5-HT3 receptors in selective animal models of cognition

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Role of 5-HT3 receptors in cholinergic hypofunctional models of cognitive impairment in the elevated plus maze model and a passive avoidance model is studied. Cognitive impairment was caused by scopolamine (1 mg/kg, ip) in mice and 5-HT3 ligands mCPBG (1 and 5 mg/kg, ip) and ondansetron (0.5 and 5 mg/kg, ip) were administered before the pre-learning phase to study the effects on acquisition, while post-learning administration was used to determine the effects on consolidation. Ondansetron improved acquisition and retention in cholinergic hypofunctional models while mCPBG potentiated selected impaired cognitive indices. The results indicate the role of 5-HT3 receptors in cognition and that an ideal evaluation of 5-HT3 ligands in cognition should distinguish true cognitive effects from locomotor, motivational and emotional effects.

It has been long established that the serotonergic system has an important role to play in learning and cognition. Though the early work put-forth several contradictions, with hindsight it can be said that the presence of distinct 5-HT receptor subtypes account for such discrepancies. Pre- and post-synaptic 5-HT1A receptors have been postulated to play a distinct role in learning and memory based on electrophysiological data, anatomical distribution and behavioural study. The role of 5-HT2A/D receptor agonists have been hampered by lack of specificity and negative intrinsic activity of ligands. Improvement of learning has been observed with 5-HT3 receptor antagonists. 5-HT3 receptors mediate a slow but long lasting excitatory response in the hippocampus suggesting modulation of learning and memory process. Anatomical data confirm the location of 5-HT3, 5-HT5 and 5-HT7 receptors on cognitive pathways, especially in the hippocampus, however lack of selective ligands have failed to demonstrate their role. Reports on 5-HT uptake blockers seems conflicting, possibly due to role of multiple 5-HT receptors.

The degeneration of central cholinergic system is widely believed to be associated with cognitive impairment in man, although other neurotransmitter systems have a significant role. Administration of 5-HT3 antagonists removes a potential inhibitory tone on the cholinergic neuron resulting in a net increase in acetylcholine-release, consistent with the action of a cognitive enhancer as defined by the cholinergic hypothesis of memory. Reports also demonstrate facilitation of acetylcholine release in the rat hippocampus following 5-HT3 receptor activation. There are several inconsistent reports of preclinical behavioural and biochemical activities involving 5-HT3 receptors demonstrated at wider dose ranges (µg/kg-mg/kg) with inverted U-shaped dose response curves.

In the present study the role of 5-HT3 receptors in cholinergic hypo functional models of the elevated plus maze and passive avoidance has been evaluated. The dose range of 5-HT3 modulators has been chosen based on their ability to demonstrate peripheral actions. 5-HT3 modulators are administered before the prelearning phase to study the effects on acquisition, while postlearning administration is used to determine the effects on consolidation.

Materials and Methods

Animals—Swiss albino male mice (Hissar Agricultural University, Haryana, India), weighing 20-25 g were housed under ideal conditions in a 12 hour light-dark cycle with free access to food and water. Three days prior to the experimentation the animals were kept in perspex cages in groups of 8 in the laboratory. All experiments were performed between 0900 to 1200 hrs.

Elevated plus maze—The elevated plus maze is normally used for assessment of anxiolytic activity. However, learning and memory can also be assessed as described below. In the training trial (first day), each mouse was placed at the end of one open arm,
which was randomly selected, facing away from the central platform. The latency time of transfer from the open arm to either of the closed arms (the transfer latency) was recorded. The criterion for entry of the mouse on the closed arm was the crossing of all four paws of the borderline separating the closed arm from the platform. After the measurement of the transfer latency, the mouse was allowed to move freely in the plus maze for 2 min. Then, the mouse was gently returned to its home cage and the maze cleaned with 20% (v/v) ethyl alcohol. On the next day, the retention trial was conducted. The mouse was again placed in the same position as in the training trial, and the transfer latency was recorded. Drug administered before the prelearning phase, allows the study of the effects on acquisition, while post learning administration is used to determine the effects on consolidation.

