Modulatory Effect of Serotonergic System in Pentylenetetrazole-Induced Seizures and Associated Memory Deficit: Role of 5-HT$_{1A}$ and 5-HT$_{2A/2C}$

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Background and Purpose: Recent studies have recognised the memory deficit as one of the most common psychiatric issues in the patients with epilepsy, which severely affects the quality of life. Our previous studies have demonstrated the possible involvement of serotonergic system in the pathogenesis of epilepsy and associated memory deficit. The possible involvement of 5-HT$_{1A}$ and 5-HT$_{2A/2C}$ receptor has not been explored yet. Therefore, this study has been envisaged to explore the effect of 5-HT$_{1A}$ and 5-HT$_{2A/2C}$ receptor modulation on epilepsy and memory deficit in pentylenetetrazole-kindled mice.

Methods: In the present experimental approach, we examined the efficacy of modulation of 5-HT$_{1A}$ and 5-HT$_{2A/2C}$ receptor in pentylenetetrazole-induced kindling in male Swiss mice ($n=75$). Mice were kindled by sub-convulsive dose of pentylenetetrazole (35 mg/kg, intraperitoneal injection), at the interval of 48±2 hours. Successfully kindled animals were treated with 5-HT$_{1A}$ and 5-HT$_{2A/2C}$ receptor modulators. The effect of different treatments on seizure severity score and memory impairment was analysed.

Results: 5-HT$_{1A}$ receptor agonist improved the memory functions while seizure severity was not improved, and the opposite effect was observed with 5-HT$_{1A}$ receptor antagonist. On the other hand, 5-HT$_{2A/2C}$ receptor agonist significantly improved memory deficit as well as seizure severity in the kindled animals.

Conclusions: The outcome of the study indicates the possible involvement of 5-HT$_{2A/2C}$ receptor in the pathogenesis of epilepsy and associated memory deficit, which can be further explored for its management.

Key words: Epilepsy, Memory disorders, Comorbidity, Pentylenetetrazole, Kindling, Serotonin, Receptor

Introduction

Recent studies have shown that there are growing concerns of cognitive problems in epileptic patients.$^{1,3}$ The quality of life in the patients with epilepsy gets compromised with associated psychiatric conditions. Therefore, several studies have been carried out to understand the pathology and possible approaches management of memory deficit in epilepsy in past decades.

In our previous studies, we explored the possible involvement of serotonergic system in epilepsy and associated memory deficit.$^{4,6}$ 5-HT receptors are involved not only in normal physiological function of brain like, sleep, memory, feeding and etc., but also in various psychiatric problems. The role of serotonergic innervations in epileptogenesis and consolidation of memory has been well documented.$^7$

The differential effect of serotonergic system is corresponded by the virtue of their receptors. Because of the putative role of the serotonergic system in central physiological and pathological functions and the crucial need of identifying a novel target to prevent memory deficit in epilepsy, some serotonergic excitatory (5-HT$_{2A/2C}$) and inhibitory receptors (5-HT$_{1A}$) were selected for the study.

5-HT$_{1A}$ receptor is predominantly distributed in limbic area and has been reported to exhibit antiepileptic effect. Differential effect of 5-HT$_{1A}$ receptor agonist has been reported in epilepsy. 5-HT$_{1A}$ receptor agonist has been reported to augment the latency to seizures in acute model of convulsions, while no effect has been observed in chronic amygdala kindling model.$^6$ Typically, 5-HT$_{1A}$ receptor antagonist has been reported to impair memory, whereas agonist has been found reverse memory deficit.$^7$ In contrast, some studies suggest that
5-HT\textsubscript{1A} receptor agonist may impair learning and memory in normal animals.\textsuperscript{10}

5-HT\textsubscript{2A/C} receptor appears to be widely expressed in cortex and hippocampus which regulates central nervous system excitability.\textsuperscript{11} Moreover, role of 5-HT\textsubscript{2A/C} receptor has been implicated in various pathological conditions like epilepsy, depression, psychosis etc. A recent study has demonstrated the anticonvulsant effect of 5-HT\textsubscript{2A/C} receptor agonist while presenting opposite effect with the antagonist.\textsuperscript{12} Activation of 5-HT\textsubscript{2A/C} receptor has been mentioned to improve memory deficit.

