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Research report

β1-noradrenergic system of the central amygdala is involved in state-dependent memory induced by a cannabinoid agonist, WIN55,212-2, in rat

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ABSTRACT

In the present study, we investigated effects of intra-central amygdala (intra-CeA) administrations of a β1-receptor agonist and antagonist, isoprenaline (isoproterenol) and atenolol respectively, on state-dependent memory induced by a cannabioid agonist, WIN55,212-2. This study used a step-through inhibitory avoidance task to assess memory in male Wistar rats. The results showed that post-training intra-CeA administrations of different doses of WIN55,212-2 (0.01, 0.05, 0.1 and 0.25 μg/rat) decreased memory as revealed by a decrease in memory retrieval on the test day. The decrease in retrieval induced by post-training WIN55,212-2 (0.25 μg/rat) was reversed by pre-test administration of the same dose of the drug, which was suggestive of drug-induced state-dependent memory. Although pre-test intra-CeA administrations of isoprenaline (0.01, 0.025 and 0.05 μg/rat) alone had no effect, its co-administrations at doses of 0.025 and 0.05 μg/rat with an ineffective dose of WIN55,212-2 (0.1 μg/rat) restored memory retrieval that impaired by post-training WIN55,212-2 (0.25 μg/rat). The results also showed that pre-test intra-CeA administrations of atenolol (0.01, 0.05 and 0.1 μg/rat) alone had no effect, but at dose of 0.1 μg/rat disrupted state-dependent memory induced by WIN55,212-2. Moreover, the improving effect of isoprenaline (0.025 μg/rat) on retrieval of state-dependent memory induced by WIN55,212-2 (0.1 μg/rat) was prevented by intra-CeA co-injections of atenolol. Taken together, our results suggest that the CeA may be potentially critical for state-dependent memory induced by WIN55,212-2 and the β1-noradrenergic receptor mechanism(s) interact with the cannabinergic system in the modulation of this kind of memory in the CeA.

1. Introduction

Memories especially those of emotional types, can depend on an endogenous state of subjects; this phenomenon is termed state-dependence [1,2]. In a state-dependent memory when pre- or post-training administrations of a drug decrease memory for a task, administration of the drug prior to testing retards the extinction of the task [3,4]. According to previous researches, variety of drugs can induce state-dependent memory in laboratory animals [5–7]. Cannabinoids has also been shown to induce state-dependent memory. Either pre- or post-training administrations of a potent synthetic cannabinoid receptor agonist, WIN55,212-2, impaired retrieval of learned tasks, which was reversible by pre-test administration of the drug [8–12].

Monoaminergic systems are crucially involved in the control of behavioural processes related to exploration, anxiety, learning and memory consolidation [13–16]. Although the expressions

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of β-adrenergic receptors in the brain are substantially lesser than the α subtype, they are crucial for cognitive functions [17]. It has been demonstrated that β-adrenergic receptors exhibit important modulatory roles in the regulation of vigilance, learning as well as memory consolidation and reconsolidation [18–21]. Administrations of norepinephrine (NE) and a β-adrenoreceptor agonist, isoprenaline (isoproterenol) enhanced retention performance in either spatial [22,23], or emotionally-motivated memory tasks [24–29]. Conversely, administration of β-adrenergic receptor antagonists impaired learning and memory in behavioural studies [30–36].

Documentation exists regarding interaction between noradrenergic and cannabinoid receptor functions [10,37–39]. According to research, genetic and pharmacological manipulation of the endocannabinoid system can influence the cerebral monoamine tone [40–42]. Norepinephrine has also been implicated in many of the same central processes that are affected by cannabinoids [43]. Previous data has indicated that acute administration of WIN55,212-2 increases NE efflux in the frontal cortex and stimulates c-Fos expression in noradrenergic neurons of the locus coeruleus [44]. We have already shown that the α-noradrenergic receptors of the hippocampus play an important role in state-dependent memory induced by WIN55,212-2 [10].

The amygdala, which has been subdivided into several nuclei, is critically involved in such behaviours as the motivation, emotional behaviour, fear recognition, and fear-motivated learning and memory [45–49]. The central nucleus of the amygdala (CeA) serves as the output for major of the amygdala functions [50,51]. The CeA receives dense noradrenergic innervations from the locus coeruleus. According to research, inhibitory avoidance memory is modulated by the amygdala through, at least in part, an adrenergic action [52–55]. It has been reported that β-adrenoceptors in the basolateral amygdala play an essential role in fear memory formation [35,56], but role of β-adrenoceptors of the CeA in memory is less clear. Therefore, the present study has aimed to investigate the effects of intra-CeA administration of β1 noradrenergic agonist, isoprenaline and its antagonist, atenolol, on state-dependent memory induced by WIN55,212-2 in rat.

2. Materials and methods

2.1. Subjects

Adult male Wistar rats (Pasteur institute, Tehran, Iran) weighing 220–270 g at time of surgery were used. They were housed upon their arrival in the laboratory (1 week before the experiments) in groups of four in each cage. The animals had free access to food and water, and kept at a constant temperature (22 ± 2 °C) under a 12/12 h light-dark cycle (light beginning at 7:00 a.m.). All experiments were carried out during the light phase between 8:00 and 12:00. Experimental groups consisted of eight animals, each animal was tested only once. All procedures were performed in accordance with international guidelines for animal care and use (NIH publication #85-23, revised in 1985).

