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The effects of histaminergic agents in the dorsal hippocampus of rats in the elevated plus-maze test of anxiety

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Abstract

High levels of histamine are found in the hippocampus. The central histamine system is involved in many physiological behavioural processes including anxiety-related behaviours both in animals and humans. In the present study, we investigated the effects of intra-hippocampal CA1 (intra-CA1) microinjection of histaminergic agents on anxiety-related behaviours in rats, using elevated plus-maze test of anxiety. Intra-CA1 administration of histamine (at the dose of 10 μg/rat) increased open arm time (%OAT) and open arm entry (%OAE) but not locomotor activity, thus showing an anxiolytic response. Intra-CA1 microinjection of pyrilamine (H_1 receptor antagonist; at the doses of 10, 20 and 40 μg/rat) in combination with histamine (10 μg/rat) showed a decrease in the %OAT and %OAE. Higher dose of the antagonist (40 μg/rat) by itself increased both %OAT and %OAE, but not locomotor activity, indicating an anxiolytic effect. Intra-CA1 microinjection of ranitidine (H_2 receptor antagonist), at the doses of 10, 20 and 40 μg/rat, also reduced the histamine response. Furthermore, the H_2 receptor antagonist by itself reduced %OAT and %OAE without affecting locomotor activity. The results may indicate an anxiogenic effect for the antagonist. Our results showed that histamine may modulate anxiety via H_1 and H_2 receptors in the CA1 region of hippocampus of the rat.

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

1. Introduction

Histaminergic neurons in the nucleus tuberomammillaris of the posterior hypothalamus innervate wide parts of the brain and spinal cord (Onodera et al., 1994; Panula et al., 1984; Watanabe et al., 1984; Inagaki et al., 1988). The central histamine system is involved in many central nervous system functions (for review see; Brown et al., 2001). It may have an important role in the modulation of several physiological processes such as learning and memory (Onodera et al., 1998) and novel environment motivated exploration (Zimmermann et al., 1999; Ikarashi and Yuzurihara, 2002). It has also been shown that lack of neuronal histamine increases emotional reactivity to aversive stimulation, which might increase learning motivation in avoidance and escape tasks (Dere et al., 2004). Behavioural changes in the H_3 receptor-deficient mice have also been suggested (Toyota et al., 2002). Moreover, the involvement of the histaminergic system in the modulation of anxiety-like behaviours in animals has been suggested previously (Privou et al., 1998; Malmberg-Aiello et al., 2002; Zarrindast et al., 2005b). Histamine is also involved in the morphine-state dependency (Zarrindast et al., 2005a), and its administration into central amygdala induces an anxiogenic response (Zarrindast et al., 2005b). A physiological study reported that destruction of the rat tuberomammillary rostro-ventral E-2 sub-region, from which histaminergic fibers arise, can induce anxiolytic-like effects in the elevated plus-maze test (Frisch et al., 1998). Anxiety-related stress may release

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histamine (Yoshiotomi et al., 1985). It has been shown that α-fluoromethylhistidine, an inhibitor of the histidine decarboxylase, the enzyme responsible for histamine biosynthesis in the brain, was able to attenuate the severity of restraint stress-induced gastric ulcerogenesis (Ray et al., 1992). Anxiolytic drugs such as diazepam, benzodiazepine, and buspirone, a serotonin (5-HT_{1A}) receptor agonist have also been found to decrease the turnover rate of brain histamine in mice and rats (Oishi et al., 1986, 1992). Moreover, it has been reported that benzodiazepines can prevent the decrease in hypothalamic histamine levels elicited by restraint and cold exposure (Privou et al., 1998).

