus accumbens administration of histamine decreased fear-

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like behaviour in the elevated plus-maze (Alvarez et al., 2001), suggesting that histamine effects is strongly dependent on the site of the drug administration. The histamine $H_3$ receptors that regulate the release and synthesis of neuronal histamine as a presynaptic autoreceptor (Arrang et al., 1987a,b) are also involved in the modulation of anxiety (Imaizumi and Onodera, 1993).

The lateral septum that is considered to interconnect with a number of limbic, diencephalic, and midbrain regions plays a critical role in the regulating processes related to mood and motivation (For review see Sheehan et al., 2004). The lateral septum may be involved in the expression of fear and anxiety on the basis of lesion studies (Treit et al., 1993; Menard and Treit, 1996). Furthermore, microinjection of the benzodiazepine anxiolytic midazolam (Pesold and Treit, 1996) or the $GABA_A$ receptor agonist, muscimol (Degroot et al., 2001) into the lateral septum produced an anxiolytic effect through facilitation of the lateral septum GABAergic transmission. On the other hand, intra-lateral septal injections of morphine decreased open-arm exploration in the elevated plus-maze, indicating an anxiogenic response (Le Merrer et al., 2006). It has been reported that morphine is able to release histamine in the brain (Brake and Hough, 1992). There is a close relationship between opioidergic and histaminergic system in some pharmacological effects of morphine. For example, our previous studies showed that histamine receptors may mediate morphine-induced decrease in water intake (Eidi et al., 2003) or morphine-induced decrease in memory retention of passive avoidance learning (Zarrindast et al., 2002, 2005a). Moreover, the administration of the histamine $H_2$ receptor antagonist, zolantidine potentiated the rewarding effect of morphine (Suzuki et al., 1995).

Our previous studies showed that intra-central amygdala injections of histamine-induced anxiogenic effects (Zarrindast et al., 2005b). Considering different sites of the brain which are involved in the histamine-induced anxiety-like behaviour, we hypothesis that the lateral septum could represent another brain site for the histaminergic system in the modulation of anxiety by morphine. Thus the lateral septum was chosen for our investigation because of its role in anxiety. Since no studies have been conducted examining the modulation of lateral septal histaminergic system in anxiety-like behaviour, the purpose of the present study is to elucidate the effects of histamine and the histamine $H_1$ or $H_2$ receptor antagonist microinjected into the lateral septum and their possible roles in anxiety-related behaviour using the elevated plus-maze test.

## 2. Materials and methods

### 2.1. Animals

Male wistar rats (Pasteur Institute; Tehran, Iran) weighing 220±20 g at the time of surgery were used. The animals were housed four/cage, in a colony room with a 12/12-h light/dark cycle (7:00–19:00 lights on) at 22±1 °C. They had free access to food and tap water except during the time of experiments. All animals were allowed to adapt to the laboratory conditions for at least 1 week before surgery. Rats were handled about 5 min each day prior to behavioural testing. Each animal was used once only. Eight animals were used in each group of experiments. A total number of 160 animals were used in the experiments. All procedures were carried out in accordance with institutional guidelines for animal care and use.

### 2.2. Stereotaxic surgery and microinjections

Rats were anesthetized intraperitoneally with ketamine hydrochloride (50 mg/kg) and xylazine (4 mg/kg) and placed in a Stoelting stereotaxic instrument (Stoelting Co, Illinois, USA). Stereotaxic coordinates for injection into the lateral septum (intra-lateral septal) was: +0.2 anterior to bregma, +0.8 mm lateral to the midline, and 4.8 mm ventral of the dorsal surface of the skull, according to Paxinos and Watson (1986). A stainless steel guide cannula (22-gauge) was unilaterally implanted in the lateral septum, 1.5 mm above the site of injection. It was then fixed to the skull with acrylic dental cement. To prevent clogging, the stainless steel stylet (27 gauge) was placed in the guide cannula until the animal was given the lateral septum injection. The animals were allowed 7 days to recover before the test. For drug infusion, the stylet was withdrawn and replaced by the injection unit (27-gauge stainless steel tubing), terminating 1.5 mm below the tip of the guide. Each injection unit has been connected by polyethylene tubing to 1-μl Hamilton syringe. Animals received an injection of 1 μl of each solution over a 60 s period (1 μl/rat). The inner cannula was left in place for an additional 60 s to allow diffusion of the solution and to reduce the possibility of reflux.

