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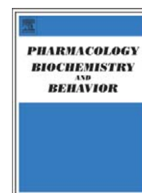

Anxiety-like behavior induced by histaminergic agents can be prevented by cannabinoidergic WIN55,212-2 injected into the dorsal hippocampus in mice

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ABSTRACT

In the present study, we investigate the effects of the histaminergic system and cannabinoid receptor agents on anxiety-related behaviors and their interactions using the hole-board test on mice. Bilateral intra-CA1 administration of the CB1/CB2 receptor agonist, WIN55, 212-2 (0.1–0.5 µg/mouse) did not modify exploratory behaviors in mice. On the other hand, intra-CA1 administration of CB1 receptor antagonist, AM251 (25 and 50 ng/mouse) or histamine, pyrilamine and ranitidine (5–10 µg/mouse) decreased the amount of head-dipping and increased the first head-dip, suggesting an anxiogenic-like response. Furthermore, our present data indicated that the co-administration of WIN55, 212-2 (0.25 µg/mouse) with histaminergic agents, decreased the anxiogenic-like response of an effective dose (5 µg/mouse) of histamine and pyrilamine, but not that of ranitidine. In addition, the results demonstrated that co-administration of an ineffective dose of AM251 (15 ng/mouse) with histaminergic drugs did not alter the response induced by an ineffective dose (3.75 µg/mouse) of either histamine or pyrilamine and ranitidine. In all experiments and doses, locomotor activity and other exploratory behaviors were not significantly changed. In conclusion, our results showed that there is a chance of partial interaction between the cannabinoidergic and the histaminergic systems of the dorsal hippocampus on anxiogenic/anxiolytic-like behaviors in hole-board test.
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1. Introduction

Psychoactive cannabinoid compounds produce a wide range of effects in different species. They may have therapeutic potential in disorders such as anxiety (Haller et al., 2007). The cannabinoid agonists have been shown to alter anxiety-related behavior in mice (Berrendero and Maldonado, 2002; Haller et al., 2004b; Patel and Hillard, 2006; Valjent et al., 2002). Three different CB1, CB2 and CB3 (non-CB1/CB2) cannabinoid receptors have been identified to date (Mackie, 2006; Ryberg et al., 2007). The CB1 receptors are located in the central nervous system such as the cortex, basal ganglia, cerebellum and hippocampus (Davies et al., 2002; Izquierdo and Medina, 1995; Nguyen et al., 1994; Wilson and Nicoll, 2002). CB2 receptors are mostly present in peripheral tissues, mainly in the immune system (Chaperon and Thiebot, 1999), but can also be found in neuronal cells (Brusco et al., 2008; Gong et al., 2006). In addition, a large number of reports describe a third type of cannabinoid receptor (CB3) that couples with the inhibitory G-protein (Vaccani et al., 2005) and decreased glutamatergic transmission in CA1 of the mouse hippocampus (Hajos and Freund, 2002; Hajos et al., 2001).

Furthermore, histamine is a neurotransmitter that functions in both the peripheral and the central nervous systems (Privou et al., 1998; Schwartz et al., 1991; Watanabe et al., 1984). It appears that the amine is involved in sleep–wake cycle, emotion, appetite control, locomotor activity, stress-related behavior, learning, and memory through different histamine receptor types (Brown et al., 2001; Leurs et al., 1994; Malmberg-Aiello et al., 2002; Privou et al., 1998; Schwartz et al., 1991; Zarrindast et al., 2005a). When the drug is administered into the central amygdala or ventral hippocampus, it induces an anxiogenic-like response (Zarrindast et al., 2005a, 2006). There is a report suggesting that the destruction of the rat tuberomammillary rostroventral 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 has also been shown to release histamine (Yamatodani et al., 1985). Histamine functions through different receptor subtypes. The histamine H1 receptors are coupled with G-protein, the activation leads to phospholipase C stimulation and also increases cAMP levels (Leurs et al., 1994). There are densities of these receptors in the limbic system, including several

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hippocampal areas (Brown et al., 2001). Furthermore, H2 receptors are also a family of G-protein receptors (Traiffort et al., 1995) whose activation leads to enhanced production of cAMP (Baudry et al., 1975; Hegstrand et al., 1976). Moreover, the regulation of the release and synthesis of histamine are also mediated by an autoreceptor, histamine H3 receptor subtype (Arrang et al., 1983). Furthermore, the hippocampal formation receives only a weak to moderate histaminergic innervation. Histamine has strong effects on excitability in the hippocampus by acting on histamine H2 receptors (Greene and Haas, 1990; Haas and Greene, 1986; Haas and Konnerth, 1983), and affects the hippocampal formation indirectly through its effects on the medial septum, which provides the cholinergic input to the hippocampus. On the other hand, it has been reported that histamine H3 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).

The activation of the cannabinoid CB1 receptors in hippocampal preparations has shown to inhibit the release of glutamate, acetylcholine, GABA and noradrenaline (Al-Hayani and Davies, 2002; Schlicker and Kathmann, 2001). It has also been demonstrated that histamine strongly depolarizes cholinergic septal neurons (Gorelova and Reiner, 1996), mainly through histamine H1 receptors, which should lead to an increased acetylcholine release in the hippocampus. However, evidence on this point is somewhat contradictory (Brown et al., 2001). Acetylcholine has been suggested to modulate anxiety (Degroot and Treit, 2002) and therefore, in the present study, we investigated the effects of intra-hippocampal CA1 (intra-CA1) microinjection of histaminergic and cannabinoid agents on anxiety-related behaviors in mice, using the hole-board test of anxiety.

2. Materials and methods

2.1. Animals

Male albino NMRI mice (Pasteur Institute; Tehran, Iran) were used, weighing 25–30 g at time of surgery. The animals were kept in an animal house with a 12/12-h light–dark cycle and controlled temperature (22 ± 2 °C). Animals were housed in groups of 10 in Plexiglas cages and food and water were available ad libitum. Ten animals were used in each group. Each animal was used only once. Behavioral experiments were performed during the light phase of the light/dark cycle. All procedures were carried out in accordance with institutional guidelines for animal care and use.

2.2. Cannula guide implantation

Mice were anesthetized with the intra-peritoneal injection of ketamine hydrochloride (50 mg/kg) plus xylazine (5 mg/kg) and placed in a stereotaxic apparatus. The skin was incised and the skull was cleaned. 22-gauge guide cannulae were placed (bilaterally) 1 mm above the intended site of injection according to the atlas of Paxinos and Franklin (2001). Stereotaxic coordinates for the CA1 regions of the dorsal hippocampus were AP: −2 mm from bregma, L: ±1.6 from the sagittal suture and V: −1.5 mm from the skull surface. The cannulae were secured with dental acrylic. Stainless steel stylets (27-gauge) were inserted into the guide cannulae to keep them free of debris. All animals were allowed 1 week to recover from surgery and from the effect of the anesthetic agents.