Histamine acts through different receptor subtypes. Histamine H_1 receptors, are a G-protein family of receptors whose activation leads to stimulation of phospholipase C and also increase in cAMP levels (Leurs et al., 1994). High densities of H_1 receptors are present in the limbic system including several hippocampal areas (Brown et al., 2001). H_2 receptors are G-protein coupled receptors (Taiffort et al., 1995), whose activation leads to enhanced production of cAMP (Baudry et al., 1975; Hegstrand et al., 1976). The histamine H_3 receptor is an autoreceptor, regulating the release and synthesis of histamine (Arrang et al., 1983). The receptors are present in all areas and layers, with high density rostally and in the deep layers (IV–VI) of the cerebral cortex (Pollard et al., 1993). It has been reported that mice lacking histamine H_1 receptors showed prolonged transfer latency in the light/dark box test indicating that mutant mice were less fearful than wildtype mice (Yanai et al., 1998a). However, the hippocampal formation receives only a weak to moderate histaminergic innervation. Histamine has strong effects on excitability in the hippocampus by acting on histamine H_2 receptors (Haas and Konnerth, 1983; Haas and Greene, 1986; Greene and Haas, 1990) and also affects the hippocampal formation indirectly through its effects on the medial septum, which provides the cholinergic input to the hippocampus. It seems that histamine strongly depolarizes cholinergic septal neurons (Gorelova and Reiner, 1996), mainly through histamine H_1 receptors, which should lead to an increased acetylcholine release in the hippocampus. However, the evidence on this point is somewhat contradictory (Brown et al., 2001). On the other hand, it has been reported that histamine H_3 receptors mediate histamine effects on spatial learning and memory (Rizk et al., 2004) and are also involved in the modulation of anxiety through inhibition of histamine synthesis and increased neuronal histamine release (Imaizumi and Onodera, 1993). Although, the effect of histamine and related compounds has been evaluated in some brain regions such as hippocampus (Alvarez and Banzan, 1986), nucleus accumbens (Alvarez et al., 1999), inferior colliculus, periaqueductal gray (Santos et al., 2003) and nucleus basalis magnocellularis (Privou et al., 1998), the effects of histaminergic system on anxiety in the CA1 of hippocampus have not been evaluated yet. Based on the previous findings, in the present study, effects of histamine and histamine H_1 and H_2 receptor antagonists microinjected into the CA1 of hippocampus and their possible roles in the modulation of anxiety-related behaviours using elevated plus-maze test of anxiety in rats have been studied.

2. Materials and methods

2.1. Animals

Male Wistar rats from Pasteur Institute (Tehran, Iran), weighing 220–270 g at the time of surgery, were used. Animals were housed four per cage, in a room with a 12:12 h light/dark cycle (lights on 07:00 h) and controlled temperature (23±1 °C). Animals had access to food and water *ad libitum* and they were allowed to adapt to the laboratory conditions for at least 1 week before surgery. Rats were handled about 3 min each day prior to behavioural testing. All experiments were performed between 9:00 h and 12:00 h and each rat was tested only once. Seven animals were used in each group of experiments. A total number of 140 animals were used in the experiments. The study was approved by the Ethics Committee of the Tarbiat Moallem University which corresponds to the national guidelines for animal care and use.

2.2. Stereotaxic surgery, microinjections and histology

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 CA1 regions of the dorsal hippocampus were: −3 to −3.5 mm (depending on body weight) anterior to bregma, ±1.8 to 2 mm lateral to the midline, and −2.8 to −3 mm ventral of the dorsal surface of the skull, according to Paxinos and Watson (1986). The stainless steel guide cannula (22-gauge, Supa; Iran) was implanted bilaterally in the right and left CA1, 1 mm above the site of injection. It was then fixed to the skull with acrylic dental cement. The animals were allowed 7 days to recover before the test. Intra-CA1 injections were performed by means of an internal cannula (27-gauge, Supa; Iran), terminating 1 mm below the tip of the guides and connected by polyethylene tubing to a 1-μl Hamilton syringe. Animals received bilateral injection of 0.5 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. Based on the method used before (Ahn et al., 2001), in case of two injections, 0.5 μl of each drug and with 5 min interval was used. At the end of the study, injecting 1 μl/rat of 1% methylene blue solution and determination of the injected dye in the right and left CA1, identified and verified the injection site. However, it is likely that the drugs diffuse into other hippocampus subfields.

2.3. Behavioural test (elevated plus-maze)

The method is the same as described previously (Rezayat et al., 2005). 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 to a height of 50 cm. Seven days after implantation, the effects of intra-CA1 injection of drugs were tested in the elevated plus-maze. At least 1 h before testing, rats

were placed in the room used for the experiments. Animals were randomly allocated to treatment conditions and tested in counterbalanced order. After injection of the drugs, the rats were placed in a wooden arena (50 × 50 × 35 cm) for 5 min prior to maze testing. Then each animal was 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 entries and open arm time as the standard anxiety indices (Rodgers and Johnson, 1995) was calculated as follows: (a) %OAT (the ratio of times spent in the open arms to total times spent in any arms × 100); (b) %OAE (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).

