m-CPP INDUCED HYPOLOCOMOTION DOES NOT INTERFERE IN THE ASSESSMENT OF MEMORY FUNCTIONS IN RATS

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ABSTRACT

Central serotonergic system plays a critical role in the regulation of memory processes in rats. Evidence suggests that a dysfunction of serotonergic system contributes to various pathological conditions. Among the multiple classes of serotonin (5-Hydroxytryptamine, 5-HT) receptors described in CNS, much attention has been devoted to the role of 5-HT2C receptor family on memory functions. A number of studies have shown that 5-HT2C receptor agonists impair memory function and also decreased locomotor activity of rats. The present study was designed to investigate the effect of different doses of 5-HT2C receptor agonist metachlorophenylpiperazine (mCPP) on locomotion and cognitive behavior in rats. Groups of adult male rats were injected mCPP intraperitoneally at doses of 1, 3 and 5 mg/kg. The learning and memory of rats were assessed by water maze (WM) and passive avoidance (PA) tests. Locomotor activity of rats was monitored by open field test. mCPP decreased locomotor activity of rats as reported earlier. A negative correlation between memory function and 5-HT2C receptor stimulation was observed in WM. Furthermore the administration of mCPP dose dependently impaired memory functions and the impairment of memory induced by mCPP was greatest at the highest dose. PA test was also performed in the present study to confirm that the decreased locomotor activity exhibited by mCPP injected rats did not affect the memory assessment in WM. Irrespective of hypolocomotion induced by mCPP, drug injected rats took less time to enter the punishable compartment which confirmed that the impairment in memory functions following mCPP was not due its effect on locomotion. It is suggested that 5-HT2C receptors might be involved in memory function probably mediating a suppressive or constraining action by decreasing dopamine levels. It can be therefore concluded that 5-HT2C receptors have a negative influence on memory function, which raises the possibility of using 5-HT2C receptor antagonists in the improvement of memory functions.

Keywords: mCPP, memory, locomotor activity.

INTRODUCTION

Metachlorophenylpiperazine (mCPP) is an active metabolite of the antidepressant trazodone and nefazodone (Caccia *et al*., 1981) which has been widely used in psychopharmacology research as a probe of 5-HT2C receptor function in vivo (Silverstone *et al*., 1994) and its administration reduces food intake and appetite in animals and human (Kennett and Curzon, 1988; Cedraz-Mercez *et al*., 2005). mCPP displays high affinity towards 5-HT2C receptors and moderate, or low, affinity towards 5-HT1A, 5-HT1B, 5-HT2A, 5-HT2B, and 5-HT3 receptors; in addition, the compound has some affinity for alpha-2-adrenoceptors (Silverstone *et al*., 1994). At the 5-HT2C receptor, mCPP seems to act as a partial agonist with relatively high intrinsic efficacy, whereas, at the other serotonin receptors, the intrinsic efficacy of mCPP is moderate or low (Kahn and Wetzler, 1991; Murphy *et al*., 1991). mCPP is also known to suppress locomotor activity in mice and rats (Meert *et al*., 1997; Stiedl *et al*., 2006). It is commonly used as an anxiety-provoking agent (Bilkei-Gorzo *et al*., 1996; Erzegovesi *et al*., 2001). The 5-HT2 receptor has been further divided into the 5-HT2A, 2B and 2C subtypes along with the advent of the selective antagonists has allowed a more detailed investigation on the role and therapeutic significance of these subtypes in cognitive functions. Specific agonists and antagonists of 5-HT2C receptor showed differential effects on acquisition, maintenance and retention of memory (Meneses and Hong, 1997; Sabbe *et al*., 2001). The 5-HT2 receptor subtypes blockade may provide some benefit to reverse poor memory consolidation conditions associated with decreased cholinergic, glutamatergic, and serotonergic neuro-transmission (Boast *et al*., 1999; Meneses, 2004).

The present study further analyzed the 5-HT2C receptor role in memory functions. The main objective of the present study was to further emphasize and confirm that the impairment in memory function following mCPP was not due to its effect on locomotion.

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MATERIALS AND METHODS

Animals

Twenty four locally bred albino Wister rats (180-200g) purchased from Aga Khan University hospital were used in the study. All animals were housed individually under a 12 h light-dark cycle (light on at 6:00h) and controlled room temperature (22 ± 2°C) with free access to cubes of standard rodent diet and tap water for at least 3-4 days before experimentation. All experiments were conducted according to a protocol approved by Local Animal Care Committee.