2.3. Intra-CA1 injections

For drug infusion, the animals were restrained gently by hand; the stylets were removed from the guide cannulae and replaced by 27-gauge injection needles (1 mm below the tip of the guide cannulae). The injection solutions were administered in a total volume of 1 µl/mouse (0.5 µl in each side) over a 60 s period, manually. Injection needles were left in place for an additional 60 s to facilitate the diffusion of the drugs.

2.4. Drugs

The drugs used in the study were WIN55, 212-2 mesylate and AM251 (Tocris, Cookson Ltd., UK), pyrilamine maleate (Osve, Tehran, Iran), ranitidine hydrochloride and histamine dihydrochloride (Sigma Chemical Co., USA). WIN55, 212-2 and AM 251 were dissolved in vehicle (water/dimethylsulfoxide; DMSO; 9/1) solvent and one drop of Tween 80. Pyrilamine, ranitidine and histamine drugs were dissolved in sterile 0.9% saline.

2.5. Apparatus and behavioral test

The hole-board test as a simple method for examining the response of an animal to an unfamiliar environment was first introduced by Boissier and Simon (1962). This test has been used to evaluate emotionality, anxiety and/or responses to stress in animals (Rodriguez Echandia et al., 1987). Different behaviors which can be observed and measured in this test, make possible a comprehensive description of the animal's behavior.

The hole-board apparatus (Borj Sanat Co, Tehran, Iran) consisted of gray Perspex panels (40 cm × 40 cm, 2.2 cm thick) with 16 equidistant holes 3 cm in diameter in the floor made on the basis of the method used previously (Vinade et al., 2003). The board was positioned 15 cm above a table. Animals were placed singly in the center of the board facing away from the observer and head-dip numbers were recorded by photocells arranged below the holes over 5 min. Furthermore, locomotor activity was measured by an observer that was unaware of the treatments measured during the testing phase. For this purpose the ground area hole-board was divided into four equal sized squares. Locomotion was measured as the number of locomotor activity crossings from one square to another. Other behavioral performances such as latency to the first head-dipping, rearing, grooming and defecation were recorded by the experimenter manually during the test.

2.6. Experiment design

Ten animals were used in each experimental group. The experiments were based on previous studies in order to obtain a maximum response (Roohbakhsh et al., 2007; Zarrindast et al., 2005a,b, 2006). The protocol is summarized in Table 1.

2.6.1. Experiment 1: effect of WIN55, 212-2 on exploratory behaviors

Four groups of mice received saline (0.5 µl/side; 1 µl/mouse), vehicle (0.5 µl/side; 1 µl/mouse) or 3 different doses of WIN55, 212-2 (0.25, 0.5 and 1 µg/mouse; 0.5 µl/side). The test session was performed 5 min after intra-CA1 injection of the drug.

2.6.2. Experiment 2: effects of AM251 on exploratory behaviors

Four groups of animals received vehicle (0.5 µl/side; 1 µl/mouse) or different doses of AM251 (25, 50 and 100 ng/mouse; 0.5 µl/side). The test session was performed 5 min after intra-CA1 drug injection.

2.6.3. Experiment 3: effects of histamine on exploratory behaviors

Five groups of animals received either saline (0.5 µl/side; 1 µl/mouse) or different doses of histamine (2.5, 5, 7.5 and 10 µg/mouse; 0.5 µl/side). The test session was performed 5 min after intra-CA1 injection of the drugs.

2.6.4. Experiment 4: effects of pyrilamine on exploratory behaviors

Four groups of animals received saline (0.5 µl/side; 1 µl/mouse) or different doses of pyrilamine (2.5, 5 and 10 µg/mouse; 0.5 µl/side). The test session was performed 5 min after intra-CA1 drug injection.

Table 1
Summary of experimental design.

Pre-testing treatment: bilateral intra-CA1 injection									
Figure		First infusion							
		Saline (μl/mice)	Vehicle (μl/mice)	AM251 (ng/mice)	WIN55,212-2 (μg/mice)	Histamine (μg/mice)	Pyrilamine (μg/mice)	Ranitidine (μg/mice)	+Second infusion
1	A	Actual image							
	B	Schematic image							
2		1	1	–	(0.25–1)	–	–	–	–
3		–	1	(2.5–10)	–	–	–	–	–
4		1	–	–	–	(2.5–10)	–	–	–
5		1	–	–	–	–	(2.5–10)	–	–
6		1	–	–	–	–	–	(2.5–10)	–
7	Left	1	–	–	–	5	5	5	Saline (1 μl/mice)
	Right	1	–	–	–	5	5	5	WIN55,212-2 (0.25 μg/mice)
8	Left	1	–	–	–	3.75	3.75	3.75	Saline (1 μl/mice)
	Right	1	–	–	–	3.75	3.75	3.75	AM251 (15 ng/mice)

2.6.5. Experiment 5: effects of ranitidine on exploratory behaviors

Four groups of animals received saline (0.5 μl/side; 1 μl/mouse) or different doses of ranitidine (2.5, 5 and 10 μg/mouse; 0.5 μl/side). The test session was performed 5 min after intra-CA1 injection of the drug.

2.6.6. Experiment 6: effects of WIN55, 212-2 in combination with histamine, pyrilamine or ranitidine on exploratory behaviors

In this experiment, eight groups of animals were used. Four groups received saline (1 μl/mouse; 0.5 μl/side), histamine (5 μg/mouse; 0.5 μl/side), pyrilamine (5 μg/mouse; 0.5 μl/side) or ranitidine (5 μg/mouse; 0.5 μl/side) in the presence of saline (0.5 μl/side) injection. The other four groups received saline (1 μl/mouse; 0.5 μl/side), histamine (5 μg/mouse; 0.5 μl/side), pyrilamine (5 μg/mouse; 0.5 μl/side) or ranitidine (5 μg/mouse; 0.5 μl/side) in the presence of ineffective doses of WIN55, 212-2 (0.25 μg/mouse 0.5 μl/side).

2.6.7. Experiment 7: effects of histamine, pyrilamine or ranitidine on exploratory behaviors in the presence or absence of AM251

Four groups of animals received saline or ineffective doses of histamine (3.75 μg/mouse; 0.5 μl/side), pyrilamine (3.75 μg/mouse; 0.5 μl/side) or ranitidine (3.75 μg/mouse; 0.5 μl/side), plus an ineffective dose of AM251 (15 ng/mouse; 0.5 μl/side).

2.7. Statistical analysis

Data are presented as the mean ± S.E.M. One-way repeated measures analysis of variance (ANOVA) was used for the statistical evaluation, followed by Dunnett's test ($p < 0.05$).

3. Results

3.1. Histology

Fig. 1 illustrates the approximate point of the drug injections in the CA1 from animals (A). The histological results were plotted on representative sections taken from the mice brain atlas of Paxinos and Franklin (2001) (B). A total number of 433 animals were implanted into the CA1 regions of the dorsal hippocampus, but only the data from 380 animals with correct cannula implants were included in the statistical analyses.

