The modulatory effect of CA1 5HT 4 receptors on memory acquisition deficit induced by harmaline

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ABSTRACT

The plethora of studies indicated that there is a cross talk relationship between harmaline and serotonergic (5-HT) system on cognitive and non-cognitive behaviors. Thus, the purpose of this study is assessment the effects of CA1 5-HT4 receptor on memory acquisition deficit induced by harmaline. Harmaline was injected peritoneally, while 5-HT4 receptor agonist (RS67333) and antagonist (RS23597-190) were injected intra-CA1. For memory measurement,a single-trial step-down passive avoidance apparatus was used. The data revealed that pre-training injection of higher dose of harmaline (1 mg/kg), RS67333 (0.5 ng/mouse) and RS23597-190 (0.5 ng/mouse)decreased memory acquisitionprocess in the adult mice. Moreover, concurrent pre-training administration of subthreshold doseof RS67333 (0.005 ng/mouse) orRS23597-190 (0.005 ng/mouse)with subthreshold dose of harmaline (0.5 mg/kg, i.p.)intensify impairment of memory acquisition. All above interventions did not change locomotion and tail flick behaviors. In conclusion, the results demonstrated that the synergistic effect between both CA1 5-HT4 receptor agonist and antagonist with impairment of memory acquisition induced by harmaline, indicating a modulatory effect for CA1 5HT4 receptor on Harmaline induced amnesia.

Keywords: Memory; 5-HT; Harmaline; Mice; Passive avoidance.

INTRODUCTION

There are severities of studies about involvement of serotonergic (5-HT) system in cognitive and non-cognitive behaviors.For instance a modulatory effect on stress, anxiety, food intake, pain perception, rhythm, learning and memory behaviors, inasmuch as dysfunction of serotonergic system can induced posttraumatic stress disorder, anxiety, and depression [1-5], which are often accompanied by impairment of learning and memory [6-8]. In this line some investigations demonstrated that 5-HT has a crucial role for learning and memory formation process via interaction on multiple receptor subtypes. For instance, it showed that blockade or activation of serotonergic system induced enhanced and impaired learning and memory process, respectively[9], while the opposite findings have also been reported[10, 11].It is clear that multiple 5-HT receptors have different responses on learning and memory, depending the drug, place of injection (focal or systemic), timing of drug injection and behavioral tests used[12].

The serotonergic axons from raphe nucleus (primarily localized of 5-HT cell bodies) projects into almost every brain region[13, 14], including hippocampus[6, 15, 16] and cerebral cortex[17], that have high expression of different 5-HT superfamily [18-21], indicating the important role of this system on cognition process such as learning and memory [18]. In according of structural, operational, and transductional properties of 5-HT receptors, seven classes for 5-HT were classified, namely 5-HT1–7superfamily[22, 23]. In among of these receptors 5-HT3 receptor is a ligand-gated ion channel, while others are G-protein coupled receptor[22, 23].

Among of 5-HT receptors 5-HT4 receptor has a relatively high expression in the limbic system and a critical role for learning and memory process[24]. This receptor seems that has variety

responses on different stages of memoryformation (acquisition, consolidation and retrieval). It seems that activation of this receptor induced a positive effect on the acquisition phase. The effect 5-HT4 on memoryconsolidation is very variation dependent the method of injection and memory assessment[25-27]. About the memory retrieval a study revealed that 5-HT4receptor did not alter this memory state [25].It showed that activation of 5-HT4 receptor via systemic infusion increased LTP phenomenon in the CA1 and dentate gyrus regions[28, 29]. Monoamine oxidase A (MAO_A), as a key enzyme in for serotonergic system,degrades the concentration level of 5-HT in the several parts of brain such as hippocampus[30, 31].

