Is the NMDA Receptor of the CA1 Region Participated in the Amnesic Effect of Harmane?

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ABSTRACT

Aims: In the present study, we investigated the influence of NMDA receptor agonist (N-methyl-d-aspartate) and antagonist (D-AP7) on amnesia induced by a β-carboline alkaloid, harmame.

Methodology: Animals implanted with bilateral cannuae at the CA1 regions of the dorsal hippocampus and microinjected with glutamatergic drugs. One-trial step-down was used to assess memory acquisition and then, the hole-board method to assess exploratory behaviors in adult male NMRI mice. Results: The results revealed that pre-training intra-CA1 administration of NMDA (0.5 ng/mouse) and D-AP7 (0.25 and 0.5 ng/mouse) improved and impaired memory acquisition, respectively. Also, pre-training intra-peritoneal (i.p.) administration of harmame (12 mg/kg) decreased memory acquisition. Furthermore, pre-training intra-CA1 injection of sub-threshold dose of NMDA (0.02 ng/mouse) reversed, while non-significant dose of D-AP7 (0.125 ng/mouse) did not change impairment of memory acquisition induced by harmame (12 mg/kg, i.p.). Conclusion: In addition, all above doses of drugs did not alter locomotor activity. These results suggest that the CA1 NMDA receptors are involved in harmame-induced amnesia.

Keywords: NMDA, D-AP7, Harmame, CA1, Memory

INTRODUCTION

Harmane is a type of β-carboline alkaloids,¹ which naturally present in the human food chain,² and supposed to occur endogenously in normal body constituents.³ β-carboline alkaloids are found in the blood plasma, heart, kidney, liver and also in the brain tissue,⁴⁵ where they have been proposed to be endogenous ligands for benzodiazepine and imidazoline receptors.⁶⁷ It seems that the biological significance of β-carboline alkaloids comprised cytotoxic as well as neuroprotective properties. On the one hand, β-carboline alkaloids have been proposed to act as endogeneous neurotoxins because of their structural similarity to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP),⁸ a compound which induced a parkinsonian-like syndrome in animals.⁹ On the other hand, increasing evidence have been indicated a protective effect of endogeneous β-carboline alkaloids on oxidative neuronal damage.¹⁰ Moreover, β-carboline alkaloids have been shown to protect neurons against the excitotoxic effects of dopamine and glutamate.¹¹ These alkaloids may interact with glutamate receptors of the type N-methyl-D-aspartate (NMDA).¹² The glutamate as an excitatory neurotransmitter is predominantly ample in the mammalian brain.¹³¹⁴ The NMDA receptors are an important mediator of synaptic plasticity which involved in the neurobiological mechanisms of emotion, as well as learning and memory processes.¹⁵¹⁶ These receptors are found in high density in the brain.
Nevertheless, the maximum distributions of the NMDA receptors are presented in the hippocampal CA1 area. The CA1 region of the dorsal hippocampus mediated neural plasticity processes involved in acquisition, storage and retrieval of memory in the hippocampus. The main glutamatergic projection to the hippocampus is originated from the pyramidal neurons of the entorhinal cortex. The NMDA receptors of the hippocampal formation play a critical role in cognitive performance, specifically in learning and memory processes. Based on the interaction of β-carbolines with NMDA receptors and considering the role of NMDA receptor and hippocampus in memory process, in this investigation, the involvement of NMDA receptor agents on memory acquisition and exploratory behavior have been examined.

MATERIALS AND METHODS

2. Experimental procedure

2.1. Animals

Adult male albino NMRI mice (Institute of Cognitive Science, Tehran, Iran) weighing 25–30 g at time of surgery were used. They were housed in Plexiglas cage kept at 22±2°C under a 12 h light: 12 h dark cycle (lights on at 07:00) with water and food freely available. Behavioral tests were carried out during the light phase of the light-dark cycle. All procedures in the current investigation are in accordance with the guidelines for the Care and Use of Laboratory Animals as adopted by the Ethics Committee of Faculty of Science, Tehran University (357: November 2000).

2.2. Stereotaxic surgery

Subjects were anesthetized using intraperitoneal (i.p.) injection of a ketamine/xylazine mixture (50 mg/kg and 5 mg/kg, respectively) and then located in a stereotaxic frame (Stoelting Co, Illinois, USA) with flat-skull position. A midline incision was made in the skin of the skull, afterward periosteum was retracted and bilateral stainless steel guide cannulae (8 mm length, 22 gauge) were implanted until 1 mm above the CA1 area. Stereotaxic coordinates were –2 mm (depending on body weight) posterior to the bregma, ±1.6 mm lateral to the midline and ±1.5 mm ventral of the dorsal surface of the skull. The cannulae were secured to the skull with a jeweler’s screw and dental cement. After the surgery, two stainless steel stylets (27 gauge) were presented into the guide cannulae to maintain patency before to microinjections. All mice were permitted approximately 5-7 days to recuperate from surgery and the influence of anesthetic drugs.

