ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 13, Issue 12, 2020

Online - 2455-3891
Print - 0974-2441

Research Article

EFFECT OF DROTAVERINE, A PHOSPHODIESTERASE 4 INHIBITOR IN SCOPOLAMINE-INDUCED COGNITIVE IMPAIRMENT IN RATS

KAYALVIZHI MK

Department of Pharmacology, Madha Medical College and Research Institute, Chennai, Tamil Nadu, India.
Email: drmkayalvizhi@gmail.com

Received: 31 August 2020, Revised and Accepted: 10 October 2020

ABSTRACT

Objective: Cognitive impairment (CI) is a progressive neurodegenerative disorder and causes significant dementia in the elderly. Intracellular cyclic AMP (cAMP) signaling has been well established in the mediation of memory. Phosphodiesterases (PDEs) are enzymes that catalyze the hydrolysis of cAMP and/or cyclic GMP. Drotaverine is a novel non-anticholinergic smooth muscle antispasmodic which acts by inhibiting PDE-4. It is now clinically used in smooth muscle spasms (intestinal, biliary and renal colic, irritable bowel syndrome, uterine spasms, etc.) without anticholinergic side effects. Since Drotaverine has PDE4 inhibition property, its role in learning and memory was evaluated in this study and found that it has memory enhancing effect comparable with donepezil in scopolamine-induced CI in rats.

Methods: Learning and memory were assessed with two behavioral models, namely, elevated plus maze (EPM) and Y maze. CI was produced by scopolamine. Rats were divided into five groups, Group I treated with normal saline, Group II treated with scopolamine, and Groups III, IV, and V were treated with donepezil, Drotaverine, and both, respectively.

Results: The result analysis revealed significant differences in transfer latency in EPM performance between Groups III, IV, V and Group II (***$p<0.001$). The results of spontaneous alternation in Y maze show that there was a significant difference among all the treatments groups ($p<0.001$).

Conclusion: Drotaverine has promising memory enhancing effect in CI induced by scopolamine in rats. Further clinical trials are needed to prove this finding which has been elicited in animal models.

Keywords: Cognition, Phosphodiesterase, Drotaverine, Donepezil, Scopolamine.

 DOI: http://dx.doi.org/10.22159/ajpcr.2020.v13i12.39589

INTRODUCTION

Cognition is the brains ability to process, retain, and use information. Cognitive disorders (CDs) include amnesia, dementia, and delirium that primarily affect learning, memory, perception, and problem solving, and come under mental health disorders. This disorder significantly impairs the cognitive function of an individual to the point where normal functioning in society is impossible without treatment [1]. Alzheimer's disease (AD) is a neurodegenerative disorder of the central nervous system (CNS) characterized by progressive cognitive dysfunction. The hallmark of AD is the excessive accumulation of neurotoxic β-amyloid peptide in the brain which causes neuropathological lesions in the brain of patients with AD. Intracellular cyclic AMP (cAMP) signaling has been well established in the mediation of memory [2]. Activation of cAMP signaling increases synaptic plasticity and produces memory-enhancing effects in rodents [3]. Phosphodiesterases (PDEs) are enzymes that catalyze the hydrolysis of cAMP and/or cyclic GMP (cGMP) and thereby regulate intracellular levels of the second messengers. PDE4, formerly known as cAMP-PDE, is a cAMP-specific PDE and is the predominant isoenzyme in the majority of inflammatory cells. It is expressed in the airways smooth muscle, brain, and cardiovascular tissues and is the largest PDE subfamily. It plays an important role in the mediation of learning and memory. Administration of PDE4 inhibitors such as rolipram enhances memory and reverses memory deficits induced by pharmacological, physical, physiological, or genetic manipulations [4,5]. Drugs that inhibit PDE4 have received a great deal of attention, partially due to their inhibitory effects on inflammation and apoptosis in various models [6,7]. Thus, there still remains a challenge to design even better PDE4 inhibitors with an improved therapeutic index. Drotaverine, which acts by inhibiting PDE 4, is a novel non-anticholinergic smooth muscle antispasmodic. It is now clinically use in intestinal, biliary and renal colic, irritable bowel syndrome, uterine spasms, etc., without anticholinergic side effects [8]. Scopolamine, a muscarinic cholinergic receptor antagonist, impairs learning and memory in rodents and humans, especially the processes of learning acquisition and short-term memory. Cholinergic neurons in the CNS are involved in learning and memory in both humans and animals. In this context, scopolamine triggers reactive oxygen species (ROS) formation and induces free radical injury [9]. Donepezil is a well-established drug for clinical treatment and scopolamine-induced Alzheimer type dementia. Donepezil ameliorated the scopolamine-induced memory impairment by reducing acetylcholine esterase activity and oxidative stress and restoring cerebral circulation [10]. With this background, this research was an attempt to investigate the memory-enhancing effect of Drotaverine for the prevention of CDs like AD.