### 2.3. Apparatus

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 have no side or end walls (open arms; 50×10 cm). The other two arms have side walls and end walls, but are open on the top (closed arms; 50×10×40 cm). Where the four arms intersect, there is a square platform of 10×10 cm. The maze was elevated 50 cm above the floor level. The room was illuminated by a 60-W bulb 1.5 m above the apparatus.

### 2.4. Behavioural testing

The method is the same as described previously (Zarrindast et al., 2001). Seven days after implantation, the effects of intra-lateral septal injection of drugs were tested in the elevated plus-maze. On the day of experiments, rats were kept in the soundproof room for at least an hour before the beginning of treatments. For testing, rats were individually placed in the center of the maze facing a closed arm and allowed 5 min of free exploration. The number of entries into open arms, the number of entries into closed arms, and the total time spent in the open arms and total time spent in the closed arms were measured. Entry was defined as all four paws in the arms. The percentage of open arm time and open arm entries as the standard anxiety indices (Rodgers and Johnson, 1995) were calculated as follow: (a) % open arm time (the ratio of time spent in the open arms to

total time spent in any arms × 100); (b) % open arm entries (the ratio of entries into open arms to total entries × 100). (c) Total closed arm entries were measured as a relative pure index of locomotor activity (Rodgers and Johnson, 1995). The apparatus was wiped clean with water and dried after each subject. All tests were performed between 9 a.m. and 1 p.m. of the light phase of the light/dark cycle.

### 2.5. Drugs

The drugs used in the present study were histamine dihydrochloride, ranitidine hydrochloride (Sigma Chemical Co., USA) and pyrilamine maleate (Osve, Tehran, Iran). All drugs were dissolved in sterile 0.9% saline, just before the experiment. The drugs were unilaterally injected in a volume of 1 μl in the site (1 μl/rat). Total doses of the drugs were expressed as μg/rat. Control animals received 0.9% saline.

### 2.6. Experimental design

#### 2.6.1. Experiment 1: effects of histamine on anxiety-like behaviour

The animals received saline (1 μl/rat) or histamine (0.1, 0.5 and 1 μg/rat). The test session was performed 5 min after intra-lateral septal injections. % open arm time, % open arm entries and locomotor activity were measured as described in the method section (Fig. 1).

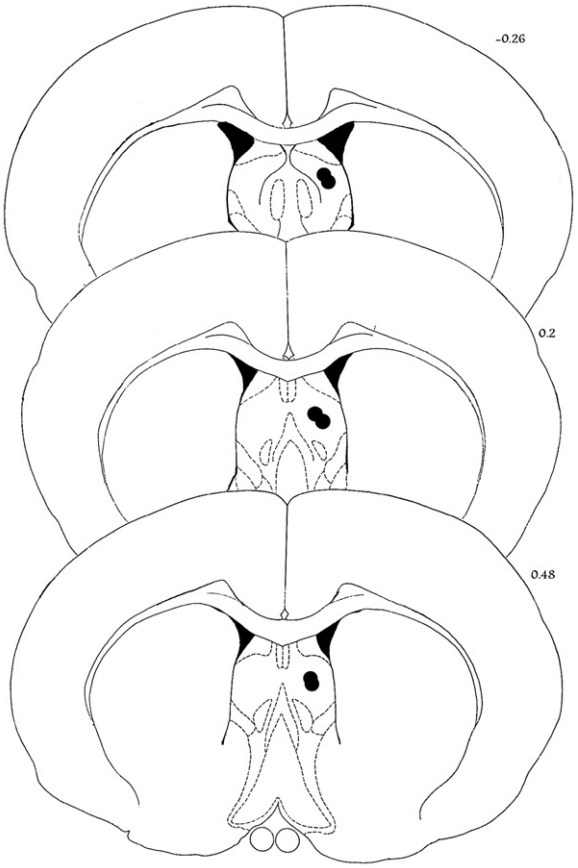


Fig. 1. Schematic illustrations of coronal sections of the rat brain showing the approximate location of the lateral septal injection sites in the experiments. The numbers indicate A–P coordinates relative to bregma. Atlas plates adapted from Paxinos and Watson (1986).