β-carbolines are a class of alkaloids which have elicited considerableresearch interest [32, 33]. β-carbolinesconsistof an indole nucleus and a pyridine ring[32, 34] that depending upon their degree of ring saturation can be dividedinto three structural groups: (a)harmane; (b)harmalane; and (c) the harmaline[32]. These compounds are endogenously produce and exist in normal body, such asblood plasma, heart, kidney, liver and brain tissue[35-37]. The biological significance of β-carbolines has been suggestedto have neuroprotective properties as well as cytotoxic properties [32, 37-39], excitation and euphoria [40, 41],analgesic effects[42], anticancerous and antibioticproperties[43, 44]. Some investigations indicated that β-carbolines bind with high affinity toa variety of different targets including MAO_A, MAO_B, benzodiazepine, imidazoline, dopamine and 5-HT receptors[45-49]. β-carbolines by inhibition of MAO_A or MAO_Bincrease the extracellular norepinephrine, dopamine and 5-HT levels in several brain regions[34, 35, 50]. As regards β-carboline could enhance 5-HT levels in several brain area through inhibition of MAO reuptake system and considering the role of β-carbolines[51], 5-HT receptors[52-57], and hippocampus[58-60], in memory process, in the present study, the effects of harmaline on memory acquisition/exploratory behaviors/pain response and involvement of 5-HT4 receptors on these behaviors in the step-down passiveavoidance, open field and tail flick tests in mice have been investigated.

MATERIALS AND METHODS

Animals

Male NMRI mice weighing 25–30 g obtainedfrom the University of Tehran (Tehran, Iran) were used. Animals were housed in groups of 10 in plastic cages and maintained at a controlled temperature of 22±2 °C under a 12/12-h light/dark cycle with water and food freely available except during the limited times of experiments. Ten animals were used in each group and each mouse was used once only. Behavioral tests were done during the light phase of the light/dark cycle. The investigation was approved by the Ethics Committee of the Faculty of Science of the University of Tehran which corresponds to the national guidelines for animal care and use.

Stereotaxic surgery

Mice were anaesthetized using a solution containing ketamine hydrochloride (50 mg/kg) plus xylazine (5 mg/kg) and positioned in a stereotaxic frame (Stoelting Co, Illinois, USA). Then, the skin was incised and skull was cleaned. Next, two stainless-steel guide cannulae (8 mm length, 22 gauge) were bilaterally implanted 1 mm above the dorsal portion of the dorsal hippocampus (CA1). The following coordinates were used based on the Paxinos and Franklin atlas) [61]. Stereotaxic coordinates for the CA1 area of the dorsal hippocampus were anteroposterior (AP)=-2 mm from bregma, mediolateral (ML) ±1.6 from the sagital suture and dorsoventral (DV)= -1.5 mm from the skull surface. Cannulae were secured to the bone with dental acrylic cement. A stylet was presented into the guide cannula to prevent possible obstruction. All mice were allowed about 5-7 days to recover from surgery and from the effect of the anesthetic agents[62, 63].

Memory testing and apparatus

The inhibitory avoidance apparatus comprised of a wooden box (30×30×40 cm^3) with a floor which consisted of parallel stainless steel rods (0.3 cm in diameter, spaced 1 cm apart). A wooden platform (4×4×4 cm^3) was set in the center of the grid floor. Electric shocks (1 Hz, 0.5 s and 50 VDC) were delivered to the grid floor via an isolated stimulator (Grass S44, Quincy, MA, USA).

For testing, each mouse was gently placed on the wooden platform. When the animal stepped down from the platform and located all its paws on the grid floor, intermittent electric shocks were delivered continuously for 15 s. This training method was carried out among 9:00 a. m. and 2:00 p.m. Twenty-four hours after training, each animal was located on the platform again, and the step-down latency was measured with a stop-watch as passive avoidance behavior. An upper cut-off time of 300 s was set. The retrieval test was also carried out among 9:00 a. m. and 2:00 p. m[64, 65].

Measurement of locomotor activity

The locomotion apparatus (BorjSanat Co, Tehran, Iran)comprised of clear perspex container box (30 cm × 30 cm × 40 cm high). The apparatus has a gray perspex panel (30 cm × 30 cm × 2. 2 cm thick) with 16 photocells which separatedthebox to 16 equal-sized squares. Locomotor activity was recorded as the number of crossings from one square to another during 5 min[66-68].

