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m-Chlorophenylpiperazine enhances neophobic and anxious behaviour in mice

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1-(3-CHLOROPHENYL)PIPERAZINE (*m*CPP) reduced novelty-seeking behaviour as well as the number of rears in mice confronted with a free exploratory test. Moreover, *m*CPP was found to decrease the time spent by mice in the lit box and the number of transitions in a two-box light/dark choice situation validated for the detection of anxiolytic or anxiogenic drugs. These results suggest that *m*CPP enhances emotional responses towards novel and aversive places. Since *m*CPP has been reported as a 5-HT_{1C} receptor agonist, it can be hypothesized that increased activity of serotonin may play a role in regulating certain forms of emotional behaviour in animals.

Key words: *m*CPP, Mice, Neophobia, Anxiety, Emotional behaviour

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Introduction

There is extensive evidence indicating an involvement of serotoninergic neuronal systems in regulating emotional behaviour in humans as well as in animals.[1,2] For instance, several authors reported that in healthy subjects and agoraphobic, panic or obsessive-compulsive disorder patients anxiogenic effects with the 5-HT_{1C} receptor agonist, *m*CPP.[3–5] Furthermore, mCPP was also found to elicit anxious responses in rats confronted with a social interaction test and with a light/dark choice situation[6] and in mice exposed to an elevated plus-maze.[7]

The purpose of the present study was to investigate the possible role of serotonin in regulating emotional responses in mice using a free exploratory situation as well as a light/dark choice procedure. In the first test, psychostimulant drugs have been found to enhance neophobia in mice,[8] while in the second procedure, several benzodiazepine inverse agonists potentiated anxious responses towards aversive stimuli.[9–11]

Materials and Methods

Male Swiss albino mice from Centre d'Elevage R. Janvier and Centre d'Elevage Iffa Credo (France), 10 weeks of age at time of testing, were used. Prior to experimental testing, they were housed five to a standard cage containing a constant supply of food pellets and water and kept on a 12/12 h light/dark cycle with lights on at 1 a.m. so that we could observe animals in their high activity period, that is when the lights were off. Each mouse was tested once in only one experiment.

1-(3-chlorophenyl)piperazine (*m*CPP),(a gift from Hoffmann-La Roche Co., Basle) was dissolved in saline and administered intraperitoneally, 30 min before testing, in concentrations giving an injection volume of 10 ml kg^{-1} body wt.

Statistical significance of differences between control and treated groups was ascertained with a combined analysis of variance and the Bonferroni's a posteriori *t*-test.

Experiment 1: The apparatus consisted of a polyvinylchloride box (30 × 20 × 20 cm) covered with plexiglas and subdivided into six equal square exploratory units, which were all interconnected by small doors. It could be divided in half lengthwise by closing three temporary partitions. The apparatus was kept on a stand in the mouse room. The experimenter stood next to the box always in the same place. Approximately 24 h before testing, each subject was placed in one half of the apparatus with the temporary partitions in place, in order to be familiarized with it. The floor of this half was covered with sawdust and the animal was given unlimited access to food and water. Next day, the subject was exposed to both familiar and novel compartments by removal of the temporary partitions. It was then observed, in red light, for 10 min. The time spent in the novel half (novelty preference), the number of units entered (locomotion) and the number of rears made by the animals were recorded.

Experiment 2: The apparatus consisted of two polyvinylchloride boxes (20 × 20 × 14 cm) covered with plexiglas. One of these boxes was darkened with cardboard. A light from a 100W desk lamp, 25 cm above the other box, provided the only room illumination. An opaque plastic tunnel (5 × 7 × 10 cm) separated the dark box, from the lit one. During the observation period, the experimenter always sat in the same place, next to the apparatus. The subjects were individually tested in 5 min sessions in the apparatus described above. The floor of the box was cleaned between test sessions. Testing was performed between 2 p.m. and 4 p.m. Mice were placed in the lit box to start the test session. The amount of time spent by mice in the lit box (TLB) and the number

