Neuroscience 175 (2011) 118–126

INVOLVEMENT OF DOPAMINERGIC AND GLUTAMATERGIC SYSTEMS OF THE BASOLATERAL AMYGDALA IN AMNESIA INDUCED BY THE STIMULATION OF DORSAL HIPPOCAMPAL CANNABINOID RECEPTORS

A. REZAYOF,[a]* P. HABIBI[a] AND M.-R. ZARRINDAST[b,c,d,e]

[a]*Department of Animal Biology, School of Biology, College of Science, University of Tehran, Tehran, Iran*

[b]*Department of Neuroscience, School of Advanced Medical Technologies and Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

[c]*Iranian National Center for Addiction Studies, Tehran University of Medical Sciences, Tehran, Iran*

[d]*School of Cognitive Sciences, Institute for Research in Fundamental Sciences (IPM), Tehran, Iran*

[e]*Institute for Cognitive Science Studies, Tehran, Iran*

Abstract—The present study intended to investigate the involvement of dopaminergic and glutamatergic systems of the basolateral amygdala in amnesia induced by the stimulation of dorsal hippocampal cannabinoid receptors in male Wistar rats. The animals were stereotaxically implanted with guide cannulas in the CA1 region of the dorsal hippocampus and basolateral amygdala (BLA), trained in a step-through type passive avoidance task, and tested 24 h after training to measure memory retrieval. Post-training intra-CA1 microinjection of the nonselective CB1/CB2 receptor agonist WIN55,212-2 (WIN) (0.1–0.5 μg/rat) dose-dependently induced amnesia. Post-training intra-BLA administration of the D1/D2 dopamine receptor agonist apomorphine (0.3 and 0.5 μg/rat) plus intra-CA1 administration of 0.1 μg/rat of WIN, which alone did not induce amnesia, inhibited memory formation. The inhibitory effect of 0.5 μg/rat of WIN (intra-CA1) on memory formation was significantly decreased by the D1 dopamine receptor antagonist SCH23390 (0.1–0.5 μg/rat, intra-BLA) or the D2 dopamine receptor antagonist sulpiride (0.02–0.5 μg/rat, intra-BLA) given 5 min before post-training intra-CA1 microinjection of WIN. It is important to note that single intra-BLA microinjection of the same doses of apomorphine, SCH23390 or sulpiride had no effect on memory retrieval in passive avoidance task. On the other hand, post-training co-administration of N-methyl-D-aspartate (NMDA; 0.03 and 0.05 μg/rat, intra-BLA) plus an ineffective dose of WIN (0.1 μg/rat, intra-CA1) induced amnesia. Furthermore, the inhibitory effect of 0.5 μg/rat of intra-CA1 microinjection of WIN on memory formation was significantly decreased by pre-treatment with intra-BLA microinjection of the NMDA receptor antagonist D-2-amino-5-phosphonopentanoic acid (D-AP5; 0.1 and 0.5 μg/rat, intra-BLA). Intra-BLA microinjection of the same doses of NMDA or D-AP5 by itself did not induce any response on memory retrieval. Taken together, these findings support the existence of a functional interaction between dorsal hippocampal and basolateral amygdaloid neural circuits during processing cannabinoid-induced amnesia.

Key words: dorsal hippocampus, basolateral amygdala, dopaminergic system, glutamatergic system, passive avoidance learning, rat(s).

It is well recognized that abuse of drugs such as morphine (Zhang et al., 1996), nicotine (Placzek et al., 2009), alcohol (Matthews and Silvers, 2004) and cannabis (Riedel and Davies, 2005) can affect the functions of the hippocampus including cognition, learning and memory. A large body of evidence suggests that cannabinoid system of dorsal hippocampus via its receptors participates in learning and memory processes (Riedel and Davies, 2005). Dorsal hippocampus expresses at least two types of cannabinoid receptors including CB1 and CB2 receptors (Brusco et al., 2008). Both CB1 and CB2 receptors which are coupled to Gi-Go heterotrimeric G proteins, induce the inhibition of adenylate cyclase, influence ion channels and activate mitogen-activated protein (MAP) kinase (Turu and Hunyady, 2010; Pertwee and Ross, 2002; Mackie, 2008). These receptors may be involved both independently and/or cooperatively in important physiological activities of the hippocampus (Brusco et al., 2008). It has been reported that hippocampal administration of cannabinoid receptor agonists impairs memory formation and retrieval (Zarrindast et al., 2010; Clarke et al., 2008; Nasehi et al., 2009). Furthermore, hippocampal long-term potentiation (LTP) has been shown to be blocked by the administration of cannabinoid receptor agonists (Fan et al., 2010; Paton et al., 1998; Abush and Akirav, 2010) and enhanced in CB1 knock-out mice (Mascia et al., 1999).

