Brain Research Bulletin 130 (2017) 101–106

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Possible involvement of the CA1 GABAergic system on harmaline induced memory consolidation deficit

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ARTICLE INFO

Article history:
Received 25 July 2016
Received in revised form
27 December 2016
Accepted 11 January 2017
Available online 19 January 2017

Keywords:
Harmaline
$GABA_B$ receptor
The CA1 region
Step-down
Memory

ABSTRACT

Activation of the $GABA_B$ receptors inhibit learning and memory processes. The current research was designed to examine the role of dorsal hippocampal (CA1) $GABA_B$ receptors on harmaline induced memory consolidation deficit in mice. For this purpose, the effects induced by the $GABA_B$ antagonist phaclofen and the $GABA_B$ agonist baclofen on memory consolidation were assessed by using the step-down inhibitory avoidance task. Furthermore, the possible involvement of harmaline on $GABA_B$ receptor's effects was also assessed through using the same behavioral procedure. In a first dose response experiments, post-training intra-CA1 injections of phaclofen did not change while baclofen (0.1 μg/mouse) impaired animals' performance in this task, suggesting a modulation of storage of information. Moreover, Post-training intra-peritoneal (i.p.) infusion of harmaline (2 and 5 mg/kg) also decreased memory consolidation. Interestingly, phaclofen at the sub-threshold dose (0.001 μg/mouse, intra-CA1), successfully antagonized the deficits on memory consolidation induced by the highest doses of harmaline (2 and 4 mg/kg, i.p.). On the other hand, non significant dose of baclofenc (0.001 μg/mouse, intra-CA1) potentiated impairment of memory consolidation induced by harmaline (2 mg/kg, i.p.). In addition in all experiments, locomotor activity did not alter significantly. These results indicate a) that the CA1 $GABA_B$ receptors are involved in memory consolidation b) that harmaline interact with the CA1 $GABA_B$ receptors in modulation of memory consolidation.

1. Introduction

γ-Aminobutyric acid (GABA), the most abundant inhibitory neurotransmitter in the brain, acts at different pharmacologically distinct receptor subtypes: the ionotropic $GABA_A$ and $GABA_C$ receptors (both of which activate Cl^- currents) and the metabotropic $GABA_B$ receptor (G-protein coupled receptor) (Couve et al., 2000; Li et al., 2004; Zarrindast et al., 2006). There are experimental studies that the $GABA_B$ receptor is participated in learning and memory processes, though its exact function is not yet explained (Mondadori et al., 1993; Pitsikas et al., 2003). It has been demonstrated that GABA plays a controlling role on the balance of excitability and inhibitory states in the cortex and hippocampus (Nazari-Serenjeh et al., 2011; Paulsen and Moser, 1998). Almost 10% of the CA1 region interneurons are related to the GABAergic systems (Buzsaki, 1997). It appears that the hippocampal output is partly controlled via $GABA_A$ or $GABA_B$ receptors (Ling and Benardo, 1994). There are studies showing that blockade of hippocampal $GABA_B$ receptor results in suppression of hippocampal long-term potentiation and spatial learning (Brucato et al., 1996).

Harmaline, a β-carboline alkaloid derived from the seeds of the plant Peganum harmala (Frostholm et al., 2000), is a monoamine oxidase inhibitor (Jimenez et al., 2008). Recently, harmaline has been revealed to lower voltage-gated calcium channel currents (Splettstoesser et al., 2005), resulting in decreased neuron excita-

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http://dx.doi.org/10.1016/j.brainresbull.2017.01.011
0361-9230/

tion. On the other hand, harmaline is known to affects non spatial and non aversive memory tasks, increasing predominantly long-term memory (LTM) (de Montigny and Lamarre, 1973). Numerous evidences have documented that harmaline has interaction with GABA-benzodiazepine receptors (Weiss et al., 1995). Moreover, there are reports showing that β-carboline alkaloids increase GABA responses in the spinal cord neurons (Skerritt and Macdonald, 1984), and cerebral cortex (Malatynska et al., 1992). In view of (1) the existence of interaction between GABA receptors and harmaline (Malatynska et al., 1992; Skerritt and Macdonald, 1984; Weiss et al., 1995), and (2) the role of hippocampus (Khakpai et al., 2012; Khakpai et al., 2013; Khakpai et al., 2016), harmaline (Nasehi et al., 2014; Nasehi et al., 2015; Nasehi et al., 2016), and GABA receptors (Nakagawa and Takashima, 1997; Shahidi et al., 2008; Yousefi et al., 2012) in learning and memory processes, the aim of the current research was to investigate the effects of harmaline and $GABA_B$ receptors on memory consolidation and locomotor activity in the step-down inhibitory avoidance and open field test. In the first set of experiments, the effect of the inhibition and stimulation of $GABA_B$ receptors in the CA1 area on memory consolidation was investigated. In the second set of experiments, we evaluated the effect of harmaline on inhibitory avoidance memory. In the third set of experiments, we examined whether the inactivation or activation of $GABA_B$ receptors of the CA1 area affect harmaline-induced memory consolidation deficit.

