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Activation of cannabinoid CB1 receptors in the central amygdala impairs inhibitory avoidance memory consolidation via NMDA receptors

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ABSTRACT

In the present study, we investigated the influence of bilateral intra-central amygdala (intra-CeA) microinjections of *N*-methyl-D-aspartate (NMDA) receptor agents on amnesia induced by a cannabinoid CB1 receptor agonist, arachydonilcyclopropylamide (ACPA). This study used a step-through inhibitory (passive) avoidance task to assess memory in adult male Wistar rats. The results showed that intra-CeA administration of ACPA (2 ng/rat) immediately after training decreased inhibitory avoidance (IA) memory consolidation as evidenced by a decrease in step-through latency on the test day, which was suggestive of drug-induced amnesia. Post-training intra-CeA microinjections of NMDA (0.0001, 0.001 and 0.01 μg/rat) did not affect IA memory consolidation. However co-administration of NMDA with ACPA (2 ng/rat) prevented the impairment of IA memory consolidation that was induced by ACPA. Although post-training intra-CeA administration of the NMDA receptor antagonist, D-(−)-2-amino-5-phosphonopentanoic acid (D-AP5; 0.01, 0.05 and 0.1 μg/rat) alone had no effect, its co-administration with an ineffective dose of ACPA (1 ng/rat) impaired IA memory consolidation. Post-training intra-CeA microinjection of an ineffective dose of D-AP5 (0.01 μg/rat) prevented an NMDA response to the impaired effect of ACPA. These results suggest that amnesia induced by intra-CeA administration of ACPA is at least partly mediated through an NMDA receptor mechanism in the Ce-A.

1. Introduction

Endocannabinoids play an important neuromodulatory role in many behavioral processes including locomotion, anxiety, learning and memory [for review see (Lichtman, Varvel, & Martin, 2002; Pacher, Batkai, & Kunos, 2006)]. Receptors CB1 and CB2, which are both members of the G-protein-coupled receptor superfamily (Howlett, 2002; Howlett, Blume, & Dalton, 2010), primarily elicit the effects of cannabinoids. Growing evidence has suggested additional receptor targets for cannabinoids not mediated by CB1 or CB2 receptors. Those receptors include the orphan G-protein-coupled receptor GPR55, referred as the novel cannabinoid receptor 3 (CB3), and transient receptor potential vanilloid 1 (TRPV1) [for review see (Begg et al., 2005; Brown, 2007; Mackie & Stella, 2006; Pertwee, 2005; Sharir & Abood, 2010)].

Cannabinoid CB1 receptors are present at higher densities in pre-synaptic terminals such as the amygdala, hippocampus, anterior cingulate cortex and prefrontal cortex (Hajos & Freund, 2002; Herkenham et al., 1990; Katona et al., 2001; Pistis et al., 2004). A range of cognitive functions including emotion, reward, learning, memory, attention and perception have been reported to be associated with the amygdala [for review see (Baxter & Murray, 2002)]. The amygdala is also central to manifesting stress-related behaviors and changes in hippocampal functioning and emotional memory (LeDoux, 2000; McGaugh, 2004; Schafe, Doyere, & LeDoux, 2005). Abundant expression of cannabinoid receptors within the amygdala and participation of the endocannabinoid system of the amygdala in the regulation of memory processes have been proposed [for review see (Casswell & Marks, 1973; Marco & Viveros, 2009)]. According to previous research, genetic deletion of the CB1 receptor can improve non-associative learning, which supports the general idea that endocannabinoids are involved in inhibitory control of learning and mnemonic functions (Degroot, Salhoff, Davis, & Nomikos, 2005).

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Expression of *N*-methyl-D-aspartate (NMDA) (Glass, 2010) and cannabinoid CB1 receptors (Roberto et al., 2010) in the central amygdala (CeA) in addition to a functional interaction between cannabinoids and glutamatergic neurotransmission have been demonstrated (Liu, Bhat, Bowen, & Cheng, 2009). An electrophysiological study by Ohno-Shosaku and co-workers (2007) showed that activation of NMDA receptors facilitated endocannabinoid release in cultured hippocampal neurons (Ohno-Shosaku et al., 2007). Conversely, documentation exists regarding the inhibition of glutamatergic neurotransmission by cannabinoid agonists in a variety of brain structures, such as the amygdala (Hajos & Freund, 2002; Piomelli, 2003). According to research, cannabinoid agonists reduce glutamatergic transmission in the amygdala of normal mice but fail to do so in CB1-deficient mutants (Azad et al., 2003).

Both NMDA (Rebola, Srikumar, & Mulle, 2010) and cannabinoid CB1 receptors (Zhu, 2006) are known to mediate synaptic plasticity necessary for learning and memory processes. Therefore, the present study has aimed to investigate the involvement of NMDA receptors in the CeA effect of arachydonilcyclopropylamide (ACPA), a selective potent agonist of cannabinoid CB1 receptors, on consolidation of inhibitory avoidance (IA) memory. After successful acquisition of a step-through type of IA task, we examined the individual effects of post-training intra-CeA administration of ACPA, NMDA and D-(−)-2-amino-5-phosphonopentanoic acid (D-AP5), respectively, on IA memory consolidation. Finally, this study evaluated the interactions of intra-CeA administration of the above-mentioned drugs on consolidation of IA memory.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (Pasteur institute, Tehran, Iran) weighing 240–280 g at time of surgery were used. They were housed four per each cage, and kept at 22 ± 2 °C under a 12/12-h light/dark cycle (light beginning at 7:00 a.m) with free access to food and water. All experiments were carried out during the light phase of the cycle between 8:00 and 12:00. All procedures were performed in accordance with institutional guidelines for animal care and use which adhered to the international principles of Laboratory Animal Care (NIH publication #85-23, revised in 1985).

