Neuroscience 212 (2012) 77–85

THE AMNESIC EFFECT OF INTRA-CENTRAL AMYGDALA ADMINISTRATION OF A CANNABINOID CB1 RECEPTOR AGONIST, WIN55,212-2, IS MEDIATED BY A BETA-1 NORADRENERGIC SYSTEM IN RAT

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Abstract—In this study, we investigated effects of intra-central amygdala (intra-CeA) administrations of a cannabinoid agonist, WIN55,212-2 by itself and its interaction with β1-adrenoceptor agents on memory consolidation. We used a step-through inhibitory avoidance (IA) task to assess memory in male Wistar rats. The results showed that post-training intra-CeA administrations of different doses of WIN55,212-2 at doses of 0.1 and 0.25 μg/rat impaired memory consolidation (or induced amnesia) as revealed by a decrease in step-through latency on the test day. Post-training intra-CeA injections of a β1-adrenoceptor agonist, isoprenaline (0.01, 0.025, 0.05 μg/rat) by itself had no significant effect on memory consolidation, while at all doses prevented the amnesia induced by post-training injections of WIN55,212-2 (0.25 μg/rat). Although, post-training intra-CeA administrations of β1-adrenoceptor antagonist, atenolol alone at different doses (0.01, 0.025, 0.05 and 0.1 μg/rat) had no significant effect, but its co-administrations at doses of 0.05 and 0.1 μg/rat along with an ineffective dose of WIN55,212-2 (0.05 μg/rat) induced amnesia, and at dose of 0.1 μg/rat along with an effective dose of WIN55,212-2 (0.25 μg/rat) increased amnesia that induced by the later drug. Moreover, the improving effect of isoprenaline (0.025 μg/rat) on amnesia induced by WIN55,212-2 (0.25 μg/rat) was prevented by intra-CeA co-injections of atenolol at doses of 0.01 and 0.025 μg/rat. The present results suggest that a β1-adrenoceptor mechanism in the central amygdala (CeA) is involved in amnesia induced by post-training intra-CeA injections of WIN55, 212-2.

Key words: cannabinoid receptor, amnesia, isoprenaline, atenolol, central amygdala, rat.

INTRODUCTION

There is considerable evidence that the amygdala, through its projections to the other areas of the brain, is involved in modulating memory consolidation (for review see Roozendaal et al., 1999; McGaugh, 2000, 2002, 2004; McIntyre et al., 2011). According to previous reports and recent updates, it is the basolateral complex of the amygdala (BLA) that serves the above important role mentioned for the amygdala (for review see McGaugh, 2002; McIntyre et al., 2011; Schwabe et al., 2011). However, the central nucleus of the amygdala (CeA) receives convergent information from several other amygdaloid regions and generates behavioral responses that presumably reflect the sum of neuronal activity produced by different amygdaloid nuclei (Pitkanen et al., 1997, 2000). Inhibitory avoidance (IA) task, which has been widely used for the study of memory consolidation (Izquierdo and McGaugh, 2000; Izquierdo et al., 2006), is also known as a form of fear conditioning (Izquierdo et al., 1992; Cammarota et al., 2004). Besides the pivotal role of the amygdala in fear conditioning (Davis, 1992; Duvarci et al., 2011), it has been reported that the amygdala is also a critical site for influencing IA memory consolidation (Izquierdo and McGaugh, 2000; Izquierdo et al., 2006).

According to anatomical data, the amygdala receives dense noradrenergic innervations from the locus coeruleus (LC) (Valentino et al., 1993; Clayton and Williams, 2000; Berridge and Waterhouse, 2003). In addition, it has been shown that norepinephrine (NE) is a key neurotransmitter involved in the processes by which the amygdala regulates memory formation (Liang et al., 1990; Williams et al., 1998; McIntyre et al., 2002; Watanabe et al., 2003; Murchison et al., 2004). According to research, IA memory consolidation is modulated

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Abbreviations: BLA, basolateral amygdala; CeA, central amygdala; IA, inhibitory avoidance; LC, locus coeruleus; mPFC, medial prefrontal cortex; NE, norepinephrine; VTA, ventral tegmental area.

0306-4522/12 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2012.04.008

through, at least in part, a noradrenergic mechanism in the amygdala (Liang et al., 1990; Davis et al., 1994; Ferry et al., 1997; Cecchi et al., 2002). Of particular interest it has been reported that there is an increase in NE release after IA training (Galvez et al., 1996; Williams et al., 1998). It has also been reported that blockade of beta-adrenoceptors in the amygdala prevents the memory-modulating effects of other treatments that enhance and impair memory (McGaugh, 2004). Beta-adrenoceptors in the BLA have been shown to play an essential role in modulating memory consolidation (Roozendaal et al., 1999; Qu et al., 2008), but role of beta-adrenoceptors of the CeA in memory has not been fully elucidated.

