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The Effect of Ketanserin and Pirenperone Injected into the CA1 Region on Spatial Discrimination

Nasser Naghdi*, Nahid Majlessi and Fahshideh Broofar

Dept. of physiology & pharmacology, Pasteur Institute of Iran, Pasteur Ave., Tehran 13164, Iran

ABSTRACT

In the present study, the effect of 5-HT_{2A} receptor blockers in CA1 region of rat hippocampus on spatial learning was assessed in a T-maze, a spatial discrimination task. Rats were canulated bilaterally and injected daily vehicle (saline), 5-HT_{2A}-selective antagonist, ketanserin (0.6, 1.2 or 2.4 µg/0.5 µl) and pirenperone (0.1, 0.3, 1.2 or 2.4 µg/0.5 µl) into the cannula 30 minutes before training. Results indicated that ketanserin and pirenperon did not affect spontaneous alternation and also did not induce a significant effect on trials to reach criterion and errors made by animals throughout spatial discrimination and reversal learning. But, in the rats that received ketanserin produced dose dependent decrease in the latencies to enter the chosen arm in both learning and reversal stages. During extinction, no change was observed in the choice of the previously reinforced arm in both ketanserin and pirenperone groups. The slope of latency in the ketanserin group that had received the highest dose of ketanserin (2.4 µg) than the sham operated group but not in the pirenperone group. These findings suggest that 5-HT_{2A} receptors blockade (ketanserin, but not pirenperone) in the CA1 region may decrease decision time and increase behavioural flexibility in T-maze. *Iran. Biomed. J. 5 (4): 141-147, 2001*

Keywords: Ketanserin, Pirenperone, CA1, T-maze, Spatial discrimination

INTRODUCTION

The role of serotonin (5-HT) on cognition has been conducted by many researches. However, the literature regarding experimental evidence on the role of 5-HT in cognitive processes is rather controversial [1, 2]. Also, some studies on the effects of serotonin on mnemonic function suggest that 5-HT exerts an inhibitory influence on learning and memory [3]. Serotonergic neurotransmission involves the action of multiple 5-HT receptor types and subtypes 5-HT_{1A}, 5-$HT_{1B\,/\,D}$, 5-$HT_{2A,\ 2C}$ and 5-$HT_{3\,-\,7}$ [4]. Previous pharmaco-logical data provided some insight into the nature of the 5-HT receptors associated with learning and memory [3, 5]. These data may suggest that stimulation of 5-$HT_{1B/1D}$ receptors produces a decrease in learning, while blockade of 5-$HT_{2A/2C}$ receptors leads to an increase in learning [3, 5, 6]. For example, ketanserin is a 5-HT_2 antagonist [6-8] which displays a high binding affinity for this receptor type [7, 8]. It is inactive at 5-HT_1 [7-9], 5-HT_3 and 5-HT_4 [9] binding sites, shows only moderate binding affinity for histamine H_1 and α_1-adrenergic receptors and binds very weakly to dopamine receptor binding sites [7, 8]. In addition, the effect of m-trifluoromethyl-phenylpiperazine (TFMPP), an agonist of 5-HT_{1B}, decreases the consolidation. This effect was reversed by (±) pindolol, ketanserin and ritanserin [7]. Pirenperone is also a potent antagonist that shows high specificity for the 5-HT_2 receptors in ligand binding. It has a little effect on 5-HT_1 ligand binding and an affinity for the dopamine receptor [10]. Some serotonergic receptors show post training adminstration of their antagonists (ketanserin, pirenperone and mianserin) improved passive avoidance retention or adminstration of serotonergic receptor antagonists prior to retention test facilitated passive avoidance retrieval [11]. The hippocampus has been traditionally linked to cognitive functions, particularly spatial memory [12]. The serotonergic innervation of the hippo-campus arises from 5-HT neurons of the median and dorsal raphe area (DR and MR) [13-15]. The CA1 region of the hippocampus is rich in 5-HT

*Corresponding Author; Tel: (98-21) 646 9871-4; Fax: (98-21) 646 5132; E-mail: naghdi@ institute.pasteur.ac.ir.

