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THE MAJOR NEUROTRANSMITTER SYSTEMS IN THE BASOLATERAL AMYGDALA AND THE VENTRAL TEGMENTAL AREA MEDIATE MORPHINE-INDUCED MEMORY CONSOLIDATION IMPAIRMENT

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Abstract—In the present study, we investigated the possible participation of the endocannabinoid system in the basolateral amygdala and N-methyl-D-aspartate (NMDA) or GABA-A receptor neurotransmission in the ventral tegmental area in the memory consolidation impairment induced by morphine administration. To measure memory formation, step-through type passive avoidance apparatus was used with adult male Wistar rats. The results showed that intraperitoneal (i.p.) administration of morphine (3 and 6 mg/kg) after the successful training phase had an amnestic effect and induced memory consolidation impairment. After training, injection of a selective cannabinoid CB1 receptor agonist, arachydonilcyclopropylamide (ACPA; 0.4–0.6 ng/rat) plus systemic injection of an ineffective dose of morphine (0.5 mg/kg, i.p.) into the basolateral amygdala impaired memory consolidation suggesting the facilitatory effect of ACPA on morphine response. Also, the results showed that the injection of bicuculline, a GABA-A receptor antagonist (0.3–0.5 μg/rat) or NMDA (0.015–0.02 μg/rat) into the ventral tegmental area reversed ACPA-induced potentiation of morphine response and improved memory consolidation. It should be considered that the injection of ACPA into the basolateral amygdala and the injection of bicuculline or NMDA into the ventral tegmental area alone could not affect memory consolidation. Taken together, it seems that there is a functional interaction between the basolateral amygdala endocannabinoid system and the ventral tegmental area GABAergic- or glutamatergic neurotransmission in the modulation of morphine-induced memory consolidation impairment.

Key words: morphine amnesia, neurotransmitter systems, basolateral amygdala, ventral tegmental area, rat(s).

INTRODUCTION

Memory formation depends on the changes in the efficacy of synaptic connections between neurons in different brain regions (Schafe et al., 1999). There is a growing body of literature that recognizes the important role of opioid neuronal system(s) in memory processes (Ukai et al., 2001; Borbély et al., 2013). Much evidence shows that systemic or intracerebroventricular injection of μ- and Δ-opioid receptor agonists has an impairing effect on memory performance in the passive avoidance task (Ragozzino and Gold, 1994; Ukai and Lin, 2002; Khavandgar et al., 2003). Based on the results which show that the stimulation (McGaugh et al., 1996) or inhibition (Parent et al., 1992) of the basolateral amygdala improve or impair the passive avoidance memory respectively, it seems that this site plays a key role in memory formation (Roozendaal and McGaugh, 1996; McIntyre et al., 2003). Even though the basolateral amygdala has neurons whose projections can undergo learning-dependent plasticity (Grundemann and Luthi, 2015), it has been shown to strongly collaborate with other limbic structures such as the ventral tegmental area to mediate learning and memory processes (Phelps, 2004; Tsoory et al., 2008). The ventral tegmental area has a key effect on inhibitory memory formation (McGaugh, 2002; Pare, 2003). The basolateral amygdala and the ventral tegmental area are the two main components of the limbic circuit (Nazari-Serenjeh and Rezayof, 2013) which have functional connections to each other and are involved in memory retrieval (Spanis et al., 1999; Mahmoodi et al., 2011). The existence of neural circuits between the basolateral amygdala and the ventral tegmental area led to the involvement of these two brain regions in different stages of learning functions at the same time (Alvarez and Ruarte, 2004). In addition to the existence of connections between these targeted sites, Lisman and Grace (2005)

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Abbreviations: ANOVA, analysis of variance; ACPA, arachidonylcyclopropylamide; GABA, gamma-Aminobutyric acid; NMDA, N-methyl-D-aspartate; S.E.M, standard error of mean.

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also suggested that there is a functional loop between the ventral tegmental area and the hippocampus to regulate the information flow into the long-term memory. It should be considered that the ventral tegmental area, through a mechanism that is mediated via the interaction between the dopamine and GABA-A receptors, can play a critical role in modulating the hippocampal memory formation (Nazari-Serenjeh et al., 2011). Although GABA is a major inhibitory neurotransmitter in the central nervous system which has destructive effects on memory formation (Myhrer, 2003), glutamate is also a main excitatory neurotransmitter that has improving effects on many different types of learning (Riedel et al., 2003). Extensive research also suggests that the glutamatergic neurotransmission might be a substantial neurochemical agent of learning and memory (Niehaus et al., 2010). Glutamate exerts its effects through the activation of its N-methyl-D-aspartate (NMDA) receptors which are widely distributed in the ventral tegmental area (Wedzony et al., 2007). Interestingly, both glutamatergic NMDA and GABAergic GABA-A receptors are involved in memory formation which are intermediated by the basolateral amygdala and the ventral tegmental area (McGaugh et al., 1996; Hansell et al., 2006; Ahmadi et al., 2007).

