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Mouse light/dark box test reveals anxiogenic-like effects by activation of histamine H_1 receptors

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Abstract

Effects of substances that are able to alter the histamine level, a histamine H_1-receptor agonist and antagonist, and a histamine H_2-receptor agonist were investigated in an anxiety-like state in mice by means of the light/dark box test. Diazepam was used as positive control. The histamine H_3-receptor antagonist, thioperamide (2, 5, and 20 mg/kg sc), showed an anxiogenic-like effect that reached a maximum with the dosage of 5 mg/kg. The histamine-*N*-methyltransferase (HMT) inhibitor, metoprine (5 and 20 mg/kg sc), also decreased the time in the light at the highest dose used and, likewise, the highly selective histamine H_1-receptor agonist, 2-(3-trifluoromethylphenyl)histamine (FMPH) (2.65 and 6.5 μg/mouse, icv). On the contrary, the histamine H_2-receptor agonist, impromidine (3, 10, 20, and 30 μg/mouse, icv), dose-dependently showed an anxiolytic-like effect. The selective histamine H_1 antagonist, pyrilamine (20 mg/kg ip) was able to prevent the anxiogenic-like effect of FMPH significantly, and that of thioperamide partially, while the effect caused by metoprine remained unvaried. It is suggested that the histaminergic system modulates anxiety-like states via the activation of both postsynaptic receptors in a contrasting manner: activation of the H_1 receptor causes an anxiogenic-like effect, while that of the H_2 receptors reduces anxiousness. However, on the basis of effects observed with the substances capable of releasing endogenous histamine, it seems likely that the anxiogenic-like effect is prevalent.

Keywords: Anxiety; Light/dark box test; Histamine H_1-receptors; Histamine H_1-receptor agonist; Histamine H_1-receptor antagonist; Metoprine; Thioperamide; 2-(3-Trifluoromethylphenyl)histamine; Pyrilamine; Impromidine

1. Introduction

Many psychopharmacological studies have been made relative to the participation of the histaminergic system in animal learning and memory (Prast et al., 1996; Malmberg-Aiello et al., 2000a) and in antidepressant-like effects (Lamberti et al., 1998; Pérez-Garcìa et al., 1999). Involvement of the histaminergic system in the animal anxiety-like state has not yet been clarified. In the acute stress situation, an increased histamine level in the rat hypothalamus was reported (Mazurkiewicz-Kwilecki and Tau, 1978) and likewise in the diencephalon and in the nucleus accumbens (Ito et al., 1999). This increased histamine level in the diencephalon has been interpreted as representing an increased histamine synthesis. The above-cited authors also observed an increased histamine-*N*-methyltransferase (HMT) activity in the nucleus accumbens and the striatum, which seems to be related to an increase in brain histamine metabolism (Ito et al., 1999). A physiological study reported that a destruction of the rat tuberomammillary rostroventral E-2 subregion, from which the histaminergic neuron fibers rise, can induce anxiolytic-like effects in the elevated plus maze test, these effects being linked to a lesion-induced reduction in histaminergic activity (Frisch et al., 1998). Furthermore, mice lacking histamine H_1 receptors showed prolonged transfer latency in the light/dark box test, indicating that mutant mice were less fearful than wild-type mice. On the contrary, in this same test, the mouse exploration time was not modified in comparison with that of the wild-type mouse (Yanai et al., 1998). The use of histamine influencing substances was also reported: α-fluoromethylhistidine, an inhibitor of the histidine decarboxylase (HDC), the enzyme, responsible for histamine biosynthesis in the brain, and compound 48/80, capable of depleting the mast cell histamine, were both able to

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attenuate the severity of restraint stress-induced gastric ulcerogenesis (Ray et al., 1992). As regards the histamine antagonists, it is reported that the H_1 receptor antagonist, chlorpheniramine (Privou et al., 1998; Hasenöhrl et al., 1999), and the H_2 receptor antagonist, ranitidine, (Privou et al., 1998) reduce fear-related behaviors in rats. As for the histamine H_3 receptors, in the light/dark box test a significant decrease in time spent in the light zone and in the number of transfers was obtained only when the animals were treated not only with thioperamide, a histamine H_3-receptor antagonist, but also with a histamine H_2-receptor antagonist, zolantidine (Imaizumi and Onodera, 1993). Those decreased parameters were reversed by pretreatment with a histamine H_1-receptor antagonist, pyrilamine. Using another test, the elevated plus maze, it was reported that neither thioperamide nor the histamine H_3-receptor agonist, *R*-α-methylhistamine, modified the state of animal anxiety (Pérez-Garcìa et al., 1999).