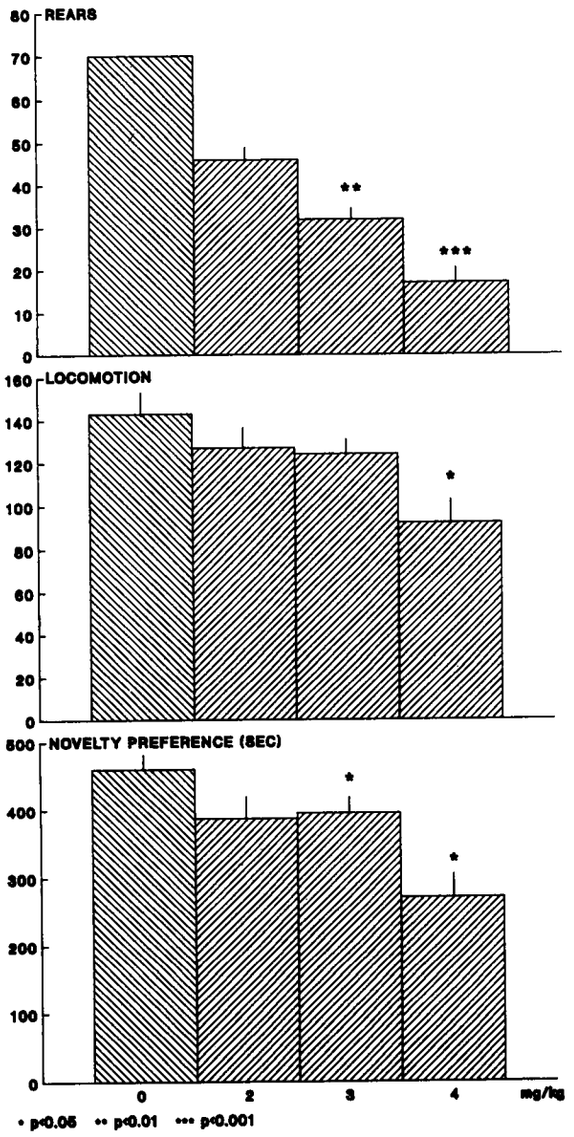

FIG. 1. Effects of mCPP on the behaviour of mice confronted with a free exploratory test (controls: $n = 14$; drug: $n = 12$; s.e.m. indicated above each bar).

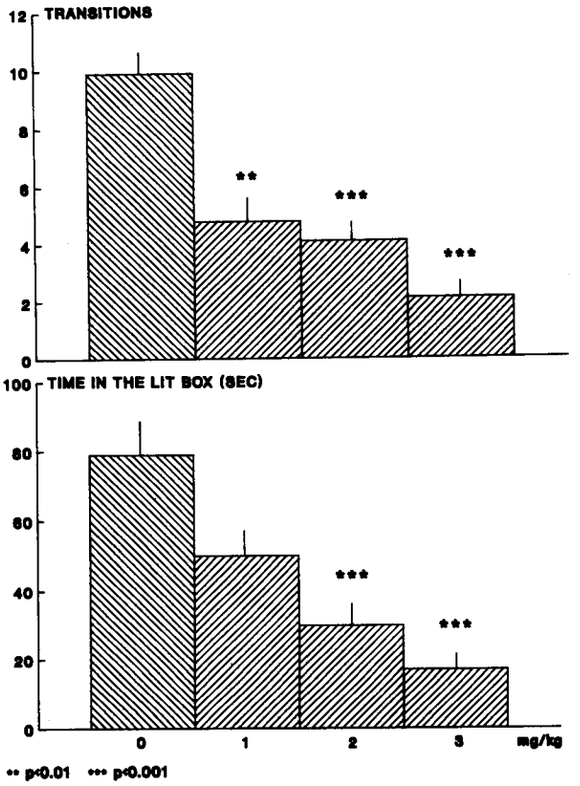

FIG. 2. Effects of mCPP on the behaviour of mice in the light/dark choice procedure ($n = 15$).

of transitions through the tunnel were recorded over a 5 min period, after the first entry in the dark box. A mouse whose four paws were in the new box was considered as having changed boxes.