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receptors [21] and 5-HT containing terminals [15]. The result of the researchers on the possible involvement of hippocampal 5-HT in the mediation of learning and memory provides evidences in support of its negative effect on one or more of hippocampal pathways involved in spatial information processing [13, 16, 17].

The present experiment was conducted to examine specifically the effect of 5-HT_{2A} receptor blockade in the CA1 region of the rat hippocampus on spatial discrimination by pirenperone and ketanserin. A T-maze was used to test spontaneous alternation, spatial and reversal learning and extinction.

MATERIALS AND METHODS

Subjects. Male albino rats (200-250 g, 3 months old) were obtained from breeding colony of the Pasteur Institute of Iran. The rats were housed five per cage before surgery and individually after surgery. Then they were maintained at room temperature of 25 ± 2°C and on a standard 12 h light-12 h dark cycle with lights on at 07.00. Food and water were available.

Surgery. Approximately 7 days prior to initiation of the behavioural experiments, the rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and were implanted with a stainless steel thin-wall cannulae (21 gauges) bilaterally into the CA1 region of the hippocampus (AP: -3.80 mm from bregma; ML: ± 2.2 mm from midline; DV: -2.4 mm below the diameter according to the atlas Paxinos and Watson) [18]. The tooth was bared at -3.3 mm. The cannula and two anchoring screws were fixed to the skull with dental cement.

Drugs. Ketanserin purchased from Sigma and pirenperone from RBI (Reasearch Biochemical International) were dissolved in 0.9% isotonic saline.

Microinjection procedure. Before injection, the animal was restrained by hands and the cannula stylet was removed and replaced with the injection needle (27 gauges) connected with a short piece of polyethylene tubing to a Hamilton syringe. The needle was inserted 0.5 mm beyond the tip of the cannula. Saline (0.5 μl) or different doses of ketanserin (0.6, 1.2 or 2.4 μg/0.5μl) or pirenperone (0.1, 0.3, 1.2 or 2.4 μg / 0.5 μl) was injected during 1 min. A total volume of 0.5 μl was injected into each side.

Behavioral assessment. One week after surgery, the animals were deprived of food to the amount of 85% of the body weight and maintained at this level throughout behavioural testing.

Apparatus. The apparatus used consisting of a wooden T-maze with 15-cm walls, start and goal boxes 16.5 cm × 16.5 cm, the length of stem was 50 cm led to L-shaped arms. The first part of each arm was 36 cm long and the second part which led to the goal box, 30 cm long. Guillotine doors separated the start box from the stem and the goal boxes from the arms.

Training procedure. The protocol used for this test was similar to that of the Annett *et al.* [19]. Briefly, preliminary training took place on day 1 to 3. On days 4 and 5 (spontaneous alternation), food was available in both of the goal boxes on every trial. Each rat was confined to the start box for 10 seconds before being allowed to choose one of the goal boxes, where it was confined for a further 20 s. Eleven consecutive trials were given per day and the choice of right or left goal box was recorded, as was the latency between leaving the start box and entering the chosen arm. On days 6 to 8 (spatial discrimination and reversal), only one of the goal boxes was rewarded and the rats had to learn which was correct. Trials continued until the criterion of 5 consecutive correct responses had been achieved. The choice of arm and latency between leaving the start box and entering the chosen arm was recorded on every trial. Immediately after reaching criterion, the contingencies were reversed so that the previously unreinforced goal box was no correct. Training continued until the new response had been learned, again to criterion of 5 consecutive correct responses. On day 9 (extinction), after a spatial discrimination had been completed to 5 consecutive correct responses, an extinction stage was introduced. The food pellets were removed from the T-maze and goal box choices and start box to arm latencies were recorded over a further 10 trials.

