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Nitric oxide in the dorsal hippocampal area is involved on muscimol state-dependent memory in the step-down passive avoidance test

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ABSTRACT

In the present study, the effects of intra-dorsal hippocampal (intra-CA1) injections of nitric oxide (NO) agents on muscimol state-dependent memory were examined in mice. A single-trial step-down passive avoidance task was used for the assessment of memory retrieval in adult male NMRI mice.

Post-training intra-CA1 administration of a $GABA_A$ receptor agonist, muscimol (0.05 and 0.1 μg/mouse) dose dependently induced impairment of memory retention. Pre-test injection of muscimol (0.05 and 0.1 μg/mouse) induced state-dependent retrieval of the memory acquired under post-training muscimol (0.1 μg/mouse, intra-CA1) influence.

Pre-test injection of a NO precursor, L-arginine (1 and 2 μg/mouse, intra-CA1) improved memory retention, although the low dose of the drug (0.5 μg/mouse) did not affect memory retention.

Pre-test injection of an inhibitor of NO-synthase, L-NAME (0.5 and 1 μg/mouse, intra-CA1) impaired memory retention, although the low dose of the drug (0.25 μg/mouse) did not affect memory retention.

In other series of experiments, pre-test intra-CA1 injection of L-arginine (0.25 and 0.5 μg/mouse) 5 min before the administration of muscimol (0.1 μg/mouse, intra-CA1) dose dependently inhibited muscimol state-dependent memory. Pre-test intra-CA1 administration of L-arginine (0.125, 0.25 and 0.5 μg/mouse) by itself cannot affect memory retention.

Pre-test intra-CA1 injection of L-NAME (0.25 μg/mouse, intra-CA1) reversed the memory impairment induced by post-training administration of muscimol (0.1 μg/mouse, intra-CA1). Moreover, pre-test administration of L-NAME (0.125 and 0.25 μg/mouse, intra-CA1) with an ineffective dose of muscimol (0.025 μg/mouse, intra-CA1) significantly restored the retrieval and induced muscimol state-dependent memory. Pre-test intra-CA1 administration of L-NAME (0.0625, 0.125 and 0.25 μg/mouse) by itself cannot affect memory retention.

It may be suggested that the nitric oxide in the dorsal hippocampal area play an important role in muscimol state-dependent memory.

1. Introduction

Nitric oxide (NO) is an intercellular retrograde messenger in the central nervous systems which has been shown to involve in certain forms of long-term potentiation (LTP), expression and synaptic plasticity (Prast and Philippu, 2001; Domek-Łopacińska and Strosznajder, 2005; Koylu et al., 2005; Edwards and Rickard, 2007; Kleppisch and Feil, 2009; Cserép et al., 2011).

In the brain, NO is generated by a series of isoenzymes of the family of NO-synthases (NOS's) from L-arginine in low nanomolar concentrations under physiological conditions (Alderton et al., 2001; Burette et al., 2002; Stuehr et al., 2004; Szabadits et al., 2007; Garthwaite, 2008; Contestabile, 2008).

Neuronal NOS (nNOS) and endothelial (eNOS) are widely distributed in the brain, prominently in the cerebellum and CA1 region of the hippocampus, which is critical in memory formation (Rodrigo et al., 1994; Pepicelli et al., 2004; Ledo et al., 2004; Feil and Kleppisch, 2008; Harooni et al., 2009).

Experiments investigating the role of hippocampal NO in memory processes have shown that intra-hippocampal injections of NO donors induce LTP in CA1 region and consequently improve memory (Arancio et al., 1996; Huang, 1997; Prast and Philippu, 2001; Majlessi et al., 2008), while NOS inhibitors eliminate or partially block hippocampal LTP and consequently impair memory formation in various behavioral tasks (Blokland et al., 1998; Kopf et al., 2001; Prast and Philippu, 2001; Bon and Garthwaite, 2003; Khavandgar et al., 2003; Yildirim and Marangoz, 2004), whereby NO may have modulatory effects on learning and memory processes.

γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system (Castellano et al., 1996) which plays a controlling role on the balance of excitability and inhibitory states in the cortex, hippocampus and the interneurons, and is involved in memory formation in the hippocampus (Paulsen and Moser, 1998).

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GABA exerts its action by binding to specific membrane receptors that are divided into three major subclasses: $GABA_A$, $GABA_B$ and $GABA_C$ (Bormann, 2000; Semyanov and Kullmann, 2002; Emson, 2007; Olsen and Sieghart, 2009).

It is well documented that activation of $GABA_A$ receptors in the hippocampus impairs memory in inhibitory avoidance tasks (Farr et al., 2000; Amaral et al., 2007; Reis et al., 2009; Jafari-Sabet and Jannat-Dastjerdi, 2009; Makkar et al., 2010; Jafari-Sabet, 2011; Jafari-Sabet et al., 2013).

Our previous studies have shown that pre-training intra-dorsal hippocampal (intra-CA1) administration of the $GABA_A$ receptor agonist, muscimol induced memory impairment which was restored when the same dose of the drug was administered 24 h later in a pre-test session in the step-down passive avoidance task (Jafari-Sabet and Jannat-Dastjerdi, 2009; Jafari-Sabet, 2011; Jafari-Sabet et al., 2013). This phenomenon has been named state-dependent learning (Izquierdo, 1980; Jafari-Sabet et al., 2005; Zarrindast et al., 2006).

These state-dependent effects are time- and dose-dependent and may be prevented or enhanced by activation of the dorsal hippocampal systems (Jafari-Sabet et al., 2005; Rezayof et al., 2008; Jafari-Sabet and Jannat-Dastjerdi, 2009; Jafari-Sabet, 2011; Jafari-Sabet et al., 2013).

Interaction between GABAergic and the NO systems have been reported in some behavioral studies (Segovia et al., 1994; Getting et al., 1996; Casamenti et al., 1999; Prast and Philippu, 2001).

Furthermore, there is evidence that the release of GABA in hippocampus (Segovia et al., 1994), striatum (Guevara-Guzman et al., 1994; Segovia and Mora, 1998), and basal forebrain (Casamenti et al., 1999) is modulated by NO.

Also there are reports indicating that the release of GABA is biphasically (i.e., both excitatory and inhibitory), dependent on the NO concentration.

Ample evidence indicates that the CA1 region of the hippocampus is essential for memory formation of one-trial avoidance and is necessary to mediate the expression of place learning (Riedel et al., 1999; Compton, 2004; Izquierdo et al., 2006; Jafari-Sabet, 2006a, 2011; Jafari-Sabet and Jannat-Dastjerdi, 2009; Jafari-Sabet et al., 2013).

One-trial avoidance has been widely used for the study of memory formation and corresponds to many important examples of learning in humans (Izquierdo et al., 2008).

One-trial step-down inhibitory (passive) avoidance in rodents has long been a favorite model for biochemical and pharmacological studies of memory (Izquierdo and Medina, 1997; McGaugh, 2000; Izquierdo and McGaugh, 2000; Szapiro et al., 2002) and induces LTP in CA1 region of the hippocampus (Whitlock et al., 2006).

However, memory formation requires not only LTP in the hippocampal CA1 region but also different sequences of the same or of related signaling pathways in other cortical regions and the amygdala (McGaugh et al., 1990; Izquierdo et al., 2006; Jafari-Sabet, 2006b).

Considering that nNOS is present in hippocampal GABAergic synapses and also NO can directly modify GABAergic synapses on hippocampal pyramidal cells in rodents (Szabadits et al., 2007; Zanelli et al., 2009), the main aim of the present study was to investigate the effects of intra-dorsal hippocampal (intra-CA1) microinjections of L-arginine (a NO precursor) and L-NAME (a non-specific inhibitor of nitric oxide synthase) on muscimol induced state-dependent memory retrieval in a passive avoidance task in mice.