2.4. Drugs

The drugs used in the present study were pyrilamine maleate (Osve, Tehran, Iran), ranitidine hydrochloride and histamine dihydrochloride (Sigma Chemical Co., USA). All drugs were dissolved in sterile 0.9% saline. The drugs were injected in a volume of 0.5 μl in each side of CA1 (1 μl/rat). Total doses of the drugs used in both sides, were expressed as μg/rat. The drug doses and the time course of the drugs' actions used in our experiments are based on a pilot study and those used previously (Privou et al., 1998; Alvarez et al., 2001; Zarrindast et al., 2005a,b).

2.5. Drug treatments

2.5.1. Experiment 1: effects of histamine on anxiety

Four groups of rats received saline (0.5 μl/side; 1 μl/rat) or 3 different doses of histamine (1, 5 and 10 μg/rat; 0.5 μl/side). The test session was performed 5 min after intra-CA1 injections. %OAT, %OAE and locomotor activity were measured as described in the Materials and methods section.

2.5.2. Experiment 2: effects of pyrilamine alone or with histamine on anxiety

In this experiment, four groups of rats received saline (1 μl/rat; 0.5 μl/side) or 3 different doses of pyrilamine (10, 20 and 40 μg/rat; 0.5 μl/side). Four other groups of rats received saline (1 μl/rat) or 3 different doses of pyrilamine (10, 20 and 40 μg/rat; 0.5 μl/side) 5 min before intra-CA1 injection of histamine (10 μg/rat; 0.5 μl/side). The test session was performed 5 min after final intra-CA1 injections. %OAT, %OAE and locomotor activity were measured.

2.5.3. Experiment 3: effects of ranitidine alone or with histamine on anxiety

In this experiment, four groups of rats received saline (0.5 μl/side) or 3 different doses of ranitidine (10, 20 and 40 μg/rat; 0.5 μl/side). Four other groups of rats received saline (1 μl/rat; 0.5 μl/side) or 3 different doses of ranitidine (10, 20 and 40 μg/rat; 0.5 μl/side) 5 min before intra-CA1 injection of histamine (10 μg/rat; 0.5 μl/side). The test session was performed 5 min after final intra-CA1 injections. %OAT, %OAE and locomotor activity were measured.

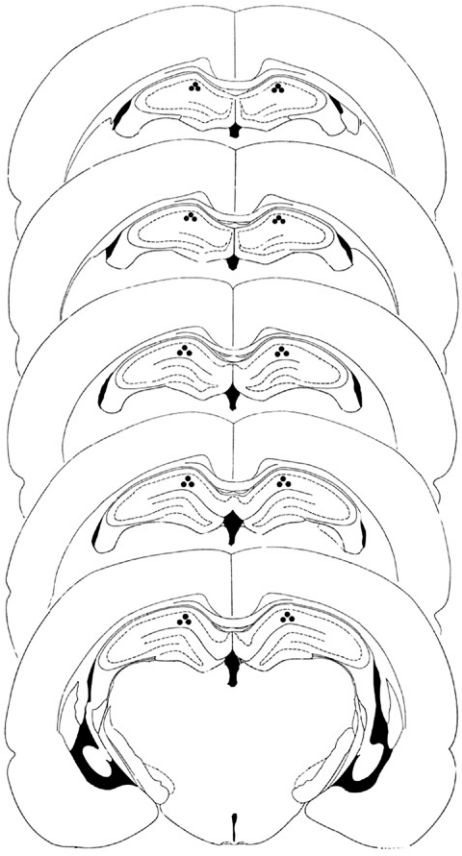

Fig. 1. The approximate placements of injection cannulae within the CA1 were indicated by the circles. Representative sections of the CA1 (−2.56, −2.8, −3.3, −3.6, −3.8 mm from bregma) were taken from the rat brain atlas of Paxinos and Watson (1986).

2.6. Statistical analysis

Since data displayed normality of distribution and homogeneity of variance, comparisons between groups were made with one- or two-way analysis of variance (ANOVA) followed by Tukey-test. A difference with $P<0.05$ between the experimental groups was considered statistically significant.