Histology. At the end of each experiment, the animals deeply anesthetized with ether, sacrificed by decapitation and the brain was removed. For histological verification of cannulae and needle places in the CA1 region, 100 μ thick sections were

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taken, mounted on slides, and stained with cresyl violet and the cannulae track was examined for each rat. The animals were accepted for data analysis only if both needles were located within the CA1 region. There were several animals whose cannulae tip was not located at the correct position and the data were not included in analysis regardless of their memory performance.

Statistics. All data initially subjected to an analysis of variance (ANOVA) were followed, where appropriate, by subsidiary, post-hoc, pair-wise comparisons using the newman-keuls procedure. The difference was considered significant at the level of $p<0.05$. A parallelism test followed by student's *t*-test was used to compare the slopes.

RESULTS

Spontaneous alternation. There were no significant differences in the percentage of alternate choices among the four ketanserin groups (F3, 16 = 2.18, n.s.) and in five pirenperone groups (F4, 28 = 1.277, n.s.). Latencies between leaving the start box and entering the chosen arm were not affected by intrahippocampal injection of ketanserin (F3, 16 = 0.47, n.s.) and pirenperone groups (F4, 28 = 0.5101, n.s.).

Spatial discrimination and reversal. There were no significant differences in trials to reach criterion among the group in learning and reversal stages in ketanserin group (ketanserin learning: F3, 16 = 0.080, n.s.; reversal: F4, 28 = 0.5845, n.s.) and pirenperone groups (learning: F4, 28 = 0.503, n.s.; reversal: F4, 28 = 0.5845, n.s.). The number of errors of the ketanserin and pirenperone groups while achieving criterion was not significantly different in the learning and reversal stages in ketanserin groups (learning: F3, 16 = 0.02, n.s.; reversal: F3, 16 = 2.15, n.s.) and pirenperone groups (learning: F4, 28 = 0.7057, n.s.; reversal: F4, 28 = 0.09729, n.s.). However, there were no significant differences in the latencies to enter the chosen arm in the groups of received pirenperone on both learning and reversal trials (learning: F4, 28 = 0.46, n.s.; reversal: F4, 28 = 1.102, n.s.) but there was significant decrease in the ketanserin groups (learning: F3, 16 = 11.56, $p<0.01$; reversal: F3, 16 = 5.34, $p< 0.01$) (Fig. 1).

Extinction. Over the 10 extinction trials, there were no significant differences in the percentage of previously reinforced arm choices among the ketanserin groups (F3, 16 = 0.49, n.s.) and pirenperone groups (F4, 28 = 0.4042, n.s.).

