INTRODUCTION

The role of serotonin (5-Hydroxytryptamine, 5-HT) in the mechanism of learning and memory is a topic of intense research. It produces its effects by binding to a number of membrane bound receptors. Increased brain 5-HT has been shown to improve cognitive performance, whereas, decreased brain 5-HT has been shown to impair cognition. 5-HT and its receptors are widely distributed and are found in both central and peripheral nervous system, as well as in the gut, heart, blood and vascular system. 5-HT2 is one of the types of 5-HT receptors and is classified into three subclasses 5-HT2A, 5-HT2B and 5-HT2C. Among the multiple classes of serotonin receptors, much attention has been focused to 5-HT2C receptor family because of its involvement in memory functions. 5-HT2C subtype corresponds to previously known 5-HT1C receptors, which are now classified as 5-HT2C. This 5-HT2C subtype is involved in the regulation of anxiety, cognition and brain plasticity.

Metachlorophenylpiperazine (mCPP) is derived from antidepressant trazodone and nefazodone which has been mostly used by the researchers in psychopharmacology research as a probe of 5-HT2C receptor function in vitro. Its administration reduces food intake and appetite in animals and human. mCPP binds to a variety of receptors but displays high affinity towards 5-HT2C receptors and moderate to 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. 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. mCPP is also known to suppress locomotion in mice and rats.

Previously, we have reported that different doses of mCPP induced memory impairment in a dose dependent manner probably by decreasing dopaminergic neurotransmission. Specific agonists and antagonists of 5-HT2C receptor showed varying effects of different types on memory. The main objective of this study was to assess the contribution of 5-HT2C receptors in the regulation of memory functions by using nonselective 5-HT2C antagonist / D2 agonist mesulergine and mCPP.

ABSTRACT

Objective: To determine the effect of non-selective 5-HT2C antagonist mesulergine and 5-HT2C agonist mCPP (meta-chlorophenylpiperazine) on learning acquisition (LA), short-term memory (STM) and long-term memory (LTM).

Study Design: Experimental study.

Place and Duration of Study: Department of Biochemistry, University of Karachi, from December 2009 to June 2010.

Methodology: Twenty-four male albino Wistar rats were used in this study. The agonist and antagonist (mCPP and mesulergine) were injected intraperitoneally at a dose 3.0 mg/kg in volumes of 1 ml/kg. Control animals were injected with saline (1 ml/kg). Animals were randomly divided into four groups (n=6). 1st being control group, 2nd being mCPP injected group, 3rd being mesulergine injected group and 4th group being injected with both mesulergine and mCPP. Behavioural activities of rats were monitored after 30 minutes of injection. For assessment of memory functions, water maze apparatus was used.

Results: Administration of mCPP impaired STM, LTM and LA of rats. Mesulergine injected rats exhibited no alteration in memory functions. However, when it was injected with mCPP then there were no memory deficits induced by mCPP.

Conclusion: Ability of 5-HT2C receptor antagonist mesulergine to block the memory impairment effect of mCPP indicated an important regulatory role of 5-HT2C receptors in cognitive processes.

Key words: mCPP (meta-chlorophenylpiperazine), Mesulergine, Memory function, Water maze, Dopamine, Learning acquisition, Short-term memory, Long-term memory.
METHODOLOGY

Twenty-four locally bred albino Wistar male rats purchased from the Aga Khan University Hospital were used in the study. Animal use protocols were approved by the Local Animal Care Committee on Animal Research. All efforts were made to minimize animal suffering, as well as to reduce the number of animals according to the guidelines recommendations. Animals were housed in individual cages for 7 days for acclimatization to the study surroundings, and allowed free access to fresh water and chow in a temperature-controlled environment of 24°C with a 12-hour light, 12-hour dark cycle before initiation of the experiment. mCPP and mesulergine were purchased from Merck (Germany).

mCPP and mesulergine at a dose 3.0 mg/kg were injected intraperitoneally in volumes of 1 ml/kg. Control animals were injected with saline (1 ml/kg). Animals were randomly divided into two groups (n=12). The first group was injected with saline and the second group of rats was injected with mesulergine (3.0 mg/kg). Thirty minutes after the first injection both groups were again subdivided into two groups (n=6), one injected with saline and other group was injected with mCPP (3.0 mg/kg). The four groups were saline + saline (control), saline + mCPP, mesulergine + saline and mesulergine + mCPP. Behavioural activities of rats were monitored 30 minutes after the second injection.