3. Results

3.1. Histology

Fig. 1 illustrates the approximate point of the drug injections in the CA1 from some animals. The histological results were plotted on representative sections taken from the rat brain atlas of Paxinos and Watson (1986). Data from the animals with injection sites located outside the CA1 were not used in the analysis.

3.2. Effects of histamine on anxiety

Fig. 2 shows the effects of histamine (1, 5 and 10 μg/rat) on anxiety-related parameters in the elevated plus-maze. A one-

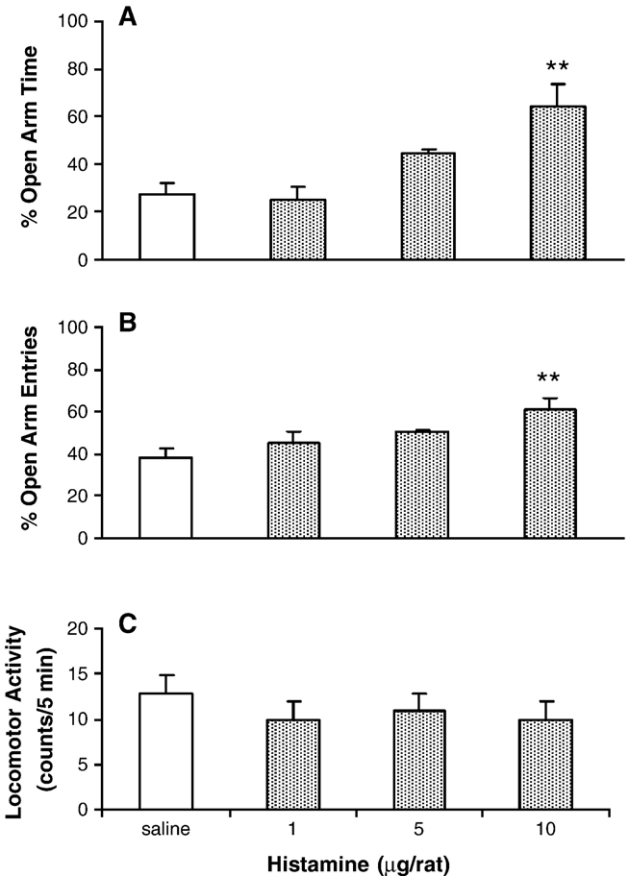

Fig. 2. The effects of intra-CA1 injection of histamine on anxiety. Rats were injected with saline (1 μl/rat; 0.5 μl/ bilateral) or histamine (1, 5 and 10 μg/rat; 0.5 μl bilateral). The test was performed 5 min after intra-CA1 injections. Each bar is mean±S.E.M. %OAT (A), %OAE (B) or locomotor activity (C). $N=7$. $^{**}P<0.01$, when compared to the saline treated rats.

way ANOVA revealed that histamine at the dose of 10 μg/rat increased %OAT [F (3,24)=8.7, $P<0.001$] and %OAE [F (3,24)=5.3, $P<0.01$] indicating an anxiolytic response by histamine. No significant change in the locomotor activity was observed following administration of histamine [F (3,24)=0.8, $P>0.05$]. The data indicates that histamine administration into CA1 is able to induce an anxiolytic effect.

3.3. The effects of pyrilamine alone or with histamine on anxiety

The effects of pyrilamine alone or with histamine on anxiety are shown in Fig. 3. A two-way ANOVA revealed a significant difference between the responses induced by pyrilamine in the absence or presence of histamine. For %OAT [F_{Drug} (1,48)=3.9, $P<0.05$; F_{Dose} (3,48)=4.1, $P<0.05$; $F_{Drug\times Dose}$ (3,48)=11.8, $P<0.0001$]. For %OAE [F_{Drug} (1,48)=4.4, $P<0.05$; F_{Dose} (3,48)=5.7, $P<0.01$; $F_{Drug\times Dose}$ (3,48)=8.1, $P<0.0001$]. For locomotor activity [F_{Drug} (1,48)=0.8, $P>0.05$; F_{Dose} (3,48)=0.7, $P>0.05$; $F_{Drug\times Dose}$ (3,48)=2.5, $P>0.05$]. Post hoc analysis revealed that pyrilamine in comparison to its saline control, at the dose of 40 μg/rat increased %OAT and %OAE but not locomotor activity. Histamine in comparison to saline control group, at the dose of 10 μg/rat (0.5 μl/side) also significantly increased %OAT and %OAE but not locomotor activity. These findings show that pyrilamine and histamine increase anxiolytic-like behaviour following intra-CA1 injection. Moreover, post hoc analysis showed that pyrilamine in combination with histamine decreased %OAT and %OAE. No interaction was found between the effects of histamine on locomotor activity and those induced by pyrilamine. Pyrilamine and histamine appear to be acting antagonistically when given together, but the effect of histamine appears to be modulatory rather than anxiolytic per se.