3.2. Effects of WIN55, 212-2 on exp1oratory behaviors in mice

Fig. 2 indicates the effect of bilateral intra-CA1 infusion of WIN55, 212-2 on exploratory behaviors. One-way ANOVA revealed that WIN55, 212-2 (0.1–0.5 μg/mouse, bilateral, intra-CA1) did not modify the number of head-dips [$F\ (4, 45) = 0.19$, $p > 0.5$], latency to head-dipping [$F\ (4, 45) = 0.52$, $p > 0.5$], locomotor activity [$F\ (4, 45) = 1.64$, $p > 0.5$], number of rearing [$F\ (4, 45) = 0.86$, $p > 0.5$], number of grooming [$F\ (4, 45) = 0.19$, $p > 0.5$] and number of defecation [$F\ (4, 45) = 0.35$, $p > 0.5$]. The data indicate that at the doses used, WIN55,212-2 did not modify exploratory behaviors. Data for rearing, grooming and defecation are not shown.

3.3. Effects of AM251 on exploratory behaviors in mice

Fig. 3 indicates the influence of bilateral intra-CA1 infusion of AM251 on exploratory behaviors. One-way ANOVA revealed that AM251 (10–50 ng/mouse) produced a dose-dependent decrease in head-dips [$F\ (3, 36) = 18.43$, $p < 0.001$] and increased in latency to head-dipping [$F\ (3, 36) = 3.9$, $p < 0.05$]. This difference was statistically significant at 25 and 50 ng/mouse and 50 ng/mouse for head-dips and latency to head-dipping, respectively. In addition, AM251 did not modify locomotor activity [$F\ (3, 36) = 1.77$, $p > 0.5$], number of rearing [$F\ (3, 36) = 0.66$, $p > 0.5$], number of grooming [$F\ (3, 36) = 2.08$, $p > 0.5$] and number of defecation [$F\ (3, 36) = 1.22$, $p > 0.5$]. The data indicate that the administration of AM251into CA1 is anxiogenic-like and non-sedative. Data for rearing, grooming and defecation are not shown.

3.4. Effects of histamine on exploratory behaviors in mice

Fig. 4 indicates the effect of bilateral intra-CA1 infusion of histamine on exploratory behaviors. One-way ANOVA revealed that histamine (2.5–10 μg/mouse) decreased head-dips [$F\ (4, 45) = 22.9$, $p < 0.001$] and increased latency to head-dipping [$F\ (4, 45) = 4.2$, $p < 0.01$] and the difference was statistically significant at 2.5, 5, 7.5, and 10 μg/mouse for head-dips and latency to head-dipping, respectively. In addition, histamine did not modify locomotor activity [$F\ (4, 45) = 0.88$, $p > 0.5$], number of rearing [$F\ (4, 45) = 0.8$, $p > 0.5$], number of grooming [$F\ (4, 45) = 0.8$, $p > 0.5$] and number of defecation [$F\ (4, 45) = 1.7$, $p > 0.5$]. The data indicate that administration of histamine into CA1 is anxiogenic-like and non-sedative. Data for rearing, grooming and defecation are not shown.

3.5. Effects of pyrilamine on exploratory behaviors in mice

Fig. 5 indicates the response of bilateral intra-CA1 infusion of pyrilamine on exploratory behaviors. One-way ANOVA revealed that pyrilamine (2.5–10 μg/mouse) decreased head-dips [$F\ (3, 36) = 19.27$, $p < 0.001$] and increased latency to head-dipping [$F\ (3, 36) = 2.8$, $p < 0.05$] and this difference was statistically significant at 5, 10 μg/mouse and 10 μg/mouse for head-dips and latency to head-dipping, respectively. On the other hand, pyrilamine did not modify locomotor activity [$F\ (3, 36) = 0.54$, $p > 0.5$], number of rearing [$F\ (3, 36) = 0.71$, $p > 0.5$], number of grooming [$F\ (3, 36) = 0.69$, $p < 0.001$] and number of defecation [$F\ (3, 36) = 0.32$, $p > 0.5$]. The data indicate that

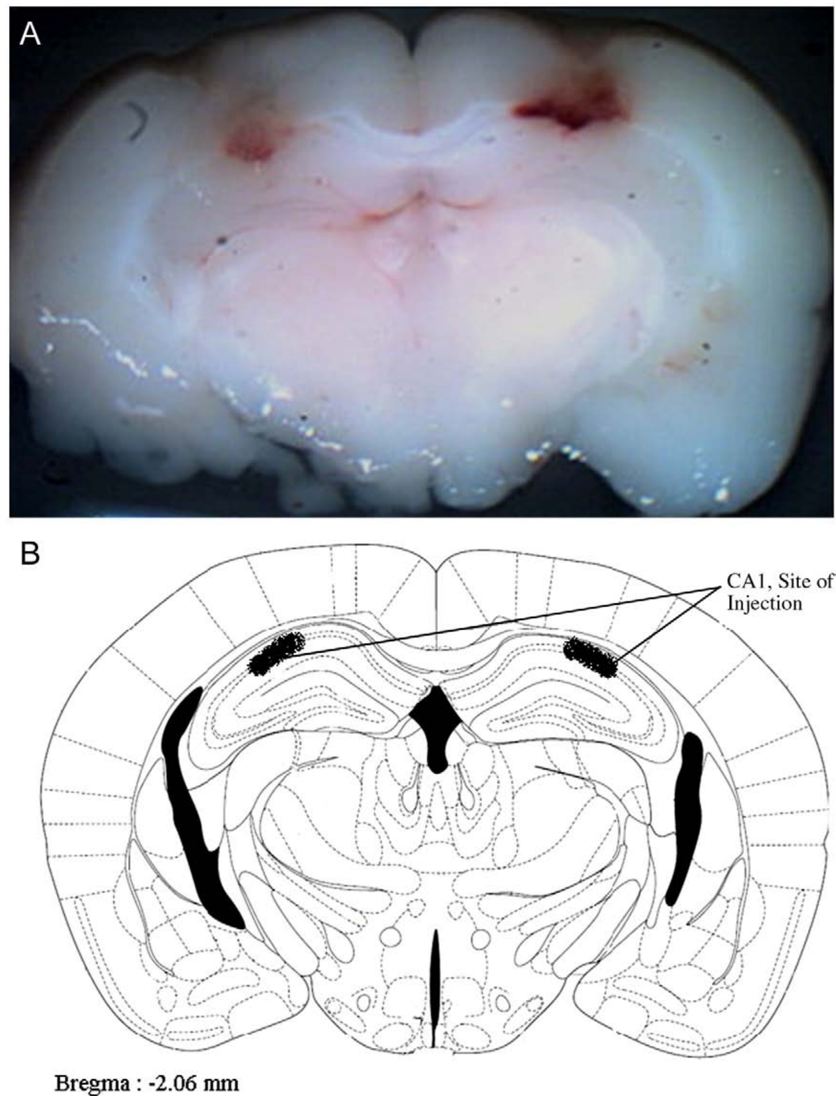

Fig. 1. (A) Location of the injection cannula tips in the CA1 regions of the dorsal hippocampus for all mice included in the data analyses. (B) Verified section was taken from the atlas of Paxinos and Franklin (2001).

administration of pyrilamine into CA1 is anxiogenic-like and non-sedative. Data for rearing, grooming and defecation are not shown.

