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Effects of URB597 as an inhibitor of fatty acid amide hydrolase on WIN55, 212-2-induced learning and memory deficits in rats

Parisa Hasanein [1], Massoud Teimuri Far

Department of Biology, School of Basic Sciences, Bu-Ali Sina University, Hamedan, Iran

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ABSTRACT

Cannabinoid and endocannabinoid systems have been implicated in several physiological functions including modulation of cognition. In this study we evaluated the effects and interaction between fatty-acid amide hydrolase (FAAH) inhibitor URB597 and CB_1 receptor agonist WIN55, 212-2 on memory using object recognition and passive avoidance learning (PAL) tests. Learning and memory impairment was induced by WIN 55, 212-2 administration (1 mg/kg, *i.p.*) 30 min before the acquisition trial. URB597 (0.1, 0.3 and 1 mg/kg, *i.p.*) or SR141716A (1 mg/kg, *i.p.*) was injected to rats 10 min before WIN 55, 212-2 or URB597 respectively. URB597 (0.3 and 1 mg/kg) but not 0.1 mg/kg induced higher discrimination index (DI) in object recognition test and enhanced memory acquisition in PAL test. The cognitive enhancing effect of URB597 was blocked by a CB_1 receptor antagonist, SR141716A which at this dose alone had no effect on cognition. WIN55, 212-2 caused cognition deficits in both tests. URB597 (0.3 and 1 mg/kg) treatment could alleviate the negative influence of WIN 55, 212-2 on cognition and memory. These results indicate URB597 potential to protect against memory deficits induced by cannabinoid. Therefore, in combination with URB597 beneficial effects, this study suggests that URB597 has recognition and acquisition memory enhancing effects. It may also constitute a novel approach for the treatment of cannabinoid induced memory deficits and lead to a better understanding of the brain mechanisms underlying cognition.

1. Introduction

It has long been known that cannabis, the most widely used illicit substance, as well as naturally occurring cannabinoids induces cognitive deficits in humans and laboratory animals (Ranganathan and D'Souza, 2006; Riedel and Davies, 2005). Similarly, synthetic cannabinoid agonists CP55, 940 or WIN55, 212-2 impair passive avoidance learning as well as spatial and working memory in different experimental tasks (Braida and Sala, 2000; Lichtman et al., 1995; Suenaga and Ichitani, 2008; Moshfegh et al., 2011). Cannabinoid CB_1 receptors appear to play an important role in mediation of cannabinoids induced memory deficits (Egashira et al., 2002). Therefore, cannabinoid-induced cognitive dysfunction is extensively used for characterizing potential cognition enhancing drugs for this model.

Recently, cannabinoid endogenous system, endocannabinoid system, has been implicated in several physiological functions including the modulation of cognition (Terranova et al., 1996; Marsicano et al., 2002; Varvel et al., 2005). Fatty-acid amide hydrolase (FAAH) is an integral membrane enzyme that is responsible for the degradation of the endocannabinoid anandamide as well as several non-cannabinoid fatty-acid amides (FAAs; Cravatt et al., 1996). Although marijuana and CB_1 agonists are well known for their amnestic effects, and FAAH inhibition increases levels of the CB_1 agonist anandamide, previous studies have indicated that FAAH inhibition might enhance learning and memory. Varvel et al. (2007) studied the effects of the FAAH inhibitor OL-135 and of genetic deletion of FAAH in mice. Both FAAH manipulations enhanced acquisition of spatial learning in a water maze, and this enhancement was blocked by treatment with the CB_1 antagonist/inverse agonist SR141716A, suggesting that the enhancement was mediated by CB_1 receptors (Panlilio et al., 2013; Varvel et al., 2006, 2007). On the other hand, it has been reported that FAAH inhibitor.

Since FAAH inhibition might have a wide range of therapeutic actions but might also share some of the adverse effects of cannabis, it is noteworthy that at least one FAAH inhibiting drug URB597 has been found to have potentially beneficial effects but no indication of liability for abuse or dependence. Therefore, URB597 has been suggested for improved therapeutic interventions in addiction and memory deficit cases (Panlilio et al., 2013).

The object recognition and PAL tests are useful as screens for testing new drugs in cognitive dysfunctions on the basis of previous studies (Hasanein and Shahidi, 2010; Jabbarpour et al., 2014; Raghavendra and Kulkarni, 2001). The object recognition task is a working memory task that primarily relies on cortical functioning and to a lesser extent, hippocampal functioning (Baek et al., 2009) while PAL and memory

E-mail address: p.hasanein@basu.ac.ir (P. Hasanein).
[1] Bu-Ali Sina University, School of Basic Sciences, Department of Biology, 65178–33391 Hamedan, Iran. Tel./fax: +98 811 8381058.

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task mainly focus on hippocampal function. As cortical and hippocampal cannabinoid CB_1 receptors play an important role in mediation of cannabinoids induced memory deficits (Wegener et al., 2008; Wise et al., 2009), these tasks are widely used for investigating cannabinoid induced learning and memory deficits.

Despite a growing consensus that the cannabinoids modulate cognition, there is no data about the effects of FAAH inhibitor URB597 on cannabinoid induced learning and memory deficits. Therefore, in the current study we sought to investigate this issue by examining the dose-dependent effects of URB597 in an object recognition task and a passive avoidance learning (PAL) test in adult rats. We also assessed the effects of URB597 at different doses on WIN55, 212-2-induced learning and memory impairment in both tests. Finally we evaluated the effects of SR141716A administration as a cannabinoid CB_1 receptor antagonist on the cognitive-altering effects of URB597.

