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Research report

Dorsal hippocampal cannabinergic and GABAergic systems modulate memory consolidation in passive avoidance task

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ABSTRACT

In the present research, we examined the possible interaction between the hippocampal CB2 and $GABA_A$ receptors on modulation of memory consolidation. In this research, step-down passive a .

voidance task was used to evaluate memory consolidation in adult male NMRI mice. The results showed that post-training intra-CA1 administration of muscimol (0.05 and 0.1 μg/mouse) impaired memory consolidation as evidenced by a decrease in step-down latency on the test day, which was suggestive of drug-produced amnesia. However co-administration of different doses of muscimol (0.01, 0.05 and 0.1 μg/mouse) along with a not-effect dose of AM630 (1 μg/mouse) or GP1a (1 μg/mouse) had no significant effect on impairment of memory consolidation induced by muscimol. Although post-training intra-CA1 administration of the cannabinoid CB2 receptor antagonist, AM630 (1, 10 and 100 μg/mouse) alone had no effect, its co-administration with an effective dose of muscimol (0.05 μg/mouse) impaired memory consolidation. Furthermore, post-training intra-CA1 microinjection of cannabinoid CB2 receptor agonist, GP1a (100 μg/mouse) impaired memory consolidation. Interestingly, post-training intra-CA1 co-injection of different doses of GP1a (1, 10 and 100 μg/mouse) along with an effect dose of muscimol (0.05 μg/mouse) significantly intensified impairment of memory consolidation induced by GP1a (10 μg/mouse). Moreover, all above doses of drugs did not significantly change locomotor activity. These findings suggest possible interaction between the CA1 cannabinoid CB2 and $GABA_A$ mechanisms on modulation of memory consolidation in mice.

1. Introduction

The CA1 areas of dorsal hippocampus participate in neural plasticity processes such as acquisition, consolidation and retrieval of memory (Okada and Okaichi, 2010; Riedel and Micheau, 2001). The hippocampal neurons are innervated by GABAergic, cholinergic, glutamatergic and serotonergic neurons, and release of these neurotransmitters play a modulatory role in hippocampal-dependent memory (Khakpai et al., 2012; Khakpai et al., 2013). GABA, γ-aminobutyric acid, is one of the most abundant inhibitory neurotransmitter in the CNS (Chandra et al., 2010; Jafari-Sabet and Jannat-Dastjerdi, 2009). This neurotransmitter elicit its function through binding to three pharmacological receptor subtypes: the ionotropic $GABA_A$ and $GABA_C$ receptors (both activate Cl^- currents) and the metabotropic $GABA_B$ receptor (G-protein coupled receptor) (Bormann, 2000; Couve et al., 2000; Li et al., 2004). In the cortex and hippocampus, GABA plays a regulatory role on the balance of excitability and inhibitory states (Paulsen and Moser, 1998). There are many GABAergic interneurons in the hippocampus and these interneurons project from the septum (Drake and Milner, 1999; Pascual et al., 2004). It has been shown that the hippocampal output is partly regulated by the $GABA_A$ or $GABA_B$ receptors (Ling and Benardo, 1994), and these receptors are involved in the hippocampal-dependent learning and memory by mediating information processing (Krebs-Kraft et al., 2007; Luft et al., 2004; Makkar et al., 2010).

The endocannabinoid system consists of endocannabinoids and cannabinoid receptors. This system modulates cognitive processes and emotional behaviors (Campolongo et al., 2009; Lichtman et al., 2002; Pacher et al., 2006). Studies have revealed that two cannabinoid receptors, namely CB1 and CB2, are participating in cannabinoids' functions (Mackie and Stella, 2006; Ryberg et al., 2007). Both CB1 and CB2 receptors belong to the G-protein-coupled receptor superfamily (Howlett, 2002; Howlett, 2005). The CB1 receptors are predominantly

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present in the brain but the CB2 receptors are principally present in the periphery (Schatz et al., 1997; Tsou et al., 1998). Nonetheless, recent evidence showed that the CB2 receptors are much more broadly found in the brain than originally thought (Bisogno et al., 2016). The cannabinoid CB2 receptors express in the hippocampus of mouse (Li and Kim, 2015), and play a role in learning and memory processes as well as the molecular mechanisms of neurodegeneration in Alzheimer's disease (Bedse et al., 2015; Koppel et al., 2014). Moreover, endocannabinoids can decrease the release of dorsal hippocampal GABA and glutamate neurotransmitters through presynaptic mechanism(s) (Hajos and Freund, 2002; Hoffman and Lupica, 2000; Shen et al., 1996), which can cause variations in the hippocampal neural networks (Hajos et al., 2000). Activation of hippocampal CB1 receptor inhibits local GABA release by acting as retrograde messengers (de Oliveira Alvares et al., 2006). Recent evidence suggests the similarity in CB1 and CB2 receptor signaling mechanisms (Khella et al., 2014), so we propose that the hippocampal CB2 receptor may inhibits GABA release, too. Considering that the hippocampus (Farr et al., 2000; Morgado-Bernal, 2011), cannabinoid (De Oliveira Alvares et al., 2008; Svizenska et al., 2008), and GABA (Collinson et al., 2002; Shahidi et al., 2008) receptors are well-known to mediate synaptic plasticity which is essential for learning and memory processes, the current study aimed to examine a possible interaction between cannabinoid and GABA mechanisms of the CA1 on memory consolidation and locomotion in the step-down inhibitory avoidance task and open field test in mice.