Results

Experiment 1: Analysis of variance revealed significant differences among groups for novelty preference in mice treated with *m*CPP ($F(3,46)=7.43$; $p < 0.001$), for locomotion ($F(3,46)=3.10$; $p < 0.03$) and for rearing behaviour ($F(3,46)=15.52$; $p < 0.001$). Figure 1 shows that *m*CPP at the highest doses induced a significant decrease in novelty preference as well as in rears, whereas locomotion was significantly decreased only at the highest dose.

Experiment 2: ANOVA revealed significant group differences for the time spent by mice in the lit box ($F(3,56)=10.69$; $p < 0.001$) and for the number of transitions between the two boxes ($F(3,56)=12.55$; $p < 0.001$). Figure 2 shows that *m*CPP decreased both behavioural parameters in a dose-dependent manner, the effect being significant at the highest doses with respect to time and at all doses with respect to the number of transitions.

Discussion

The data obtained here support the hypothesis that the selective 5-HT_{1C} receptor agonist *m*CPP possesses anxiogenic properties in mice. We examined this hypothesis in both a free exploratory situation and in the light/dark choice procedure. Experiment 1 indicates that *m*CPP reduced exploratory parameters such as time spent by animals in the novel compartment and the number of times they reared, while it decreased locomotion only at the highest dose. These findings partly parallel those which were found for methamphetamine in the same test.[8] The experimental situation lacks constraining and aversive components in so far as the animals are free to enter a novel compartment or to stay in a familiar one.

In this paradigm, control mice exhibited a significant preference for the novel compartment since they were seen more than 75% of time in this half of apparatus. Thus, it can be suggested that *m*CPP produced neophobic responses in mice rather than exacerbating them. In addition, the hypolocomotor effects of *m*CPP at the highest dose seem to be merely secondary to neophobia rather than to sedation in so far as neophobic-like effects of *m*CPP already occur at doses which have little action on locomotion. These effects closely resemble those found with high doses of methamphetamine which were also able to increase neophobic responses of mice in a dose-dependent manner and to reduce locomotor activity at high doses.[8] By contrast, the light/dark procedure can be considered as an aversive situation in so far as the animals were placed by force into a novel enclosure half of which was brightly illuminated.[12] It has been shown that benzodiazepine receptor agonists tend to increase the time spent by mice in the lit box as well as the number of transitions, while inverse agonists decrease these parameters.[9–11,13] Our results indicate, therefore, that the anxious responses of mice towards this situation became more pronounced after administration of *m*CPP.

The present findings may be viewed as consistent with the anxiogenic effects induced by *m*CPP in rats[6,7] as well as with the observed anxiogenic properties of this drug in humans.[3–5]

Results suggest that the *m*CPP anxiogenic action is mediated by 5-HT_{1C} receptor stimulation.[6] Recently, we found that eltoprazine, like mCPP, was able to induce neophobic responses and to potentiate anxiety in mice.[14] As eltoprazine has been reported to act as a postsynaptic 5-HT_1 agonist,[15,16] we can tentatively suggest the hypothesis that increased activity of ascending serotonin pathways may produce and/or potentiate emotional behaviour in humans as well as in animals. This view is further substantiated by the observation that drugs which reduce 5-HT activity such as buspirone, 8-OH-DPAT or MDL 73005EF possess anxiolytic properties.

Conclusion

The present findings confirm earlier reports describing anxiogenic-like effects of *m*CPP and furthermore suggest that increased 5-HT activity leads to increased arousal as well as emotional behaviour.

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