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Glucocorticoid receptors in the basolateral amygdala mediated the restraint stress-induced reinstatement of methamphetamine-seeking behaviors in rats

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ABSTRACT

Methamphetamine (METH) addiction is a growing epidemic worldwide. It is a common psychiatric disease and stress has an important role in the drug seeking and relapse behaviors. The involvement of the basolateral amygdala (BLA) in effects of stress on the reward pathway has been discussed in several studies. In this study, we tried to find out the involvement of glucocorticoid receptors (GRs) in the BLA in stress-induced reinstatement of the extinguished METH-induced conditioned place preference (CPP) in rats. The CPP paradigm was done in eighty-one adult male Wistar rats weighing 220–250 g. The animals received a daily injection of methamphetamine (0.5 mg/kg), during the conditioning phase. In extinction phase, the rats were put in the CPP box for 30 min per day for 8 days. After the extinction, the animals were exposed to acute restraint stress (ARS), 3 h before subcutaneous administration of sub-threshold dose of methamphetamine (0.125 mg/kg), based on our previous study, in reinstatement phase. In separated groups, the rats were exposed to chronic restraint stress (CRS) for 1 h each day during the extinction phase. To block the GRs in BLA, the animals unilaterally received RU38486 as GRs antagonist (10, 30 and 90 ng/0.3 µl DMSO) in all ARS groups on reinstatement day. In separated experiments, RU38486 (3, 10 and 30 ng/0.3 µl DMSO) was microinjected into the BLA in CRS groups prior to exposure to stress every day in extinction phase. The results revealed that intra-BLA RU38486 in ARS (90 ng) and CRS (10 and 30 ng) groups significantly prevented the stress-induced reinstatement. It can be proposed that stress partially exerts its effect on the reward pathway via GRs in the BLA. This effect was not quite similar in acute and chronic stress conditions.

1. Introduction

Traditionally, the amygdala has been assigned a crucial role in learning, memory, vigilance, attention and motivation [1]. It may act to interface external and internal sensory information with the mesocorticolimbic dopamine system, which is critically involved in addiction [2]. The amygdala is a forebrain structure that has been divided into several nuclei based on cytoarchitectural, histochemical, connectional, and functional criteria and consists of the basolateral (BLA), central (CeA) and lateral amygdaloid nuclei [3]. BLA acts as a critical gateway in mediating stress effects on the other aspects of memory formation by sending projections to structures such as the medial prefrontal cortex (mPFC) and hippocampus [4]. Moreover, the BLA is stimulated by fearful or anxiety-inducing stimuli, drug-related cues and/or stress coupled with insufficient inhibition from the mPFC [5]. BLA stimulation can induce overexpression of conditioned responses that may produce drug-seeking behavior [6], and craving in addiction [7,8] and increase the likelihood of relapse [2].

It has been shown that a response to stress begins with the activation of the hypothalamic pituitary-adrenal (HPA) axis which then leads to an increase in glucocorticoid (GC) release [4]. The binding of GCs the glucocorticoid receptors (GRs) mediates the adaptation to stress and regulates the termination of the stress response through negative feedback at the HPA axis level [9]. Stress or impaired GR feedback has been proposed as the leading cause of dysregulation of the HPA axis activity [4]. BLA is a brain region that is abundant in GRs [10] and critical for cue-induced drug-seeking behaviors [6]. Stress is a significant contributing factor to the initiation and maintenance of psychostimulant use, craving, and relapse [11,12]. Methamphetamine (METH) is a potent psychostimulant that reinforces behavioral response and leads to compulsive drug use and vulnerability to relapse [13,14]. There are no effective medications (FDA approved) to treat METH addiction [15,16,14]. Several reports indicate that use of METH associated with stress in humans, there are both clinical and laboratory evidences which indicated that stress could increase drug-using [17,18]. More recent studies have focused on the neurobiological and

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molecular mechanisms of stress, which are the core endogenous motivational factors of compulsive drug-taking and intense drug craving [19,20]. However, the mechanisms of stress-induced drug relapse have been discussed for many years [21]. Exposure to stressors can augment the rewarding properties of drugs of abuse in both animals and humans [22–24]. In a study on humans it was found that stress provoked a craving for cocaine under controlled laboratory conditions [17].

Preclinical studies on rodents demonstrate that chronic or repeated stress can result in increased rates of psychostimulant self-administration, like those of cocaine and amphetamine [25]. Thus, a better understanding of the susceptibility of stress-induced METH relapse may provide more effective treatment strategies for METH-induced psychiatric disorders [26]. Several studies have examined the involvement of glucocorticoids in drug-seeking behavior [27–29,6]. Our previous study showed that physical stress can significantly induce reinstatement of extinguished morphine-CPP, while the blocking of the glucocorticoid receptor in BLA prevents stress-induced reinstatement [4]. Reductions of craving and relapse are the most important aspects of therapeutic strategies for the treatment of psychostimulant addiction, while relapse still remains a major challenge in drug therapy. Therefore, the current study was conducted to investigate the involvement of BLA glucocorticoid receptors in acute and chronic stress-induced reinstatement of extinguished METH-induced CPP.