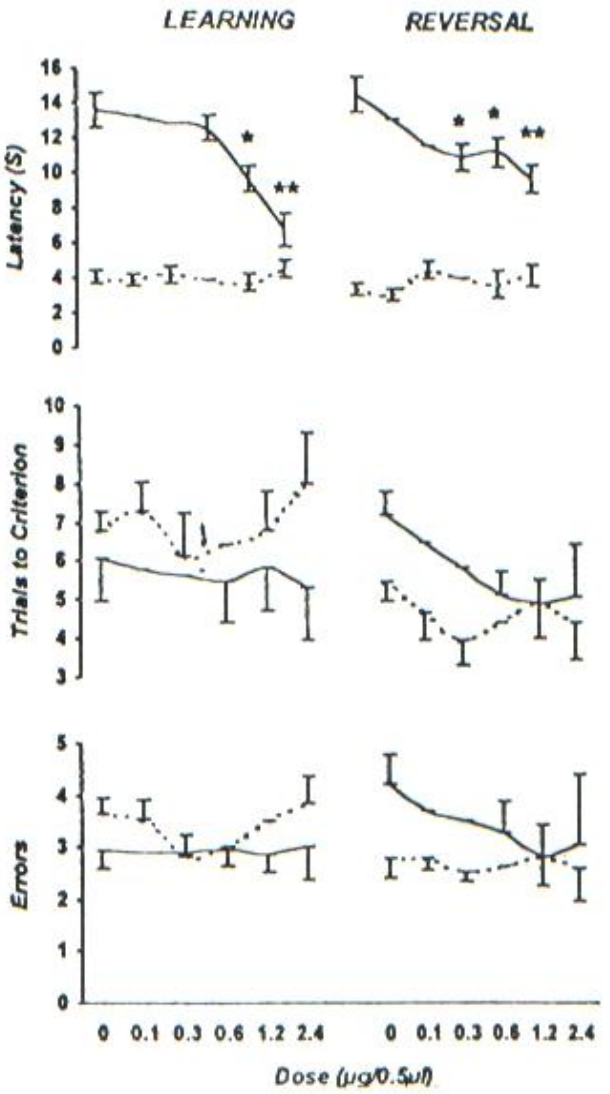

Fig. 1. Effect of different doses of ketanserin (_____) and pirenperone (……) injected into the CA1 region on spatial learning and reversal of rat performance in T-maze, over days 6-8. A, Mean latency (s) from leaving the start box to entering the chosen arm; B, Mean number of trials to reach criterion.; C, Mean number of errors while reaching criterion. (Ketanserin, n = 5 for each dose-Pirenperone, n = 7 for control, 0.3 and 2.4 µg/0.5µl; n = 6 for 0.1 and 1.2 µg/0.5 µl). Each point represents the mean ± SEM. $*p<0.05$, $**p<0.01$.

The latencies of both ketanserin and pirenperone groups to reach the chosen arm from the start box

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were not significantly different in learning (ketanserin: F3, 16 = 2.27, n.s.; pirenperone: F4, 28 = 2.323, n.s.) and extinction (ketanserin: F3, 16 = 0.51, n.s.; pirenperone: F4, 28 = 1.828, n.s.) stages (Fig.2). On the extinction trials, there is no significant differences in the latencies of the ketanserin and pirenperone groups from leaving the start box to entering the chosen arm (Fig. 2), but the slope of the latency increase was higher in ketanserin received groups and there was a significant difference between the group which had received 2.4 μg ketanserin and the sham operated group (t = 2.266, 96 d.f., $P< 0.05$) but not in pirenperone groups (Figs.3 and 4).

DISCUSSION

The results indicated that ketanserin and pirenperone injected into the CA1 region of rat hippocampus had no significant effect on spontaneous alternation. Ketanserin but not pirenperone could reduce the latencies to enter the chosen arm throughout spatial discrimination and its reversal. At the extinction stage, ketanserin affected the slope of latency increase and caused it to be significantly higher in the group which had received the highest dose of ketanserin (2.4 μg) compared to the sham operated group. But there are no significant changes in slope of latency in pirenperone groups.

It has been suggested that activation of 5-HT_{2A} receptor maybe involved in impairment of memory in mice [20] and may play an inhibitory role in memory consolidation [5]. Furthermore it has been demonstrated that administration of ketanserin can