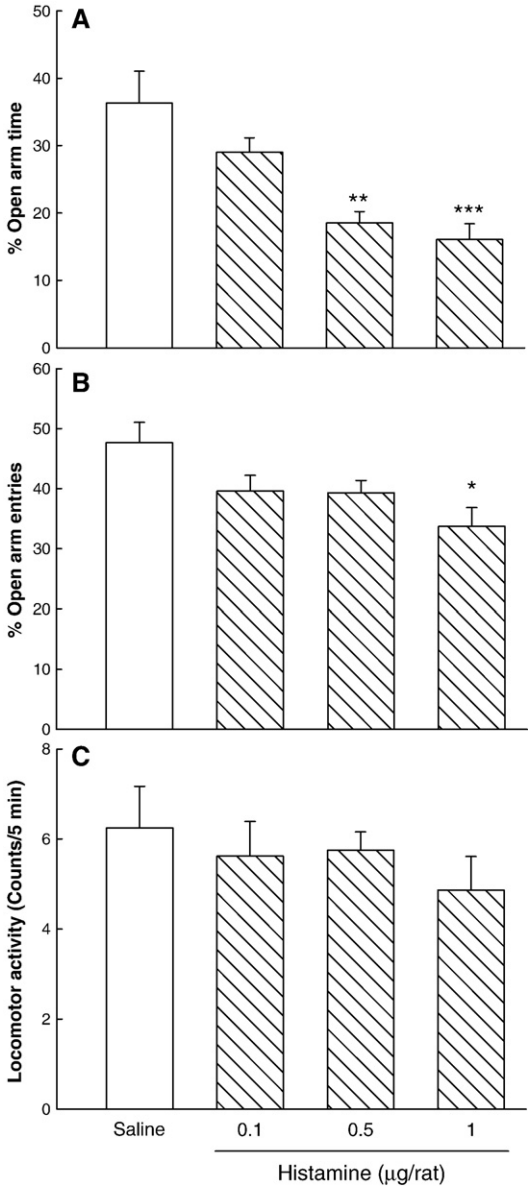


Fig. 2. The effects of intra-lateral septal injection of histamine on anxiety-like behaviour. Rats were injected saline (1 μl/rat) or histamine (0.1, 0.5 and 1 μg/rat). The test was performed 5 min after intra-lateral septal injections. Each bar is mean ± S.E.M. of 8 animals. % open arm time (A), % open arm entries (B) or locomotor activity (C). *$P<0.05$, **$P<0.01$, ***$P<0.001$, compared with the saline treated rats.

#### 2.6.2. Experiment 2: effects of pyrilamine alone or with histamine on anxiety-like behaviour

In this experiment, four groups of animals received saline (1 μl/rat; intra-lateral septal) or the different doses of the histamine $H_1$ receptor antagonist, pyrilamine (5, 10 and 20 μg/rat; intra-lateral septal). Four other groups of animals received saline (1 μl/rat) or the different doses of pyrilamine (5, 10 and 20 μg/rat) 5 min before intra-lateral septal injection of histamine (1 μg/rat). The test session was performed 5 min after final

intra-lateral septal injections. % open arm time, % open arm entries and locomotor activity were measured (Fig. 2).

*2.6.3. Experiment 3: effects of ranitidine alone or with histamine on anxiety-like behaviour*

In this experiment, four groups of rat received saline (1 μl/rat; intra-lateral septal) or the different doses of the histamine $H_2$ receptor antagonist, ranitidine (5, 10 and 20 μg/rat; intra-lateral septal). Four other groups of animals received saline (1 μl/rat) or the different doses of ranitidine (5, 10 and 20 μg/rat) 5 min before intra-lateral septal injection of histamine (1 μg/rat). The test session was performed 5 min after final intra-lateral septal injections. % open arm time, % open arm entries and locomotor activity were measured (Fig. 3).