We therefore considered it worthwhile to investigate the molecules capable of modifying the endogenous histamine level, not only by acting on the histamine H_3 receptor with thioperamide, which is able to stimulate histamine release and synthesis (Arrang et al., 1987), but also by altering the brain histamine level with the specific HMT, [EC 2.1.1.8] inhibitor metoprine (Duch et al., 1978). In order further to investigate the possible implications of the histaminergic system in anxiety-like states, the aim of our study also included an elucidation of the exact role of histamine postsynaptic receptors. For this purpose, three key molecules were used: a selective receptor agonist, 2-(3-trifluoromethylphenyl)histamine (FMPH) being 2138-fold more selective in H_1- than in H_2-receptors (Leschke et al., 1995), impromidine, in order to study the participation of H_2-receptors and pyrilamine, the most selective H_1-receptor antagonist (Hill, 1990). The worth of our procedure was evaluated using the known anxiolytic drug diazepam.

Preliminary data were presented at the XXIXth Annual Meeting of the European Histamine Research Society (Malmberg-Aiello et al., 2000b).

2. Methods

The experiments were carried out in accordance with the Animal Protection Law of the Republic of Italy, DL n. 116/1992, based on the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize animal suffering and to reduce the number of animals used. Male CD-1 albino mice (22–24 g) were used. Twelve mice were housed per cage and fed a standard laboratory diet, with tap water ad libitum for 12/12 h light/dark cycles (lights on 7:00). The cages were brought into the experimental room the day before the experiment for purposes of acclimatization. All experiments were performed between 10:00 and 15:00.

2.1. Mouse light/dark box test

The apparatus (length 50 cm, width 20.5 cm, and height 19 cm) consisted of two equal acrylic compartments, one dark and one white, illuminated by a 60-W bulb lamp and separated by a divider with a 10×3.2 cm opening at floor level. Each mouse was tested by placing it in the center of the white area, facing away from the dark one, and was allowed to explore the novel environment for 5 min. The number of transfers from one compartment to the other and the time spent in the illuminated side were measured. This test exploited the conflict between the animal's tendency to explore a new environment and its fear of bright light.

2.2. Drugs

The following drugs were used: Impromidine 3HCl (SmithKline Beecham), metoprine (Burroughs Wellcome), pyrilamine maleate (RBI), thioperamide maleate (RBI), FMPH, (Institut für Pharmazie, Freie Universität Berlin), and diazepam—Valium (Roche). Metoprine was dissolved in 10% aqueous lactic acid and then diluted with saline solution (1:30). Other drugs were dissolved in isotonic (NaCl 0.9%) saline solution immediately before use, except Valium which was suspended in 1% sodium carboxymethylcellulose (Fluka). Drug concentrations were prepared so that the necessary dose could be injected in a volume of 10 ml/kg, using sc, ip, and po routes. For icv administration, a short ether anesthesia was adopted. FMPH and impromidine, drugs that are not capable of passing the blood–brain barrier, were injected in the necessary dosage, dissolved in 5 μl per mouse, according to the method proposed by Haley and McCormick (1957) but with certain modifications. In brief, the injection site was 1.5 mm from either side of the midline, on a line drawn through the anterior base of the ears. The head of the anesthetized mouse was grasped firmly, and the needle of a 10-μl microsyringe was inserted perpendicularly 2 mm through the skull into the brain. Five microliters of solution were then injected slowly, in 20 s, into a lateral ventricle. Immediately after removal of the needle, the animal remained quiet for approximately 1 min and then resumed its normal activity. To ascertain the exact site of the icv injection, some mice were icv injected with 5 μl of 1:10 diluted India ink, and their brains were examined macroscopically after sectioning. This kind of injection procedure was compared in a previous study (Malmberg-Aiello et al., 1994) with the one performed using permanent icv cannulae without ether anesthesia. No effects due to the anesthesia were observed.

