

Evidence that tolerance to the anxiogenic-like effects of mCPP does not involve alteration in the function of 5-HT_{2C} receptors in the rat choroid plexus

G. Griebel¹, J.-L. Moreau², F. Jenck², V. Mutel², J.R. Martin² and R. Misslin³

¹Békésy Laboratory of Neurobiology, University of Hawaii, 1993 East-West Road, Honolulu, HI 96822, USA, ²Pharma Division, Preclinical Research, F. Hoffmann-La Roche Ltd, CH-4002, Basel, Switzerland, and ³Laboratoire de Psychophysiologie, 7 rue de l'Université, F-67000 Strasbourg, France

Correspondence to: G. Griebel at above address

The mechanisms by which 1-(3-chlorophenyl) piperazine (mCPP) causes anxiety are unclear, but it has been suggested that the serotonin 5-HT_{2C} receptor subtype may be involved in this effect. We have therefore studied the effect of chronic treatment (3 weeks) with mCPP in two animal models of anxiety (light/dark choice task in mice and elevated plus-maze test in rats) and subsequently assessed the function of 5-HT_{2C} receptors (measured by maximal stimulation of 5-HT_{2C} receptor-mediated phosphoinositide hydrolysis) in rat choroid plexus, where the receptor is present at very high levels. mCPP treatment regimens led to a tolerance to the anxiogenic-like action of the drug, but failed to alter the second messenger coupling of the 5-HT_{2C} receptors in the choroid plexus, thereby suggesting the involvement of different mechanisms in this behavioral effect.

Keywords: Anxiety – Choroid plexus – Chronic treatment – Elevated plus-maze test – 5-HT_{2C} receptor – Light/dark choice procedure – mCPP – Mouse – Phosphoinositide hydrolysis – Rat

INTRODUCTION

Numerous clinical studies have demonstrated that the serotonin (5-HT) agonist 1-(3-chlorophenyl) piperazine (mCPP) causes anxiety in healthy volunteers and potentiates anxious reactions in agoraphobic, obsessive-compulsive disorder and panic disorder patients (e.g. Kahn *et al.*, 1990). Acute administration of the compound in animals has also been reported to produce anxiogenic-like effects (e.g. Kennett *et al.*, 1989; Griebel *et al.*, 1991; Rodgers *et al.*, 1992), whereas chronic mCPP treatment led to a tolerance to the anxiogenic-like action of the drug (Kennedy *et al.*, 1993).

mCPP binds preferentially to the 5-HT_{2C} receptor with lower affinity for 5-HT_{1A}, 5-HT_{1B} and 5-HT_{2A} receptors in rat brain tissue (Hoyer, 1991). One major hypothesis to explain the anxiogenic action of mCPP is its effects on 5-HT_{2C} receptors (Kennett *et al.*, 1989; Gibson *et al.*, 1992). It has also been shown that in rats, chronic mCPP downregulates cortical 5-HT_{2A} receptors as well as 5-HT_{2C} sites in cortex, hippocampus and choroid plexus and finally, upregulates hippocampal 5-HT_{1A} sites (Ulrichsen *et al.*, 1992; Pandey *et al.*, 1993).

The purpose of this study was to further characterize the behavioral and biochemical effects of mCPP after repeated administration. The behavioral effects were evaluated using the light/dark choice paradigm in mice (Misslin *et al.*, 1989) and the elevated plus-maze test in rats (Pellow and File, 1986). As the 5-HT_{2C} site is particularly dense in the choroid plexus (Pompeiano *et al.*, 1994), we investigated the effect of chronic mCPP on the function of this receptor by measuring the maximal stimulation of 5-HT_{2C} receptor-mediated phosphoinositide (PI) hydrolysis in rat choroid plexus.

METHODS

Subjects

Male Swiss albino mice (Ibm:MoRo), 10 weeks old at the time of testing, were used in the light/dark choice procedure. Male Wistar rats (Ibm:RoRo) weighing 150–200 g at the time of testing were used in the elevated plus-maze. All animals were housed in groups of five with free access to food and water. They were bred and provided by

Biological Research Laboratories (Füllinsdorf, 4414, Switzerland).

Apparatus

The light/dark choice paradigm consisted of two polyvinylchloride boxes ($20 \times 20 \times 14$ cm) separated by an opaque plastic tunnel ($5 \times 7 \times 10$ cm). One of the boxes was darkened. The amount of time spent by mice in the lit box (TLB) and the number of transitions through the tunnel were recorded. Mice were assigned randomly to treatment with either mCPP (2 mg/kg; $n = 15$) or saline ($n = 28$). Twenty-four hours after the last injection, mice from the saline group were further divided into saline (Group A; $n = 15$) and mCPP (Group B; $n = 13$) groups. Animals chronically treated (20 days) with mCPP (group C) were challenged with an acute mCPP administration.