2.2. Surgery

Animals were anaesthetized using intraperitoneal injection of a ketamine/xylazine mixture (100 mg/kg and 10 mg/kg, respectively) and placed in a stereotaxic frame (Stoelting Co., Wood Dale, Illinois, USA) with flat-skull position. A midline incision was made in the skin of the skull, then underlying periosteum was retracted and bilateral stainless steel guide cannulae (22 gauge) were implanted until 2 mm above the central amygdala. Stereotaxic coordinates were; −2.2 mm posterior to the bregma; ±4.2 mm lateral from midline; −6 mm ventral relative to the dura (Paxinos & Watson, 2007). The cannulae were anchored to the skull with a jeweler's screw and dental cement. After the surgery, two stainless steel stylets (27 gauge) were inserted into the guide cannulae to maintain patency prior to microinfusions.

2.3. Drugs and microinjections

All of drugs were purchased from Tocris (Tocris, Cookson Ltd., UK), included: ACPA (arachidonylcyclopropylamide; N-(2-cyclopropyl)-5Z,8Z,11Z,14Zeicosatertraenanmide), *N*-methyl-D-aspartate (NMDA) and D-(−)-2-amino-5-phosphonopentanoic acid (D-AP5). All of drugs were dissolved in sterile saline, except for ACPA, which was supplied dissolved in anhydrous ethanol at a concentration of 5 mg/ml and was diluted to the required volume using saline. Bilateral microinjections of drugs into the central amygdala (Intra-CeA injections) were in a volume of 0.5 μl/rat (0.25 μl/each side) and were done by lowering a 27-gauge injection cannula. The injection cannula was 2 mm longer than the guide cannulae to extend 2 mm beyond the tip of the guide cannulae into the center of the CeA (−8 mm from the skull). The injection cannula was attached with a polyethylene tube to a 1-μl Hamilton syringe. Intra-CeA injections were carried out over 30 s first in one side then the other one. The injection cannula was left in the place for an additional 30 s to facilitate diffusion of the drugs from the tip of the injection cannula.

2.4. Inhibitory avoidance apparatus

The inhibitory avoidance (IA) apparatus consisted of two compartments of the same size ($20 \times 20 \times 30\ cm^3$), divided by a wall that in the middle of it a guillotine door ($7 \times 9\ cm^2$) could be lifted manually. The walls and floor of one compartment consisted of white opaque Plexiglas and were lit with a 20 W electric bulb placed approximately 50 cm above the floor of the apparatus. The walls of the other compartment were dark but its floor was stainless steel grids (3 mm in diameter), which were placed at 1-cm intervals (distance between the centers of the grids). Intermittent electric shocks (50 Hz, 3 s, 1 mA intensity) were delivered to the grid floor of the dark compartment by an isolated stimulator (Borj Sanat Apparatus, Tehran, Iran).

2.5. Behavioral procedures

Training was based on the protocol used in our previous studies (Ahmadi, Zarrindast, Haeri-Rohani, Rezayof, & Nouri, 2007b; Ahmadi, Zarrindast, Nouri, Haeri-Rohani, & Rezayof, 2007a). First, all animals were allowed to habituate in the experimental room (with light and sound attenuated) for at least 30 min prior to the experiments. Then, each animal was gently placed in the brightly white compartment of the apparatus, after 5 s the guillotine door was opened and the animal was allowed to enter into the dark compartment. The latency to enter the dark compartment was recorded for each animal. Animals that waited more than 100 s to cross to the dark compartment were eliminated from the experiments (in the present experiments, all of the animals entered the dark compartment before 100 s). Once the animal crossed with all four paws to the next compartment, the guillotine door was closed and immediately was withdrawn from the compartment into its home cage.

After 30 min, the trial was repeated for each subject as in the acquisition trial except that as soon as the animal crossed to the dark compartment, the door was closed and a foot shock (50 Hz, 1 mA and 3 s) was immediately delivered to the grid floor. After 20 s, the subject was removed from the apparatus and placed temporarily into its home cage. Two minutes later, the animal was retested in the same way as in the previous trials and a successful acquisition of IA memory was recorded for animals with 120 s latency to enter into the dark compartment. Otherwise, when the rat entered the dark compartment (before 120 s) a second time, the door was closed and the animal received the shock again. After retesting and successful acquisition, the animal was removed from the apparatus and immediately received post-training injection of drugs. The aim of all experiments with post-training drug administration was to evaluate effects of drugs on IA memory consolidation.

Retention session was done 24 h after training. Each subject was gently placed in the light compartment, after 5 s the guillotine door was opened and the step-through latency with which the animal crossed to the dark compartment with all four paws was