2.3. Memory testing

Inhibitory avoidance apparatus compose of a woody box (30×30×40 cm³) with a floor which made of parallel stainless steel rods (0.3 cm in diameter, space 1 cm apart). A woody platform (4×4×4 cm³) was placed in the middle of the grid floor. Electric shocks (1 Hz, 0.5 s and 50 VDC) were provided to the grid floor by an isolated stimulator (Grass S44, Quincy, MA, USA). In order to testing, each subject was gently placed on the woody platform. Once mice stepped down from the platform and located all its paws on the grid floor, intermittent electric shocks were provided constantly for 15 s. This training technique was done among 8:00 a.m. and 12:00 p.m. Retention test session was done 24 h after training and was procedurally alike to training, except that no shock was presented. Step-down latency was recorded as memory retention. An upper cut-off time of 5 min was set. The retention test was also performed among 8:00 a.m. and 12:00 p.m.

2.4. Measurement of locomotor activity

Locomotor apparatus (Borj Sanat Co, Tehran, Iran) composed of clear perspex container box (30×30×40 cm³). The apparatus has a gray perspex section (30×30 cm², 2.2 cm thick) with 16 photocells which separated the box to 16 equal-sized squares. The locomotor activity was recorded as the quantity of crossings from one square to another in the course of 5 min.

2.5. Drugs

The following drugs were used in the experiments were ketamine, xylazine (For surgical procedure; Alfasan Chemical Co, Woerden, and Holland), N-methyl-d-aspartate (NMDA receptor agonist), dl-2-amino-7-phosphonoheptanoate (D-AP7, NMDA receptor antagonist; Tocris, Bristol, UK) and harmane (1-methyl-9H-pyridol [3,4-b] indole, C12H10N2) from Sigma (St. Louis, MO). The time of infusion and doses of the drugs used in the experiments were selected according to pilot and published work in the scientific literature. Harmane was dissolved in 0.9% physiological saline and the compound was moved for 1 h prior to obtaining the ultimate solution; other drugs were solved in sterile 0.9% NaCl solution, just previous the experiments.

2.6. Drug treatment

In order to drug injection, animals were controlled mildly by hand; the stylets were separated from the guide cannulae and substituted by 27-gauge infusion needles (1mm beneath the tip of the guide cannulae). The injection solution was manually infused over a 60 s period. The infusion needles were removed in place for an extra 60 s to expedite diffusion of the drugs. The protocol has been described in Table 1.

2.7. Statistical analysis

Since individual differences in the step-down apparatus data, we analyze data by Kruskal–Wallis nonparametric one-way analysis of variance (ANOVA) followed using a two-tailed Mann–Whitney’s U-test. Holmes Sequential Bonferroni Correction Test was done for paired assessments once appropriate. The median as well as interquartile ranges of the step-down latencies were recorded for 10 mice in every experimental group. One/two-way ANOVA followed by post-hoc test was performed for the statistical assessment in the locomotor activity. In all assessments p<0.05 was shown statistically significant.

2.8. Experimental design
Ten animals were used in every experimental group and every animal was tested once. Harmane administrated i.p. but NMDA and D-AP7 administrated intra-CA1. All infusions were done 5 min before training. In experiments which animals received two infusions, harmane was injected, followed by administration of NMDA or D-AP7. Interval time between two injections was 5 min.

### 2.8.1. Experiment 1

This experiment examined the influence of pre-training intra-CA1 injection of NMDA and D-AP7 on memory acquisition and locomotor activity. In this test, eight groups of subjects were used. The mice were divided into two sets of four groups, received pre-training injections of saline (1 µl/mouse) or different doses of NMDA (0.02, 0.1 and 0.5 ng/mouse) or D-AP7 at different doses (0.125, 0.25 and 0.5 ng/mouse) plus saline (1 µl/mouse). The exploratory behaviors of mice were recorded via locomotion apparatus, 5 min afterward memory testing.

### 2.8.2. Experiment 2

Experiment 2 evaluated the effects of pre-training intra-CA1 injection of NMDA receptor drugs on memory acquisition and locomotor activity under the amnesia induced by harmane. Twelve groups (three arms) of subjects were used. The animals received saline (1 µl/mouse) or diverse doses of harmane (4, 8 and 12 mg/kg; i.p.) 5 min previous training. These animals received intra-CA1 infusion of saline (1 µl/mouse, four groups), not effect dose of NMDA (0.02 ng/mouse, four groups) or D-AP7 (0.125 ng/mouse, four groups) 5 min earlier training. The exploratory activities of animals were measured through locomotion apparatus, 5 min afterwards memory testing.