Tail flick test

The tail flick test is a test of the pain responsein animals, alike to the hot plate test. It is used in basic painexamination and to measure the effectiveness of analgesics, through observing the reaction to heat. It was first presentedby[66, 69, 70]. A light beam is focused on the animal's tail and a timer starts. Once the animal flicks its tail, the timer stops and the recorded time is a measure of the pain threshold. This behavior testing was done 5 min after training.

Drugs

The drugs used in the present study were Harmaline (1-methyl-7-methoxy-3, 4-dihydro-bcarboline)from Sigma (St. Louis, MO), 5-HT4 receptor agonist (RS67333)and 5-HT4 receptor antagonist (RS23597-190) from (Tocris Bioscience United Kingdom). The time ofadministration and doses of drugs used in the experiments were chosenaccording to pilot studies and publishedwork in scientific literature[33, 34, 64, 71]. The compounds were tested at doses: harmaline:0.25, 0.5 and 1mg/kg, RS67333: 0.005, 0.05 and 0.5 ng/mouse, RS23597-190: 0.005, 0.05 and 0.5 ng/mouse. Harmaline was dissolved in sterile0.9% saline solution and the compound was stirred for 1h beforeobtaining the final solution; other drugs were dissolved in 0.9% saline, just before the experiments.

Drug treatment

For drug administration, the animals were restrained gently by hand; the stylets were removed from the guide cannulae and substituted by 27-gauge infusion needles (1mm below the tip of the guide cannulae).

The injection solutions were administered in a total volume of 1 µl/mouse (0.5 µl in each side) over a 60 s period, manually. Injection needles were left in place for an extra 60 s to facilitate the diffusion of the drugs[71, 72]. The protocol has been illustrated in Table 1.

Table 1. illustrates all experiments, groups of animals, time of drugs infusion and doses of drugs.

	Figure 1	**Pre-training treatment (i.p.)**	**Pre-training treatment (intra-CA1)**	**Step-through latency (panel A)**	**locomotor activity (panel B)**	**Tail flick (panel C)**
Experiment 1	**Left**	-	RS67333 (0.005, 0.05 and 0.5 ng/mouse)	Decrease	No effect	No effect
	Right	-	RS23597-190(0.005, 0.05 and 0.5 ng/mouse)	Decrease	No effect	No effect
Experiment 2	**Figure 2**					
	Left	harmaline (0.25, 0.5 and 1 mg/kg)	Saline (1 µl/mouse)	Decrease	No effect	No effect
	Middle	harmaline (0.25, 0.5 and 1 mg/kg)	RS67333 (0.005 ng/mouse)	Potentiated amnesia by harmaline	No effect	No effect
	Right	harmaline (0.25, 0.5 and 1 mg/kg)	RS23597(0.005 ng/mouse)	Potentiated amnesia by harmaline	No effect	No effect

Statistical analysis

We chose to analyze data using the Kruskal-Wallis nonparametric one-way analysis of variance (ANOVA) followed by a two-tailed Mann-Whitney's U-test for because individual variations in step-down apparatus data. The median as well as interquartile ranges of the step-down latencies were recorded for ten mice in each experimental group. One/two way ANOVA followed by post-hoc test was used for statistical evaluation in the tail flick and open filed tasks. In all evaluations $p<0.05$ was considered statistically significant. All statistical analysis results have been summarized in the table 2.

Experiment1: effects of pre-training 5-HT4 drugs administration on memory acquisition

In this experiment, eight groups of mice were used. Four groups of animals received saline (1µl/mouse) or different doses of RS67333 (0.005, 0.05 and 0.5 ng/mouse)5 min before training. The other four groups received saline (1 µl/mouse)ordiverse doses of RS23597-190 (0.005, 0.05 and 0.5 ng/mouse)5 min prior training.