3.6. Effects of ranitidine on exploratory behaviors in mice

Fig. 6 shows the effect of bilateral intra-CA1 infusion of ranitidine exploratory behaviors. One-way ANOVA revealed that ranitidine (2.5–10 µg/mouse) reduced head-dips [$F(3, 36) = 5.73$, $p < 0.01$] and increased latency to head-dipping [$F(3, 36) = 5.9$, $p < 0.05$] and this difference was statistically significant at 5 µg/mouse and 2.5, 5 µg/mouse for head-dips and latency to head-dipping, respectively. On the other hand, ranitidine did not modify locomotion [$F(3, 36) = 0.2$, $p > 0.5$], number of rearing [$F(3, 36) = 0.54$, $p > 0.5$], number of grooming and number of defecation [$F(3, 36) = 0.41$, $p > 0.5$]. The data indicate that administration of ranitidine into CA1 is anxiogenic-like and non-sedative. Data for rearing, grooming and defecation are not shown.

3.7. Effects of WIN55, 212-2 plus histamine, pyrilamine and ranitidine on exploratory behaviors

Fig. 7 shows influence of bilateral intra-CA1 infusion of WIN55, 212-2 plus histamine, pyrilamine or ranitidine on exploratory behaviors. One-way ANOVA revealed that head-dips induced by histamine (5 µg/mouse) and pyrilamine (5 µg/mouse) but not by ranitidine (5 µg/mouse) [$F(7, 72) = 9.1$, $p < 0.001$] were prevented in the presence of an ineffective dose of WIN55, 212-2 (0.25 µg/mouse). On the other hand, WIN55, 212-2 did not modify other behavior aspects such as latency to head-dipping [$F(7, 72) = 8.30$, $p < 0.001$], locomotor activity [$F(7, 72) = 5.16$, $p < 0.001$], number of rearing [$F(7, 72) = 0.2$, $p > 0.05$], number of grooming [$F(7, 72) = 0.22$, $p > 0.05$] and number of defecation [$F(7, 72) = 0.77$, $p > 0.05$] induced by histamine, pyrilamine or ranitidine. The behavior induced by histamine (2.5 µg/mouse), pyrilamine (2.5 µg/mouse) or ranitidine (2.55 µg/mouse) indicates that WIN55, 212-2 reduced anxiogenic-like induced-behaviors by histamine and pyrilamine but not ranitidine. Data for rearing, grooming and defecation are not shown.

3.8. Effects of AM251 plus histamine, pyrilamine and ranitidine on exploratory behaviors

Fig. 8 shows the effect of bilateral intra-CA1 infusion of AM251 plus histamine, pyrilamine and ranitidine on exploratory behaviors. One-way ANOVA revealed that an ineffective dose of AM251 (15 ng/mouse) did not modify behaviors such as numbers of head-dips [$F(7, 72) = 1.52$, $p > 0.5$], latency to head-dipping [$F(7, 72) = 0.92$, $p > 0.5$], locomotor activity [$F(7, 72) = 3.4$, $p > 0.5$], number of rearing [$F(7, 72) = 1.2$,

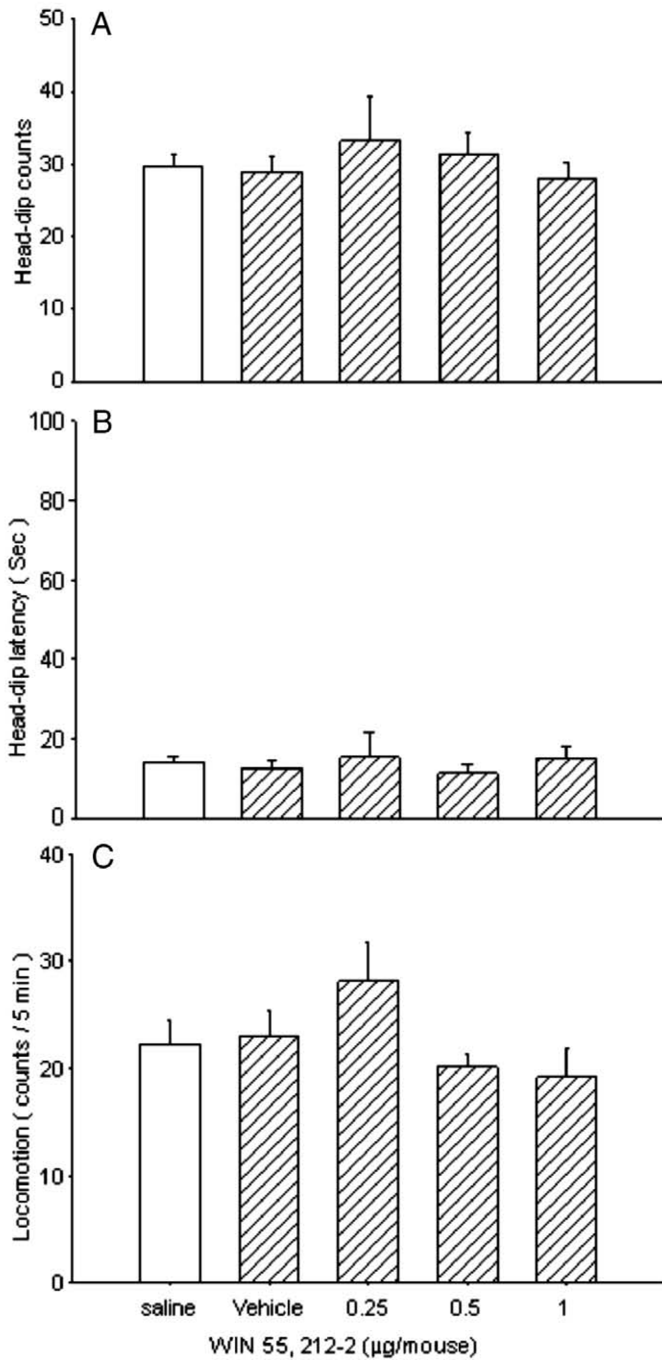

Fig. 2. The effects of intra-CA1 injection of WIN55, 212-2 on exploratory behaviors. Animals were injected with saline (1 µl/mouse), vehicle (saline/DMSO, 9/1; 1 µl/mouse) or WIN55, 212-2 (0.25, 0.5 and 1 µg/mouse; 0.5 µl bilateral). The tests were performed 5 min after intra-CA1 injections. Each bar is mean ± S.E.M. number of head-dips (A) and latency to head-dipping (B) and locomotors activity (C).