Chemicals

mCPP was purchased from Merck (Germany). All the other chemicals used were also of the highest quality available.

Drug administration

mCPP at doses of 1, 3 and 5 mg/kg were injected intraperitoneally (i.p.) in volumes of 1ml/kg. Control animals were injected with saline (1ml/kg).

Experimental protocol

Animals were randomly divided into control and three test groups. Control rats were injected with saline (0.9% NaCl) and other 3 test groups were injected with different doses of mCPP (1mg/kg, 3mg/kg and 5mg/kg). Weighed amount of food was placed in the hopper of all the cages. Behavioral activities of rats were monitored after 30 minutes of injection.

Behavioral tests

Water Maze Test

The effects on spatial memory were examined by assessing performance in a Water Maze (WM) test designed in our laboratory. Actual Morris Water Maze is circular while we used rectangular maze that has been used before by Plech *et al* (2000**)**. The method is not same as that described by them. It is a modification of their method. Dimensions of the WM are same as described by them. The WM apparatus used in the present study consisted of a transparent rectangular glass tank (60 x 30cms) filled with room temperature-water opacified with powder milk, to the depth of 12cm. A wooden platform (15 x 13cms) was hidden 2cm below the surface of water in a fixed location. The experiment was performed after 30 minutes of injections. Initially the rats were trained and during the training session each rat was placed into the water facing the wall of the tank and allowed 120 seconds to locate and climb onto the submerged platform. The rat was allowed to stay on the platform for 10 seconds. If it failed to find the platform within the allowed time it was guided gently onto the platform. Memory functions of rats were tested by recording the retention latency (RL; the time taken by each rat to locate the hidden platform 24h after training). The cut off time for each session was 2 minutes.

Locomotor and exploratory activity

The activity of control and mCPP treated rats were monitored in an open field apparatus which consisted of a square area measuring 76 x 76 cm with walls 42 cm high. The floor was divided by lines into 25 equal squares. To determine activity, a rat was placed in the central square of the open field and the number of square crossed with all four paws was scored for 5 min as described earlier (Haider *et al*., 2004). Activities of control and mCPP-treated rats were monitored in a balanced design to avoid order effect.

Passive avoidance test

Passive avoidance paradigm consists of two compartments as an illuminated ‘safe’ and a dark 'punishable' one. Both compartments were connected with a door that enable free crossing from one compartment to another. Both compartments had a grid floor. The diameter of rods was 5 mm with 0.5 cm distance between the rods. In the training session, rat was placed in an illuminated box. Once the rats prompted by their instinct stepped its four paws into the dark compartment, rats received 1.5 mA foot shock through the grid floor to its paws for 5 seconds. After receiving the foot shock, it immediately came back to illuminated safe compartment. During the test period (24 hour later), rats were placed in the bright compartment again for a maximum of 5 minutes. The step- through latency that indicates the time elapsed before the rat entered the dark compartment was recorded in the test session as described earlier (Khaliq *et al*., 2006).

STATISTICAL ANALYSIS

Neurochemical and behavioral data were analyzed by one-way ANOVA. Post hoc comparisons were made using the Newman-Keuls test; p values < 0.05 were considered significant.

RESULTS

Fig. 1A shows the effect of mCPP (1mg/kg, 3mg/kg and 5mg/kg) on training of rats in WM. Data analyzed by one-way ANOVA exhibited non-significant treatment effect.

Fig. 1B shows the effect of mCPP (1mg/kg, 3mg/kg and 5mg/kg) on memory functions in rats using water maze. Analysis by one-way ANOVA showed a significant effect of drug on memory functions ($F=41.9$ df 20,3 $P<0.01$). Post hoc analysis by Newman-Keuls test showed that memory of rats was significantly ($P<0.01$) impaired following 1 mg/kg (182%), 3 mg/kg (395%) and 5 mg/kg mCPP (457%). A dose-dependent increase in time to reach the platform was seen with increasing doses of

mCPP showing a dose dependent impairment in spatial navigational skills.