2. Materials and methods

2.1. Animals

Male, five-eight weeks old NMRI mice (University of Tehran, Tehran, Iran), weighing 25–30 g were used in this study. The subjects were housed in polypropylene cages, ten per cage, in a regulated environment (22 ± 2 °C; 50–55% relative humidity; 12-h light:dark cycle, lights on at 07:00 h), with free access to food and water. Experiments were carried out in the room where only these subjects were housed, and took place between 9:00 h and 12:00 h. Behavioral researches and assessments were done by experimenters who were unconscious of the pharmacological treatment.

Procedures including subjects and their care were conducted in according with the international guidelines in agreement with National and International laws and policies (the Ethics Committee of the Faculty of Science of the University of Tehran).

2.2. Cannula guide implantation

All surgical procedures were carried out under ketamine/xylazine (50 mg/kg and 5 mg/kg, respectively) anesthesia. Two stainless steel, 22-gauge guide cannulas were bilaterally implanted 1 mm above the intended site of injection according to the atlas of Paxinos and Franklin (Paxinos and Franklin, 2001). Stereotaxic coordinates for the CA1 area of the dorsal hippocampi were: anteroposterior (AP) = −2 mm from the bregma, mediolateral (ML) = ±1.6 from the sagittal suture and dorsoventral (DV) = −1.5 mm from the skull surface. Cannulas were secured to anchor jeweler's screws with dental acrylic cement. To avoid clogging, stainless steel stylets (27 gauge) were located in the guide cannulas until the subjects were given the CA1 injections. All subjects were allowed 5–7 days to recover from surgery and clear anesthetic.

2.3. Memory testing and apparatus

The inhibitory avoidance behavior was studied in one-trial learning, step-down type, which uses the natural preference of mice for a dark location. The inhibitory avoidance apparatus consisted of a dark compartment ($20 \times 20 \times 15\ cm^3$) with a stainless steel grid floor and a descending door opened in its front center communicating with a small illuminated platform ($5 \times 5\ cm^2$) attached to it and elevated 1 m from the floor. The subjects were not exposed to the inhibitory avoidance apparatus before the learning trial. During training session, each mouse was quietly placed in the illuminated platform. As it stepped all paws into the dark compartment received a footshock of 1.2 mA, 50 Hz, 1 s, which produces median retention scores at the ceiling. The retention test session was done 24 h after training. Therefore, each mouse was located on the platform again, and the step-down latency was measured. When the mouse stepped into the dark compartment or failed to cross in five min, the retention test was finished. In the second case the mouse was instantly removed from the platform and assigned a score of five min. The retention test session was performed with no footshock.

2.4. Measurement of locomotor activity

Locomotor activity was measured using an locomotion apparatus (Borj Sanat Co, Tehran, Iran) that consists of clear perspex container box ($30 \times 30 \times 40\ cm^3$) with wire meshed floor equipped with two arrays of 16 infrared photocells. The number of movement from one photocell to another was measured as the locomotor activity within five min period.

2.5. Drugs

The drugs used in the present study were harmaline (1-methyl-7-methoxy-3,4-dihydro-b-carboline) from Sigma (St. Louis, MO), phaclofen (Sigma, St. Louis, CA, USA) and baclofen (Tocris, UK). The time of injection and doses of the drugs was selected based on our previous studies (Khanegheini et al., 2015; Nasehi et al., 2014; Nasehi et al., 2015; Nasehi et al., 2016). The compounds were tested at doses: harmaline: 1, 2 and 4 mg/kg, phaclofen: 0.001, 0.01, and 0.1 μg/mouse, baclofen: 0.001, 0.01, and 0.1 μg/mouse. The injected doses of the drugs were diverse due to diverse site of administration. The utilized doses of harmaline were given in mg/kg because this drug injected i.p. On the other hand the utilized doses of GABAergic agents (phaclofen and baclofen) were given in μg/mouse because these drugs injected intra-CA1. Harmaline was dissolved in 0.9% physiological saline and the compound was stirred for 1 h previous obtaining the final solution; phaclofen and baclofen were dissolved in 0.9% physiological saline, just previous the experiments.

2.6. Drug treatment

For drug injection (immediately after training), the mice were gently restrained by hand; the stylets were removed from the guide cannulas and substituted by 27-gauge administration needles (1 mm below the tip of the guide cannulas). Each administation unit was connected via polyethylene tubing to 1 μl Hamilton syringe. The left and right CA1 areas were injected with a 0.5 μl solution on each side (1 μl/mouse) over a 60 s period. The infusion needles were left in place an extra 60 s to allow diffusion and then the stylets were reinserted within the guide cannulas. The procedure has been explained in Table 1.

2.7. Statistical analysis

The data of step-down apparatus show individual variations, hence we analyze data by Kruskal–Wallis nonparametric one-way analysis of variance (ANOVA) followed via a two-tailed Mann–Whitney's *U* test. Holmes Sequential Bonferroni Correction