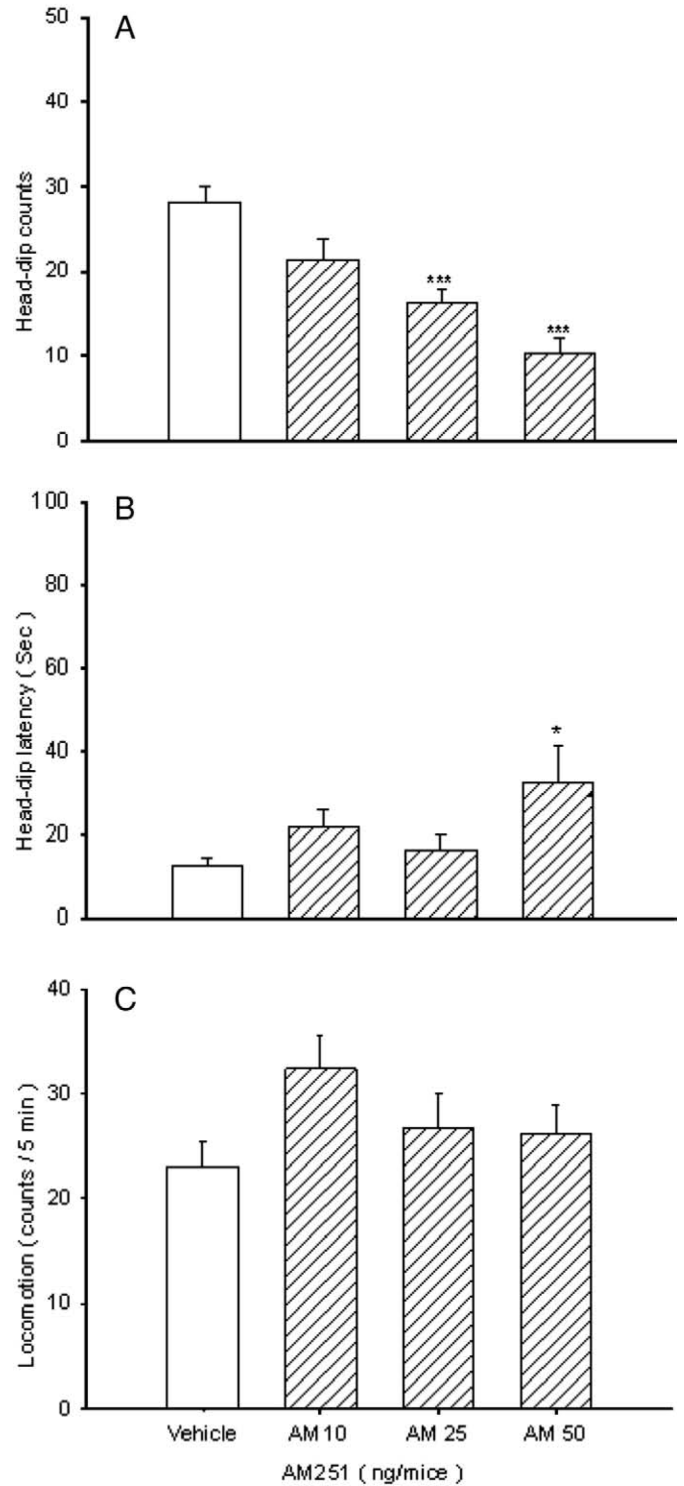

Fig. 3. The effects of intra-CA1 injection of AM 251 on exploratory behaviors. Animals were injected with vehicle (1 µl/mouse) or AM251 (10, 25 and 50 ng/mouse; 0.5 µl bilateral). The tests were performed 5 min after intra-CA1 injections. Each bar is mean ± S.E.M. number of head-dips (A) and latency to head-dipping (B) and locomotors activity (C). $^{*}p<0.05$ and $^{***}p<0.001$ when compared to the vehicle treated mice.

$p>0.5$], number of grooming [F (7, 72) = 0.3, $p>0.5$] and number of defecation [F (7, 72) = 2.4, $p>0.5$] induced by ineffective doses of histamine (3.75 µg/mouse), pyrilamine (3.75 µg/mouse) and ranitidine (3.75 µg/mouse). The data indicate that AM251 had no effects on behavioral patterns induced by histamine, pyrilamine and ranitidine. Data for rearing, grooming and defecation are not shown.

4. Discussion

In the present study, the effects of cannabinoidergic and histaminergic agents on anxiety-related behaviors and the interactions between them in mice have been investigated. The hole-board, which is one of the many tests for the identification of anxiolytic or anxiogenic-like effect of a drug (Rodriguez Echandia et al., 1987) has been used to test anxiety-like behavior.

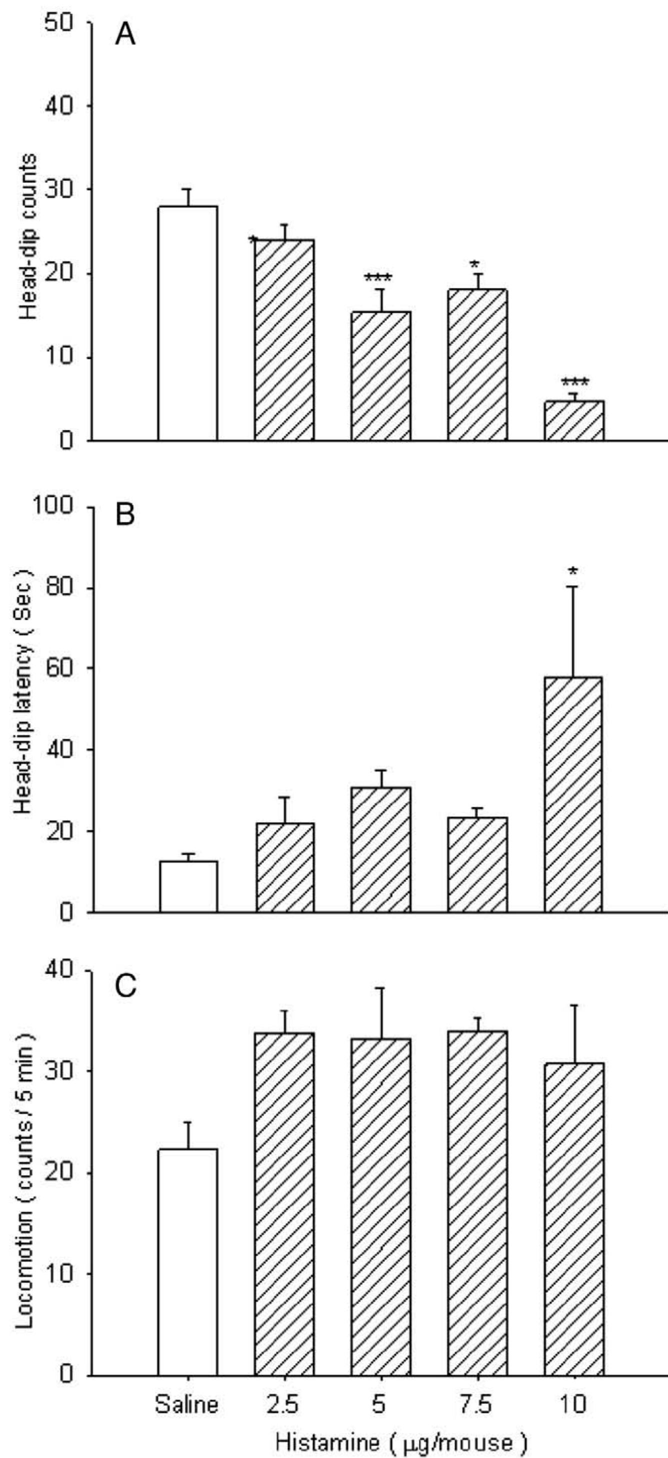

Fig. 4. The effects of intra-CA1 injection of histamine on exploratory behaviors. Animals were injected with saline (1 μl/mouse) or histamine (2.5, 5, 7.5 and 10 μg/mouse; 0.5 μl bilateral). The tests were performed 5 min after intra-CA1 injections. Each bar is mean ± S.E.M. number of head-dips (A) and latency to head-dipping (B) and locomotors activity (C). $*p<0.05$ and $***p<0.001$ when compared to the saline treated mice.