The hippocampus and the amygdaloid complex especially the basolateral amygdala (BLA) are critically correlated in mediating learning and memory processes (Richter-Levin and Akirav, 2000; Alvarez and Ruarte, 2002, 2004). This was shown by lesions of the amygdaloid nuclei or injections of drugs into the amygdaloid nuclei that impair or enhance hippocampal-dependent learning (Ikegaya et al., 1994; McGaugh et al., 1996; Vazdarjanova and McGaugh, 1999; Abe, 2001). There seems to be a consensus that the BLA facilitates memory consolidation by emotional

*Corresponding author. Tel: +9821-61112483; fax: +9821-66405141.
E-mail address: rezayof@khayam.ut.ac.ir (A. Rezayof).

Abbreviations: ANOVA, analysis of variance; BLA, basolateral amygdala; CA1, CA1 region of dorsal hippocampus; D-AP5, D-2-amino-5-phosphonopentanoic acid; LTP, long-term potentiation; NMDA, N-methyl-D-aspartate; SCH23390, R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-3,4,5-tetrahydro-1H-3-benzazepine hydrochloride; SEM, standard error of mean; WIN, WIN55,212-2.

0306-4522/11 $ - see front matter © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2010.12.006

arousal (Pelletier et al., 2005; Paz et al., 2006). The BLA can influence synaptic plasticity, which is necessary for consolidation and modulation of memories in other brain regions such as hippocampal formation (Johnson et al., 1996). For instance, the BLA lesions block the induction of LTP in the dentate gyrus (Ikegaya et al., 1994, 1995), while stimulating the BLA modulates hippocampal LTP (Frey et al., 2001). Since the BLA is involved in hippocampal LTP, which appears to underlie some types of hippocampus-dependent learning (Pastalkova et al., 2006; Minichiello, 2009), the present study intended to test whether the activation of dopaminergic or glutamatergic system in the BLA is necessary for the amnesia which is induced by intra-CA1 microinjection of a non-selective CB1/CB2 receptor agonist WIN 55,212-2 (WIN). A step-through type passive avoidance task was used for measuring memory retrieval in the animals which were stereotaxically implanted with guide cannulas in the CA1 region of the dorsal hippocampus and the BLA. Since both the hippocampus (Cimadevilla et al., 2007) and the amygdala (Jellestad and Bakke, 1985; Izquierdo et al., 1999) play important roles in memory formation of the passive avoidance learning task which is an approved model to explore long term memory in a simple conditioning task, the experiment examined if there is any functional connection between hippocampus and BLA in cannabinoid receptor activation-induced amnesia.

EXPERIMENTAL PROCEDURES

Subjects

Adult male Wistar rats (Pasteur Institute; Tehran, Iran), weighing 200–240 g at the time of the surgery were used as subjects. Animals were housed four per standard rat cage, in a room with a 12/12 h light:dark cycle (lights on 7:00 h) and controlled temperature (22±1 °C). Commercial rodent pellets and tap water were available *ad libitum*. They were allowed to adapt to the laboratory conditions for at least 1 week before surgery. All experiments were performed between 9:00 h and 13:00 h. Each rat was tested only once. There were eight rats per group in each experiment. The procedures were performed in accordance with institutional guidelines for animal care and use. The Research and Ethics Committee of the School of Biology, University of Tehran approved the experimental protocol.

