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Possible involvement of the CA1 $GABA_A$ receptors upon acquisition and expression of the ACPA-induced place preference in mice

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HIGHLIGHTS

- Intraperitoneal injection of ACPA induced CPP
- Intra-CA1 injection of muscimol (Mus)/bicuculline (Bic) fail to induce CPP or CPA.
- Mus and Bic decreased and did not alter CPP acquisition by ACPA, respectively.
- Intra-CA1 administration of Mus and Bic prior to testing, did not induce CPP or CPA.
- Mus and Bic increase and decrease CPP expression induced by ACPA, respectively.

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ABSTRACT

A plethora of investigations has substantiated a close relationship between cannabinoidergic and GABAergic systems in hippocampal CA1. The crucial role of these two systems in regulation of the addictive behaviors is well described. The aim of the current study was to investigate whether the CA1 $GABA_A$ receptors are involved in ACPA (a selective CB1 cannabinoid receptor agonist)-induced rewarding effects using the condition place preference (CPP) protocol. Moreover, the hole-board paradigm was used to measure the exploratory behaviors which may potentially influence this phenomenon. Results showed that ACPA (0.02 mg/kg, i.p.) induced CPP. Applying a 3-day conditioning schedule, we found that the sole administration of the $GABA_A$ receptor agonist, muscimol (0.125, 0.25 and 0.5 μg/mouse; intra-CA1), or the $GABA_A$ receptor antagonist, bicuculline (0.0635, 0.125 and 0.25 μg/mouse; intra-CA1), fail to induce CPP or CPA (condition place aversion). Similarly, injection of the subthreshold dose of muscimol (0.125 μg/mouse, intra-CA1) decreased the CPP acquisition induced by ACPA. Similar intervention with the subthreshold dose of bicuculline (0.125 μg/mouse, intra-CA1) did not alter the CPP acquisition induced by ACPA. Furthermore, the sole intra-CA1 administration of muscimol (0.125, 0.25 and 0.5 μg/mouse) and bicuculline (0.0635, 0.125 and 0.25 μg/mouse; intra-CA1) prior to testing, did not induce CPP or CPA. Similar interventions revealed that muscimol and bicuculline increase and decrease CPP expression induced by ACPA, respectively. The ACPA- and muscimol-induced CPP could be blocked by bicuculline. Taken together, the CA1 $GABA_A$ receptors seem to be possibly involved in the modulation of acquisition or expression process upon ACPA-induced CPP.

1. Introduction

Marijuana (common name for cannabis) is a famous recreational drug with mind-altering effects, used worldwide today [1]. This agent's effective compound has also been used for therapeutic purposes as an

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analgesic, antiemetic and anticonvulsant. It is also shown to lower the intraocular pressure [2] and also stimulate appetite [3]. According to several studies on ligand-binding properties and signal transduction systems, three cannabinoid receptors have been discovered, namely the CB1, CB2 and CB3 receptors [1,4]. CB1 receptors are shown to be predominantly localized in the brain and testis (similar to levels of GABA and glutamate-gated ion channels) [5]. CB2 receptors however, are mainly localized peripherally in cells and tissues derived from the immune system [1]. Since, CB2 [6,7] and CB3 [4] receptors do exist in the brain, the involvement of these receptor subtypes in various cognitive functions cannot be excluded.

Given the high densities of CB1 receptors in the hippocampus, this receptor type suggested to have a crucial role in the hippocampus-regulation processes such as memory, learning, anxiety and reward [8–11]. The presynaptic CB1 cannabinoid receptors are known to regulate GABA release from the axon terminals of specific hippocampal interneurons [12,13]. This may propose the function of endocannabinoid compounds in neurotransmitter release regulation.

GABA (the most abundant inhibitory neurotransmitter involved in almost 30% of the mammalian cerebral cortex synapses) is synthesized by glutamic acid decarboxylase [14] and take a role in the regulation of neuronal excitability. From the three identified types of GABA receptors in the brain (i.e. $GABA_A$, $GABA_B$ and $GABA_C$) [15], $GABA_A$ receptors which belong to the ligand-gated ion channel (coupled with Cl-channel) superfamily [16] can be modulated by cannabinoids [17] and benzodiazepines [18]. As such, some studies have postulated the critical role of this receptor in cannabinoids-induced rewarding effects [19,20].

Furthermore, it is believed that the conditioned place preference (CPP) paradigm reflects a preference for a context due to the contiguous association between the context and the drug stimulus [21]. CPP can then be used as a model for studying the reinforcing effects of drugs with dependent liability [22]. Several studies have indicated that ACPA (selective CB1 cannabinoid receptor agonist) may induce CPP or CPA (conditioned place aversion). Rezayof et al. showed that the injection of ACPA into ventral tegmental area (VTA) induced CPA, while its injection into the basolateral and central amygdala induced CPP in rats [23,24]. Similar study in mice demonstrated that intraperitoneal injection of ACPA induce CPP [8].