Experiment2: effects of pre-training 5-HT4 receptor drugs administration on memory acquisition under the disruptive influence of harmaline

In this experiment, twelve groups (three arms) of mice were used. The animals received saline (1 µl/mouse) or different doses of harmaline (0.25, 0.5 and 1mg/kg; i.p.)15 min before training. These mice received intra-CA1 pre-training saline (1µl/mouse, for groups), subthreshold does of RS67333 (0.005ng/mouse, for groups) or RS23597-190 (0.005 ng/mouse, for groups) 5 min earlier training.

Histology

Histological results were plotted on representative sections taken from the mice brain atlas of Paxinosand Franklin(Paxinos and Franklin, 2001)[61][61]. Cannulae were implanted into the CA1 regions of dorsal hippocampus of a total of 214 mice, however only the data from 200 animals with correct cannulae implants were included in statistical analyses.

RESULTS

Effects of pre-training intra-CA1 administration of 5-HT4 drugs on memory acquisition, locomotor activity and tail flick

Kruskal-Wallis and Mann-Whitneydatadisplays that the infusion of RS67333 (0.5ng/mouse, figure 3A; left panel) and RS23597-190 (0.5ng/mouse, figure 1A; right panel), 5 min before training, reduced memory acquisition. In addition, one-way ANOVA postulates that all interventions did not alter locomotor activity and tail flick behaviors (figure 1B and C; left panels for RS67333, meanwhile figure 1B and C; right panels for RS23597-190).

Table 2. describe Kruskal–Wallis and one/two-way ANOVA analyses results for all experimental groups.

	Step-through latency(panel A)				Locomotor activity (panel B)						Tail flick (panel C)					
					Treatment effect		dose effect		dose interaction		treatment effect		dose effect		dose interaction	
Experiment 1	Figure 1	Drug	H (3,36)	P	F (3, 36)	P	-	-	-	-	F (3, 36)	P	-	-	-	-
	Left	RS67333	10.45	<0.01	1.19	>0.05	-	-	-	-	1.17	>0.05	-	-	-	-
	Right	RS23597	10.12	<0.01	0.79	>0.05	-	-	-	-	3.06	>0.05	-	-	-	-
Experiment 2	Figure 2	Drug	H (3,36)	P	F (1, 72)	P	F (3, 72)	P	F (3, 72)	P	F (1, 72)	P	F (3, 72)	P	F (3, 72)	P
	Left	Harmaline + Saline	8.936	<0.001	4.28	>0.05	0.39	>0.05	0.2	>0.05	4.8	>0.05	1.89	>0.05	0.29	>0.05
	Middle	harmaline + RS67333	2.416	<0.01	0.35	>0.05	0.73	>0.05	0.26	>0.05	1.82	>0.05	1.37	>0.05	0.12	>0.05
	Right	harmaline + RS23597	20.732	<0.001	0.58	>0.05	0.42	>0.05	0.11	>0.05	0.13	>0.05	0.31	>0.05	0.18	>0.05