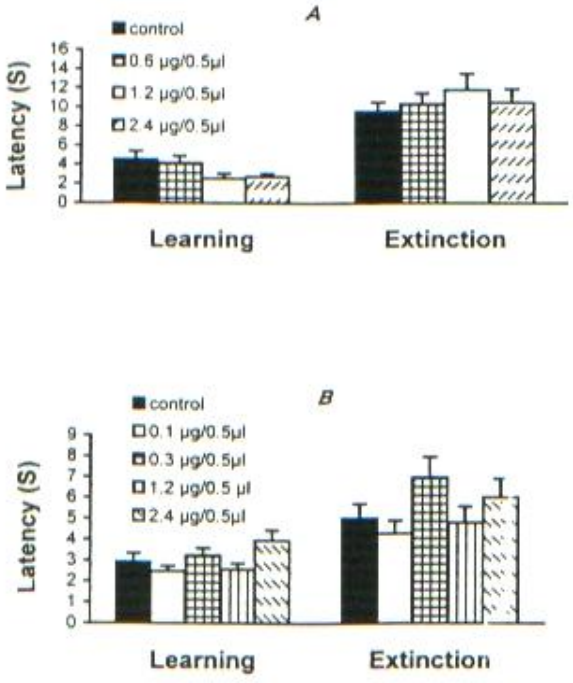

Fig. 2. Effect of ketanserin (A) and pirenperone (B) on mean latency (s) from leaving the start box to entering the chosen arm in learning and extinction phases (9th day). (Ketanserin, n = 5 for each dose-Pirenperone, n =7 for control, 0.3 and 2.4 μg/0.5 μl; n = 6 for 0.1 and 1.2 μg/0.5 μl). Each bar represents the mean ± SEM.

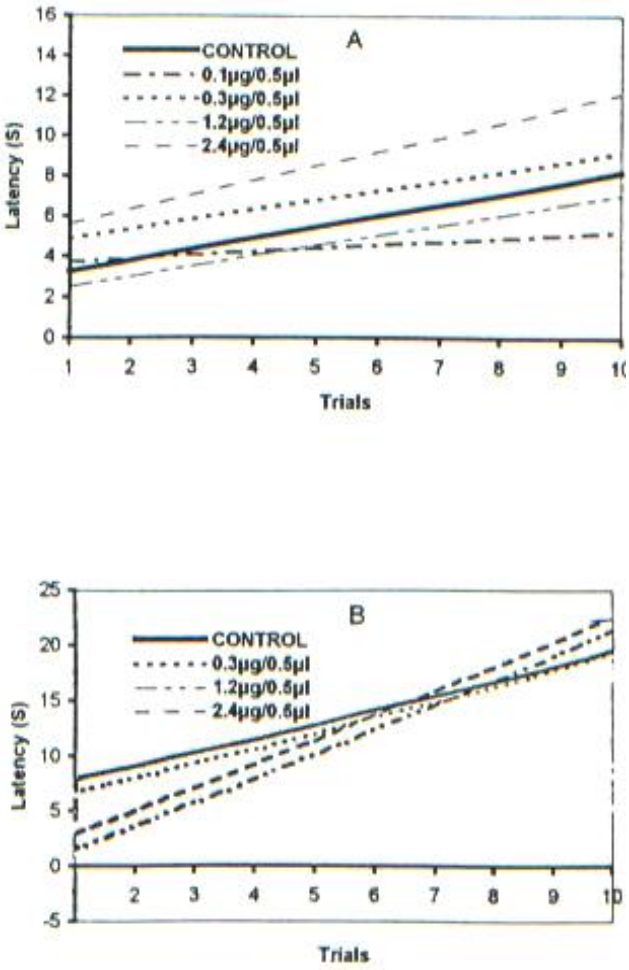

Fig. 3. Effect of different doses of pirenperone (A) and ketanserin (B) on increasing the mean latency (s) from leaving the start box to entering the chosen arm in extinction phase (9th day). (Ketanserin, n = 5 for each dose-Pirenperone, n = 7 for control, 0.3 and 2.4 μg/0.5μl; n = 6 for 0.1 and 1.2 μg/0.5 μl).