Results are presented as the mean ± S.E.M. Statistical analysis was performed by means of ANOVA followed by Scheffe's test. Student's two-tailed *t* test was used to verify the significance between two means. *P* values of less than .05 were considered significant. Data were analyzed with

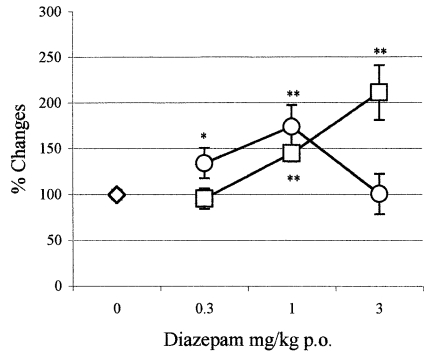

Fig. 1. Anxiolytic-like effects of diazepam in the mouse light/dark box test. Diazepam (0.3, 1, and 3 mg/kg p.o.) was administered 30 min before the test. The % time in the light (□) and the % number of transfers (○) from one compartment to the other during a 5-min observation period with respect to controls (◇). Each point represents the mean of 10–15 animals. Vertical lines give S.E.M. * $P<.05$, ** $P<.001$ versus control mice (ANOVA followed by Scheffe's test).

the aid of a computer program (Number Cruncher Statistical System, Version 5.03 9/92).

3. Results

Diazepam in doses of 0.3, 1 and 3 mg/kg po was used as a reference molecule. It was able dose-dependently to prolong the time spent in the lighted compartment, thus evidencing its anxiolytic-like properties and validating our experimental approach (Fig. 1). On the contrary, both substances, i.e., thioperamide and metoprine, which augmented the endogenous histamine and the H_1-receptor agonist increased the mouse's anxiety-like state, thus shortening the time spent in the light during a 5-min observation period with respect to the controls.

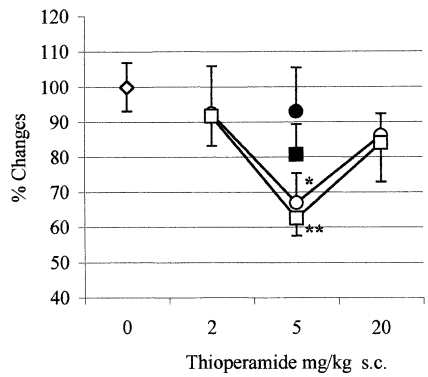

Fig. 2. Anxiogenic-like effect of thioperamide in the mouse light/dark box test. The % time in the light (□) and the % number of transfers (○) during a 5-min observation period with respect to controls (◇). Thioperamide maleate (2, 5, and 20 mg/kg sc) was injected 15 min before the test. Pyrilamine maleate 20 mg/kg sc + thioperamide 5 mg/kg ip (■, ●). Pretreatment with pyrilamine was performed 10 min before treatment with thioperamide. Each point represents the mean of 12–14 animals. Vertical lines give S.E.M. * $P<.05$ ** $P<.001$ versus control mice (ANOVA followed by Scheffe's test).