METHODS

Wistar albino rats of either sex weighing between 150 and 200 g were randomly selected from central animal facility. The animals were fed with Purina Chow diet, water *ad libitum,* and maintained under standard conditions of temperature, humidity, and light (12 h light/12 h dark cycle). All the experiments were conducted after the approval of Institutional Animal Ethical Committee. Pregnant animals, animals with injuries, disease, infection, and deformity were excluded from the study. All the animals were maintained in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experimentation on Animals. Scopolamine, donepezil, and Drotaverine were used. All the chemicals were obtained from Sigma-Aldrich.

Experimental design

Learning and memory were assessed with two behavioral models, namely, elevated plus maze (EPM) and Y maze. Rats were divided into

five groups (n=6/group). The grouping for pharmacological screening models was as follows:

- Group I (Positive control): Rats received only distilled water
- Group II (Negative control): Rats received scopolamine (3 mg/kg) [11]
- Group III: Rats were treated with donepezil (3 mg/kg) + scopolamine (3 mg/kg) [11]
- Group IV: Rats were treated with Drotaverine (8 mg/kg) [12] + scopolamine (3 mg/kg)
- Group V: Rats were treated with donepezil (3 mg/kg) + Drotaverine (8 mg/kg) + scopolamine (3 mg/kg).

Groups III, IV, and V rats were dosed orally every 24 h interval with donepezil (3 mg/kg), Drotaverine (8 mg/kg), and donepezil (3 mg/kg) + Drotaverine (8 mg/kg), respectively, for 14 consecutive days. The acquisition trail for EPM was carried out on the 14th day, and scopolamine (3 mg/kg, i.p.) was administered on the 14th day after the acquisition trail to all groups except normal control group, which provoked the cognitive impairment (CI) in rats. Retention of memory was tested on the 15th day.

EPM test

EPM served as the exteroceptive behavioral model to evaluate long-term memory in rats. Maze consisted of two open (16 × 5) and two closed arms (16 × 5 × 20). All treatments were given for 14 days. On the 14th day, after 60 min of administration of treatments, scopolamine (3 mg/kg, i.p.) was injected to all groups except Group 1. After 30 min of scopolamine injection, animals were placed individually at the end of either of the open arm, facing away from the center and the time taken by the animal to move from open-to-closed arm, that is, transfer latency (TL) was noted for the acquisition trial. TL was defined as the time (in seconds) taken by the animals to move from the open arm into one of the covered arms with all its four legs. If the rats were unable to reach the closed arm within allotted period, that is, 180 s, it was placed into one of the closed arms for 15 s, so as to explore it and the TL was taken as 180 s. After 24 h of exposure, the procedure was again repeated and TL was recorded as the parameter for memory on the day of retention. Reduction in TL values of the 2nd day of the test in comparison to the 1st day test indicates improvement in memory [13,14].

Y maze test

The experimental design was same as EPM. The Y maze is a simple two trial recognition test for measuring spatial recognition memory, it does not require learning of a rule, and thus is useful for studying memory in rodents, and in particular for the study of genetic influences on the response to novelty and recognition processes. Y maze made of wood consists of three arms with an angle of 120° between each of the two arms. The arm dimensions are 8 cm × 30 cm × 15 cm (width × length × height). The three identical arms will be randomly designated: Start arm, in which the rat started to explore (A), novel arm (B, with food stimuli), and the other arm (C). Rats tend to explore the maze systematically, entering each arm in turn. The ability to alternate requires that the rats know, which arm they have already visited. On the 1st day, all the rats were allowed to explore the Y maze apparatus for a period of 10 min each. From the 2nd to 5th day, the rats received four consecutive trials of training per day in the maze of 8 min duration. In each trial, the rats were placed in the entry chamber (A) and the series of arm entries in all the three arms, including possible return into the same arm is recorded visually. An alternation is defined as entry into all three arms consecutively, for instance, if the animal makes the following arm entries; ACB, CA, B, C, A, CAB, C, and A, in this example, the animal made 13 arm entries 8 of which are correct alternations. The percentage of alteration is calculated as the total number of arm entries minus two and multiplied by 100.