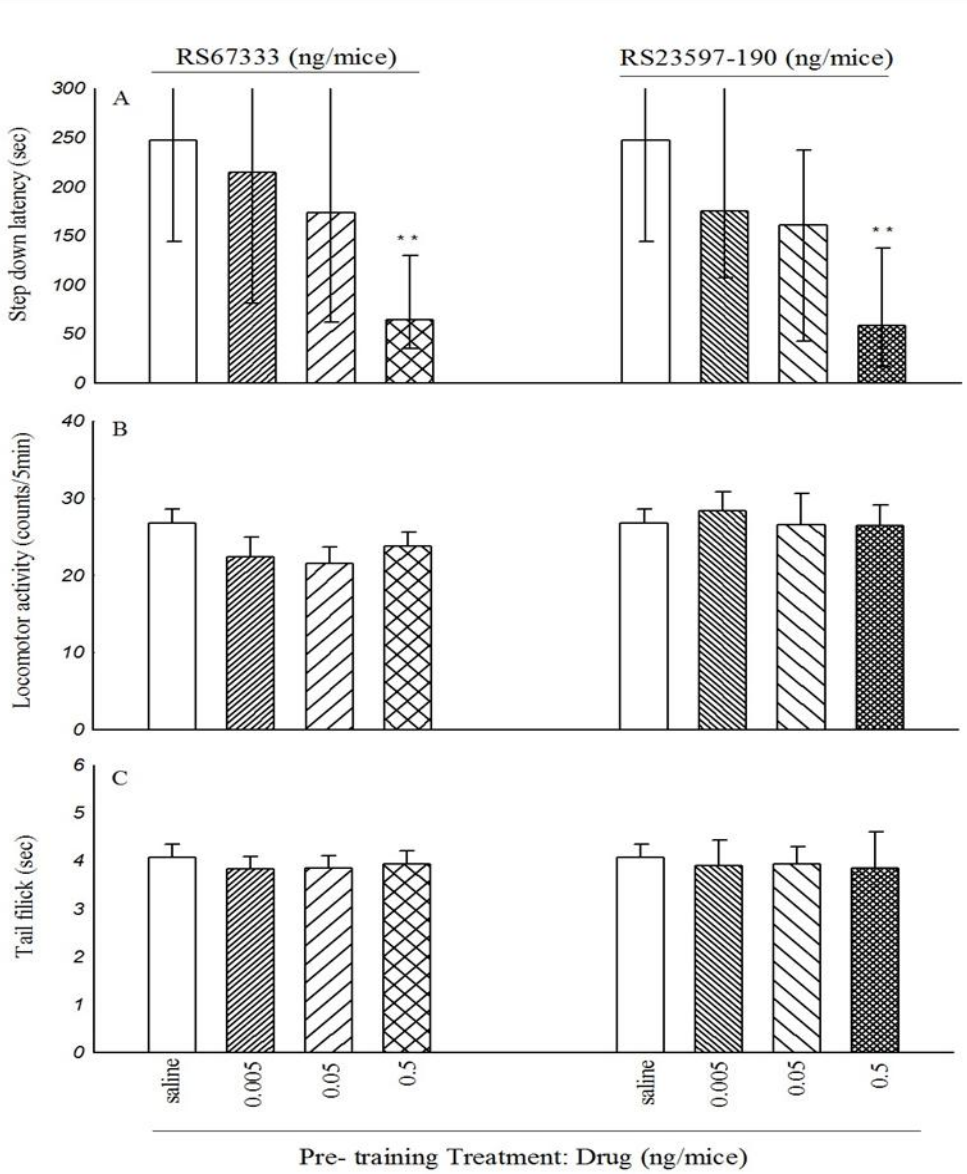

Figure 1. the effects of pre-training intra-CA1 administration of saline, RS67333 and RS23597-190 on memory acquisition, locomotor activity and tail flick. A left and right panels exhibit the effects of pre-training administration of RS67333 (0.005, 0.05 and 0.5 ng/mouse) and RS23597-190 (0.005, 0.05 and 0.5 ng/mouse) on memory acquisition, respectively. Test session step-down latencies are expressed as median and quartile for 10 animals. Similarly, locomotor activity in panel B was examined 5 min after memory testing and tail flick in panel C was tested 5 min after training. Each bar is mean±S.E.M. **P<0.01 when compared to saline/saline group.

Effects of pre-training 5-HT4 receptor drugs administration on memory acquisition, locomotor activity and tail flick under the amnesia induced byharmaline

Kruskal-Wallis and Mann-Whitneyresults in according of harmaline-treated groups exhibit that a subthreshold dose of RS67333 (0.005 ng/mouse, figure 2A, middle panel) or RS23597-190 (0.005 ng/mouse, figure 2A, right panel)potentiated memory impairment induced by harmaline. Moreover, two-way ANOVA postulates that these interventions did not alter both locomotor activity (figure 2B; middle panel for RS67333, meanwhile figure 2B; right panel for RS23597-190) and tail flick (figure 2C; middle panel for RS67333, meanwhile figure 2C; right panel for RS23597-190) behaviors.