3.4. The effects of ranitidine alone or with histamine on anxiety

The effects of ranitidine alone or with histamine on anxiety are shown in Fig. 4. A two-way ANOVA revealed a significant difference between the responses induced by ranitidine in the absence or presence of histamine. For %OAT [F_{Drug} (1,48)=

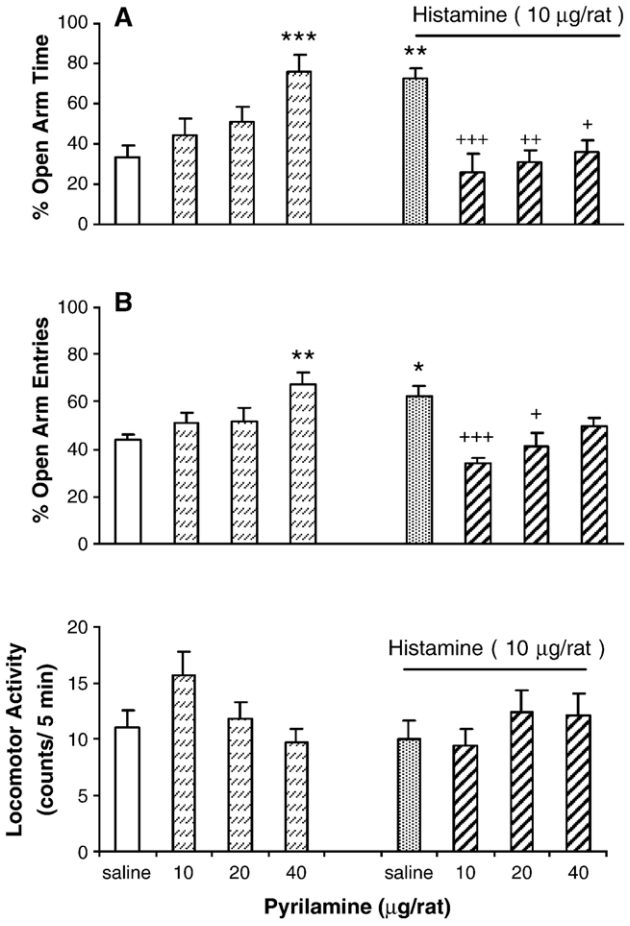

Fig. 3. The effects of intra-CA1 injection of pyrilamine alone or with histamine on anxiety. Rats were injected with saline (1 μl/rat) or pyrilamine (10, 20 and 40 μg/rat; 0.5 μl bilateral) alone or 5 min before injection of histamine (10 μg/rat; 0.5 μl bilateral). The tests were performed 5 min after intra-CA1 injections. Each bar is mean±S.E.M. %OAT (A), %OAE (B) or locomotor activity (C). $N=7$. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ when compared to the saline treated rats. $^{+}P<0.05$, $^{++}P<0.01$ and $^{+++}P<0.001$ when compared to the saline/ histamine treated rats.