*2.7. Verification of cannula placements*

After completion of the experimental sessions, each animal was killed with an overdose of chloroform. Subsequently, 1.0 μl of ink (1% aquatic methylene blue solution) was injected into the lateral septum by a 27-gauge injection cannula, which projected a further 1.5 mm ventral to the tip of the guide to aid in histological verification. The brains were removed and fixed in a 10% formalin solution 10 days before sectioning. Sections were examined to determine the location of the cannula aimed for the lateral septum. The cannula placement was verified using the atlas of Paxinos and Watson (1986). Data from rats with cannula placement outside the lateral septum were excluded from the analyses.

*2.8. Statistical analysis*

Data were expressed as mean±S.E.M. Analysis of data was performed using one-or two-way analysis of variance (ANOVA). 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.

## 3. Results

*3.1. Histology*

Fig. 1 shows the approximate point of the drug injections in the lateral septum. The histological results were plotted on the representative section taken from the rat brain atlas of Paxinos and Watson (1986). Data from the animals with the injection sites located outside the lateral septum were not used in the analysis.

*3.2. Effects of histamine on anxiety-like behaviour*

Fig. 2 shows the effects of intra-lateral septal injections of histamine on anxiety-related parameters in the elevated plus-maze. A one-way ANOVA revealed that histamine decreased % open arm time [$F(3,28)=9.92$, $P<0.001$] and % open arm entries [$F(3,28)=3.20$, $P<0.05$] indicating an anxiogenic response by histamine. No significant change in the locomotor activity was observed following administration of histamine [$F(3,28)=0.60$, $P>0.05$].

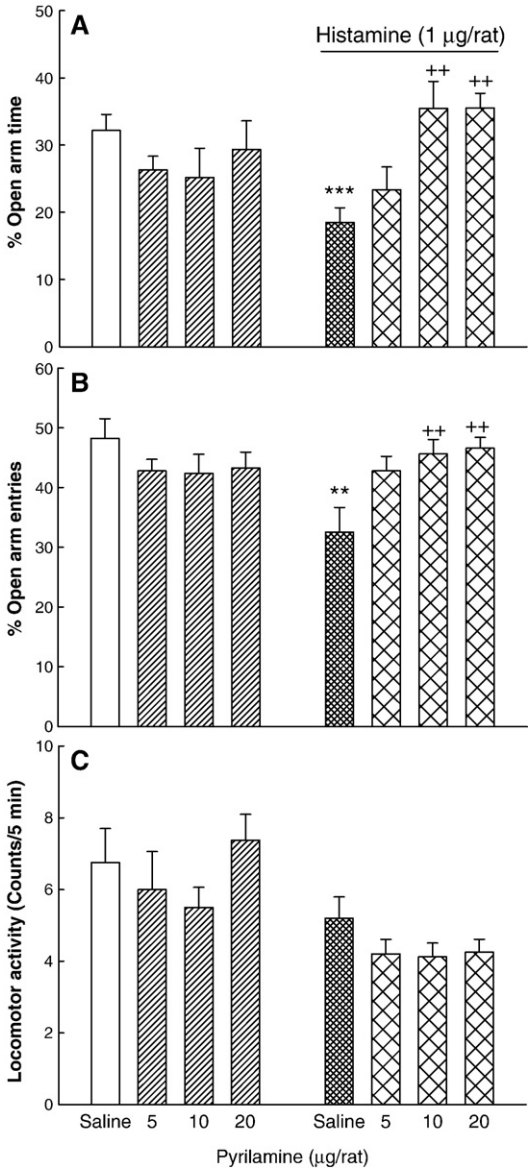


Fig. 3. The effects of intra-lateral septal injections of pyrilamine alone or with histamine on anxiety-like behaviour. Four groups of animals received saline (1 μl/rat) or pyrilamine (5, 10 and 20 μg/rat) and another four groups received saline or pyrilamine 5 min before histamine (1 μg/rat). The test was performed 5 min after intra-lateral septal injections. Each bar is mean±S.E.M. of 8 animals. % open arm time (A), % open arm entries (B) or locomotor activity (C). **$P<0.01$, ***$P<0.001$, compared with the saline treated rats. ++$P<0.01$, compared with the histamine treated rats.