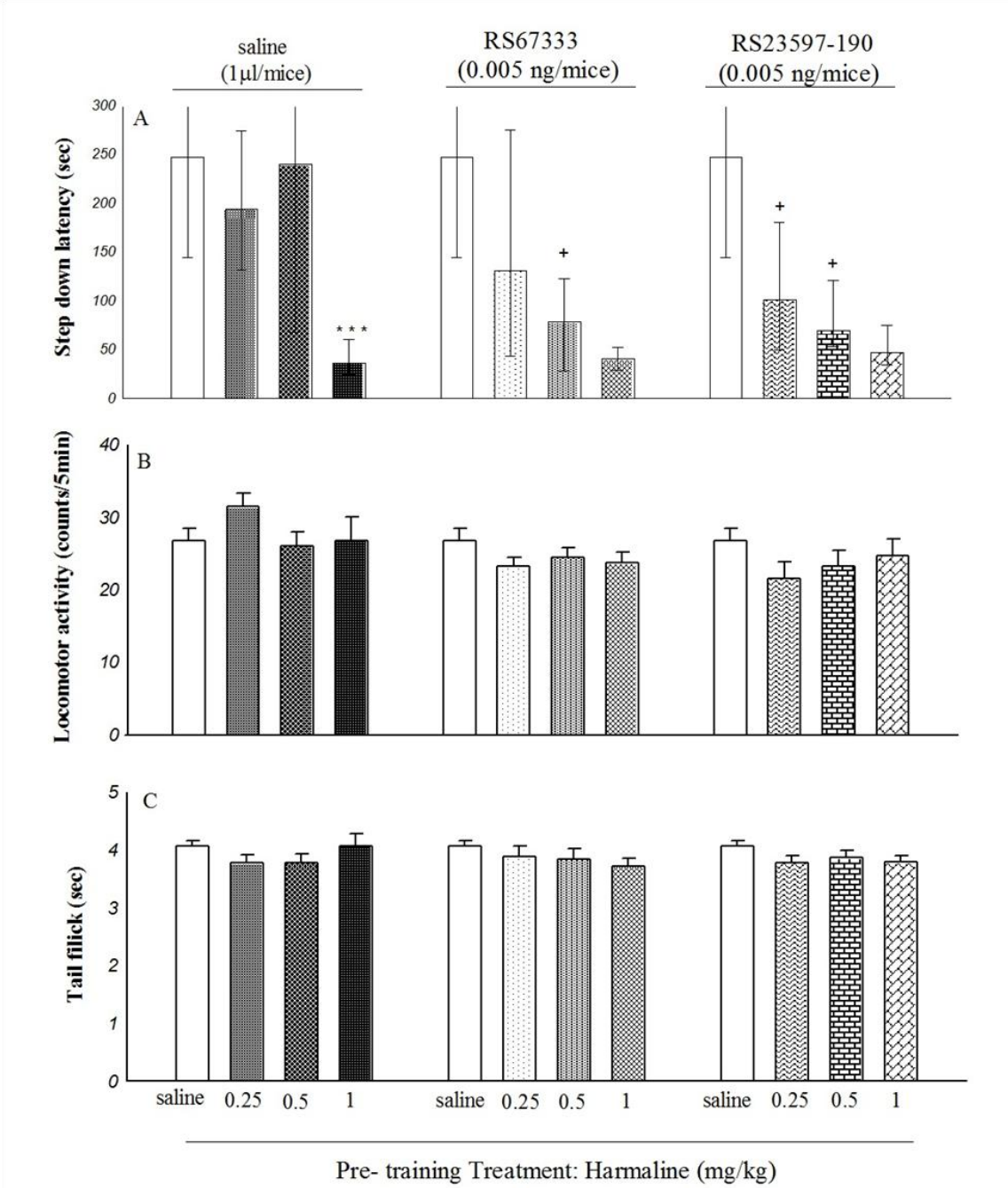

Figure 2. the effects of 5-HT4 receptor drugs on memory acquisition, locomotor activity and tail flick in the present and absence of harmaline. Figure 4A indicates the effects of pre-training infusion of harmaline (0.25, 0.5 and 1 mg/kg, i.p.; left panel) on animals which were trained under the effect of saline (1 µl/mouse; intra-CA1; left panel), RS67333 (0.005 ng/moue, intra-CA1; middle panel) or RS23597-190 (0.005 ng/mouse, intra-CA1; right panel). Test session step-down latencies are expressed as median and quartile for 10 animals. Furthermore, locomotor activity in panel B was measured 5 min after memory testing and tail flick in panel C was tested 5 min after training. Each bar is mean±S. E. M. ***$p<0.001$ when compared with saline/saline group. +$p<0.05$ when compared with respective harmaline/saline group.

DISCUSSION

The data revealed that pre-training intra-CA1 injection of higher dose of 5-HT4 receptor agonist (RS67333) and 5-HT4 receptor antagonist (RS23597-190)impaired memory acquisition by itself, while did not alter locomotion and tail flick behaviors. The previous reports indicated that 5-HT receptors depletion or activation of serotonergic system (i.e., tryptophan) impaired [74] memory formation process respectively, indicating direct participation of 5HT in learning and memoryformation[75].The responses of 5-HT receptors on learning and memory are very variable, because: 1- differentialaffinity from 5-HT to its receptors[76] and 2- expression of 5-HT receptors on pre- and postsynaptic membranes of neurons[75, 76]. Both of these points can be mention as two critical factors inlearning and memory mechanisms[75, 76]. 5-HT4 receptors displays constitutive (ligand independent) activity, even if it contributes to function of the receptor only in a small extent. This activity clarifies the differences between expected and observed effects of agonists and antagonists of 5-HT4 receptors. Some expected agonists shown rather silent or antagonistic effects depending on the level of ligand independent activity [77]. In consistent our data, some evidences revealed that 5-HT4 receptor agonist [25] and antagonist [78] impaired memory. Some studies indicated that 5-HT4 agonists enhance learning and memory[79-82], or has no effect