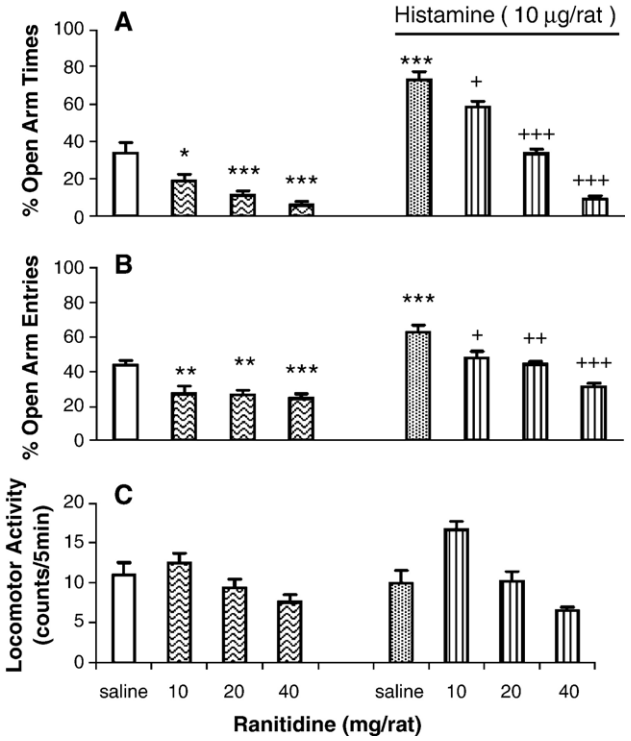

Fig. 4. The effects of intra-CA1 injection of ranitidine alone or with histamine on anxiety. Rats were injected with saline (1 µl/rat) or ranitidine (10, 20 and 40 µg/rat; 0.5 µl bilateral) alone or 5 min before injection of histamine (10 µg/rat; 0.5 µl bilateral). The tests were performed 5 min after intra-CA1 injections. Each bar is mean±S.E.M. %OAT (A), %OAE (B) or locomotor activity (C). $N=7$. *$P<0.05$, **$P<0.01$, and ***$P<0.001$, when compared to the saline treated rats. $^{+}P<0.05$, $^{++}P<0.01$ and $^{+++}P<0.001$ when compared to the saline/histamine treated rats.

117.6, $P<0.0001$; F_{Dose} (3,48)=67.9, $P<0.0001$; $F_{Drug\times Dose}$ (3,48)=12.7, $P<0.0001$]. For %OAE [F_{Drug} (1,48)=53.6, $P<0.0001$; F_{Dose} (3,48)=23.3, $P<0.0001$; $F_{Drug\times Dose}$ (3,48)=2.4, P>0.05]. For locomotor activity [F_{Drug} (1,48)=0.8 $P>0.05$; F_{Dose} (3,48)=13.1, $P<0.0001$; $F_{Drug\times Dose}$ (3,48)=2.0, $P>0.05$]. Post hoc analysis indicate that ranitidine decreases %OAT and %OAE, but not locomotor activity. Histamine at the dose of 10 µg/rat (0.5 µl/side) could significantly increase both %OAT and %OAE but not locomotor activity. These results show an anxiolytic response following administration of histamine into the CA1 while administration of ranitidine induced anxiety-related parameters. Moreover, two-way ANOVA revealed that ranitidine could decrease %OAT, but not %OAE induced by histamine. On the other hand, ranitidine did not affect the locomotor activity in the presence of histamine. These results may imply that ranitidine could not affect all anxiety-related parameters of histamine in the CA1.

4. Discussion

In the present study, involvement of H_1 and H_2 histamine receptor systems in the CA1 of dorsal hippocampal region in the elevated plus-maze has been investigated. The elevated plus-maze is one of the many tests for the identification of anxiolytic or anxiogenic effect of a drug in rodents (Pellow et al., 1985). However, there may be other method such as the Vogel conflict test (Umezu, 1999). Present results show that CA1 microinjection of higher dose of histamine increased %OAT (% Open Arm Times) and %OAE (% Open Arm Entries), the parameters of anxiety-related behaviour, without locomotor impairment in the elevated plus-maze. This may indicate that the higher dose of histamine exerts its anxiolytic effect in CA1 region.