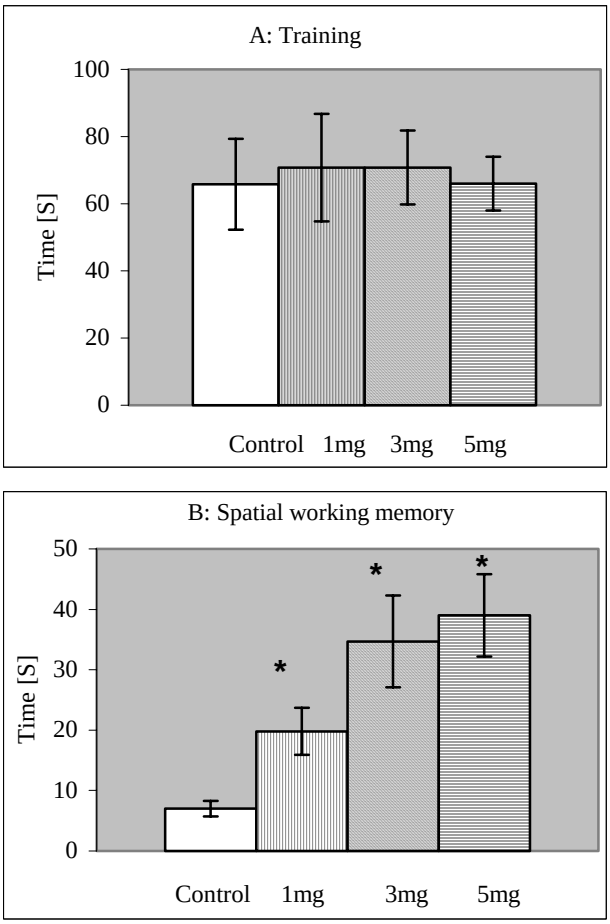

Fig. 1A and **1B**: Effect of mCPP administration (1mg/kg, 3mg/kg and 5mg/kg) on training (a) and memory functions (1b) of rats using Water Maze. Values are means ± SD (n=6). Significant differences by Newman keuls test:*P < 0.01 compared with control rats.

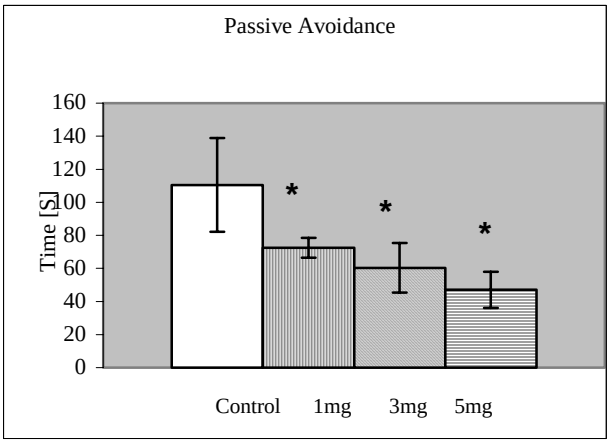

Fig. 2: Effect of mCPP administration (1mg/kg, 3mg/kg and 5mg/kg) on passive avoidance response in rats in the bright and dark compartment. Values are mean ± S.D (n=6). Significant difference by Newman Keuls test; *P<0.01 compared with controls.

Fig. 2 shows the effects of increasing doses of mCPP on the performance of rats in PA test. One-way ANOVA revealed significant impairment in memory (F=15 df 20,3 P<0.01). Post hoc comparison by Newman-keuls test showed that retention of Passive Avoidance was impaired in test rats than in controls. A dose-dependent decrease in retention of Passive Avoidance was seen in the rats that were administered with the drug. It was seen that the rats given the highest dose 5mg/kg (57%) showed greater impairment than the rats given 3mg/kg (45%) and 1mg/kg (34%) doses. Despite the hypolocomotary effects of mCPP the rats still made attempts to enter the dark box and this effect was seen to increase with the dose of the drug. This shows the dose-dependent impairment in memory following mCPP administration.

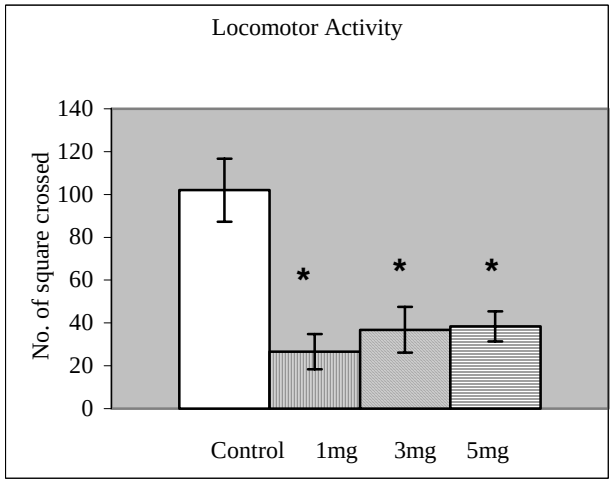

Fig. 3: Effect of mCPP administration (1mg/kg, 3mg/kg and 5mg/kg) on locomotion of rats using Open Field Apparatus. Values are means ± SD (n=6). Significant differences by Newman keuls test:*P < 0.01 compared with controls.