2. Materials & methods

2.1. Animal

The experiments were carried out on 81 male adult Wistar rats weighing 220–250 g (Pasteur Institute, Tehran, Iran). Animals were acclimated to the vivarium (a climate-controlled environment on a 12 h light/dark cycle), for at least one week prior to the onset of the experiments [30,31]. The animals were randomly assigned to different experimental groups, habituated to their new environment and handled for one week prior to the experimental treatment. Each treatment plan group contained of 5–8 animals. The tests were done between 9:00 a.m. and 3:00 p.m. Animal care types of procedures were conducted in compliance to the guide for the care and use of laboratory animals (National Institutes of Health Publication No. 80–23, revised 1996) and were conducted with the approval associated with an institutional animal care and use committee at the investigation and Ethics Committee of Hamadan University of Medical Sciences. Additionally, all efforts were made to minimize animals' suffering and to use only the number of animals necessary to produce reliable scientific data.

2.2. Drugs

In the present study, the following drugs were used: METH (Purity $>$ 98%, gift from the Iran drug control headquarters) that was dissolved in sterile saline, and RU38486, as a GRs antagonist (Tocris Bioscience, Bristol, United Kingdom) dissolved in 12% dimethyl sulfoxide (DMSO; Sigma-Aldrich, Germany). Control animals received either saline or 12% DMSO.

2.3. Surgical and microinjection procedures

All surgical procedures were conducted under ketamine 10% (100 mg/kg) and xylazine 2% (10 mg/kg) [32,33]. Stainless steel, 23-gauge guide cannulae were unilaterally implanted 1 mm above the intended site of injection according to atlas of rat brain. Stereotaxic coordinates for the BLA were 2.6 mm posterior to bregma, +4.5 mm lateral to the sagittal suture and 8.7 mm down from top of the skull. Cannulae were secured to anchor jewelers' screws with dental acrylic. To prevent clogging, stainless steel stylets were placed in guide cannulae until the animals were given BLA injection. Penicillin-G 200,000 IU/ml (0.2–0.3 ml/rat, single dose, intramuscular) were immediately administered after surgery. All animals were individually housed and allowed 5–7 days to recover from surgery [34,35]. For drug infusion, the animals were gently restrained by hand; the stylets were removed from the guide cannulae and replaced by 30-gauge injection needles (1 mm below the tip of guide cannula). Each injection unit was connected by polyethylene tubing (PE-20) to 1-μl Hamilton syringe. The RU38486 solutions or vehicle (12% DMSO) were administered slowly in a volume of 0.3 μl over a period of 60 s. Injection needles were left there for an additional 60 s to facilitate diffusion of the drugs, and then the stylets were reinserted into the guide cannulae.

2.4. Conditioning place preference apparatus and paradigm

2.4.1. Apparatus

The testing apparatus consisted of three plexiglas compartments. Two large compartments were identical in size (30 cm × 30 cm × 40 cm) but differed in covering and texture. Compartment A was white with black-colored horizontal stripes 2 cm wide on walls and also had a textured floor. Compartment B was black with vertical white stripes 2 cm vast and also had a smooth floor. The third compartment C was a red tunnel (30 cm × 15 cm × 40 cm) as start (null) box. It protruded from the rear of two large compartments and linked the entrances to them. In this apparatus, rats showed no consistent preference for none of the large compartments, which supports our unbiased CPP paradigm [36].

2.4.2. Behavioral testing

The CPP procedure consists of a 5-day schedule with three distinct phases: pre-conditioning, conditioning and post-conditioning. This method (unbiased design) was similar to that used in previous studies, Fig. 1A [36,4,3].

Pre-conditioning phase. During this phase (day 1), each animal was placed in start box (compartment C) and the guillotine door removed to allow access to entire apparatus for 10 min. Each animal displacement was recorded.

Conditioning phase. This phase started 1 day after pre-conditioning phase. It consisted of six, 30-min sessions (three saline and three drug pairing) in a 3-day schedule. These sessions were conducted twice each day (from day 2 to day 4) with a 6-h interval. On each day, separate groups of animals received a conditioning session with METH and another with saline instead of METH. During 30-min session intervals for METH/saline, the animals were confined to one compartment by closing the removable wall. Treatment compartment and order of presentation of METH /saline were counterbalanced for either group. In control group animals receive saline in two compartments.

Post-conditioning or testing phase. This phase was carried out on day 5 (the preference test day), 1 day after the last conditioning session, in a drug free state. Each animal was tested only once. For testing, the removable wall was raised and rat could access the entire apparatus for 10-min period. The time spent for each rat in both compartments during a 10-min period was recorded by a 3CCD camera (Panasonic Inc., Japan) and analyzed using the Maze router software, a video tracking system for automation of behavioral experiments (Science Beam company, Iran) in order to calculate the conditioning score (CPP score) as the preference criteria; the time spent in the drug-paired place minus the time spent in saline-paired place. No injection was given in the acquisition tests on post-conditioning day (test day). Total distance traveled for each animal was also recorded in control and experimental groups.

2.4.3. Methamphetamine extinction phase

Pursuing the preference test day, the animals were exposed to extinction training with access to all compartments in the CPP apparatus without any drug injection for a 30-min period each day. This process was repeated for each animal in the control and experimental groups until the calculated CPP scores in two consecutive times in extinction