In the present study, H_1 and H_2 receptor antagonists were microinjected into the dorsal region of hippocampus of rats in order to specify the receptor type involved in the histamine response in this region. However, the use of specific H_1- and H_2-receptor agonists may be needed to show the histamine receptor type(s) in this respect. When H_1 histamine receptor antagonist, pyrilamine was challenged against histamine in the present study, a decrease in the %OAT and %OAE was shown, indicating that H_1 histamine receptor in the CA1 region may be involved in anxiety-related behaviour. The present data is opposed to that obtained in our previous study in the amygdala (Zarrindast et al., 2005b), ventral hippocampus (Rostami et al., 2006) and that by Malmberg-Aiello et al. (2002) in which it has been reported that activation of histamine H_1 receptors could reduce the time spent in the lighted compartment of light/dark box, indicating a probable anxiogenic effect for H_1 receptors. It seems possible that based on sites of injections or histamine doses, it causes different effects, or induces a modulatory influence on anxiety-related behaviour (Bannerman et al., 2004), but further experiments may be needed to support this hypothesis. However, another possibility may be that the CA1 region is not the main site for modulation of anxiety-related behaviours, even though, it seems likely that either ventral hippocampus or other sites of hippocampus may be involved in anxiety. Moreover, in agreement with our present data, it has also been reported that microinjection of histamine into the ventral hippocampus of rats decreased fear-like behaviours in the elevated asymmetric plus-maze (Ruarte et al., 1997). In contrast, Alvarez and Banzan (1986) reported an anxiogenic effect following administration of histamine into the ventral hippocampus of the rats. Furthermore, intra-accumbens microinjection of histamine produced a dual effect in asymmetric plus-maze (Alvarez et al., 1999). These findings may suggest that the effect of histaminergic system in the modulation of anxiety is strongly dependent on the site of drug administration. There may be the possibility that the higher dose of histamine used, acts on pre-synaptic H_3 receptors and induces anxiolytic response. The higher dose of pyrilamine also induced anxiolytic response by itself in the present study. Moreover, an important interaction between neuronal histamine and acetylcholine levels and also change in the 5-HT turnover has been suggested (Dere et al., 2004). Furthermore, the H_1 receptor activation by histamine, may also inhibit 5-HT release (Yanai et al., 1998b). Therefore, it may be possible that higher dose of pyrilamine acts indirectly through acetylcholine or 5-HT receptor mechanisms and alters anxiety-related behaviour. This issue of the antagonist response needs more experiments to be clarified. The present results also show that the histamine H_2 receptor antagonist ranitidine, reduced %OAT and %OAE induced by histamine. However, it had no interaction with %OAE induced by histamine. The H_2 receptor antagonist by itself decreased both %OAT and %

OAE. These results indicate that the response of histamine may also be mediated by H_2 receptor system in the CA1 region. Although activation of histaminergic receptors induces anxiolytic effects, the opposite intrinsic behavioural effects of ranitidine and higher dose of pyrilamine used by us, may indicate that the response of histamine in the CA1 is physiological or modulatory. Even though the interaction of the antagonists with other neurotransmitters i.e. 5-HT and acetylcholine should be also considered (Yanai et al., 1998b; Dere et al., 2004). Furthermore, there are reports showing either anxiogenic or anxiolytic effects following administration of H_1 and H_2 blockers applied to different brain regions. It has been reported that intra-nucleus basalis magnocellularis administration of both H_1 receptor antagonist, chlorpheniramine and the H_2 receptor antagonist ranitidine, reduces anxiety in rats (Privou et al., 1998). Santos et al. (2003) showed that administration of ranitidine into the periaqueductal gray and inferior colliculus induced fear-like behaviours. The authors reported a significant difference between the effects of H_2 receptor blockade in the periaqueductal gray and inferior colliculus. Such controversial results have also been reported for histamine. Our data show that pyrilamine could significantly reverse the anxiolytic effect of histamine without impairing the locomotor activity. Only a lower dose of ranitidine but not higher doses in combination with histamine increased locomotion. Therefore, the influence of locomotion in the response induced by the drugs cannot be involved. Alvarez et al. (2001) have shown that behavioural effects of histamine following intra-accumbens administrations are H_1 but not H_2 receptor mediated. However, it has been reported that both pyrilamine and ranitidine could reduce the anxiogenic effect of histamine microinjection into the ventral hippocampus of rats (Orofino et al., 1997). There is a report indicating that H_3 receptor blockade could be devoid of anxiolytic potential (Perez-Garcia et al., 1999). Thus, although in the present study, the effect of H_3 receptor blockade was not investigated, it may however be interesting to be clarified in the future work. In conclusion, based on our results, the H_1 and H_2 receptors in hippocampus may be involved in the modulation of anxiety-related behaviours.

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