Fig. 3 shows the effect of mCPP(1mg/kg, 3mg/kg and 5mg/kg) on locomotor activity of rats was assessed by open field apparatus. Analysis by one-way ANOVA showed a significant treatment effect (F=63.4 df 3, 20 P<0.01). Post hoc comparison by Newman keuls test showed a decrease in the number of squares crossed by mCPP injected rats at doses of 1 mg, 3mg and 5 mg as compared to control.

DISCUSSION

Studies have reported that 5-HT2C receptor antagonists modulate storage and retrieval of information (Pitsikas and Sakellaridis, 2005). mCPP has been shown to potentiate the cognitive deficits produced by scopolamine in healthy elderly volunteers (Little *et al*., 1995) and the cognitive deficit found in Alzheimer disease (Lawlor *et al*., 1989). In current study we chose passive avoidance

and water maze tests to measure changes in memory functions following mCPP administration at various doses. Memory of rats was tested in the Water Maze in which the motivating stimulus was a hidden fixed platform. This task requires subject to use the spatial arrangement of cues outside of the rectangular pool to swim to the hidden fixed located platform. It was seen that mCPP increased the latency time to reach the platform as compared to control rats and this effect was more pronounced at 3 and 5 mg/kg doses i.e. higher doses of mCPP resulted in more impairment in spatial working memory, which showed that mCPP induced dose related impairment of spatial memory functions. mCPP also decreased the locomotor activity of rats in the novel environment of open field which is consistent with previous reports (Haleem, 1993; Meert *et al*., 1997). In the present study mCPP injected rats exhibited a decrease in Passive Avoidance learning. This memory impairing effect of mCPP on PA was again dose dependent as the rats injected with 3 and 5 mg/kg took lesser time to reach the dark box than 1mg/kg dose. It was seen that despite the hypolocomotor effects of mCPP, the rats still made effort to enter the dark punishable compartment which showed that decreased locomotor activity of mCPP injected rats did not interfere with cognitive impairment.

Central serotonergic and dopaminergic systems play a critical role in the regulation of normal and abnormal behavior. There are reports which showed that mCPP increased extracellular concentration of 5-HT (Baumann *et al*., 1993; Eriksson *et al*., 1999) and decreased dopamine levels (Matteo and Esposito, 2003; Alex and Pehek, 2006). Memory impairment effect of mCPP in the present study may be due to decreased DA levels as there is an overwhelming evidence for a critical role of dopamine in cognition (Wilkerson and Levin, 1999; Liao *et al*., 2002). Cognitive symptoms have been associated with dopamine disregulation in numerous diseases including schizophrenia (Knable and Weinberger, 1997), depression (Jimerson, 1987), drug addiction (Wise, 1996) and Parkinson disease (Gotham *et al*., 1988). Support for a role of dopamine in cognition also comes from studies of dopaminergic drug in normal subject. Luciana *et al* (1998) showed facilitation of working memory with the D2 agonist, bromocriptine. There is also evidence for a cognitive enhancing effect of the D1/D2 agonist pergolide (Muller *et al*., 1998). The 5-HT2C receptor mediates an inhibitory effect on DA release and inhibits tonic as well as evoked DA release (Alex and Pehek, 2006). It is reported that mCPP inhibits dopaminergic activity via 5HT2C receptors as it has a high affinity for this receptor subtype (Baxter *et al*., 1995). The involvement of 5-HT2C receptors in the modulation of DA activity was demonstrated by Goldstein *et al* (1987), which showed that at low doses 5-HT2C receptor antagonists increased firing rates of dopamine neurons in the mesocorticolimbic system. The above studies suggest that mCPP decreases DA firing via the 5-HT2C receptor, which could be the cause of impaired memory function exhibited by the mCPP injected rat.

Previously we reported that 5-HT enhanced memory function in rats (Haider *et al*., 2006) whereas decreased 5-HT levels decreased memory functions (Haider *et al*., 2005). Lack of improvement in memory function following 5-HT agonist mCPP in the present study suggests the involvement of neurotransmitter other than 5-HT such as dopamine in cognitive processes. The present finding therefore suggests that impairment in memory exhibited by the mCPP injected rats may be due to decreased dopaminergic function. Moreover the assessment of memory by two different methods WM and PA further confirms that mCPP induced hypolocomotion does not interfere with the effects of drug on memory function. Further experiments are needed to confirm the role of DA following mCPP administration.

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