Scopolamine (1mg/kg, ip, RBI, USA), an anticholinergic drug was used to prevent the retention of learning. Interaction studies with scopolamine were conducted by pretreatment (30 min) with 5-HT₃ antagonist ondansetron (0.5 and 5mg/kg, ip, Natco Pharma, India) and agonist mCPBG (1 and 5mg/kg, ip, Lancaster, UK). The latency periods were measured 30 min after scopolamine administration. The drug treatments were given either before the learning session or before retention session.

Passive avoidance test—The apparatus consists of an electrical grid (24 x 30cm) having perspex enclosure. A small wooden platform in the center formed the shock free zone (SFZ; 10x7x1cm). Mice were put individually on the electric grid and were allowed to explore for 1 min. The stimulus (20V) was then applied and the latency to reach SFZ was recorded, three consecutive times at basal readings. Animals that reached SFZ in 2 min in the first trial were selected for the study. The reading obtained in the last trial was taken for evaluation. After one day of training, each animal was again put on the electric grid, and latency in reaching the SFZ and the number of mistakes (descents) made in 15 min were recorded both in the control and drug treated groups. After each trial the floor and the safe zone was wiped with 20% (v/v) ethyl alcohol.

Scopolamine (1mg/kg, ip), was used to prevent the retention of learning. Interaction studies with scopolamine were conducted by pretreatment (30 min) with the 5-HT₃ antagonist ondansetron (0.5 and 5mg/kg, ip, Natco Pharma, India) and agonist mCPBG (1 and 5mg/kg, ip). The latency periods were measured 30 min after scopolamine administration. The drug treatments were given either before the learning session or before retention session.

Statistical analysis—The difference in training and retention period latencies were compared for both pre learning and pre retention administrations of saline and scopolamine using a 't' test. One-way ANOVA was performed for the elevated plus maze and the passive avoidance test to evaluate the following:

to compare the training period latencies of various treatments on pre learning administration; and
to compare the retention period latencies of various treatments on pre learning administration; to compare the training period latencies of various treatments on pre retention administration; and
to compare the retention period latency of various treatments on pre retention administration.

In passive avoidance test the following were also evaluated:
to compare the number of 2 paw descents of various treatments (both before and after a 24 hr period) on pre retention administration; and
to compare the number of 4 paw descents of various treatments (both before and after a 24 hr period) on pre retention administration.

Post-hoc comparisons were made using a Dunnet’s test after ANOVA resulted in a significant F-test. Data obtained from the experiments carried out have been expressed as mean ± SE. The significance level for all tests was chosen, a priori, as P<0.05.

Results

Elevated plus maze—The difference in training and retention period latencies on both prelearning and pre retention in saline administered controls were significant (t test, P<0.05), while scopolamine (1 mg/kg, ip) did not produce a significant difference. (Table 1).

The difference in training and retention period latencies were compared for both prelearning and preretention administrations of ondansetron and mCPBG in comparison to scopolamine using Dunnet’s test (P<0.05) after one way ANOVA resulted in significant F-test (P<0.05) (Table 1). Prelearning administration of ondansetron (5 mg/kg, ip) significantly
decreased the retention period in comparison to scopolamine control animals. Pretreatment administration of ondansetron (0.5 and 5 mg/kg, ip) significantly decreased the retention period latency. Pre learning administration of mCPBG (1 and 5 mg/kg, ip), significantly increased the training period and retention period latencies in comparison to scopolamine control animals. Pretreatment administration of mCPBG (5 mg/kg, ip), significantly increased the retention period latency in comparison to scopolamine control animals.

Passive avoidance test—Scopolamine (1 mg/kg, ip), significantly (t test \( P < 0.05 \)) increased training period latency and retention period latency on prelearning administration in comparison to saline control animals, while pretreatment administration of scopolamine (1 mg/kg, ip), significantly increased only the retention period latency (Tables 2 and 3).

The difference in training and retention period latencies were compared for both prelearning and pre retention administrations of ondansetron and mCPBG in comparison to scopolamine using Dunnet’s test \( (P < 0.05) \) after one way ANOVA resulted in a significant F-test \( (P < 0.05) \) (Tables 2 and 3). Pre learning administration of ondansetron (0.5 and 5 mg/kg, ip), significantly decreased the training period and retention period transfer latencies in comparison to scopolamine control animals. mCPBG (1 and 5 mg/kg, ip), significantly increased training period latency and retention period latency in comparison to scopolamine control animals. Pre retention administration of ondansetron (0.5 and 5 mg/kg, ip), significantly decreased the retention period transfer latency in comparison to scopolamine control while mCPBG (1 and 5 mg/kg, ip), significantly increased the retention period latency (Tables 2 and 3).