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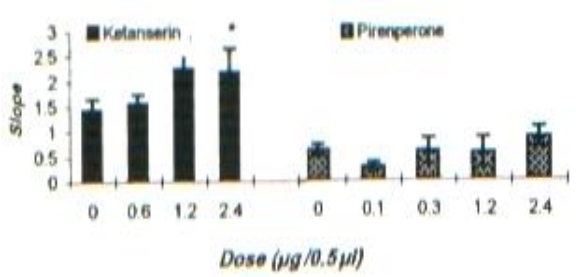

Fig. 4. Effect of different doses of ketanserin and pirenperone on slope of time increasing lines in extinction phase (9^{th} day). (Ketanserin, n = 5 for each dose-Pirenperone, n =7 for control, 0.3 and 2.4 μg/0.5μl; n = 6 for 0.1 and 1.2 μg/0.5μl). $*p<0.05$. Each bar represents the mean ± SEM.

enhance the memory of a previously learned inhibitory avoidance response in mice [21], attenuate hypoxia-induced [5] or age-related [22] passive avoidance retention deficits in rats and improve cognitive performance in squirrel monkeys [23]. It also enhances protective effect of other agents against amnesia [24]. Pirenperone, cianserin and ritanserin significantly reduced the increase in errors expected to occur 24 h after the 5 min of ischemia in a three-panel runway task [25]. These studies provide strong support for the notion that blockade of the 5-HT_2 receptors can enhance memory retrieval and protect against an experimentally induced amnesia and prevents of working memory following transient forebrain ischemia [25].

The results of a number of studies show the negative influence of 5-HT on the processing of spatial information within the hippocampus. An inverse correlation has been observed between serotonergic content in the hippocampus and retention of conditioned reflexes in rats [16]. It has been reported that intrahippocampal 5-HT injection immediately after training, impaired retention of a Y-maze brightness discrimination task in rats, while infusion of mianserin, a 5-HT_2 receptor antagonist enhanced it [17]. Ketanserin and pirenperone were found to be ineffective in a step-through passive avoidance test in the male rats [26] and also in rat elevated plus-maze [27]. The rats trained in the stone 14-unit T-maze learned significantly faster and also they had significantly fewer errors throughout training following selective neurotoxic (5, 7 = DHT) differentiation of the 5-HT in spatial learning within the hippocampus [13]. There are different effects on spatial discrimination which 5-HT_2 receptor antagonists generally produce [13]. The effect of serotonin on long-term potentiation (LTP) has been assessed in a number of *in vitro* studies, and most of the reports have suggested that 5-HT inhibits induction of LTP in the CA1 region [28] and commissural synapses [29] of rat hippocampus. In the CA1 region, it has been demonstrated that 5-HT_2 receptor blockade significantly enhances hippo-campal LTP [30].

The result of this study indicated that ketanserin and pirenperone injected into the CA1 region did not affect spontaneous alternation, since no difference was seen in the percentage of alternate choices and latency to choose an arm on days 4 and 5. There was also no significant effect on the number of trials to reach criterion and errors made by animals while achieving criterion in learning spatial discrimination task and its reversal. Ketanserin, but not piren-perone reduces the latencies to enter the chosen arm in both learning and reversal stages in a dose-dependently. So, the ketanserin received rats would be able to choose their way on each trial faster than sham operated group. On the other hand their decision time decreased following ketanserin injection. Since ketanserin did not affect the latencies on day 4 and 5, it could be concluded that latency decrease on days 6-8 was due to ketanserin effect on spatial discrimination and reversal. But, pirenperone had no effect on latency error and trial to criterion in learning spatial discrimination task and its reversal on days 6-8.

Over the 10 extinction trials, ketanserin caused the slope of latency increase to be higher in the ketanserin received group but pirenperon did not change the slope of latency. At the extinction stage, when food reward is omitted, the rats reduce the pace at which they run the maze, i.e. in absence of reinforcer, they do not choose an arm with the same pace as before and the latency to enter the chosen arm increases. However, the slope of latency increase was higher in the ketanserin received groups and there was a significant difference between the group which had received 2.4 μg ketanserin and the sham operated group.