2. Materials and methods

2.1. Animals

Adult male NMRI mice (University of Tehran, Tehran, Iran) weighing 25–30 g (5–8 weeks old animals) at the time of surgery were used. They were housed ten per each cage, and kept at 22 ± 2 °C under a 12-h light: 12-h dark cycle (lights on at 07:00 a.m.) with free access to food and water. All experiments were done during the light phase of the cycle among 8:00 a.m. and 12:00 p.m. All animal procedures reported in this research, were conducted in agreement with the guidelines laid down by the NIH (NIH Guide for the Care and Use of Laboratory Animals) in the USA. The Research and Ethics Committee of the Faculty of Science of the University of Tehran confirmed the experimental procedure.

2.2. Surgery

Subjects were anaesthetized by intra-peritoneal administration of a ketamine/xylazine mixture (50 mg/kg and 5 mg/kg, respectively) and located in a stereotaxic frame (Stoelting Co., Wood Dale, Illinois, USA) with flat-skull location. A midline incision was create in the skin of skull, then underlying periosteum was retracted and bilateral stainless steel guide cannulae (22 gauge, 0.7 mm diameter) were placed until 1 mm above the intended site of injection according with the atlas of Paxinos and Franklin. Stereotaxic coordinates for the CA1 areas 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 (Paxinos and Franklin, 2001). Cannulae were anchored to the skull using a jeweler's screw and dental cement. Afterward the surgery, two stainless steel stylets (27 gauge) were located into the guide cannulae to maintain patency previous to microinjections. All mice were allowed a week to recover from surgery and clear anesthetic agents.

2.3. Drugs and microinjections

The drugs used in the current research were ketamine and xylazine (Alfasan Chemical Co, Woerden, Holland) for mice anesthesia. Other drugs which were supplied by Tocris, Bristol, UK were: muscimol was used as $GABA_A$ receptor agonist and dissolved in physiological saline. GP1a [N-(piperidin-1-yl)-1-(2,4-dichlorophenyl)-1,4-dihydro-6-methylindeno [1,2-c] pyrazole-3-carboxamide] and AM630 [6-Iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H–indol-3-yl](4-methoxyphenyl)methanone] as CB2 receptor agonist and antagonist were used and dissolved in 20 or 10% dimethyl sulphoxide (DMSO), respectively. This solvent has been used previous as vehicle for cannabinoids with no significant effects by itself on behavior (Nasehi et al., 2017d). In the current research all drugs were injected just after training, consequently drug effects can be recognized to influences on memory consolidation, a process that takes place instantly after the training experience and lasts for some time (Abel and Lattal, 2001). Though there is a paucity of data on the half-lives of used drugs in mice, some works suggest that the behavioral half-lives of these drugs. Disorbo et al. reported that muscimol has relatively short half-life (administration time-point 30 min before test) (Disorbo et al., 2009). Soethoudt et al. indicated that AM630 and Gp-1a have moderately short half-life (injection time-point 30 min before test) (Soethoudt et al., 2017). Consequently, all drugs could affect consolidation of memory.

The drugs were tested at doses: muscimol, 0.01, 0.05 and 0.1 μg/mouse; AM630, 1, 10 and 100 μg/mouse; and Gp1a, 1, 10 and 100 μg/mouse. The anesthetic drugs were injected intra-peritoneally (i.p.) and the other drugs were administered into the CA1 areas (intra-CA1). Bilateral microinjections of drugs into the CA1 were in a volume of 1 μl/mouse (0.5 μl/each side) and were performed using lowering a 27-gauge injection cannula. The injection cannula was 1 mm longer than the guide cannulae to extend 1 mm beyond the tip of the guide cannulae into the CA1 areas. The injection cannula was attached with a polyethylene tube to a 1 μl Hamilton syringe. Intra-CA1 injections of drugs were done over 60 s first in one side then the other one. The injection cannula was left in the place for an extra 60 s to facilitate diffusion of the drugs from the tip of injection cannula. The time of injection and doses of the drugs used in the experiments were chosen based on pilot and published work in the scientific literature (Nasehi et al., 2016; Nasehi et al., 2017b). The protocol has been explained in Table 1.

2.4. Memory testing and apparatus

The inhibitory avoidance apparatus made of a wooden box (30 cm × 30 cm × 40 cm) with a floor which made of parallel caliber stainless steel bars (0.3 cm in diameter, spaced 1 cm apart). A wooden

Table 1
The table clarifies experimental design.

Experiment	Figure	Post-training treatment (intra-CA1)	Step-down latency (panel A)	locomotor activity (panel B)
1	Left	Muscimol (Saline, 0.01, 0.05 and 0.1 μg/mouse)	Decrease	No effect
	Middle	Muscimol (Saline, 0.01, 0.05 and 0.1 μg/mouse) + AM630 (1 μg/mouse)	Decrease	No effect
	Right	Muscimol (Saline, 0.01, 0.05 and 0.1 μg/mouse) + GP1a (1 μg/mouse)	Decrease	No effect
2	Left	AM630 (1, 10 and 100 μg/mouse)	No effect	No effect
	Right	AM630 (1, 10 and 100 μg/mouse) + Muscimol (0.05 μg/mouse)	Decrease	No effect
3	Left	GP1a (1, 10 and 100 μg/mouse)	Decrease	No effect
	Right	GP1a (1, 10 and 100 μg/mouse) + Muscimol (0.05 μg/mouse)	Decrease	No effect