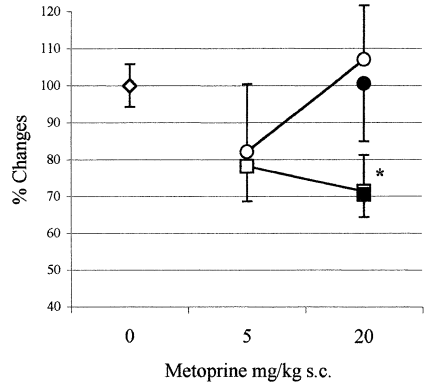

Fig. 3. Anxiogenic-like effect of metoprine in the mouse light/dark box test. The % time at light (□) and the % number of transfers (○) during a 5-min observation period with respect to controls (◇). Metoprine (5 and 20 mg/kg sc) was injected 30 min before the test. Pyrilamine maleate 20 mg/kg sc + metoprine 20 mg/kg ip (■, ●). Pretreatment with pyrilamine was performed 10 min before treatment with metoprine. Each point represents the mean of 10–18 animals. Vertical lines give S.E.M. * $P<.05$ versus control mice (ANOVA followed by Scheffe's test).

The administration of thioperamide 15 min before the test, in doses of 2 and 5 mg/kg sc, dose-dependently diminished the time spent in the light, while the dose of 20 mg/kg was less effective than the former (Fig. 2). With the dose of 5 mg/kg, a statistically significant lowering in the number of transfers was also observed.

Metoprine also dose-dependently (5 and 20 mg/kg sc) shortened the time in the light, demonstrating a statistically significant effect with the dose of 20 mg/kg (Fig. 3).

Similarly to thioperamide and metoprine, the histamine H_1-receptor agonist, FMPH, administered 15 min before the test with the doses of 2.65 and 6.5 μg/mouse icv, reduced dose-dependently the time spent in the lighted compartment (Fig. 4).

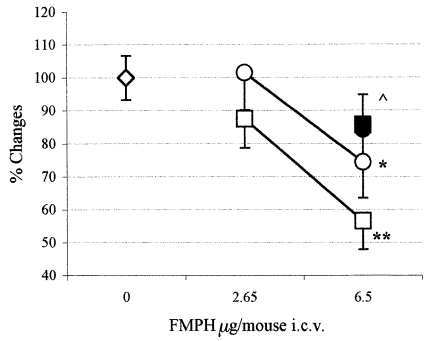

Fig. 4. Anxiogenic-like effect of 2-(3-trifluoromethylphenyl)histamine dihydrogenmaleate (FMPH) and its antagonism by pyrilamine maleate in the mouse light/dark box test. The % time at light (□) and the % number of transfers (○) during a 5-min observation period with respect to controls (◇). FMPH (2.65 and 6.5 μg/mouse icv) was injected 15 min before the test. Pyrilamine 20 mg/kg ip + FMPH 6.5 μg/mouse icv (■, ●). Pretreatment with pyrilamine was performed 10 min before treatment with FMPH. Each point represents the mean of 10–16 animals. Vertical lines give S.E.M. * $P<.05$ ** $P<.001$ versus control mice (ANOVA followed by Scheffe's test) ^ $P<.05$ versus FMPH (6.5 μg/mouse icv)-treated mice (Student's t test).

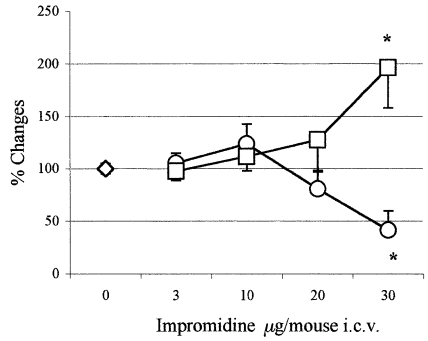

Fig. 5. Effect of impromidine in the mouse light/dark box test. Impromidine 3HCl (3, 10, 20, and 30 μg/mouse icv) was administrated 15 min before the test. The % time at light (□) and the % number of transfers (○) during 5-min observation period with respect to controls (◇). Each point represents the mean of 9–12 animals. Vertical lines give S.E.M.; $*P<.01$ (ANOVA followed by Scheffe's test).

On the contrary, a histamine H_2-receptor agonist, impromidine (3, 10, 20, and 30 μg/mouse icv), prolonged the time spent in the light and decreased the number of transfers from one compartment to the other (Fig. 5).