The effects on learning acquisition, short-term memory and long-term memory were examined by assessing performance in a Water Maze (WM) test designed in our laboratory. The actual Morris Water Maze is circular while a rectangular maze was employed in this study that has been used before by Plech and coworkers.10 The WM apparatus used in the present study consisted of a transparent rectangular glass tank (60 cm x 30 cm) filled with room temperature-water opacified with powder milk, to a depth of 12 cm. A wooden platform (15 cm x 13 cm) was hidden 2 cm below the surface of water in a fixed location. The experiment was performed after 30 minutes of drug administration. 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. After training animals learning acquisition was tested immediately by noting the initial latency (IL; the time taken by each rat to relocate the hidden platform immediately after training). STM was tested 60 minutes after training and LTM 24 hours after training by recording the retention latency (RL; the time taken by each rat to locate the hidden platform 1 hour and 24 hours after training). The cut off time for each session was 2 minutes.

Data was analyzed by two-way ANOVA. Post hoc analysis was done by Newman-Keuls test; p-values < 0.05 were considered significant.

RESULTS

Table I shows the effect of mesulergine and mCPP on LA, STM and LTM of rats. Values are presented as mean ± standard deviation.

Figure A shows the effect of mesulergine and mCPP on LA of rats. Data analyzed by two-way ANOVA revealed a significant effect of mesulergine (F=83.8, df: 23,1, p < 0.01), mCPP (F=98.6, df: 20,1, p < 0.01) and interaction between the two agents (F=55.1, df: 23,1, p < 0.01). Post hoc analysis by Newman-Keuls test showed that mCPP significantly (p < 0.01) impaired LA of rats in WM. Mesulergine itself had no effect on LA. As compared to mCPP injected rats, impaired LA induced by mCPP was completely reversed (p < 0.01) in this group.

Figure B shows the effect of mesulergine and mCPP on STM of rats. Data analyzed by two-way ANOVA revealed a significant effect of mesulergine (F=18.3, df: 23,1, p < 0.01), mCPP (F=63.7, df: 20,1, p < 0.01) and interaction between the two agents (F=26.2, df: 23,1, p < 0.01). Post hoc analysis by Newman-Keuls test showed that administration of mCPP significantly (p < 0.01) impaired STM of rats in WM. Mesulergine

<table>
<thead>
<tr>
<th>LA</th>
<th>Saline+Saline</th>
<th>Saline+mCPP</th>
<th>Saline+Mesulergine</th>
<th>Mesulergine+mCPP</th>
<th>2 Way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>16 ± 4.8</td>
<td>68 ± 12.2 **</td>
<td>10.8 ± 2.9</td>
<td>18.3 ± 5.7++</td>
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<tr>
<td>STM</td>
<td>5.8 ± 0.9</td>
<td>37.6 ± 11.1 **</td>
<td>7.8 ± 1.3</td>
<td>14.8 ± 3.86++</td>
<td></td>
</tr>
<tr>
<td>LTM</td>
<td>3.4 ± 0.8</td>
<td>45 ± 10**</td>
<td>7 ± 1.7</td>
<td>19.8 ± 5.1**++</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation (n=6). Significant difference by Newman-Keuls test; **p<0.01 vs. respective controls; +p<0.05, ++p<0.01 vs. mCPP treated rats following two-way ANOVA.
itself had no effect on STM of rats. When both were administered, impairment of STM that was induced by mCPP was 60.6% reversed (p < 0.01).