2.2. Surgery

Animals were anaesthetized intraperitoneally (i.p.) with a ketamine/xylazine mixture (50 and 5 mg/kg, respectively) and placed in a stereotaxic frame (Stoelting Instruments, USA) with flat-skull position. A midline incision was made then the skin and underlying periosteum retracted. Bilateral stainless steel guide cannulae (22 gauge) were implanted 2 mm above the CeA according to the stereotaxic coordinates: AP, −2.2 mm posterior to the bregma; L, ±4.2 mm from midline; V, −8 mm relative to dura [57]. The cannulae were anchored to the skull with a small screw and dental cement. Then, stainless steel stylets (27 gauge) were inserted into the guide cannulae to maintain patency prior to microinfusions. Following all surgical preparations, animals were spent five days of recovery period before being submitted to behavioural testing.

2.3. Drugs and microinfusions

The drugs used in the present study were included: atenolol (Daroopakhsh, Tehran, Iran), isoprenaline (isoproterenol) (Sigma, Poole, Dorset, UK), WIN55,212-2 (R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo[1,2,3-de)-1,4-benzoxazin-6-yl]-1-napthalenylmethanon) (Tocris, Cookson Ltd., Bristol, UK). All drugs were dissolved in sterile saline except for WIN55,212-2, which was dissolved in a mixture of sterile saline and dimethyl sulphoxide (DMSO, 9:1 v/v), and one drop of 0.4% Tween oil-80. Fresh solutions of all drugs were prepared prior to the experiments.

Bilateral microinfusions of all dugs into the central amygdala (intra-CeA) were in a volume of 0.6 μL/rat (0.3 μL/side). Intra-CeA infusions were given by lowering a 27-gauge injection cannula to extend 2 mm beyond the tip of the guide cannulae to the site of infusion (−8 mm from the skull). The injection cannula was attached with a polyethylene tube to a 1 μL Hamilton syringe. Intra-CeA injections (0.3 μL/side) were carried out over 60 s, first into one side then the other. The infusion cannula was left in place for an additional 30 s to facilitate diffusion of the drugs from the tip of the injection cannula.

2.4. Inhibitory avoidance apparatus

A step-through inhibitory avoidance apparatus consisted of two compartments of the same size ($20 \times 20 \times 30\ cm^3$) was used. In the middle of the separating wall, a guillotine- like door ($7 \times 9\ cm^2$) could be lifted manually. The walls and floor of one compartment consisted of white opaque resin and were lit with a 20 W electric bulb placed ∼50 cm above the floor of the apparatus. The walls of the other compartment were black and its floor was consisted from stainless steel bars (3 mm in diameter and 1 cm intervals). Intermittent electric shocks (50 Hz, 3 s, 1 mA intensity) were delivered to the grid floor of the dark compartment through an isolated stimulator (Borj Sanat Co., Tehran. Iran).

2.5. Behavioural procedures

2.5.1. Training

All the behavioural testing sessions were performed between 8.00 and 12.00 a.m. Animals were allowed to habituate in the experimental room (with light and sound attenuated condition) for at least 30 min prior to the experiments. Training was based on the protocol used in our previous studies. In brief, each animal was gently placed in the brightly lit compartment of the apparatus; after 5 s the guillotine door was lifted and the animal was allowed to enter the dark compartment. The latency for crossing into the dark compartment was recorded for each animal. Those animals that waited more than 100 s were eliminated from the experiments. In the present study, 272 rats were used and all of them successfully passed the above criterion.

Once the animal crossed with all four paws to the next compartment, the guillotine door was closed and rat was immediately removed from the compartment to its home cage. Acquisition trial was repeated after 30 min as the first trail, but as soon as the animal crossed to the dark (shock) compartment, the door was closed and a foot shock (50 Hz, 1 mA and 3 s) was delivered to the grid floor of the dark room. After 20 s, the rat was taken from the apparatus and placed temporarily in its home cage. Two minutes later, the animal was retested in the same manner as in the prior trials and a successful acquisition of inhibitory avoidance (IA) memory was recorded if the rat did not enter the dark compartment during 120 s. 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 successful acquisition each animal received post-training injection of WIN55,212-2 immediately.

2.5.2. Testing

The testing session was performed 24 h after the training session for assessment of memory retrieval. On the test day, intra-CeA infusions were performed 5 min prior to the test. Following drug administrations, each animal was gently placed in the light compartment and after 5 s the door was opened, the step-through latency with which the animal crossed to the dark compartment was recorded. The testing session was ended when the animal entered the dark compartment or remained in the light compartment for 300 s. During testing sessions no electric shock was applied.

2.6. Experimental design

2.6.1. Experiment 1: effects of post-training and pre-test intra-CeA administration of WIN55,212-2 on retrieval of IA memory

Five groups of animals received intra-CeA injections of vehicle or different doses (0.01, 0.05, 0.1and 0.25 μg/rat) of WIN55,212-2 immediately after training. On the test day, all of these animals received intra-CeA injections of vehicle, 5 min before the test. Other four groups of the animals received post-training administration of WIN55,212-2 at dose of 0.25 μg/rat and pre-test (5 min before the test) injections of different doses of WIN55,212-2 (0.01, 0.05, 0.1 and 0.25 μg/rat).