Although our knowledge on the mechanisms of action of cannabinoids has been greatly increased during the last few years, the actual mechanism(s) of these compounds on several brain sites and their effects on neurotransmitter release has not been fully understand. Therefore, the aim of the present study was to investigate the possible involvement of CA1 $GABA_A$ receptors upon acquisition and expression of ACPA-induced place preference with exploratory behaviors in mice.

2. Materials and methods

2.1. Animals

Male NMRI mice weighing 25–30 g were used. Animals were housed in standard polypropylene cage colonies maintained at 22 ± 2 °C under a 12:12-h light–dark cycle (lights on at 07:00 h) having free access to food and water. Mice were allowed to adapt to laboratory conditions for at least 1 week before surgery. Eight animals were used in each experimental group and each animal was used once only. The experiments were carried out during the light phase of the cycle. Animal treatment and maintenance were conducted in accordance with the Principles of Laboratory Animal Care (NIH 98 publication No. 85-23, revised 1985) and in line with the "Guidelines of the Animal Care and Use" laid down by Tehran University of Medical Sciences.

2.2. Drugs

The drugs used in the study were ACPA (Tocris, UK), muscimol (Tocris, UK) and bicuculline (Sigma, St. Louis, CA, USA). All drugs except bicuculline were dissolved in sterile 0.9% saline, just before the experiment. Bicuculline was dissolved in 1 drop of glacial acetic acid using a Hamilton microsyringe, then made up to a volume of 5 ml with sterile 0.9% saline and then diluted to the required volume. Muscimol and bicuculline were administered into the dorsal hippocampus (CA1) while ACPA was injected intraperitoneally (i.p.). For each drug, three doses were applied: ACPA were given at 0.01, 0.02 and 0.04 mg/kg, bicuculline at 0.0625, 0.125 and 0.25 μg/mouse and muscimol at 0.125, 0.25 and 0.5 μg/mouse. Control groups received saline (1 μl/mouse) or vehicle (1 μl/mouse). The administered drug doses were selected according to the pilot and previous study [8,17].

2.3. Cannula guide implantation

The animals were anesthetized using the 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. 22-Gauge guide cannulae (0.7 mm diameter) were implanted (bilaterally) 1 mm above the intended site of injection according to the atlas of Paxinos [25]. Stereotaxic coordinates for the CA1 regions of the dorsal hippocampus were AP: −2 mm from bregma, L: ±1.6 from the sagital suture and V: −1.5 mm from the skull surface. The implanted cannulae were secured in place using dental acrylic. Stainless steel stylets (27-gauge, 0.41 mm in diameter) were inserted into the guide cannulae to keep them free of debris. All animals were allowed 5–7 days to recover from surgery and get cleared from the anesthetics' effects.

2.4. Intra-CA1 injections

For drug infusion, animals were gently restrained in hand; stylets were removed from the guide cannulae and replaced by 27-gauge (0.41 in mm diameter) injection needles (1 mm below the tip of the guide cannulae). Injections were given at a total volume of 1 μl/mouse (0.5 μl in each side) over a 60 s period. Injection needles were left in place for an additional 60 s to facilitate drug diffusion.

2.5. Apparatus

The employed modified CPP apparatus consisted of three wooden compartments as described in earlier reports [26]. Compartments A and B were identical in size (40 × 30 × 30 cm) while different in shading pattern. The (A) compartment was white with 2 cm thick horizontal black stripes on its walls, having a textured floor. Meanwhile, the (B) compartment was black with 2 cm thick vertical white stripes on the walls, having a smooth floor. Compartment C (40 × 15 × 30 cm) which was placed next to the compartments (A) and (B) had a removable wooden partition separating that from the other two compartments. The inner walls of this compartment were in red. When the partition was removed, the animal could freely move between the two compartments (A and B) via compartment C.

2.6. Place conditioning

One week after surgery, conditioned place preference protocol was applied using an unbiased procedure like the method described previous studies [8,26]. This protocol was a 5-day schedule comprising three distinct phases, i.e. preconditioning, conditioning, and testing.

2.6.1. Preconditioning

Mice were placed at the middle of the apparatus and allowed to freely explore the three compartments for 15 min (900 s). Within the allotted time span, the time animal spent in each compartment was separately recorded (mice were considered in a compartment based on the position of their front paws). Animals showing strong unconditioned aversion (<33% of the session time, i.e., 300 s) or preference (>67%,