onmemory[12]. Therefore, the actual effect of 5-HT receptors on memory formation process has remained unclear in order to many contradictory findings. It seems that different brain regions, systemic or intra-focal injections, nature and degree of difficulty of behavioral tasks induced a critical role for the effect of 5-HT on learning and memory [57, 83-85]. Moreover, the data revealed that pre-training systemic infusion of harmaline reduced memory acquisition, while did not change locomotion and tail flick behaviors. Harmaline induce several effects on cognitive and non-cognitive behaviors, includingeuphoria[40] and impair both associative and motor learning[32, 34, 50]. On the other hand, several report showed that harmaline improved learning and memory[32, 50]. It seems that the doses of drugs and route/methods of injections are very important for the effects of these compounds [50]. A report showed that harmaline decrease current of voltage-gated calcium channel, herein decrease neuron excitation. In the synaptic communication, calcium has a vital role, which controlling many cellular processes, inasmuch as increase of sytoplasmic calcium level concentration stimulates cellular signaling pathways involved in memory processes[32]. In conclusion, we can propose that the memory acquisition deficit induced by harmaline is in order to decrease calcium level and reduction of neuron excitation.

In continue of this study, we assess the effect of 5-HT4 receptors on harmaline induced avoidance memory deficit. The present study indicated that RS67333 and RS23597-190 strengthen amnesia induced by harmaline. It seems that harmaline by reducing neuron excitation[32], and 5-HT4 agents via constitutive (ligand independent) activity [77], causes impairment of memory acquisition. Since, co-administration of harmaline and 5-HT4 agents impaired memory acquisition; we proposed that these drugs have a synergistic effect on memory acquisition. However, further experiments are required to clarify the exact mechanisms involved, but it seems that harmaline induced its interaction effect via two mechanisms: 1-directly binding to 5-HT receptors[86] and 2-enhancement of extracellular concentration 5-HT levels via inhibition of MAO_Aenzyme in different brain region[51].

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