However, there are only limited data available regarding the effect of 5-HT\textsubscript{1A} and 5-HT\textsubscript{2A/C} receptor in memory deficit in epilepsy. Converging evidence suggests that modulation of 5-HT\textsubscript{1A} and 5-HT\textsubscript{2A/C} receptors might exhibit ameliorative effect on epilepsy and associated memory deficit. Therefore, this study was envisaged to explore the effect of 5-HT\textsubscript{1A} and 5-HT\textsubscript{2A/C} Receptor ligands in pentylenetetrazole-kindling and associated memory deficit in mice.

**Methods**

**Chemicals**

Pentylenetetrazole, 8-OH-DPAT (5-HT\textsubscript{1A} receptor agonists), WAY-100,635 (5-HT\textsubscript{1A} receptor antagonists), R (-) DOI (5-HT\textsubscript{2A/C} receptor agonist) and other chemicals were procured from Sigma-Aldrich, Co. (St. Louis, MO, USA). Olanzapine (5-HT\textsubscript{2A/C} receptor antagonist) was received as a gift sample from Q. P. Pharmachem, Derabassi, India.

**Animals**

The study was carried out on male Swiss mice (22-28 g weight), obtained from the approved breeder (Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India). Animals were housed in standard cages at room temperature (22°C±2°C) under natural light/dark cycle, and the cage had free access to water and food (standard laboratory pellets). The animals were acclimatized to lab conditions for seven days before starting experiment. All the experimental work had been carried out from 8:00 am to 4:00 pm. The experimental protocol was duly approved by the Institutional Animal Ethics Committee (IAEC) and the care of the animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India vide protocol approval No. 107/99/CPCSEA/-2009-4.2.

**Development of kindling**

Kindling in mice was induced by the method, which was previously validated in our laboratory.\textsuperscript{4-6,13,14} Briefly, pentylenetetrazole (dissolved in warm saline) was injected (sub-convulsive dose of 35 mg/kg, intraperitoneal route, at 48±2 hours interval) for 9 to 11 weeks until the animal shows appearance of tonic-clonic convulsion after two consecutive pentylenetetrazole administrations.

**Experimental protocol**

A total of 75 animals were employed in this study. The group I, naïve animals, consisted of untreated animals (n=8) and rest of the animals were subjected to pentylenetetrazole (PTZ)-kindling. Successfully kindled animals were randomly divided into seven groups: group II (vehicle control group) consisted of kindled animals receiving normal saline (10 mL/kg/day; i.p.; n=8); group III consisted of kindled animals treated with 8-OH-DPAT (1 mg/kg/day; subcutaneous route [s.c.]; n=7);\textsuperscript{15} group IV consisted of kindled animals receiving WAY-100,635 (0.3 mg/kg/day; s.c.; n=8);\textsuperscript{15} group V consisted of kindled animals receiving WAY-100,635+8-OH-DPAT (n=7); group VI consisted of kindled animals receiving R (-) DOI (1 mg/kg/day; s.c.; n=7);\textsuperscript{15} group VII consisted of kindled animals receiving olanzapine (2.5 mg/kg/day; s.c.; n=8);\textsuperscript{16} and group VIII consisted of kindled animals receiving olanzapine+R (-) DOI (n=7) (Fig. 1).

The above-mentioned treatment schedule was followed up to twenty days. Except naïve ones, all kindled animals were challenged with additional pentylenetetrazole challenging dose (35 mg/kg; i.p.; just to mimic the clinical situation of occasional epileptic seizure) on a day 5, 10, 15, and 20 of treatment schedule and the seizure severity score was recorded using a modified Racine’s scale.\textsuperscript{4-6,13,14}

**Behavioral assessments**

After 2 hours of pentylenetetrazole challenging dose, once their locomotor activity became normalized (as analysed by the open field test and actophotometer), animals were evaluated for their performance in the elevated plus maze and passive shock avoidance paradigm on day 20.

**Transfer latency in the elevated plus maze**

Spatial memory was evaluated recording transfer latency with the elevated plus maze on day 20, following the procedure previously standardized in our laboratory.\textsuperscript{4-6,13,14}
Number of mistakes and step-down latency in passive shock avoidance paradigm

For the evaluation of contextual fear memory, the modified passive shock avoidance paradigm, which was previously standardized in our laboratory, was used. On day 0 animals were trained to stay on shock free zone for at least 120 seconds and the number of trials required were recorded. Further retrieval of a learned task was evaluated recording the changes in the number of mistakes and step-down latency on day 20.