*3.3. Effects of pyrilamine alone or with histamine on anxiety-like behaviour*

The effects of pyrilamine alone or with histamine on anxiety have been shown in Fig. 3. Two-way ANOVA indicated an interaction between the effects of pyrilamine (5, 10 and 20 μg/rat, intra-lateral septum) alone and pyrilamine plus histamine (1 μg/kg) on anxiety-like behaviour. For % open arm time

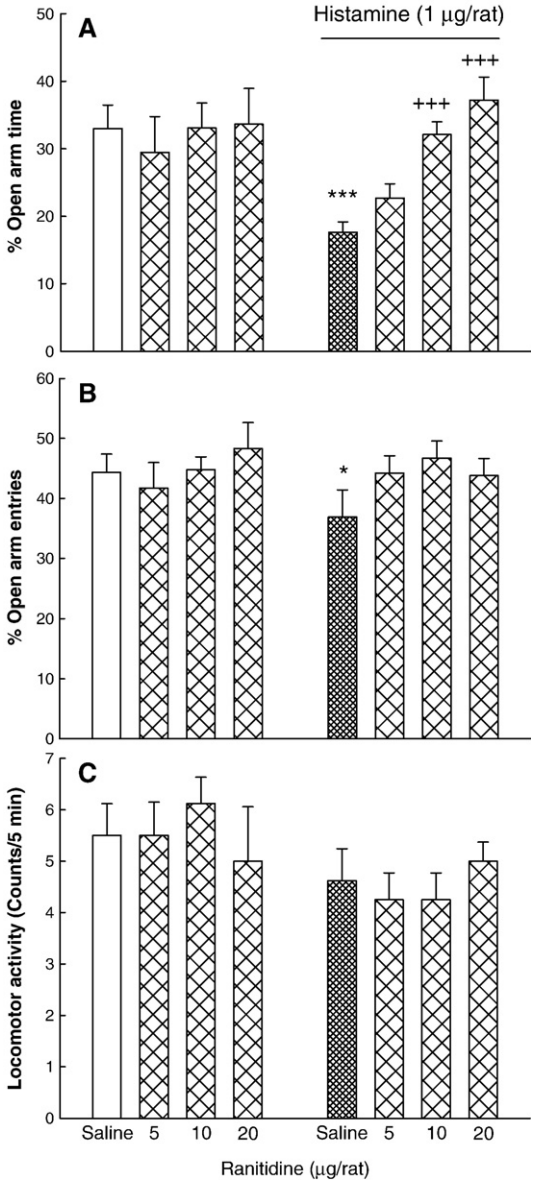


Fig. 4. The effects of intra-lateral septal injections of ranitidine alone or with histamine on anxiety-like behaviour. Four groups of animals received saline (1 μl/rat) or ranitidine (5, 10 and 20 μg/rat) and another four groups received saline or ranitidine 5 min before histamine (1 μg/rat). The tests were performed 5 min after intra-xlateral septal injections. Each bar is mean±S.E.M. of 8 animals. % open arm time (A), % open arm entries (B) or locomotor activity (C). *$P<0.05$, ***$P<0.001$, compared with the saline treated rats. +++$P<0.001$, compared with the histamine treated rats.

[$F_{\text{Treatment}}(1,56)=0.54$, $P>0.05$; $F_{\text{Dose}}(3,56)=3.0$, $P<0.05$; $F_{\text{Treatment}\times\text{Dose interaction}}(3,56)=4.70$, $P<0.01$]. For % open arm entries [$F_{\text{Treatment}}(1,56)=1.30$, $P>0.05$; $F_{\text{Dose}}(3,56)=0.97$, $P>0.05$; $F_{\text{Treatment}\times\text{Dose interaction}}(3,56)=5.30$, $P<0.01$]. For locomotor activity [$F_{\text{Treatment}}(1,56)=16.11$, $P<0.001$; $F_{\text{Dose}}(3,56)=1.35$, $P>0.05$; $F_{\text{Treatment}\times\text{Dose interaction}}(3,56)=0.69$, $P>0.05$]. Post-hoc analysis revealed that intra-lateral septal injections of different doses of pyrilamine in comparison to its saline control could not significantly altered the anxiety-like parameters in the plus-maze test. Further analysis also showed that the anxiogenic effect of 1 μg/rat of histamine was significantly reversed by pyrilamine (10 and 20 μg/rat), given 5 min before histamine. It should be noted that no significant change in the locomotor activity was observed following administration of pyrilamine or pyrilamine plus histamine.