Since Annett *et al.* [19] have attributed this parameter to behavioural flexibility, it can be concluded that ketanserin could probably increase behavioural flexibility. Taken together, it seems that 5-HT_{2A} receptor blockade in the CA1 region of rat hippocampus may decrease decision time and increase behavioural flexibility in T-maze and it seems that ketanserin is more effective than pirenperone. However, the effect of intrahippo-campal injection of ketanserin and pirenperone on

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spatial discrimination should be assessed using other types of learning tasks and additional studies warranted in order to determine the exact role of 5-HT_{2A} receptors in this region in spatial learning and memory.

REFERECES

1. Stancampiano, R., Stefania, C., Melis, F., Caugusi, C., Sarais, L. and Fadda, F. (1997) The decrease of serotonin release induced by a triptophan-free amino acid diet does not affect spatial and passive avoidance learning. *Brain Res. 762: 269-274*.
2. Dugar, A. and Lakoski, J.M. (1997) Serotonergic function of aging hippocampal CA3 pyramidal neurons: Electrophysiological assessment following adminstration of 5, 7-DHT in the Fimbria-Fornix and Cingulum bundle. *J. Neurosci. Res. 47:58-67*.
3. McEntee, W.J. and crook, T.H. (1991) Serotonin, memory, and the aging brain. *Psychopharmacology (Berl) 103: 143-149*.
4. Hong, E. and Meneses, A. (1996) Systemic injection of P-chloroamphetamine eliminates the effect of the 5-HT_3 compounds on learning. *pharmacol. Biochem. Behav. 53: 765-769*.
5. Strek, K.F., Spencer, K.R. and DeNoble, V.J. (1989) Manipulation of serotonin protects against an hypoxia-induced deficit of a passive avoidance response in rats. *Pharmacol. Biochem. Behav. 33: 241-244*.
6. Frazer, A., Maayani, S. and Wolfe, B.B. (1990) Subtypes of receptors for serotonin. *Annu. Rev. Pharmacol. Toxicol. 30: 307-348*.
7. Leysen, J.E., Awouters, F., Kennis, L., Laduron, P.M., Vandenberk, J. and Janssen, P.A.J. (1981) Receptor binding profile of R 41 468, a novel antagonist at 5-HT_2 receptors. *Life Sci. 28: 1015-1022*.
8. Leysen, J.E., Niemegeers, C.J.E., VanNueten, J.M. and Laduron, P.M. (1982) [3H] Ketanserin (R 41 468), a selective 3H-ligand for serotonin-2 receptor binding sites. *Mol. Pharmacol. 21: 301-314*.
9. Hoyer, D., Clarke, D.E., Fozard, J.R., Harting, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R. and Humphrey, P.A.A. (1994) VII. International union pharmacology classification of receptors for 5-hydroxytriptamine (serotonin). *Pharmacol. Rev. 46: 157-203*.
10. Green, A.R., O'shaughnessy, K., Hammond, M., Schachter, M. and Grahame-Smith, D.G (1983) Inhibition of 5-hydroxytriptamine-mediated behavior by the putative 5-HT_2 antagonist pirenperone. *Neuropharmacol. 573-578*.
11. Sarihi, A., Motamedi, F., Rashidy-Pour, A., Naghdi, N. and Behzadi, G. (1999) Reversible inactivation of the median raphe nucleus enhances consolidation and retrieval but not acquisation of passive avoidance learning in rats. *Brain Res. 817: 59-66*.
12. Carli, M., Bonalumi, P. and Samanin, R. (1997) WAY 100635, a 5-HT_{1A} receptor antagonist, prevents the impairment of spatial learning caused by intrahippocampal adminstration of scopolamine or 7-chloro-kynurenic acid. *Brain Res. 774: 167-174*.
13. Altman, H.J., Normile, H.J., Galloway, M.P., Ramirez, A. and Azmitia, E.C. (1990) Enhanced spatial discrimination learning in rats following 5, 7-DHT-induced serotonergic deafferentation of the hippocampus. *Brain Res. 518: 61-66*.
14. Kohler, C. (1984) The distribution of serotonin binding sites in the hippocampal region of the rat brain. An autoradiographic study. *Neurosci. 13: 667-680*.
15. Oleskevich, S. and Descarries, L. (1990) Quantified distribution of the serotonin innervation in adult rat hippocampus. *Neurosci. 34: 19-33*.
16. Kruglikov, R.I., Uniyal, M. and Gestova, V.M. (1976) Relationship between peculiarities of elaboration and retention of brightness discrimination and the serotonin content in the rat brain. *Acta Neurobiol. Exp. 36: 417-425*.
17. Wetzel, W., Getsova, V.M., Jork, R. and Matthies H. (1980) Effect of serotonin on Y-maze retention and hippocampal protein synthesis in rats. *Pharmacol. Biochem. Behav. 12: 319-322*.
18. Paxinos, G. and Watson, C. (1986) The rat brain in stereotaxic coordinates, 2nd ed., Academic Press, Orlando, pp. 32-34.
19. Annett, L.E., McGrogor, A. and Robbins, T.W. (1989) The effects of ibotenic acid lesions of nucleus accumbens on spatial learning and extinction in the rat. *Behav. Brain Res. 31: 231-242*.
20. Nabeshima, T., Itoh, K. and Kawashima, T. (1989) Effects of 5-HT_2 receptor antagonists on cycloheximide-induced amnesia in mice. *Pharmacol. Biochem. Behav. 32: 787-790*.
21. Altman, H.J. and Normile, H.J. (1987) Different temporal effects of serotonergic antagonists on passive avoidance retention. *Pharmacol. Biochem. Behav. 28: 353-359*.
22. Normile, H.J. and Altman, H.J. (1988) Enhanced Passive avoidance retention following posttrain serotonergic receptor antagonist adminstration in middle-aged and aged rats. *Neurobiol. Aging 9: 377-382*.
23. DeNoble, V.J., Schrack, L.M. and DeNoble, K.F. (1991) Visualrecognition memory in squirrel monkeys: Effects of serotonin antagonists on baseline and hypoxia-induced performance deficits. *Pharmacol. Biochem. Behav. 39: 991-996*.
24. DeNoble, V.J., DeNoble, K.F. and Spencer, K.R. (1991) Protection against hypoxia-induced passive avoidance deficits: Interaction between DUP 996 and ketanserin. *Brain Res. Bull. 26: 817-820*.