Figure C shows the effect of mesulergine and mCPP on LTM of rats. Data analyzed by two-way ANOVA revealed a significant effect of mesulergine (F=21.2, df: 23,1, p < 0.01), mCPP (F=135.4, df: 20,1, p < 0.01) and interaction between the two agents (F=37.8, df: 23,1, p < 0.01). Post hoc analysis by Newman-Keuls test showed that mCPP significantly (p < 0.01) impaired LTM of rats in WM. Mesulergine itself had no effect on LTM of rats. When both were administered, impairment of LTM that was induced by mCPP was 56% reversed.

DISCUSSION

Metachlorophenylpiperazine (mCPP) binds potently to various 5-HT receptors in rat brain with strongest affinity at 5-HT2C sites. It was reported by many authors that mCPP potentiated the cognitive deficits.11,12 In the present study water maze test were chosen to measure changes in memory functions following mCPP and mesulergine administration. The present study reported that mCPP impaired LA, STM and LTM of rats. Mesulergine injected rats had no effect on memory functions. However, when mesulergine was injected with mCPP, it significantly antagonized the memory deficits induced by mCPP.

Role of 5-HT 2C antagonists on memory function is not well established. Some authors reported memory impairment following mesulergine administration which is 5-HT2C antagonist, while others reported memory enhancement following the blockage of 5-HT2C receptors. No effect of 5-HT2C antagonist by itself on memory functions has been reported.13-15 The present study reported that antagonism of 5-HT2C receptor by mesulergine produced no effect on LA, STM and LTM of rats. However, when mesulergine was injected with mCPP, it significantly antagonized the memory deficits induced by mCPP.

Previously, brain serotonergic system has been implicated in the control of learning and memory. Increased circulating tryptophan availability and resultant increase in brain serotonin content enhanced memory functions in rats of either gender. Memory enhancement response in rats accompanied by a greater increase in serotonin release in the hippocampus played a key role in memory functions. The Hippocampus is a major limbic target of the brain stem serotonergic neurons that is known to modulate learning and memory. In the short-term learning process, the modifications are due to the changes in protein phosphorylation whereas, in long-term memory new proteins are formed.16 Previously, it was reported that increased 5-HT levels enhanced memory function in rats whereas, decreased 5-HT levels impair memory functions.17 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 (DA) 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.
The exact role of the serotonergic system on memory is complex. Among many other functions, 5-HT neurotransmission plays an important role in the mechanism of learning and memory. 5-HT receptors show regional distribution in brain areas involved in learning and memory.\textsuperscript{18} 5-HT\textsubscript{2C} receptors have a role in learning and memory but the literature concerning the role of this receptor in cognition remains unclear. Specific agonists and antagonists of 5-HT\textsubscript{2C} receptors showed different effects on acquisition, maintenance and retention of memory.\textsuperscript{8,9}

Previous study on SR46349, a 5-HT\textsubscript{2A/2C} receptor antagonist enhanced DA release in prefrontal cortex.\textsuperscript{19} There are at least 14 5-HT receptor subtypes, including 5-HT\textsubscript{1A}, 5-HT\textsubscript{1B}, 5-HT\textsubscript{2A}, 5-HT\textsubscript{3} and 5-HT\textsubscript{4} receptors act to facilitate DA release, while the 5-HT\textsubscript{2C} receptors mediates an inhibitory effect of 5-HT on DA release.\textsuperscript{20} Previous study on mCPP reported decreased DA levels following its administration\textsuperscript{21} while, mesulergine a nonselective 5-HT\textsubscript{2A/2B/2C} antagonist significantly increases DA release,\textsuperscript{22} DA is a neurotransmitter that is involved in cognitive function\textsuperscript{23,24} so decreased DA levels may be the reason of alteration in memory functions.

CONCLUSION

Mesulergine when given with mCPP can block the 5-HT\textsubscript{2C} receptors in the brain so mCPP is unable to bind to these receptors and impairment induced by mCPP was not seen. Mesulergine itself has no effect on memory functions. Ability of 5-HT\textsubscript{2C} receptor antagonist mesulergine to block the inhibitory effect of mCPP emphasizes the role of 5-HT\textsubscript{2C} receptors in the regulation of memory functions. However, further studies are warranted to determine neurochemical mechanism involved following mCPP and mesulergine administration.

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