### *3.4. Effects of ranitidine alone or with histamine on anxiety-like behaviour*

The effects of ranitidine alone or with histamine on anxiety have been shown in Fig. 4. Two-way ANOVA revealed an interaction between the effects of ranitidine (5, 10 and 20 μg/rat, intra-lateral septum) alone and ranitidine plus histamine (1 μg/kg) on % open arm time [$F_{\text{Treatment}}(1,56)=3.29$, $P>0.05$; $F_{\text{Dose}}(3,56)=5.06$, $P<0.01$; $F_{\text{Treatment}\times\text{Dose interaction}}(3,56)=3.64$, $P<0.05$]. No significant difference between % open arm entries [$F_{\text{Treatment}}(1,56)=0.63$, $P>0.05$; $F_{\text{Dose}}(3,56)=1.21$, $P>0.05$; $F_{\text{Treatment}\times\text{Dose interaction}}(3,56)=1.03$, $P>0.05$] and locomotor activity [$F_{\text{Treatment}}(1,56)=5.30$, $P<0.05$; $F_{\text{Dose}}(3,56)=0.10$, $P>0.05$; $F_{\text{Treatment}\times\text{Dose interaction}}(3,56)=0.81$, $P>0.05$] induced by ranitidine in the absence or the presence of histamine were observed. Post-hoc analysis revealed that intra-lateral septal injections of histamine decreased %open arm time, but different doses of ranitidine in comparison to its saline control could not significantly altered the anxiety-like parameters in the plus-maze test. Further analysis showed that the anxiogenic effect of 1 μg/rat of histamine was significantly reversed by ranitidine (10 and 20 μg/rat), given 5 min before histamine. It should be noted that no significant change in the locomotor activity was observed following administration of ranitidine or ranitidine plus histamine.

## 4. Discussion

The elevated plus-maze has been employed as an animal model for investigating and understanding the brain areas related to fear/anxiety (Jinks and McGregor, 1997; File et al., 1998; Carobrez et al., 2001; Jardim and Guimaraes, 2001). It seems that different neurochemical mechanisms may modulate anxiety and fear (McNaughton and Corr, 2004). Anxiety is attenuated by anxiolytic drugs, which is controlled by the amygdala and the hippocampus, while fear is resistant to anxiolytics and the periaqueductal gray may be the main site for it (for review see Brandao et al., in press). It should be considered that electric or chemical stimulation of the dorsal periaqueductal gray as a model of panic or the elevated plus-maze are tests that do not implicate the same brain structure and the same system of fear/anxiety.

We previously showed that histamine $H_1$ and $H_2$ receptors in some specific brain sites such as the central amygdala (Zarrindast et al., 2005b), the dorsal (Zarrindast et al., 2006) and ventral hippocampus (Rostami et al., 2006) may be involved in control of anxiety-like behaviour. Since other investigations have suggested that the lateral septum could be another brain site associated with the modulation of anxiety (Gavioli et al., 2002; Le Merrer et al., 2006), the aim of the present study was to investigate the possible role of the lateral septal histaminergic receptors on anxiety-like behaviour, using the elevated plus-maze and the microinjection technique.

The data of the present study show that microinjections of histamine (0.5 and 1 μg/rat) into the lateral septum decreased the percentage of time spent in the open arms (% open arm time) and caused a decrease in the percentage of open arm entries (% open arm entries), whereas no significant effect could be detected for locomotor activity in the elevated plus-maze. These results may indicate that histamine exerts an anxiogenic-like effect in the lateral septum. In agreement with the present data, it has been shown that intra-ventral hippocampal administration of histamine induces an anxiogenic effect (Alvarez and Banzan, 1986; Rostami et al., 2006). Furthermore, Kumar et al. (2007) reported that peripheral administration of L-histidine dose-dependently decreased the exploration time in open arms and the number of entries into open arms, showing an anxiogenic response. It is important to note that either lesions (Menard and Treit, 1996; Pesold and Treit, 1992) or benzodiazepine receptor stimulation (Pesold and Treit, 1996) of the lateral septum increase rats' open-arm exploration in the elevated plus-maze test, producing anxiolytic-like effects. Taken together, the present data may suggest that the lateral septum is strongly involved in mediating anxiety-like behaviour.