The elevated plus-maze test consisted of two open arms (50×10 cm) and two enclosed arms of the same size with walls 50 cm high, elevated 50 cm above the ground. The

time spent in open arms and the number of open-arm entries were scored. The results were expressed as mean ratio of open arm to total arm entries and mean ratio of time spent in open arms to total time for individual rats. Rats were assigned randomly to treatment with either mCPP 1 mg/kg ($n = 10$) or saline ($n = 20$). Twenty-four hours after the last injection, rats from the saline group were further divided into saline (Group D; $n = 10$) and 1 mg/kg mCPP (Group E; $n = 10$). Animals chronically treated with mCPP (Group F) were challenged with an acute mCPP administration.

In both experiments, animals were observed for 5 min on a video monitor from an adjacent room.

Drug

mCPP (Research Biochemicals Inc., Wayland, MA, USA) was dissolved in saline. Animals received 20 daily injections (i.p.) of mCPP in an injection volume of 10 ml/kg body weight in mice and 5 ml/kg body weight in rats.

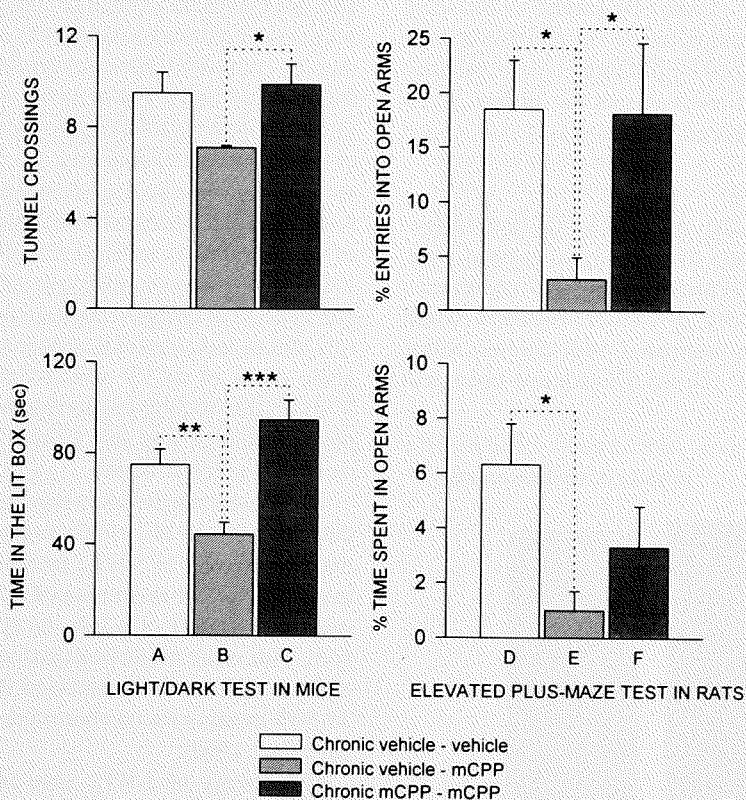


FIG. 1. Effects of acute and repeated administration of mCPP on the behavior of mice in the light/dark choice paradigm and of rats in the elevated plus-maze test. Groups A, B, D and E were treated chronically with saline, and Groups C and F with mCPP (2 and 1 mg/kg respectively) for 20 days. At day 21, and 30 min before the test, groups A and D received a saline injection, Groups B and C were challenged with a dose of 2 mg/kg of mCPP, and Groups E and F with a dose of 1 mg/kg of mCPP. Data are mean and S.E.M. * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ (Bonferroni's *t*-test).

Biochemical assay

5-HT_{2C} receptor-mediated phosphoinositide hydrolysis response in the rat choroid plexus was measured using 10 μ M of 5-HT, as preliminary findings showed that this concentration of 5-HT caused a maximal phosphoinositide hydrolysis response in the rat choroid plexus. The assay was performed as described elsewhere (Kuoppamäki *et al.*, 1993).

Statistical analysis

Statistical significance of differences between groups was ascertained by a combined analysis of variance (ANOVA) and a Bonferroni's *a posteriori* *t*-test.

RESULTS

Light/dark choice paradigm

ANOVA revealed a reliable main effect for time spent in the lit box [$F(2,40) = 13.47$; $p < 0.001$] and for tunnel crossings [$F(2,40) = 4.45$; $p < 0.02$]. Figure 1 (left panel) shows that acute mCPP (Group B) tended to decrease activity in the lit box in comparison with control group. In contrast, chronic mCPP treatment (Group C) significantly increased the behavioral measures in the lit box when compared with acute treatment (Group B).