There are considerable number of reports indicating that WIN55,212-2 as a CB1 cannabinoid agonist and other cannabinergic agents can influence memory functions (Ferrari et al., 1999; Beinfeld and Connolly, 2001; Antonelli et al., 2005).Cannabinoid receptors have been shown to be expressed abundantly within the amygdala. In addition, participation of the endocannabinoid system of the amygdala in the regulation of memory processes has been proposed (for review see Casswell and Marks, 1973; Marco and Viveros, 2009). In many studies, interaction between adrenoceptors and cannabinoid receptor functions has been reported (Singh and Das, 1976; Kataoka et al., 1987; Lichtman and Martin, 1991; Moshfegh et al., 2011). According to research, acute administration of WIN55,212-2 increased NE efflux in the frontal cortex and stimulates c-Fos expression in noradrenergic neurons of the LC (Oropeza et al., 2005). Ghiasvand et al. (2011) have recently reported that beta1-adrenoceptors of the CeA are involved in state-dependent memory induced by WIN55,212-2 in rat (Ghiasvand et al., 2011). The present study has aimed to investigate the effects of intra-central amygdala (intra-CeA) administrations of beta-1 noradrenergic agonist, isoprenaline and its antagonist, atenolol, on amnesic effect of WIN55,212-2 in rat. First, we examined amnesic effect of post-training intra-CeA injections of WIN55, 212-2. Then, the effects of intra-CeA administrations of isoprenaline and atenolol on the amnesia induced by WIN55,212-2 were evaluated.

EXPERIMENTAL PROCEDURES

Subjects

Adult male Wistar rats (Pasteur institute, Tehran, Iran) weighing 220–270 g at time of surgery were used. The animals were housed four per each cage upon their arrival in the laboratory (1 week before the experiments), with free access to food and water. They were kept at a constant temperature (22 ± 2 °C) under a 12/12 h light- dark-cycle (light beginning at 7:00 am). All experiments were carried out during the light phase of the cycle between 9:00 and 13:00. Each experimental group consisted of eight animals and each animal was tested only once. All efforts were made to minimize the number of animals used and their suffering. All procedures were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication 80–23, revised in 1996).

Surgery

A mixture of ketamine/xylazine (50 and 5 mg/kg, respectively) were injected intraperitoneally (ip) to induce anesthesia, then each rat was placed in a stereotaxic frame (Stoelting Instruments, USA) with flat-skull position. After a midline incision in the skin of the skull and retraction of underlying periosteum, bilateral stainless steel guide cannulae (22 gauge) were implanted until 2 mm above the CeA. Stereotaxic coordinates for the CeA were as follows: AP, −2.2 mm posterior to the bregma; L, ±4.2 mm lateral from the midline; V, −6 mm relative to the dura (Paxinos and Watson, 2007). The cannulae were anchored to the skull with a small screw and dental cement. Stainless steel stylets (27 gauge) were also inserted into the guide cannulae to maintain patency prior to microinfusions. Following all surgical preparations, animals were allowed to spend five days of recovery period before being submitted to behavioral testing.

Drugs and microinfusions

Atenolol was purchased from Daroopakhsh (Tehran, Iran), isoprenaline (isoproterenol) was a gift of Sigma (Poole, Dorset, UK), and WIN55,212-2 (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo[1,2,3-de)-1,4-benzoxazin-6-yl]-1-napthalenylmethanon) was purchased from 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 80. Fresh solutions of all drugs were prepared prior to experiments.

Bilateral microinfusions of all dugs into the central amygdala (intra-CeA) were done in a volume of 0.6 μl/rat (0.3 μl/side). An injection cannula (27-gauge), which was attached with a polyethylene tube to a 1 μl Hamilton syringe, lowered to extend 2 mm beyond the tip of the guide cannulae to the site of infusion (−8 mm from the skull). 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 the place for an additional 30 s to facilitate diffusion of the drugs from the tip of the injection cannula.

IA apparatus

A step-through IA apparatus consisted of two compartments of the same size (20 cm × 20 cm × 30 cm) was used. In the middle of the separating wall, a guillotine-like door (7 cm × 9 cm) 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 consisted of 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).

Behavioral procedures

All the behavioral testing sessions were performed between 8.00 and 12.00 (am). 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. 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