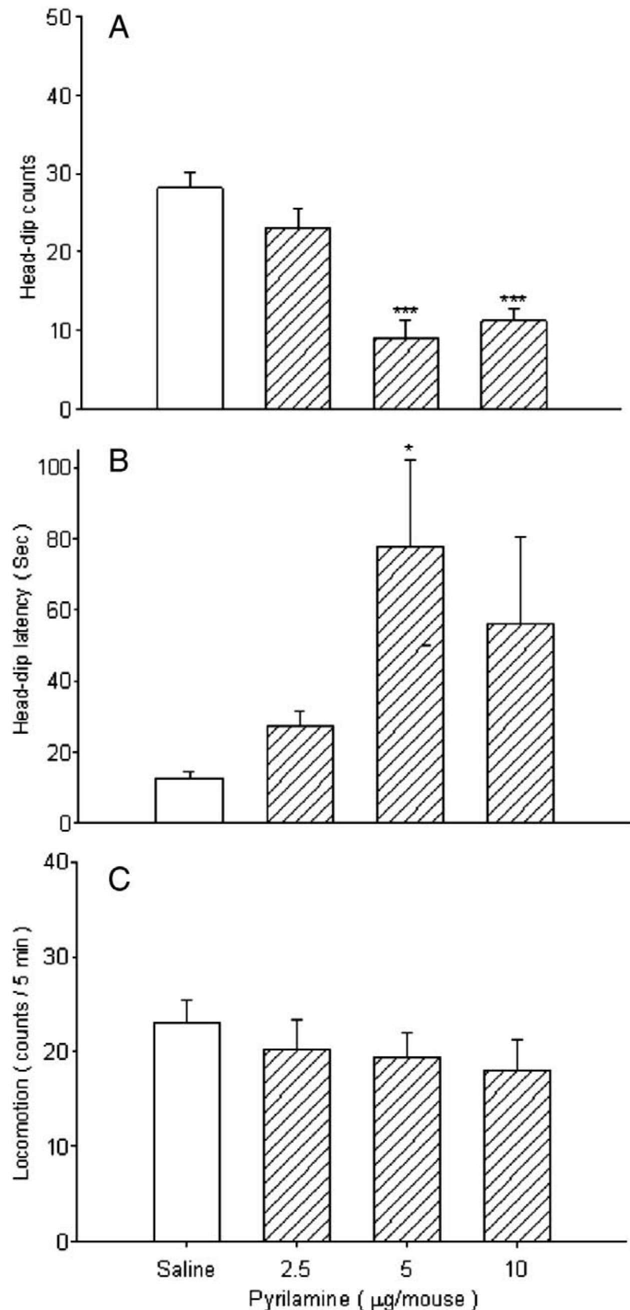

Fig. 5. The effects of intra-CA1 injection of pyrilamine on exploratory behaviors. Animals were injected with saline (1 μl/mouse) or histamine (2.5, 5, 7.5 and 10 μg/mouse; 0.5 μl bilateral). The tests were performed 5 min after intra-CA1 injections. Each bar is mean ± S.E.M. number of head-dips (A) and latency to head-dipping (B) and locomotors activity (C). $*p<0.05$ and $***p<0.001$ when compared to the saline treated mice.

4.1. The effects of cannabinoid receptor agents upon anxiety-like behavior

Our present data indicated that a bilateral intra-CA1 microinjection of WIN55, 212-2, CB1/CB2 receptor agonist, in the doses used had no significant effect on exploratory anxiety-behaviors in mice. However, previous results showed that the cannabinoid receptor agonists induced anxiogenic-like behaviors in the elevated plus-maze task in rat (Arevalo et al., 2001; Roohbakhsh et al., 2007), light dark in mice (Valjent et al., 2002) and humans (Zuardi et al., 1982), whereas other investigators have proposed anxiolytic-like effects for these compounds in light/dark task in mice (Berrendero and Maldonado, 2002; Sulcova et al., 1998) and plus maze in rat (Marco et al., 2004). Cannabis may produce a sense of euphoria, which may be accompanied by decreased anxiety and increased sociability (Ashton, 2001; Patton et al., 2002). The substance may induce its effect depending on the previous history of the individual, the animals, testing methods and doses used. Moreover,