Prelearning administration of scopolamine (1 mg/kg, ip) significantly increased the number of 2 paw and 4 paw descents after 24 hr period, while the 2 paw descents before 24 hr period were also significantly different from saline controls \( (t \text{ test}, P < 0.05) \). The difference in 2 paw and 4 paw descents were compared for prelearning administrations of ondansetron and mCPBG in comparison to scopolamine using Dunnet's test \( (P < 0.05) \) after one way ANOVA resulted in significant F-test \( (P < 0.05) \) (Tables 2 and 3). Ondansetron (0.5 and 5 mg/kg, ip), and mCPBG (1 and 5 mg/kg, ip), significantly decreased the number of 2 paw and 4 paw descents after 24 hr period and only 2 paw descents before 24 hr.

Pretention administration of scopolamine (1mg/kg, ip), significantly increased the number of 2 and 4 paw descents (after a 24 hr period) while producing no effect on the measures before 24 hr \( (t \text{ test}, P < 0.05) \) (Tables 2 and 3). The difference in 2 paw and 4 paw descents were compared for pre retention administration of ondansetron and mCPBG in comparison to scopolamine using Dunnet’s test \( (P < 0.05) \) after one way ANOVA resulted in a significant F-test \( (P < 0.05; \text{ Tables 2 and 3}) \). Ondansetron (0.5 and 5 mg/kg, ip), and mCPBG (1 and 5 mg/kg, ip), decreased the number of 2 paw descents (after 24 hr period) in comparison to scopolamine control animals. The number of 4 paw descents was significantly decreased by ondansetron (5 mg/kg, ip), and mCPBG (1 and 5 mg/kg, ip) after a 24 hr period.
Table 2 — Effect of ondansetron on scopolamine induced cognitive impairment in the passive avoidance test

[Values are mean ± SE from 8 animals in each treatment]

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TT (sec)</th>
<th>Number of descents/15 min</th>
<th>RT (sec)</th>
<th>Number of descents/15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 paws</td>
<td>4 paws</td>
<td></td>
</tr>
<tr>
<td>Saline (pre-learning)</td>
<td>25.375 ± 0.6</td>
<td>5.00 ± 0.65</td>
<td>1.375 ± 0.38</td>
<td>24.75 ± 0.75</td>
</tr>
<tr>
<td>Saline (pre-retention)</td>
<td>24.25 ± 1.83</td>
<td>4.25 ± 1.66</td>
<td>1.125 ± 1.12</td>
<td>23.875 ± 2.03</td>
</tr>
<tr>
<td>Scopolamine (1 mg/kg, ip) (pre-learning)</td>
<td>62.875 ± 3.17*</td>
<td>8.875 ± 0.74*</td>
<td>1.878 ± 0.3</td>
<td>55.75 ± 1.79*</td>
</tr>
<tr>
<td>Scopolamine (1 mg/kg, ip) (pre-retention)</td>
<td>26.75 ± 8.11</td>
<td>5.375 ± 1.68</td>
<td>1.125 ± 1.12</td>
<td>69.625 ± 6.25*</td>
</tr>
<tr>
<td>Ondansetron (0.5 mg/kg, ip) + Scopolamine (1 mg/kg, ip) (pre-learning)</td>
<td>48.5 ± 2.23*</td>
<td>3.625 ± 0.38*</td>
<td>1.375 ± 0.33</td>
<td>45.375 ± 2.12*</td>
</tr>
<tr>
<td>Ondansetron (0.5 mg/kg, ip) + Scopolamine (1 mg/kg, ip) (pre-retention)</td>
<td>28.875 ± 6.57</td>
<td>1.5 ± 1.85</td>
<td>3 ± 1.51</td>
<td>51.5 ± 4.98*</td>
</tr>
<tr>
<td>Ondansetron (5 mg/kg, ip) + Scopolamine (1 mg/kg, ip) (pre-learning)</td>
<td>36.5 ± 2.56*</td>
<td>3 ± 0.47*</td>
<td>1.5 ± 0.33</td>
<td>36.5 ± 2.6*</td>
</tr>
<tr>
<td>Ondansetron (5 mg/kg, ip) + Scopolamine (1 mg/kg, ip) (pre-retention)</td>
<td>27 ± 2.04</td>
<td>0.875 ± 0.41</td>
<td>3.25 ± 0.23</td>
<td>33 ± 1.17*</td>
</tr>
</tbody>
</table>

P values: *p < 0.05, *with respect to respective saline control, # with respect to respective scopolamine controls.