[DOR: 20.1001.1.1028852.2001.5.4.5.5]

25. Ohono, M., Yamamoto, Y. and Watanabe, S. (1991) Blockade of 5-HT_2 receptors protects agonist impairment of working memory following transient forebrain ischemia in the rat. *Neurosci. Lett. 129: 185-188*.
26. Misane, I., Johansson, C. and Ogren, S.O. (1998) Analysis of the 5-HT_{1A} receptor involvement in passive avoidance in the rat. *Br. J. Pharmacol. 125: 499-509*.
27. Griebel, G., Rodgers, R.J., Perrault, G. and Sanger, D.J. (1997) Risk assessment behaviour: Evaluation of utility in the study of 5-HT-related drugs in the rat elevated plus-maze test, Pharmacol. *Biochem. Behav. 57: 499-509*.
28. Corradetti, R., Ballerini, L., Pugliese, A.M. and Pepeu, G. (1992) Serotonin blocks the long-term potentiation induced by primed burst stimulation in the CA1 region of rat hippocampal slices. *Neurosci. 46: 511-518*.
29. Villani, F. and Johnston, D. (1993) Serotonin inhibits induction of long-term potentiation at commersial synapses in hippocampus. *Brain Res. 606: 304-308*.
30. Naghdi, N., Neveill G. and Pocket S. (1998) Block of 5-HT_2 receptors enhances hippocampal long-term potentiation. *Iran. Biomed. J. 2: 129-132*.

[DOR: 20.1001.1.1028852.2001.5.4.5.5]