Endocannabinoids as retrograde signaling molecules (Alger, 2002) have a significant neuromodulatory role in some behavioral actions including locomotion, anxiety, learning and memory (for review see Lichtman et al., 1995; Pacher et al., 2006). These compounds carry out their functions through cannabinoid CB1 and CB2 receptors which are members of the G-protein-coupled receptor superfamily (Howlett et al., 2010). Cannabinoid CB1 receptors are widely distributed in neuronal pre-synaptic membrane at various brain regions such as the amygdala, the hippocampus and the prefrontal cortex (Pistis et al., 2004). Evidence suggests that memory consolidation can be enhanced through the activation of the basolateral amygdala cannabinoid CB1 receptors, while inhibition of these receptors impairs memory formation (Campolongo et al., 2009). Previous literature has emphasized the existence of a functional interaction between endocannabinoid and GABAergic systems in cognition. For example, McGaugh et al. (2002) reported that the endocannabinoids may increase memory consolidation through regulating the activity of GABAergic system in the basolateral amygdala (McIntyre et al., 2002). Moreover, the activation of cannabinoid CB1 receptors led to a decrease in GABA release in the basolateral amygdala (Katona et al., 1999). Several studies have shown that NMDA and GABA-A receptors in the basolateral amygdala and the ventral tegmental area are involved in learning and memory processes (Salinas and McGaugh, 1996; Roozendaal et al., 1996; Ferreira et al., 2005). Considering that GABA-A and NMDA receptors have a high expression in both the basolateral amygdala and the ventral tegmental area (Saito et al., 2010) and with regard to the existence of a cooperative interaction between these two regions in mediating memory consolidation (Nazari-Serenjeh and Rezayof, 2013), the main purpose of the present study was to assess: (1) the effect of the activation of the basolateral amygdala endocannabinoid system in morphine-induced memory consolidation impairment and also, (2) the existence of any interaction between the ventral tegmental area NMDA or GABA-A receptors and the basolateral amygdala endocannabinoid system in the memory impairment induced by morphine administration.

EXPERIMENTAL PROCEDURES

Animals

The experiments were carried out using adult male Wistar rats (from Faculty of Pharmacy, Tehran University of Medical Sciences) weighting 220–240 g at the time of the surgery. Each 4 rats were kept in standard cages with free access to food and water and in a temperature-controlled environment (22 ± 2 °C) under a 12:12-h light/dark cycle (lights on at 07:00 AM). The experiments were done during the light phase between 09:00 h and 12:00 h. Each group consisted of seven animals. All experimental procedures were conducted in accordance with the guidelines for the care and use of laboratory animals observed at the School of Medicine, Tehran University of Medical Sciences and were in agreement with institutional guidelines for the care and use of laboratory animals (NIH, publication No. 85-23, revised 2010; European Communities Directive 86/609/EEC).

Surgical and infusion procedures

The rats were anesthetized intraperitoneally (i.p.) with ketamine-xylazine mixture (100 mg/kg and 5 mg/kg, respectively). The skull was fixed via ear bars in a stereotaxic apparatus (Stoelting Co., Wood Dale, Illinois, USA) in a flat-skull position, and two 22-gauge stainless steel guide cannulas were implanted in the basolateral amygdala and the ventral tegmental area unilaterally in the right hemisphere according to the atlas of Paxinos and Watson (2007). Periosteum was retracted after making a midline incision on head skin. Stereotaxic coordinates for the basolateral amygdala were AP: −2.8 mm; ML: ±5 mm; and DV: 8.5 mm and for the ventral tegmental area were AP: −6 mm; ML: ±0.7 mm; and DV: 8.7 mm from the top of the skull (Paxinos and Watson, 2007). The cannulas were affixed to the skull with dental cement. The stainless steel stylets (27 gauge) were placed in the guide cannulas to prevent clogging until the injection day. One week before the experiments, the rats were allowed to recover from the surgery and handled 5 min during the recovery period. For drug injections, the stylets were removed from the guide cannulas and replaced by 27-gauge injection needle (1 mm below the tip of the guide cannula). The injection needles should be 1 mm longer than the guide cannulas because the guide cannulas were implanted 1 mm above the basolateral amygdala and the ventral tegmental area. The injection unit was connected to a 2-μl Hamilton syringe through a polyethylene tube. The basolateral amygdala was injected with a 0.3-μl drug and the ventral tegmental area with a 0.2-μl drug for over a 60-s period. To prevent backflow of the

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