Statistical analysis

The statistical analysis was performed using the Sigma Stat Statistical software version 3.5 (Systat Software Inc., San Jose, CA, USA). Statistical significance in behavioural evaluations was calculated using one-way analysis of variance (ANOVA) followed by Tukey’s test. Each value was expressed as mean±standard error of means (S.E.M.) and statistical significance was considered at $\rho<0.05$.

Results

Animals were considered kindled when they show tonic clonic seizures upon two consecutive PTZ injections. Around 13±3 PTZ injections were administered to kindle the animals. Only successfully kindled animals (n=52) were included in the study, while animals showing mortality and resistance against PTZ kindling were excluded.

Effect on seizure severity score

There was a significant difference observed on seizure severity in different groups on day 20 ($F_{(7,52)}=32.930$, $\rho<0.001$). Naïve animals did not receive PTZ challenging dose, therefore they did not show convulsions. However, vehicle treated kindled animals have shown significant increase ($\rho<0.001$) in the seizure severity, upon administration of PTZ challenging dose, as compared to naïve animals. The treatment with 8-OH-DPAT did not change ($\rho=0.271$) the seizure severity score as compared to vehicle treated animals. However, the treatment with WAY-100,635 and WAY-100,635 in combination...
with 8-OH-DPAT significantly reduced ($p<0.001$) the seizure severity score as compared to vehicle treated animals (Fig. 2A).

The treatment with DOI significantly reduced ($p<0.001$) the seizure severity score as compared to vehicle treated animals. The treatment with olanzapine did not change ($p=1.000$) the seizure severity score as compared to vehicle treated animals. The DOI and the olanzapine combined treatment significantly reduced ($p<0.001$) the seizure severity score as compared to vehicle treated animals (Fig. 2A).

**Effect on transfer latency**

The treatment with 8-OH-DPAT significantly reduced ($p<0.001$) transfer latency as compared to vehicle treated animals. The treat-
ment with WAY-100,635 did not change \( (p=0.087) \) transfer latency as compared to vehicle treated animals. However, the combined treatment of WAY-100,635 and 8-OH-DPAT significantly reduced transfer latency as compared to vehicle treated animals (Fig. 2B).

The treatment with DOI significantly reduced \( (p<0.001) \) transfer latency as compared to vehicle treated animals. The treatment with olanzapine did not change \( (p=0.117) \) transfer latency as compared to vehicle treated animals. The DOI and Olanzapine combined treatment significantly reduced \( (p<0.001) \) transfer latency as compared to vehicle treated animals (Fig. 2B).

**Effect on number of mistakes and step-down latency**

The treatment with 8-OH-DPAT significantly reduced \( (p<0.001) \) the number of mistakes and significantly increased \( (p<0.001) \) step-down latency as compared to vehicle treated animals. However, the treatment with WAY-100,635 did not change number of mistakes \( (p=0.471) \) and step-down latency \( (p=0.404) \) as compared to vehicle treated animals. However combined treatment of WAY-100,635 and 8-OHDPAT significantly reduced the number of mistakes \( (p=0.002) \) and significantly increased step-down latency \( (p<0.001) \) as compared to vehicle treated animals (Fig. 2C, D).

The treatment with DOI significantly reduced the number of mistakes \( (p<0.001) \) and increased step-down latency \( (p<0.001) \) as compared to vehicle treated animals. Treatment with olanzapine did not change the number of mistakes \( (p=0.403) \) and the step-down latency \( (p=0.680) \) as compared to vehicle treated animals. DOI and olanzapine combined treatment significantly reduced the number of mistakes \( (p<0.001) \) and step-down latency \( (p=0.035) \) as compared to vehicle treated animals (Fig. 2C, D).

**Discussion**

In this study, the treatment with 8-OH-DPAT (5-HT_{1A} receptor agonist) did not change the elevated seizure severity score, suggesting no effect on convulsions in pentylenetetrazole-kindled animals. However, the WAY-100,635 (5-HT_{1A} receptor antagonist) treatment was found to reduce the incidences of seizures in the pentylenetetrazole-kindled animals. High density of 5-HT_{1A} receptors as somadendritic autoreceptor and postsynaptic receptor has been reported to be found in hippocampus. \(^{17}\) The role of 5-HT_{1A} receptor in epilepsy appears intriguing as reports suggest their pro-convulsant \(^{18}\) and anticonvulsant \(^{18,19}\) potential in experimental models of convulsion. In our study treatment with 8-OH-DPAT did not improved seizure severity possibly due to reduction in hippocampal GABAergic tone. The inhibition of GABA release might be caused by stimulation of the G protein-coupled presynaptic 5-HT_{1A} receptors mediated inactivation of the adenylyl cyclase/cAMP signal transduction pathway.\(^{20}\) This might be a speculation for negligible effect of 5-HT_{1A} receptor agonist and anticonvulsant effect of 5-HT_{1A} receptor antagonist.