Intra-lateral septal administration of different doses of pyrilamine, histamine $H_1$ receptor antagonist or ranitidine, histamine $H_2$ receptor antagonist could not significantly alter the anxiety-like parameters in the plus-maze test. Furthermore, no significant change in the locomotor activity was observed following intra-lateral septal administration of the antagonists. As previously mentioned, intracerebroventricular injections of mepyramine, the histamine $H_1$ receptor antagonist or cimetidine, the histamine $H_2$ receptor antagonist did not affect the anxiety-related behaviours in mice (Yuzurihara et al., 2000a). Furthermore, we previously reported that microinjection of pyrilamine or ranitidine into the central amygdala did not change anxiety-related parameters in the elevated plus-maze (Zarrindast et al., 2005b). The present results may indicate the lack of implication of natural active physiological histaminergic system in the lateral septum. On the other hand, either anxiogenic or anxiolytic effects following microinjections of histamine $H_1$ or $H_2$ receptor antagonists into the different brain site has been reported. Intra-periaqueductal gray or inferior colliculus administration of ranitidine induced fear-like behaviours (Santos et al., 2003). Moreover, intra-ventral hippocampal injections of ranitidine or pyrilamine increased anxiety-related behaviours (Rostami et al., 2006). Privou et al. (1998) implicated that the injections of the $H_1$ and $H_2$ histamine blocker chloropheniramine and ranitidine into the nucleus basalis magnocellularis region exert anxiolytic-like effects in the elevated plus-maze. Thus, it seems that the involvement of histamine $H_1$ and $H_2$ receptors in the modulation of anxiety-like behaviour can be related to the different brain sites.

Considering that the lateral septum lies in close apposition to the lateral ventricles (Gavioli et al., 2002), one may suggest that the effect of histamine is due to the possible leakage of the drug from the lateral septum to lateral ventricles. Our previous study showed that intracerebroventricular injection of histamine-induced anxiogenic-like effect. However, the dose of the drug elicited the maximum response was twenty times more than that used in the present study. On the other hand, pyrilamine and ranitidine showed anxiolytic and axiogenic-like effects respectively in the previous work (Zarrindast et al., 2006). However, neither ranitidine nor pyrilamine by themselves induced any response in the present study.

The present data provides not only evidence that intra-lateral septal injection of histamine has an anxiogenic effects but also shows that it may act through histamine $H_1$ and $H_2$ receptors. Based on this hypothesis, our results showed that intra-lateral septal administrations of different doses of pyrilamine or ranitidine significantly reversed histamine-induced anxiogenic effect without altering locomotor activity. In accordance with the present results, there are reports indicating that intra-ventral hippocampal injections of either pyrilamine or ranitidine reverse anxiogenic response of histamine (Rostami et al., 2006; Orofino et al., 1997). Since, pyrilamine reversed the histamine effects on % open arm time and % open arm entries, the potency of the drug may be more than ranitidine which antagonized the effect of histamine only on % open arm time. Our previous studies also showed that pretreatment with pyrilamine but not ranitidine could significantly reverse the anxiogenic effect of intra-central amygdala injection of histamine (Zarrindast et al., 2005b). There is a report revealing that histamine receptors are existed the most parts of rat brain including at least in the medial septum (Panula et al., 1989). Therefore, based on our present results, one may speculate that histamine acts at these receptor sites and induces angiogenic-like behaviour. Whether these receptors involved in the behaviour individually or interact with other neurotransmitter systems in the lateral septum remains to be evaluated. Since the histamine $H_1$ and $H_2$ receptor antagonists did not induce response by themselves, the existence of a histaminergic system in this site seems unlikely.

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