In order to assess whether the effects observed using thioperamide, metoprine and FMPH were due to an agonist action on H_1-receptors, a second set of experiments was performed during which animals received treatments combining the substances with the H_1-receptor antagonist pyrilamine. Mice treated with pyrilamine, 20 mg/kg ip, showed no statistically significant differences in the time spent in the lighted compartment (105.8 ± 17.2%) or in the number of transfers (96.8 ± 14.6), in comparison with the controls (100 ± 8.5% and 100 ± 6.9%, respectively). Conversely, when pyrilamine was administered 10 min before thioperamide the time spent in the light was prolonged by 18%, but not significantly (Fig. 2), and in the metoprine-treated group the time in the light after pyrilamine-pretreatment showed no variation (Fig. 3). Contrarily to this, pyrilamine was able to antagonize the anxiogenic-like effect of FMPH (6.5 μg/mouse icv) in a statistically significant manner (Fig. 4).

4. Discussion

Diazepam, used as a positive control in our conditions, resulted anxiolytic, as expected. In this regard, it is interesting to note that diazepam is able to decrease the histamine turnover rate in the central nervous system of mice (Oishi et al., 1986). In our study, in fact, substances able to enhance histaminergic transmission reduced the time spent in the lighted compartment, indicating a probable anxiogenic-like effect. This anxiogenic-like effect might be due to the activation of histamine H_1-receptors, since the selective H_1-receptor agonist caused reduction in the time spent in the light, while an H_2-receptor agonist prolonged this time.

The histaminergic system in anxiety-like states was studied by first altering histamine brain levels. The choice of metoprine as a tool for studying the role of endogenous histamine in anxiety was made on the basis that, as has been demonstrated, metoprine is a highly potent, competitive HMT inhibitor devoid of any action on HDC (Duch et al., 1978). Also, it has been found to be capable of enhancing histamine release in in vivo microdialysis studies following intraperitoneal administration (Itoh et al., 1991). A dose of 5 mg/kg ip of metoprine was able to cause a 70% reduction in whole brain HMT activity, while a 90% reduction could be obtained with 20–30 mg/kg ip (Hough et al., 1986). In our study, the dose-dependence of the metoprine effect reflected the degree of inhibition of the enzyme. A 70% reduction was not sufficient to cause an anxiogenic-like effect, while 20 mg/kg sc significantly shortened the time spent in the light.

A further approach for studying the effects of endogenous histamine was to use thioperamide. Since it has been described as a selective antagonist for histamine H_3 presynaptic autoreceptors (Arrang et al., 1987), the reduction in the time spent in the lighted compartment may have been due to the increased release of histamine from synaptic terminals. In our test, it was observed that the anxiogenic-like effect diminished with the highest dose (20 mg/kg sc) used. Since thioperamide has been reported to have a K_i of 4 nM on the H_3-receptor and >10,000 nM on the H_1- and H_2-receptors (Schwartz et al., 1990), the hypothesis of a postsynaptic antagonism could be ruled out. Instead, the less evident effect was probably due to competition between thioperamide and the endogenous histamine released in the H_3-receptor. When the dose of thioperamide was sufficiently strong, the amount of histamine released was high enough to compete with thioperamide, thus activating the negative feedback mechanism on the release and, consequently, decreasing the thioperamide anxiogenic-like effect. Such a partial reversal of the thioperamide effect with increasing doses was also observed while tests were performed for antidepressant-like activity (Lamberti et al., 1998), antinociception (Malmberg-Aiello et al., 1994), and locomotor activity (Sakai et al., 1991).

The administration time for thioperamide also seems to be critical. In a previous work we demonstrated that thioperamide caused the maximum effect 15 min after administration of the drug in both mice and rats (Malmberg-Aiello et al., 1994). This may be the reason why some authors, although adopting our range of doses but a different time schedule, 30 min (Pérez-Garcìa et al.,1999) or 60 min (Imaizumi and Onodera, 1993), could not detect any statistically significant alteration in animal state of anxiety with thioperamide alone.