TT=Training period latency (sec), The latency time of transfer from the open arm to either of the closed arms (the transfer latency) on the first day of trial.

RT=Retention period latency (sec), The latency time of transfer from the open arm to either of the closed arms (the transfer latency) on the second day of trial.

Table 3 — Effect of mCPBG on scopolamine induced cognitive impairment in the passive avoidance test

[Values are mean ± SE from 8 animals in each treatment]

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TT (sec)</th>
<th>Number of descents/15 min</th>
<th>RT (sec)</th>
<th>Number of descents/15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (pre-learning)</td>
<td>25.375 ± 0.6</td>
<td>5.00 ± 0.65</td>
<td>1.375 ± 0.38</td>
<td>24.75 ± 0.75</td>
</tr>
<tr>
<td>Saline (pre-retention)</td>
<td>24.25 ± 1.83</td>
<td>4.25 ± 1.66</td>
<td>1.125 ± 1.12</td>
<td>23.875 ± 2.03</td>
</tr>
<tr>
<td>Scopolamine (1 mg/kg, i.p) (pre-learning)</td>
<td>62.875 ± 3.17*</td>
<td>8.875 ± 0.74*</td>
<td>1.878 ± 0.3</td>
<td>55.75 ± 1.79*</td>
</tr>
<tr>
<td>Scopolamine (1 mg/kg, i.p) (pre-retention)</td>
<td>26.75 ± 8.11</td>
<td>5.375 ± 1.68</td>
<td>1.125 ± 1.12</td>
<td>69.625 ± 6.25*</td>
</tr>
<tr>
<td>mCPBG (1 mg/kg, i.p) + Scopolamine (1 mg/kg, i.p) (pre-learning)</td>
<td>73.25 ± 1.89*</td>
<td>4.25 ± 0.5*</td>
<td>1.25 ± 0.25</td>
<td>29.00 ± 1.6*</td>
</tr>
<tr>
<td>mCPBG (1 mg/kg, i.p) + Scopolamine (1 mg/kg, i.p) (pre-retention)</td>
<td>30.25 ± 1.5</td>
<td>0.875 ± 0.62</td>
<td>3.25 ± 0.22</td>
<td>62 ± 3.03*</td>
</tr>
<tr>
<td>mCPBG (5 mg/kg, i.p) + Scopolamine (5 mg/kg, i.p) (pre-learning)</td>
<td>79 ± 1.49*</td>
<td>3.25 ± 0.37*</td>
<td>1.13 ± 0.3</td>
<td>76.125 ± 1.73*</td>
</tr>
<tr>
<td>mCPBG (5 mg/kg, i.p) + Scopolamine (5 mg/kg, i.p) (pre-retention)</td>
<td>30.375 ± 1.52*</td>
<td>1.38 ± 0.59</td>
<td>3.88 ± 0.32</td>
<td>82.625 ± 2.2*</td>
</tr>
</tbody>
</table>

P values: *p < 0.05, *with respect to respective saline control, # with respect to respective scopolamine controls.

TT=Training period latency (sec), The latency time of transfer from the open arm to either of the closed arms (the transfer latency) on the first day of trial.

RT=Retention period latency (sec), The latency time of transfer from the open arm to either of the closed arms (the transfer latency) on the second day of trial.
Discussion

Use of different experimental conditions and different behavioural tasks in various studies complicate the nature of involvement of serotonergic receptors in cognition and memory. Animal models in which drugs are administered before the prelearning phase, allow the study of the effects on acquisition, while postlearning administration can be used to determine the effects on consolidation. When administration is performed during preretention, the effect is ascribed to the retrieval stage of learning. The actions of agonists and antagonists of serotonergic receptors, administered during prelearning, postlearning or preretention, in different behavioural tasks indicate their role in cognitive processes. The effects of these compounds could either be facilitative or disruptive, or they may allow recovery from impaired cognitive performance following the activation or blockade of serotonergic receptors.