$$\% \text{ alternation} = [(\text{number of alternations})/(\text{total arm entries}-2)] \times 100$$

For each animal, the Y-maze testing was carried out for 5 min. The apparatus was cleaned with 5% alcohol and allowed to dry between sessions [15,16].

Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA) followed by *post hoc* tests (Student–Newman–Keuls) carried out to determine the source of a significant effect. Results were expressed as Mean±S.E.M., $p<0.05$ was taken as accepted level of significant difference from control.

RESULTS

EPM

The effect of the vehicle, scopolamine control, and donepezil (2.5 mg/kg) and Drotaverine (8 mg/kg) were evaluated at the end of day 14. The scopolamine control group showed a significant increase in TL values on the acquisition as well as on the retention days as compared with vehicle control rats, indicating impairment in learning and memory. In the retention trial on day 15, the result analysis revealed significant differences in TL in EPM performance between Groups III, IV, V and NC group (Group II) animals (***$p<0.001$). The results were comparable with donepezil (2.5 mg/kg). TL of the 2nd day (day 15) reflected retention of learned task or memory (Table 1).

Y maze

The results of spontaneous alternation show that there was a significant difference among all the treatment groups ($p<0.001$). Scopolamine reduced the spontaneous alternation. Treatment groups significantly reversed the effect of scopolamine and increased the spontaneous alternation percentage ($p<0.001$), when compared to scopolamine alone treated group. Donepezil reversed the effects of scopolamine at the percentage of 80% and Drotaverine alone at a percentage of 76.9%. Group V (donepezil 2.5 mg/kg + Drotaverine 8 mg/kg) reversed the scopolamine effect at a percentage of 84.6% (Fig. 1.)

DISCUSSION

The present study investigated the memory enhancing property of Drotaverine in rat models. The process by which the brain absorbs information and then analyzes this information in the present context to respond and plan for the future is called cognition. Strength of the synapse mediates this process. Many neuronal functions at the molecular level are mediated by cAMP and cGMP signaling, support from the regulation of neurotransmitter release to the firing properties of neurons. The PDEs are a superfamily of enzymes that metabolize the ubiquitous intracellular second messengers cAMP and cGMP. The PDEs are encoded by 21 genes that are classified into 11 different families based on amino acid sequence similarity, catalytic characteristics, and regulatory properties. Some PDEs specifically degrade cGMP (PDE5, 6, and 9), some specifically degrade cAMP (PDE4, 7, and 8), and some have a dual specificity (PDE1, 2, 3, 10, and 11) [17]. PDE families are further divided into isoforms, more than 100 PDE isoforms can be distinguished. PDE isoforms have distinct localization at the tissue, cell, and subcellular levels, with extensive overlap being the rule rather than exception. Thus, the PDEs are essential to coordinate optimal cAMP or cGMP concentrations in both spatial and temporal dimensions and PDE inhibition offers a means for specific manipulation of cyclic nucleotide signaling for therapeutic benefit [18]. The cyclic nucleotides play critical roles in regulating synaptic plasticity, and, consequently, PDE inhibitors are of considerable interest as treatments for cognitive dysfunction. Many studies have been shown to improve learning by PDE inhibitors in rodent models of impaired cognition. The second messengers in the mature brain that are directly involved in time-dependent events of memory consolidation are cAMP and cGMP. Activation of the cAMP-PKA (protein kinase A) pathway cascade triggers the activation of transcription factors such as cAMP response element-binding protein (CREB) inducing the gene transcription required to consolidate learning and memory [19]. Moreover, recent findings have also linked the cGMP pathway to cognition. Basal levels of cGMP are higher in the new born brain than in the adult brain, and they decrease with age, a decline thought to be the consequence of increased expression of cGMP-dependent PDEs. Inhibition of PDEs that specifically mediate the hydrolysis of cGMP in the brain (mainly isoforms 2, 5, and 9) results