2. Material and methods

2.1. Animals

Locally produced male Wistar rats (250–280 g) were used in the present experiments. All animals were housed in a room maintained at a constant temperature (22 ± 0.5 °C) with a 12 h light/dark cycle. They had free access to laboratory chow and tap water. Each experimental group consisted of seven animals that were chosen randomly from different cages and each was used only once. Animals were handled in accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health (NIH) publication 86-23; revised 1985; http://www.oacu.od.nih.gov/regs/guide/guidex.htm). The protocols were also approved by the institutional ethics committee of Bu-Ali Sina University.

2.2. Drugs

(*R*)-(+)-WIN 55, 212-2 mesylate salt ((*R*)-(+)-[2,3-dihydro-5-methyl-3-(4 morpholinylmethyl) pyrrolo [1,2,3,-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate) and URB597(cyclohexylcarbamic acid 3′-carbamoylbiphenyl-3-yl ester) were purchased from Sigma (Sigma Chemical Co.; St. Louis, MO, USA). SR141716A (5-(4-chlorophenyl)-1-(2, 4-dichloro-phenyl)-4-methyl-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide) was supplied by Sanofi Synthelabo Recherche (Sanofi-Synthélabo Recherche, Montpellier, France). All drugs were dissolved in 10% DMSO and sterile water and injected in a volume of 1 ml/kg (*i.p.*).

2.3. Experimental design

Rats received URB597 (0.1, 0.3 and 1 mg/kg, *i.p.*) or vehicle 10 min before WIN 55, 212-2 administration. Learning and memory impairment was induced by WIN 55, 212-2 treatment (1 mg/kg, *i.p.*) 30 min before the acquisition trial. To examine the effects of SR141716A on memory-ameliorating properties of URB597, SR141716A was administered to rats 10 min before URB597 treatment.

$$\text{SR141716A} \xrightarrow{10\ \text{min}} \text{URB597} \xrightarrow{10\ \text{min}} \text{WIN55, 212-2} \xrightarrow{10\ \text{min}} \text{Acquistion trial}$$

To test the effects of SR141716A alone on PAL and memory in this study, we administered SR141716A (1 mg/kg, *i.p.*) 10 min before the acquisition trial. The operator was unaware of the specific treatment groups to which an animal belonged. Behavioral tests were conducted in the animal groups between 12:00 and 3:00 PM of the test day.

2.4. Object recognition test

The object recognition task is a working memory task that primarily relies on cortical functioning and to a lesser extent, hippocampal functioning (Baek et al., 2009). The utilized setup consists of a cubic open arena (50 cm × 45 cm × 35 cm) and a video recording system. The test was modified from Zheng et al. (2004) and lasted for 3 days. On day 1, each animal was placed in the box for 10 min for exploration. No object was placed in the box at this time. On day 2, each animal had 2 identical 10 min sessions exploring 4 similar sized objects placed in the box. The inter-trial interval was 30 min. The location of each object was kept constant for each rat and counterbalanced across the rats and groups. All objects and the test arena were cleaned with disinfectant and thoroughly dried before each session. On day 3, animals were tested for the reaction to novelty. On the first session of day 3 (sample trial), animals were exposed to the identical condition to the previous day. After the first session, a single *i.p.* injection of URB597, at one of the 3 doses, or the vehicle, was given to each rat and 40 min later, the second session (choice trial) was run, in which one of the sample objects was replaced with a novel one. The location of the novel object was randomized across the groups in order to prevent location effects for particular groups. WIN 55, 212-2 (1 mg/kg, *i.p.*) was injected 10 min after URB597 administration.

$$\text{URB597} \xrightarrow{10\ \text{min}} \text{WIN55, 212-2} \xrightarrow{10\ \text{min}} \text{Choice trial}$$

Exploration was defined as the animal directing its nose toward an object within a distance of 2 cm and/or touching the object. A discrimination index (DI) was represented by subtracting the mean exploration time of the familiar object from the mean exploration time of the novel object (Akirav and Maroun, 2006; Broadbent et al., 2004; Jabbarpour et al., 2014).

2.5. PAL and memory test (step through test)

The apparatus and procedure were basically the same as our previous studies (Hasanein and Shahidi, 2010; Shahidi et al., 2004). Briefly, the step-through passive avoidance apparatus consisted of a lighted chamber (20 cm × 20 cm × 30 cm) made of transparent plastic and a dark chamber whose walls were made of dark opaque plastic (20 cm × 20 cm × 30 cm). The floor of both chambers was made of stainless steel rods (3 mm diameter) spaced 1 cm apart. The floor of the dark chamber could be electrified using a shock generator. A rectangular opening (6 cm × 8 cm) was located between the two chambers and could be closed by an opaque guillotine door.

2.6. Training

First, all experimental groups were given two trials to habituate them to the apparatus. For these trials, the rats were placed in a lighted compartment of the apparatus facing away from the door and 5 s later the guillotine door was raised. The rat has native preference to the dark environment. Upon the rat entering the dark compartment, the door was closed and after 30 s the rats were taken from the dark compartment into their home cage. The habituation trial was repeated after 30 min and followed after the same interval by the first acquisition trial. The entrance latency to the dark compartment (step-through latency, STLa) was recorded when the animal had placed all 4 paws in the dark compartment. After the animal had spontaneously entered the dark compartment, the guillotine door was lowered and a mild electrical shock (0.5 mA) was applied for 3 s. After 30 s, the rat was taken from the dark compartment into their home cage. Then after 2 min, the procedure was repeated. The rat received a foot-shock each time it reentered the dark and had placed all 4 paws in the dark compartment. Training was terminated when the rat remained in the light compartment for 120

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