### RESULTS

#### 3.1. Effects of NMDA receptor drugs on memory acquisition and locomotor activity

Fig. 1 shows the influence of pre-training infusion of NMDA and D-AP7 on the step-down latency and locomotor activity. Kruskal–Wallis ANOVA revealed that pre-training injection of NMDA (H(3)=8.195, P<0.05, Fig. 1A; left panel) and D-AP7 (H(3)=17.987, P<0.001, Fig. 1A; right panel) increased and decreased the step-down latency in the one-trial passive avoidance task, respectively. The post hoc analysis by Mann–Whitney's U-test indicated that NMDA (0.5 ng/mouse) and D-AP7 (0.25 and 0.5 ng/mouse) improved and impaired memory acquisition, respectively. Moreover, one-way ANOVA exhibited that both NMDA [F (3, 28) =0.094, P>0.05, Fig. 1B; left panel] and D-AP7 [F (3, 28) =0.089, P>0.05, Fig. 1B; right panel] did not change locomotor activity.

#### 3.2. Effects of NMDA receptor drugs on memory acquisition and locomotor activity under amnesia induced by harmane

The data of Fig. 2A, left panel indicated that i.p. infusion of harmane at a dose of 12 mg/kg impaired memory acquisition process [Kruskal–Wallis ANOVA analysis (H (3) =18.147, P<0.001) followed by Mann–Whitney’s U-test]. Moreover, one way ANOVA revealed that harmane had no effect on locomotor activity [F(3, 28)=0.101, P>0.05, Fig. 2B; left panel].
Furthermore, Kruskal–Wallis analysis revealed that a meaningless dose of NMDA (0.02 ng/mouse) weakened the amnesic effect caused by harmaline (12 mg/kg) [Kruskal–Wallis ANOVA, H(3)=7.875, P<0.05, Fig. 2A; middle panel]. Also, two-way ANOVA displayed that these interventions did not alter locomotor activity [F (7, 56)=0.269, P>0.05, Fig. 2B; middle panel]. In Fig. 2A, right panel the effects of D-AP7 on memory impairment induced by harmaline [Kruskal–Wallis ANOVA, H(3)=17.918, P>0.001] can be seen. Mann–Whitney’s U-test analysis indicated that a sub-threshold dose of D-AP7 (0.125 ng/mouse) had no significant effect on the amnesia induced by harmaline (12 mg/kg). Moreover, two-way ANOVA exhibited that these interventions did not modify locomotor activity [F (7, 56)=0.421, P>0.05, Fig. 2B, right panel].

**DISCUSSION**

Memory is not a process which categorized consistent with their neurobiological basis, content and time. For example, concerning duration or time, short term memory was detected to continue from seconds to hours. Short term memory was demonstrated to be labile (that is, sensitive to distraction). On the other hand, long term memory was identified to continue from days to weeks or years. Long term memory on being once consolidated or inactive become indifferent to disturbance. Furthermore, memory is thought to be a process that has numerous stages, containing acquisition, consolidation and retrieval.

Our data showed that pre-training intra-CA1 administration of NMDA improved, while D-AP7 impaired memory acquisition, but they did not affect locomotor activity. The NMDA receptors of the hippocampus involved in the control of synaptic plasticity e.g. LTP, short- and long-term memories, learning, spatial and non-spatial learning, formation of aversive memory, object recognition memory, working memory, and cognitive processes. Many forms of the hippocampal-dependent learning need the activation of NMDA receptors. Decrease in the NMDA receptors of the hippocampus are linked to deficits of LTP, learning and memory processes. Therefore, NMDA receptor antagonists may inhibit the hippocampal LTP and intensely disturb the hippocampal-dependent learning. It has been displayed that NMDA receptor antagonists impaired
memory through reduction in levels of nitric oxide and then cGMP production in the brain.\[^{[31]}\] In contrast, there are evidences exhibited that NMDA receptor antagonists can improve memory processes.\[^{[32]}\] However, other investigations offered that NMDA receptor antagonists, D-AP7 or MK-801 facilitated retention in a step-down inhibitory avoidance task, but weakened retention in step-through dark avoidance tasks and place navigation.\[^{[33]}\] Consequently, it has been proposed that the influence of NMDA receptor antagonist on learning and memory processes is dependent on the style of task.\[^{[34]}\] Additionally, our data exhibited that while pre-processes is dependent on the style of task, evidences exhibited that NMDA receptor antagonists can reveal the interaction between harmane and the CA1 NMDA receptors in modulation of the impairment of memory acquisition induced by harmane. Taken the recent data, it becomes obvious that further researches are required to clarify the exact interaction between harmane and the CA1 NMDA receptors in the memory processes.

CONCLUSION

The current investigation showed that pre-training intra-CA1 infusion of NMDA and D-AP7 increased and decreased memory acquisition, respectively but did not modify locomotor activity. Moreover, pre-training i.p. administration of harmane elicits amnesia but had no effect on locomotor activity. Furthermore, pre-training intra-CA1 injection of sub-threshold dose of NMDA reversed, but sub-threshold dose of D-AP7 did not change impairment of memory acquisition induced by harmane. Consequently, harmane and glutamate receptors interact with each other in the CA1 area. However, more experiments are required to clarify the exact interaction between harmane and glutamatergic system in the modulation of memory in the CA1 region.

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