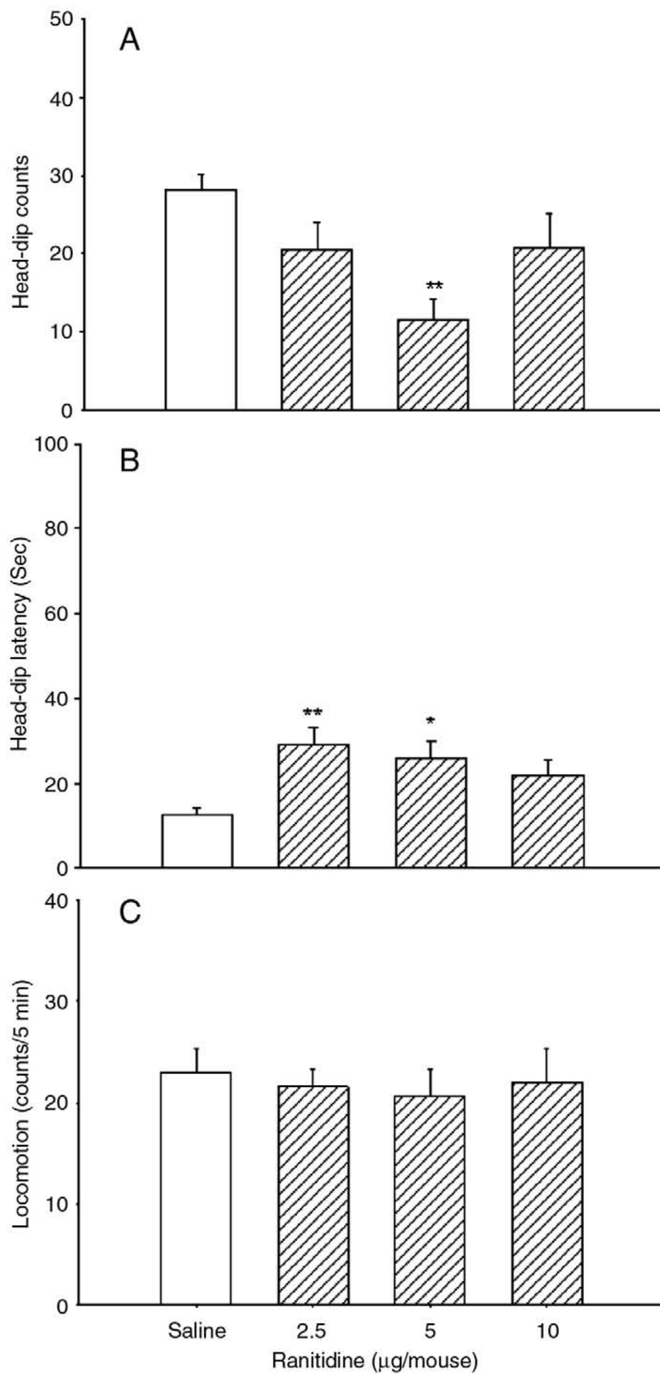

Fig. 6. The effects of intra-CA1 injection of ranitidine on exploratory behaviors. Animals were injected with saline (1 μl/mouse) or ranitidine (2.5, 5, 7.5 and 10 μg/mouse; 0.5 μl bilateral). The tests were performed 5 min after intra-CA1 injections. Each bar is mean ± S.E.M. number of head-dips (A) and latency to head-dipping (B) and locomotors activity (C). $*p<0.05$ and $**p<0.01$ when compared to the saline treated mice.

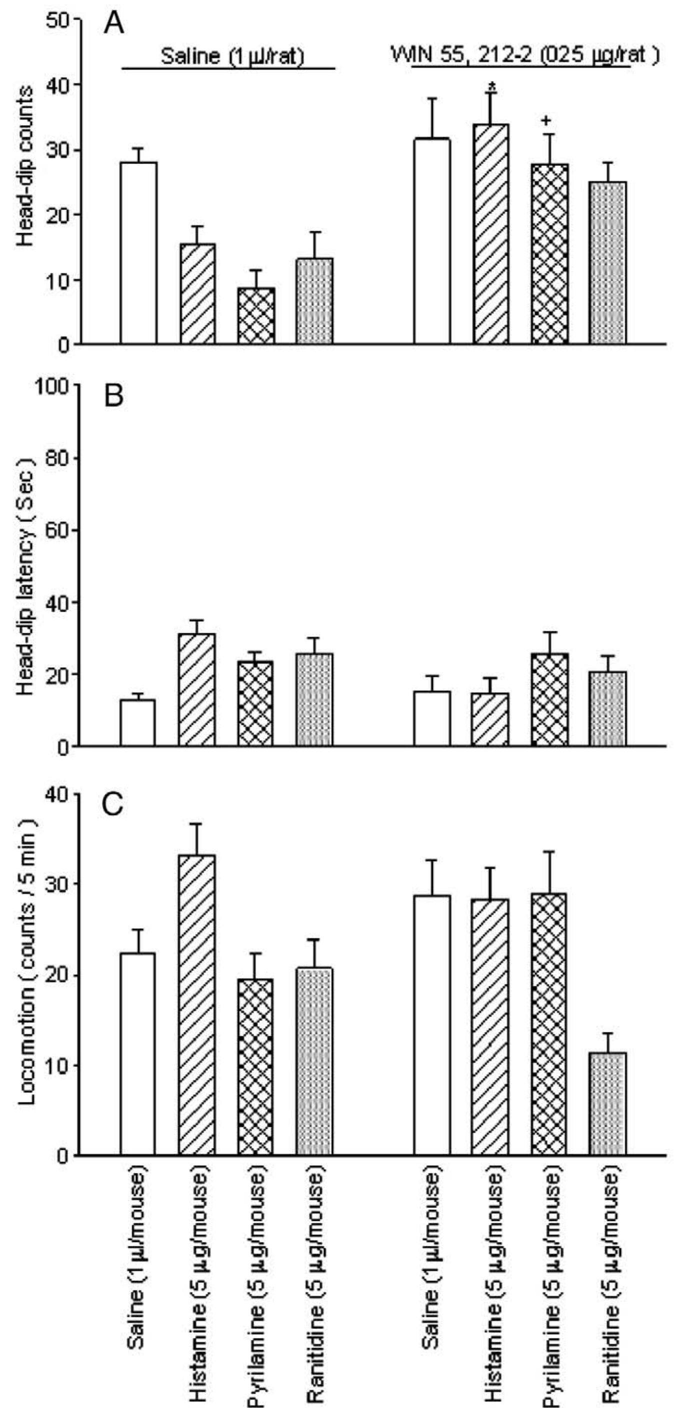

Fig. 7. The effects of WIN55, 212-2 plus histamine, pyrilamine and ranitidine on exploratory behaviors. In this experiment, eight groups of animals were used. Two four groups of animals received saline (1 μl/mouse) or same dose of histamine, pyrilamine or ranitidine (5 μg/mouse; 0.5 μl bilateral) plus saline (1 μl/mouse) or WIN55, 212-2 (0.25 μg/mouse; 0.5 μl bilateral), respectively. Every group of animals was compared to respective group.

two distinct cannabinoid sensitive presynaptic receptors may regulate network activity in the hippocampus. The CB1 receptor activation decreases GABA release from presynaptic terminals, and thus increases the excitability of principal cells in the pyramidal and dentate granule cells. On the other hand, novel, non-CB1 cannabinoid sensitive receptors (CB3) are present on the hippocampal excitatory axon terminals, which suppress glutamate release. The two receptors have different pharmacological features. Therefore, activation of the two kinds of cannabinoid receptor types may account for two different effects induced by the compounds. It has also been demonstrated that the administration of WIN 55,212-2 in lower and a higher doses induced a transient stimulation and a prolonged inhibition of hippocampal acetylcholine (Ach) release, respectively (Tzavara et al., 2003). Considering the modulatory role of Ach in the anxiety-related behavior (Degroot and Treit, 2002), this can be related to the contradictory responses of different doses of cannabinoids.