Table 1: Effect of Drotaverine and other drugs employed on TL of rats using EPM

Groups	Treatment	TL in sec day 1	TL in sec day 2
Group I	Positive control (distilled water)	52±0.6	41.65±0.32
Group II	Negative control (scopolamine 20 mg/kg)	83.34±1.2	96.45±0.23
Group III	Scopolamine (20 mg/kg)+donepezil (2.5 mg/kg)	27.54±0.40	18.23±0.82***
Group IV	Scopolamine (20 mg/kg)+Drotaverine (8 mg/kg)	32.56±0.32	23.54±0.32***
Group V	Scopolamine (20 mg/kg)+donepezil (2.5 mg/kg)+Drotaverine (8 mg/kg)	24.35±0.42	14.32±0.42***

TL: Transfer latency, EPM: Elevated plus maze, Values are expressed as mean±SEM (n=6). ***p<0.001 as compared with negative control group

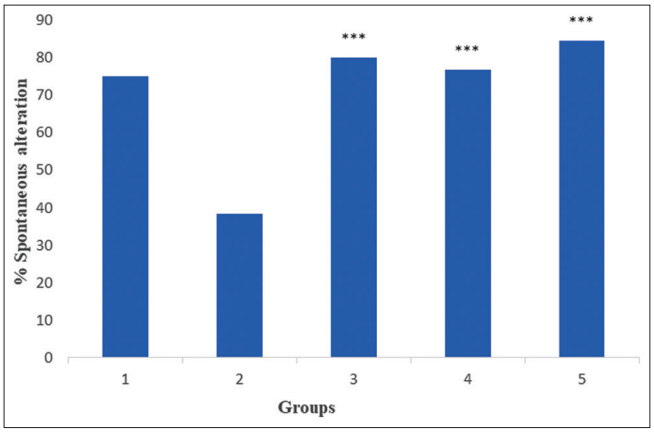

Fig. 1: Effect of Drotaverine on Y maze test showing % spontaneous alterations. (Group 1 – positive control, Group 2 – negative control – scopolamine 3 mg/kg, Group 3 – Donepezil 3 mg/kg, Group 4 – Drotaverine 8 mg/kg, Group 5 – Donepezil 3 mg/kg + Drotaverine 8 mg/kg) ***p<0.001 as compared with negative control group)

in increases in the levels of this cyclic nucleotide, which, in turn, may prevent the onset of senile dementia. In addition to inhibitors of PDE2, PDE5, and PDE9, which are specific for cGMP, inhibitors of the cAMP-specific PDEs (PDE1, PDE4, and PDE10) have been shown to boost memory in certain animal models [20,21]. There has been a strong interest in developing PDE inhibitors for the treatment of CIs. Preclinical studies have shown clear beneficial effects of various PDE inhibitors in models of learning, memory, and schizophrenia. Several studies have suggested that PDE5 inhibitors elevate cGMP levels in various brain regions, such as the cortex, hippocampus, and cerebellum, in transgenic AD mice or unimpaired rats, and cGMP elevation promotes CREB phosphorylation, thus improving memory performance in different types of behavior tests [22].