Surgery and microinjection

Under deep anesthesia (50 mg/kg of ketamine and 5 mg/kg of xylazine), animals were placed in a stereotaxic frame. The animals were unilaterally implanted with 22-gauge guide steel cannulas into the CA1 region of dorsal hippocampus, and simultaneously into the basolateral nucleus of the amygdaloid complex (BLA) according to the atlas of Paxinos and Watson (2007). Stereotaxic coordinates for the CA1 region of the dorsal hippocampus were: AP: −3.3; ML: 2; DV: −2.8 and for the BLA were AP: −2.8; ML: 5; DV: −8.5. Cannulas were secured to anchor jewelers' screws with dental acrylic. Stainless steel stylets (27 gauge) were placed in the guide cannulas in order to prevent clogging until each animal was given the CA1 and/or BLA injections. All animals were allowed a 7 day recovery period from surgery and to clear the anesthetic. During the recovery period rats were handled about 5 min each day prior to the behavioural testing.

For drug injection, the stylets were gently removed from the guide cannulas and replaced by 27-gauge injection needles. Considering that the guide cannulas were implanted 1 mm above the CA1 and BLA, the injection needles were 1 mm longer than those. Each injection unit was connected by polyethylene tubing to 2 μl Hamilton syringe. The CA1 was injected with a 0.5 μl solution for over a 60 s period and the BLA was injected with a 0.3 μl solution for over a 60 s period. In order to prevent backflow through the needle track, the injection needles were left in place for an additional 60 s to allow for diffusion. The stylets were subsequently reinserted into the guide cannulas.

Drugs

The drugs used in the present study were WIN mesylate (Tocris, Bristol, UK), apomorphine, SCH23390 (R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-3,4,5-tetrahydro-1H-3-benzazepine hydrochloride), sulpiride (Sigma Chemical Co., St Louis, CA, USA), NMDA (N-methyl-D-aspartic acid) and D-AP5 [D-(−)-2-amino-5-phosphonopentanoic acid] (Tocris, Bristol, UK). WIN was dissolved in dimethylsulphoxide (DMSO; up to 10% v/v) and sterile 0.9% saline and a drop of Tween 80, which also was used as vehicle. Apomorphine, SCH23390, NMDA and D-AP5 were dissolved in sterile 0.9% saline and sulpiride was dissolved in vehicle (the vehicle was one drop of glacial acetic acid with a Hamilton microsyringe and made up to a volume of 5 ml with sterile 0.9% saline and then diluted to the required volume) just before the experiment. Control animals received either saline or appropriate vehicle. WIN mesylate was injected into the dorsal hippocampal CA1 region (intra-CA1). Dopaminergic and glutamatergic agents were injected into the BLA (intra-BLA).

Passive avoidance apparatus

The animals were trained and tested in a step-through type passive avoidance apparatus which consisted of two compartments, one light (20×20×30 cm^3 high) and one dark, of the same size connected via a guillotine door (7×9 cm^2). The floor of the dark compartment was made of stainless steel rods (2.5 mm in diameter) separated by a distance of 1 cm. Intermittent electric shocks (50 Hz, 3 s, 1 mA) were delivered to the floor of the dark compartment by an insulated stimulator.

Behavioral testing

Training. The animals were allowed to habituate in the experimental room for 1 h prior to experiments. Then, each animal was gently placed in the brightly lit compartment of the apparatus; after 5 s the guillotine door was opened and the animal was allowed to enter the dark compartment. The latency with which the animal crossed into the dark compartment was recorded. Animals that waited more than 100 s to cross to the dark compartment were eliminated from the experiments. Once the animal crossed with all four paws to the next compartment, the guillotine door was closed and the rat was taken into its home cage. The trial was repeated after 30 min as in the acquisition trial where after 5 s the guillotine door was opened and as soon as the animal crossed to the dark (shock) compartment the door was closed and a foot shock (50 Hz, 1 mA, 3 s) was immediately delivered to the grid floor of the dark room. After 20 s, the rat was removed from the apparatus and placed temporarily into its home cage. Two min later, the animal was retested in the same way as the prior trials; if the rat did not enter the dark compartment during 120 s, successful acquisition of passive avoidance response was recorded. Otherwise, when the rat entered the dark compartment (before 120 s) a second time, the door was closed and the animal received the same shock again. After retesting, if the rat acquired acquisition of passive avoidance successfully, it was removed from the apparatus and received post-training injections of drugs immediately.