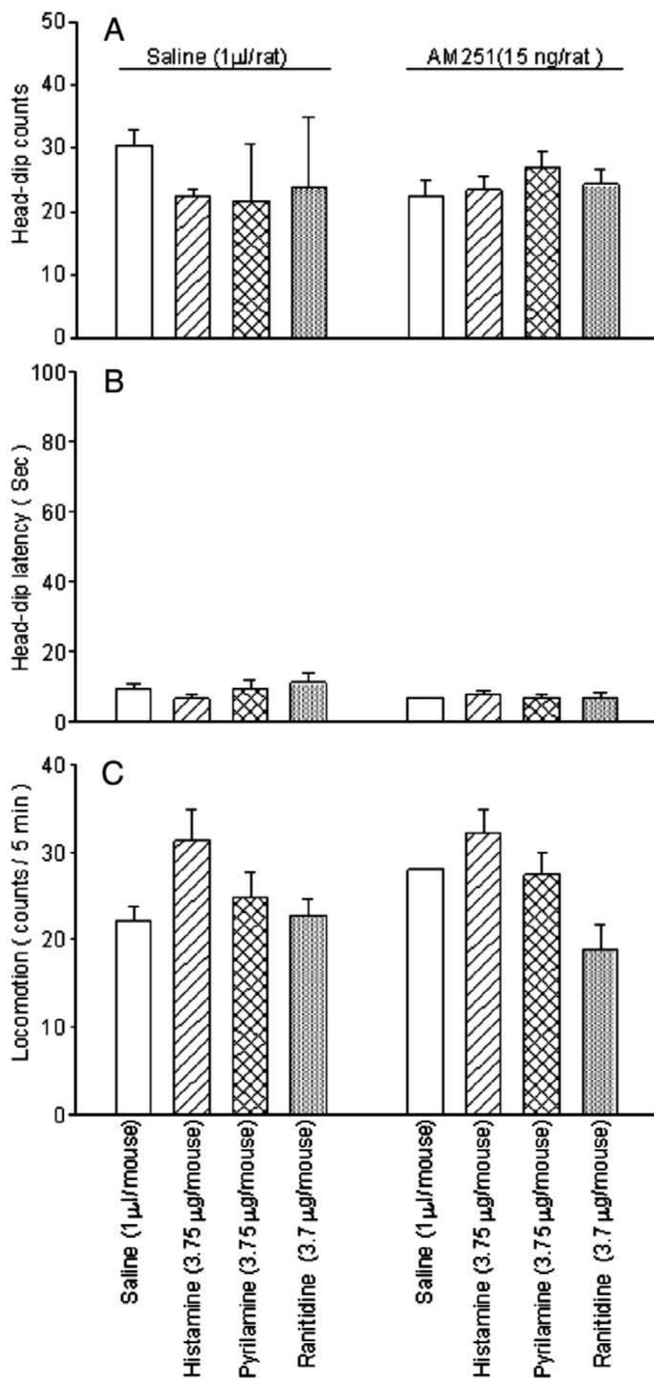

Fig. 8. The effects of AM251 plus histamine, pyrilamine and ranitidine on exploratory behaviors. In this experiment, eight groups of animals were used. Two four groups of animals received saline (1 µl/mouse) or same dose of histamine, pyrilamine or ranitidine (3.75 µg/mouse; 0.5 µl bilateral) plus saline (1 µl/mouse) or AM251 (15 ng/mouse; 0.5 µl bilateral), respectively. Every group of animals was compared to respective group.

Our present data indicate that the administration (intra-CA1) of AM251, a CB1 receptor antagonist, decreased the number of head-dippings, while increased the first head-dipping, but did not affect locomotor activity, grooming, rearing and defecation, suggesting an anxiogenic-like behavior. These results are in agreement with previous studies that showed that the peripheral injection of AM251 resulted in anxiogenic effects in mice. The authors also indicated that the cannabinoids affect both GABAergic and glutamatergic functions, which play opposite roles in anxiety (Berrendero and Maldonado, 2002; Haller et al., 2002, 2004a,b; Maccarrone et al., 2002; Patel and Hillard, 2006; Rodgers et al., 2005; Uriguen et al., 2004; Valjent et al., 2002). Thus, one can propose that the antagonist increases anxiety in rats via suppression of the GABAergic system (Haller et al., 2007). There are also reports indicating that both the genetic disruption of the CB1 receptors and its pharmacological blockade by AM251 increased anxiety behavior in mice (Haller et al., 2002, 2004b; Maccarrone et al., 2002; Martin et al., 2002; Patel and Hillard, 2006; Rodgers et al., 2005; Uriguen et al., 2004), which may support the effect of the drug to be mediated through CB1 receptor mechanism.

4.2. The effects of histaminergic agents on anxiety-like behavior

Another part of the present experiments showed the effect of bilateral intra-CA1 injection of histamine on anxiety-like behavior. The results indicated that intra-CA1 microinjection of histamine, an H1/H2 receptor agonist, decreased head-dipping, while increased first head-dipping, but did not affect other exploratory behaviors, indicating anxiety-like behavior in mice. An important role for involvement of histamine in inducing anxiety has been shown previously (Yuzurihara et al., 2000). Previously we have also shown that administration of histamine into central amygdala and ventral hippocampus induced anxiety-like behavior in the elevated plus maze (Rostami et al., 2006; Zarrindast et al., 2005a,b), while injection of histamine in the dorsal hippocampus of rats showed anxiolytic-like effects in the elevated plus-maze test (Zarrindast et al., 2006).

The present data revealed that administration of H1 receptor antagonist, pyrilamine or H2 receptor antagonist, ranitidine decreased the number of head-dipping and increased the first head-dipping without affecting locomotor activity and other parameters in the hole-board test, indicating anxiogenic-like behavior. This is in agreement with our previous data that intracerebroventricular administration of pyrilamine causes anxiogenic-like behavior in the plus-maze test (Zarrindast et al., 2005b). The intrinsic behavioral effects of pyrilamine and ranitidine in doses used in the present study may show the physiological potential of the histamine system in the dorsal hippocampus. However, there are reports indicating that intracerebroventricular administration of H1 and H2 receptor antagonists in light/dark test (Yuzurihara et al., 2000), in plus-maze and hole-board (Yanai et al., 1998) could not affect the anxiety-related behaviors in mice, other reports showed that the antagonists injected into the nucleus basalis magnocellularis region reduced anxiety-like effects in rats (Privou et al., 1998). Moreover, the administration of ranitidine into the periaqueductal gray and inferior colliculus induced fear like behaviors, while intracerebral injection of the histamine H2 receptor agonist, impromidine produced an anxiolytic-like effect dose-dependently in mice by means of the light/box test (Malmberg-Aiello et al., 2002). These contradictory findings may suggest that the effect of histaminergic system in the modulation of anxiety-related behavior may be dependent on the site of injection, the animals and drug used, and the methods of testing. There is a report showing that H1 receptor antagonists increase extracellular acetylcholine in rat hippocampus (Poli et al., 1990). Furthermore, H2 receptor antagonists can inhibit acetylcholinesterase (Hansen and Bertl, 1983) and also release acetylcholine (Poli et al., 1990). Since acetylcholine may modulate anxiety-related behaviors (Degroot and Treit, 2002), one may expect that this response to the higher doses of antagonists is mediated through changes in the acetylcholine levels.

4.3. Interactions between cannabinoids and histaminergic agents

In the rest of the experiments, we tested the effect of the co-administration of cannabinoidergic with histaminergic agents on anxiety-related behavior. Although, WIN55, 212-2 (0.25 µg/rat) administration by itself did not elicit any response in the present study, but

prevented anxiety-like behavior induced by histamine or pyrilamine and had no effect on ranitidine.

Furthermore, co-administration of ineffective doses of AM251 before threshold but ineffective histaminergic agents did not modify behaviors induced by these drugs. The H1 receptor antagonist increases extracellular acetylcholine in the hippocampus (Dringenberg et al., 1998), whereas cannabinoids can inhibit the release of acetylcholine via CB1 receptors in hippocampal preparations. In conclusion, cannabinoid system activation may decrease the anxiety-like behavior induced by histamine H1 receptors by such a mechanism.

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