The PDE4 subfamily comprising over 20 splice variants, which permits a variety of regional and subcellular localizations is quite complex. PDE4A and B isoforms are expressed at high levels in the frontal and temporal cortex, and in the hippocampus of humans [23]. The previous studies with a selective and brain-penetrant PDE4 inhibitor, rolipram, identified this isoform as a promising target for the treatment of the cognitive dysfunction associated with AD. Many previous studies indicated that rolipram can reverse the cholinergic deficit caused by scopolamine in the object recognition memory which is mainly due to cholinergic mechanism. However, serious side effects associated with PDE4 inhibitors have hampered their development [24]. Nevertheless, in 2011, the Food and Drug Administration approved the first and only selective PDE4 inhibitor, roflumilast, to reduce the exacerbation and worsening of symptoms associated with severe chronic obstructive pulmonary disease. Given the potential risks identified in clinical trials, roflumilast was approved with a medication guide informing patients of side effects. A recent report highlighted the severity of this drug's side effects and determined that its benefits are minimal at best: "Even a small increase in adverse effects would tilt the risk/benefit ratio against the use of roflumilast" [25]. The development of PDE4 inhibitors lacking side effects is critical for therapeutic applications that require chronic treatment, such as AD. In addition to the selective PDE4D inhibitor GEBR-7B, genetics has used structural information to develop a series of PDE4 allosteric modulators that serve as a molecular glue to close a regulatory domain. These compounds do not completely abolish PDE4 enzymatic activity in cellular and *in vivo* models [26]. Interestingly, the occurrence of emesis, a dose-limiting side effect of existing active site-directed PDE4 inhibitors, is also significantly reduced by some clinical trial drugs like D159687. Thus, modulation of PDE4 may, therefore, lead to the development of pharmacotherapeutic compounds in the near future that will ultimately become available to the patient. Thus, the previous studies have shown that the acetylcholinesterase inhibitor metrifonate ameliorates the effects of advanced tool design as well and improves performance in scopolamine-deficit models of the object recognition test. This suggests that the cholinergic and serotonergic systems act in a synergistic manner in memory performance [27]. There has been a strong interest in developing PDE inhibitors for the treatment of CIs. Preclinical studies have shown clear beneficial effects of various PDE inhibitors in models of learning, memory, and schizophrenia. Our study also confirms the memory enhancing effect of Drotaverine, a PDE4 inhibitor. Donepezil, an antidementia drug, increases acetylcholine levels by inhibition of acetylcholinesterase. Pre-treatment with donepezil prevented the inhibitory effect of scopolamine. Since the spontaneous alternation performance of the Drotaverine and scopolamine group was comparable to that of the donepezil pre-treated group, it proves the cognition enhancing effect of Drotaverine.

CONCLUSION

PDE inhibitors have demonstrated promising effects in animal models of CI, which will soon be investigated in clinical trials. The proven safety of several PDE inhibitors already in clinical use (often administered chronically) has created high expectations regarding their use for the treatment of AD. At present, many clinical trials are already ongoing with an inhibitor of PDE4, 9, and 10. Other promising PDE types are 1B, 2A, 4B, and 8B but these are still in an early preclinical phase of development. From this study, we expect that PDE4 inhibitor Drotaverine could be considered for treating cognitive dysfunction. In this regard, further clinical trials are needed to prove its worth.

AUTHORS' CONTRIBUTIONS

The authors contributed fully in experimental work and manuscript preparation.

CONFLICTS OF INTEREST

The authors declared there are no conflicts of interest.

AUTHORS' FUNDING

Self.

REFERENCES

1. Trivedi JK. Cognitive deficits in psychiatric disorders: Current status. Indian J Psychiatry 2006;48:10-20.
2. Suh YH, Checler F. Amyloid precursor protein, presenilin, and alpha-synuclein: Molecular pathogenesis and pharmacological applications in Alzheimer's disease. Pharmacol Rev 2002;54:469-525.