In our experiments, thioperamide decreased the number of transfers from one compartment to the other, while diazepam increased this number at doses of 0.3 and 1 mg/kg po. Nevertheless, a decrease was observed at the highest diazepam dosage used. However, at this high dosage (3 mg/kg po) the mice were no longer able to coordinate their movements well, and they fell off a speedy rotating rod

(24 rpm) (Selleri et al., 1997). Despite this, they chose to remain in the lighted zone.

Due to its inability to cross the blood–brain barrier, FMPH was administered icv. On a molar basis, 2.65 μg of FMPH dihydrogenmaleate correspond to 1 μg of histamine 2HCl. In the dosages used, this substance did not cause any motor impairment, as evidenced by the rotating rod test (Malmberg-Aiello et al., 1998). This selective H_1-receptor agonist dose-dependently and significantly diminished both the time spent in the light and the number of transfers from one compartment to the other. These observations were consistent with results obtained with a less selective molecule, betahistine, which stimulates histamine H_1-receptors and blocks, like thioperamide, histamine H_3-presynaptic receptors (Imaizumi et al., 1996).

As regards the receptor that might have been involved in mediating the anxiogenic-like effects, the fact that the selective histamine H_1-receptor agonist FMPH caused the most powerful effect, significatively antagonizable by pyrilamine, supports the idea that the H_1-receptor may have mediated the anxiogenesis. In our experiments, pyrilamine, intraperitoneally, demonstrated no effect on its own, while it has been reported that by means of continuous intraventricular infusion in aged rats (Hasenöhrl et al., 1999) or by injection in the vicinity of the nucleus basalis magnocellularis, another histamine H_1 receptor antagonist (chlorpheniramine) exerted an anxiolytic-like effect in the elevated plus-maze task. Contrary to case of the H_1 receptor agonist, the H_2-receptor agonist impromidine caused an anxiolytic-like effect similarly to diazepam. At the highest dosage used (30 μg/mouse icv), the number of transfers was diminished and the mice's performance on the rotating rod was weakened (Lamberti et al., 1996), as was the case with diazepam. Also in this case, the mice did not hide in the dark compartment, but chose the lighted, more adverse situation, which seemed not to disturb them. Interestingly enough, on the basis of their observations, Young and Johnson (1991) concluded that simply the measurement of the time spent in the lighted area, but not the number of transfers, was the most consistent and useful parameter for assessing anxiolytic-like action. Furthermore, Lepicard et al. (2000) reported that the time spent in the light was a stronger indication in the study of anxiety, whereas the number of transfers reflected both anxiety and exploration. These observations are in good agreement with our results.

Both thioperamide and metoprine cause the release of endogenous histamine, which in turn stimulates both histamine postsynaptic receptors. It seems reasonable to assume that a greater stimulation took place in the H_1 receptors, since a decrease in the time spent in the light was prevalent. Nevertheless, also the activation of histamine H_2 receptors is intuitable, since when the increase of histamine release and synthesis is modest, as in the case of thioperamide (25%) (Arrang et al., 1987), the anxiogenic-like effect was partially antagonized by pyrilamine. On the contrary, when the output of endogenous histamine is massive, as in the case of metoprine, the anxiogenic-like effect was no longer antagonizable by a relatively low dose of pyrilamine. In fact, in the rat metoprine produced a dose-dependent 2.5–5-fold elevation of brain histamine levels (Duch et al., 1980) and a twofold increase in histamine output with a dose of 5 mg/kg ip (Itoh et al., 1991). In explaining the probable mechanism of anxiogenic-like effect of thioperamide, we also have to take into account its possible effects through the H_3-heteroreceptors, the existence of which is well demonstrated by Cumming et al. (1991) and by Pollard et al. (1993). However, the eventual participation of other neurotransmitters (serotonin, noradrenaline, acetylcholine, GABA, etc.) in thioperamide-induced anxiogenic-like effects has still to be studied. The H_2 receptor also needs more profound attention.

At present, our data suggest that stimulation of the two histamine postsynaptic receptors can mediate contrasting effects in anxiety-like states in the mouse, with the anxiogenic-like action being prevalent, through the activation of histamine H_1-receptors.

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