Elevated plus-maze test

ANOVA indicated a reliable main effect for both behavioral measures: percentage of time spent in open arms [$F(2,27) = 4.25$; $p < 0.02$] and percentage of entries into these arms [$F(2,27) = 4.48$; $p < 0.02$]. Figure 1 (right panel) shows that when compared with chronic saline (Group D), acute mCPP (Group E) reduced open arm

investigations. In comparison with animals treated chronically with mCPP (Group F), rats receiving a single injection of this drug (Group E) made significantly fewer entries into open arms. Unlike a single injection, repeated administration of mCPP did not significantly alter animals' performances when compared with the control group.

PI hydrolysis measurements in rats

As shown in Fig. 2, the stimulation of inositol phosphate production with 10 μ M 5-HT led to the same level of [3 H]inositol phosphate production in the different groups tested. Under these conditions of maximal stimulation, neither acute nor chronic treatment with mCPP significantly modified the apparent efficacy of 5-HT to stimulate this second messenger pathway.

DISCUSSION

In the present study, a single injection of mCPP in mice and rats enhanced avoidance responses to aversive situation in the light/dark task and the elevated plus-maze test, thereby confirming previous reports of an anxiogenic-like action of the drug. Furthermore, repeated administration of mCPP induced tolerance to the anxiogenic-like effects of the drug in both paradigms. These data are also consistent with a recent finding showing that chronic treatment with mCPP produces tolerance to anxiogenic-like effects of mCPP in the rat social interaction test (Kennedy *et al.*, 1993). Furthermore, analysis of the 5-HT_{2C} receptor-mediated PI hydrolysis response performed in the rat choroid plexus indicated that neither acute nor chronic anxiogenic doses of mCPP affected the apparent efficacy of 5-HT in this second messenger pathway, indicating that neither regimen was able to alter the function of 5-HT_{2C} receptors in this structure.

The present results taken together with receptor binding data (Pandey *et al.*, 1993) suggest that changes in the maximal density of 5-HT_{2C} receptors after chronic mCPP treatment are not accompanied by similar changes in the maximal response of 5-HT_{2C} receptor-mediated PI hydrolysis. This result might be explained by a putative receptor reserve, as it has been reported that a 5-HT_{2C} reserve of 30-50% exists in the rat choroid plexus (Sanders-Bush and Breeding, 1990).

The density of 5-HT_{2C} sites in the choroid plexus is extremely high, whereas in other brain areas (e.g. in the frontal cortex and in limbic structures), the density of 5-HT_{2C} receptors is considerably lower (Pompeiano *et al.*, 1994). Therefore, it is not known what the relationship is between the 5-HT_{2C} receptor occupancy and functional responses in other brain areas (e.g. hippocampus) which, compared with choroid plexus, are more likely to mediate anxiogenic-like effects of mCPP (Whitton and Curzon,

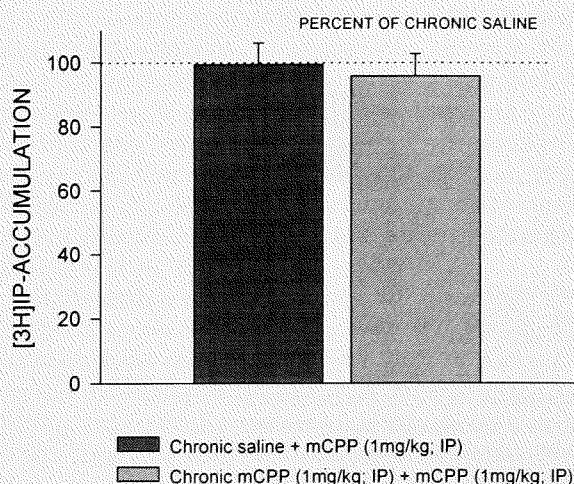


FIG. 2. 5-HT (10 μ M)-stimulated inositol phosphate production in the choroid plexi of control rats or rats treated acutely or chronically with mCPP. Data are mean and S.E.M.

1990). In addition to the well-established interaction of mCPP with various 5-HT receptor subtypes, the drug has also been shown to increase extracellular 5-HT in rat brain by a presynaptic mechanism involving 5-HT transporters and this effect must be taken into account in the pharmacological analysis of its central effect (e.g. Baumann *et al.*, 1993).

In conclusion, in spite of the high density of 5-HT_{2C} sites in the choroid plexus, there is no evidence from the results of this study that the tolerance to anxiogenic action of mCPP emerges from changes in the function of this receptor in this particular area.

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