Involvement of 5-HT3 receptors in learning and memory was first studied using the antagonist, ondansetron. Pretraining injections of ondansetron (10 ng/kg) in mice on a habituation test, facilitated the reversal of impairment in habituation induced by scopolamine. In the Morris water-maze spatial navigation task, ondansetron (0.03-1 mg/kg, ip) reversed the longer latencies to find the submerged platforms caused due to treatment with atropine (30 ng/kg, ip). In aversive experiments using 5-HT3 antagonists, granisetron (1-10 mg/kg) given as pre training injection, increased the step-down latency when mice were tested 24 hr after foot shock on a passive avoidance task. Tropisetron was found to improve the retrieval of a previously learned aversive habit in mice at doses of 1, 10, 100 mg/kg and pretreatment with itasetron (1, 10, 100 mg/kg) antagonized a deficit in the acquisition of a passive avoidance response caused by scopolamine administration in rats. In aged animals, itasetron, at 10 mg/kg, injected for 3 weeks, significantly improved task performance compared to that displayed by old rats treated with the vehicle in the Morris water-maze task. Posttraining ip injection of the 5-HT3 agonist mCPBG (1-10 mg/kg) has been shown to impair consolidation in the learning of a lever-press response in autoshaping, tropisetron (0.01 to 0.1 mg/kg) and ondansetron (0.1 to 1 mg/kg) improved it, and the effect induced by mCPBG (1 mg/kg) was prevented by each of these 5-HT3 antagonists at the lower dose. Ondansetron, over a large dose range (1 ng-1 mg/kg) injected during the pre-session for 10-15 days failed to attenuate a scopolamine induced impairment in the stone maze, which is considered to be a complex spatial memory task.

The elevated plus maze is normally used to evaluate anxiety in animals, as they prefer closed arms due to an inherent fear of height. The transfer latency, number of transitions between open and closed arms and the time spent in open and closed arms are used as a measure to evaluate anxiogenic and anxiolytic properties of drugs. This has been modified to evaluate learning by placing pre-trained animals on the maze and the transfer latency from the open arm where it is placed first, to the closed arm quantified as a measure of learning. In the present study, the period of transfer latency in the elevated plus maze was decreased by the pretreatment of mice with ondansetron before scopolamine injection (injections after the learning session) in comparison with scopolamine treated control mice. Data suggest that ondansetron may have cognition enhancing properties in cholinergic hypofunctional models. However, the influence of the possible anxiolytic properties of ondansetron could influence these findings. mCPBG was found to have significant increase, under the same test protocol, on transfer latency (injections prior to learning session).

In the passive avoidance test, the transfer latency was decreased by the pretreatment of mice with ondansetron before scopolamine injection (injections either prior to or after the learning session) in comparison with scopolamine treated control mice. mCPBG was found to significantly increase transfer latency at a higher dose while at low doses, the effect was reversed (injections prior to or after the learning session). Ondansetron was found to decrease the number of descents in the passive avoidance test an apparent contradiction. A similar pattern was found with mCPBG in comparison with scopolamine treated control mice.

Taking into account the preclinical data available, the role of serotonergic receptors in learning and memory has always been controversial. Various studies as well as the present data suggest that the action of 5-HT3 antagonists may be task dependent and hence it is highly imperative to use experimental models that distinguish true cognitive effects from locomotor, motivational and emotional effects. The individual effects of 5-HT3 receptor agonists and antagonists are significantly decreased by para chloro phenyl alanine, implying that pre-synaptic 5-HT3 receptors may be involved in the impairment and enhancement of learning. Long term potentiation
(LTP) provides a cellular basis for memory and 5-HT3 receptor activation has been proved electrophysiologically to inhibit the induction of LTP. This response is presumed to be through the activation of 5-HT3 receptors present on GABAergic interneurons. The role of pre and post-synaptic 5-HT3 receptors and their distribution may throw more light on this aspect and the interaction between GABA and 5-HT3 also needs further scrutiny. Recent data suggests that the native 5-HT3 receptor is a heteromer and the 5-HT3B subunit contributes to tissue specific functional changes in 5-HT3 mediated signalling and/or modulation. The nature of regulation of these receptors in brain areas associated with cognition and the binding of various 5-HT3 ligands has to be further probed for their role in cognition.

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References