3. Ortega-Martínez S. A new perspective on the role of the CREB family of transcription factors in memory consolidation via adult hippocampal neurogenesis. Front Mol Neurosci 2015;8:46.
4. Sapio L, di Maiolo F, Illiano M, Esposito A, Chiosi E, Spina A, *et al*. Targeting protein kinase A in cancer therapy: An update. EXCLI J 2014;13:843-55.
5. Rutten K, Basile JL, Prickaerts J, Blokland A, Vivian JA. Selective PDE inhibitors rolipram and sildenafil improve object retrieval performance in adult cynomolgus macaques. Psychopharmacology (Berl) 2008;196:643-8.
6. Rutten K, Prickaerts J, Blokland A. Rolipram reverses scopolamine-induced and time-dependent memory deficits in object recognition by different mechanisms of action. Neurobiol Learn Mem 2006;85:132-8.
7. Zhang HT, O'Donnell JM. Effects of rolipram on scopolamine-induced impairment of working and reference memory in the radial-arm maze tests in rats. Psychopharmacology (Berl) 2000;150:311-6.
8. Bolaji OO, Onyeji CO, Ogundaini AO, Olugbade TA, Ogunbona FA. Pharmacokinetics and bioavailability of drotaverine in humans. Eur J Drug Metab Pharmacokinet 1996;21:217-21.
9. Klinkenberg I, Blokland A. The validity of scopolamine as a pharmacological model for cognitive impairment: A review of animal behavioral studies. Neurosci Biobehav Rev 2010;34:1307-50.
10. Tiseo PJ, Perdomo CA, Friedhoff LT. Metabolism and elimination of 14C-donepezil in healthy volunteers: A single-dose study. Br J Clin Pharmacol 1998;46:19-24.
11. Pattanashetti LA, Taranalli AD, Parvatrao V, Malabade RH, Kumar D. Evaluation of neuroprotective effect of quercetin with donepezil in scopolamine-induced amnesia in rats. Indian J Pharmacol 2017;49:60-4.
12. Shin JW, Seol IC, Son CG. Interpretation of animal dose and human equivalent dose for drug development. J Korean Orient Med 2010;31:1-7.
13. Nigam A, Kulshreshtha M, Panjwani D. Pharmacological evaluation of *Hibiscus abelmoschus* against scopolamine-induced amnesia and cognitive impairment in mice. Adv Hum Biol 2019;9:116-23.
14. Kamila S, Madhav NV, Sarkar CN. Safety and efficacy study of herbal polyphyto formulations: For its learning and memory enhancing properties. Int J Pharm Pharm Sci 2016;8:119-23.
15. Zaki HF, Abd-El-Fattah MA, Attia AS. Naringenin protects against scopolamine-induced dementia in rats. Bull Fac Pharm 2014;52:15-25.
16. Benatoui R, Bairi A, Tahraoui A. Estimation of the anxiolytic-like effect of the β-carboline alkaloid harmine on stressed pregnant rats. Int J Pharm Pharm Sci 2017;9:166-72.
17. Reneerkens OA, Rutten K, Steinbusch HW, Blokland A, Prickaerts J. Selective phosphodiesterase inhibitors: A promising target for cognition enhancement. Psychopharmacology (Berl) 2009;202:419-43.
18. Blokland A, Menniti FS, Prickaerts J. PDE inhibition and cognition enhancement. Expert Opin Ther Pat 2012;22:349-54.
19. Francis SH, Blount MA, Corbin JD. Mammalian cyclic nucleotide phosphodiesterases: Molecular mechanisms and physiological functions. Physiol Rev 2011;91:651-90.
20. Boess FG, Hendrix M, van der Staay FJ, Erb C, Schreiber R, van Staveren W, *et al*. Inhibition of phosphodiesterase 2 increases neuronal cGMP, synaptic plasticity and memory performance. Neuropharmacology 2004;47:1081-92.
21. van der Staay FJ, Rutten K, Barfacker L, Devry J, Erb C, Heckroth H, *et al*. The novel selective PDE9 inhibitor BAY 73-6691 improves learning and memory in rodents. Neuropharmacology 2008;55:908-18.
22. Jin F, Gong QH, Xu YS, Wang LN, Jin H, Li F, *et al*. Icariin, a phosphodiesterase-5 inhibitor, improves learning and memory in APP/PS1 transgenic mice by stimulation of NO/cGMP signalling. Int J Neuropsychopharmacol 2014;17:871-81.
23. Wang H, Peng MS, Chen Y, Geng J, Robinson H, Houslay MD, *et al*. Structures of the four subfamilies of phosphodiesterase-4 provide insight into the selectivity of their inhibitors. Biochem J 2007;408:193-201.
24. Gong B, Shelanski M, Arancio O. Persistent improvement in synaptic and cognitive functions in an Alzheimer mouse model after rolipram treatment. J Clin Invest 2004;114:1624-34.
25. Vanmierlo T, Creemers P, Akkerman S, van duinen M, Sambeth A, de Vry J, *et al*. The PDE4 inhibitor roflumilast improves memory in rodents at non-emetic doses. Behav Brain Res 2016;303:26-33.
26. Bruno O, Fedele E, Prickaerts J, Parker LA, Canepa E, Brullo C, *et al*. GEBR-7b, a novel PDE4D selective inhibitor that improves memory in rodents at non-emetic doses. Br J Pharmacol 2011;164:2054-63.
27. Lieben CK, Blokland A, Sik A, Sung E, van Nieuwenhuizen P, Schreiber R. The selective 5-HT6 receptor antagonist Ro4368554 restores memory performance in cholinergic and serotonergic models of memory deficiency in the rat. Neuropsychopharmacology 2005;30:2169-79.