8-OH-DPAT has also been reported to bind with 5-HT_{7} receptor.\(^{21}\) Therefore the possible interaction of 8-OH-DPAT with 5-HT_{7} receptor in this study cannot be neglected. The activation of 5-HT_{7} receptor by selective agonist has been reported to increase seizures in pilocarpine induced rat model of temporal lobe epilepsy.\(^{22}\) In contrast, antagonism of 5-HT_{7} receptor has been reported to reduce spontaneous seizures in the WAG/Rij rat model of absence epilepsy\(^{19}\) and pilocarpine induced spontaneous seizures.\(^{22}\) Therefore activation of 5-HT_{7} receptor might be another hypothesis in support of negligible/proconvulsant nature of 8-OH-DPAT in our study. Generally, depletion of 5-HT level has been found to be associated with reduced seizures threshold\(^{23}\) while agents which elevate extracellular serotonin level have been found to have anticonvulsant effect.\(^{24}\) WAY-100,635 has been reported to increase extracellular 5-HT level\(^{25}\) and might suggest another hypothesis for its anticonvulsant effect of WAY-100,635 and opposite/negligible effect of 8-OH-DPAT, in our study.

Behavioural findings of this study suggested that the 8-OH-DPAT treatment improves memory function by reducing transfer latency in elevated plus maze and by increasing step-down latency in the passive shock avoidance paradigm. However, the WAY-100,635 treatment impaired memory in the pentylenetetrazole-kindled animals, as observed by increased transfer latency and reduced step-down latency. The protective effect of the 8-OH-DPAT treatment was significantly reversed by co-administration of WAY-100,635 in this study. Systemically administered 5-HT_{1A} receptor agonists, either by inhibitory somadendritic 5-HT_{1A} autoreceptor or by the inhibitory 5-HT_{1A} receptor on GABAergic interneurons, might be involved in the indirect facilitation of acetylcholine (ACh) release in hippocampus, and thus helps in improvement in memory.\(^{26}\) However, 5-HT_{1A} receptor antagonist might result in depletion of hippocampal ACh level, and thus producing memory deficit in the pentylenetetrazole-kindled animals. The protective effect of 8-OH-DPAT on memory can also be supported by another hypothesis which includes stimulation of 5-HT_{7} receptor and the stimulation has been documented to improve hippocampal based cognitive process.\(^{27}\)

The treatment with DOI (5-HT_{2A/2C} receptor agonist) has shown
with 5-HT2 receptor agonist has also been reported to inhibit glutamatergic release (via inhibitory presynaptic receptors) from cerebellar mossy fibre terminals, which could be speculated as another possible anticonvulsant mechanism of 5-HT2 receptor agonist. Olanzapine has been reported to have proconvulsant nature, which might be attributed due to their antagonistic effect on dopaminergic D2 receptor.

Behavioural evaluation suggested that DOI treatment significantly improved memory by reducing transfer latency in elevated plus maze and by increasing step-down latency in passive shock avoidance paradigm. The opposite effect was observed with Olanzapine treatment. Activation of 5-HT2A2C receptor has long been reported to improve memory functions, possibly via enhancing glutamate and acetylcholine release in prefrontal cortex and hippocampus. These can be speculated as possible mechanisms for the memory improvement effect in DOI treated animals and vice-versa in Olanzapine treated animals.

In conclusion, this study demonstrates the protective effect of 5-HT2A2C receptor agonist on seizure severity and associated memory deficit in pentylenetetrazole-kindled animals. On the other hand, modulation of 5-HT1A resulted in improving either seizures or memory impairment in animals. Furthermore, findings of this study may also suggest possible involvement of 5-HT2A2C receptor in the development and management of epilepsy associated memory deficit. However, therapeutic application of 5-HT2A2C receptor hypothesis for the management of epilepsy associated memory deficit warrants further studies to confirm its other psychiatric effects.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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