2. Materials and methods

2.1. Animals

Male albino NMRI mice, weighing 24–30 g at the time of the surgery were used. The animals were kept in an animal house with a 12-h light/12-h dark cycle and controlled temperature (22 ± 2 °C). Food and water were available ad libitum. Animals were housed in groups of 10 in Plexiglas animal cages. Each animal was used once only. Ten animals were used in each group. Training and testing were done during the light phase of the cycle. All procedures were carried out in accordance with institutional guidelines for animal care and use. Behavioral tests and animal care were conducted in accordance with the standard ethical guidelines (NIH, publication no. 85–23, revised 1985; European Communities Directive 86/609/EEC) and approved by the local ethical committee.

2.2. Surgical and infusion procedures

Mice were anesthetized with intra-peritoneal injection of ketamine hydrochloride (50 mg/kg) plus xylazine (5 mg/kg) and placed in a stereotaxic apparatus. The skin was incised and the skull was cleaned. Two 27-gauge guide cannulae were placed (bilaterally) 1 mm above the intended site of injection according to the atlas of Paxinos and Franklin (2001). Stereotaxic coordinates for the CA1 regions of the dorsal hippocampi were AP: −2 mm from bregma, L: ±1.6 from the sagittal suture and V: −1.5 mm from the skull surface. The cannulae were secured to anchor jewelers' screws with dental acrylic. Stainless steel stylets (30-gauge) were inserted into the guide cannulae to keep them free of debris. All animals were allowed 1 week to recover from surgery and clear anesthetic.

For drug infusion, the animals were gently restrained by hand; the stylets were removed from the guide cannulae and replaced by 30-gauge injection needles (1 mm below the tip of the guide cannulae). The injector cannula was attached to a polyethylene tube fitted to a 2-μl Hamilton syringe. The injection solutions were administered in a total volume of 1 μl/mouse (0.5 μl in each side, intra-CA1) over a 60 s period. Injection needles were left in place for an additional 60 s to facilitate the diffusion of the drugs.

2.3. Passive avoidance apparatus

Animals were submitted to the behavioral procedure 7 days after surgery. The apparatus was a (30 cm × 30 cm × 40 cm high) wooden box; the floor of which consisted of parallel stainless steel bars (0.3 cm diameter spaced 1 cm apart). A wooden platform (4 cm × 4 cm × 4 cm) was placed on the center of the grid floor. In the training session the animals were placed on the platform and their latency to step down on the grid with all four paws was measured. Immediately after stepping down on the grid, animals received electric shocks (1 Hz, 0.5 s, 45 V DC) continuously for 15 s. The shocks were delivered to the grid floor by an isolated (Harvard Stimulator 6002, England) stimulator. If any animal stayed on the platform more than 20 s or stepped up to the platform before the end of 15 s of electric shocks, it was omitted from the experiments. Retention test session was carried out 24 h after training and was procedurally identical to training, except that no shock was delivered to the animals. Step-down latency was used as a measure of memory retention. An upper cut-off time of 300 s was set (Jafari-Sabet and Jannat-Dastjerdi, 2009; Jafari-Sabet, 2011). The retention test was carried out between 8:00 a.m. and 3:00 p.m.

2.4. Drugs

The drugs used in the present study were muscimol (a $GABA_A$ receptor agonist), L-arginine (a nitric oxide precursor) and L-NAME (N^G-nitro-L-arginine methyl ester hydrochloride, a non-specific inhibitor of nitric oxide synthase) were purchased from Tocris (Cookson Ltd, UK). All drugs were dissolved in sterile 0.9% saline just before the experiments and were injected into the dorsal hippocampal CA1 regions (intra-CA1) 1 μl/mouse (0.5 μl per each side). Control animals received sterile 0.9% saline. The doses of muscimol were those used in our previous studies (Jafari-Sabet and Jannat-Dastjerdi, 2009; Jafari-Sabet, 2011; Jafari-Sabet et al., 2013).