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Research report

Activation of endocannabinoid system in the rat basolateral amygdala improved scopolamine-induced memory consolidation impairment

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HIGHLIGHTS

- Intra-peritoneal injection of scopolamine impaired memory consolidation.
- Intra-BLA injections of ACPA or AM251 had no effect on memory consolidation alone.
- Intra-BLA injection of ACPA reversed the amnesic effect of scopolamine.
- Intra-BLA injection of AM251 potentiated the amnesic effect of scopolamine.
- Blockade of the BLA CB1 receptors, inhibited the reversal effect of ACPA.

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ABSTRACT

The current study was designed to examine the involvement of cannabinoid CB1 receptors in the basolateral amygdala (BLA) in scopolamine-induced memory impairment in adult male Wistar rats. The animals were bilaterally implanted with the cannulas in the BLA and submitted to a step-through type passive avoidance task to measure the memory formation. The results showed that intraperitoneal (i.p.) administration of different doses of scopolamine (0.5–1.5 mg/kg) immediately after the training phase (post-training) impaired memory consolidation. Bilateral microinjection of the cannabinoid CB1 receptor agonist, arachydonilcyclopropylamide (ACPA; 1–4 ng/rat), into the BLA significantly improved scopolamine-induced memory consolidation impairment. On the other hand, co-administration of AM251, a cannabinoid CB1 receptor antagonist (0.25–1 ng/rat, intra-BLA), with an ineffective dose of scopolamine (0.5 mg/kg, i.p.), significantly impaired memory consolidation and mimicked the response of a higher dose of scopolamine. It is important to note that post-training intra-BLA microinjections of the same doses of ACPA or AM251 alone had no effect on memory consolidation. Moreover, the blockade of the BLA CB1 receptors by 0.3 ng/rat of AM251 prevented ACPA-induced improvement of the scopolamine response. In view of the known actions of the drugs used, the present data pointed to the involvement of the BLA CB1 receptors in scopolamine-induced memory consolidation impairment. Furthermore, it seems that a functional interaction between the BLA endocannabinoid and cholinergic muscarinic systems may be critical for memory formation.

Abbreviations: CB1, cannabinoid receptor; i.p., intraperitoneal; BLA, basolateral amygdala; ACPA, arachydonilcyclopropylamide; ACh, acetylcholine; AD, alzheimer disease; LTP, long-term potentiation; AP, anterior-posterior; AM251, (N-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide); DMSO, dimethylsulphoxide; STL, step-through latency; ANOVA, analysis of variance; SEM, standard error of mean.

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1. Introduction

Extensive evidence indicates that cholinergic system is critically involved in cognitive processes including memory consolidation and retrieval (for review see [1]). It seems that the learning of new information functionally modifies cholinergic neurons via increasing the release of acetylcholine (ACh) in the hippocampus for memory formation in a spatial memory task [2]. In addition, the inhibition of acetylcholine esterase, which breaks ACh into choline and acetic acid, has been suggested to decrease the acetylcholine turnover and enhance the synaptic efficiency for improving cognitive functions (for review see [3]). Increasing the concentrations of ACh in the synaptic cleft via activating muscarinic M1 receptors triggers phospholipase C-activating G-protein coupled signaling pathway [4] to facilitate the induction of long-term potentiation [5]. There are a large number of reports indicating that scopolamine, as a muscarinic receptor antagonist, induces synaptic dysfunction and cognitive deficit [6] that may be considered as a useful pharmacological tool to produce an animal model of Alzheimer disease (AD; [7,8]). In order to support the hypothesis that muscarinic receptor-mediated activation may be necessary for the regulation of cognitive processes, Ferrari-DiLeo and co-workers [9] using immunoblotting method showed that the number of cortical muscarinic receptors decreased in AD patients. A recent systematic literature review of preclinical and clinical studies concluded that M_1- and M_4-selective modulators may be potential new treatments for cognitive disorders [10].

A great deal of previous research into the basolateral amygdala (BLA), as a main part of the amygdaloid complex, has focused on its involvement in emotional memory processes that are special category of memory involving the storage of the emotional event into long-term memory (for review see [11,12]). The available evidence suggests that the consolidation of emotional memories transiently increased the BLA ACh concentrations through mediating the muscarinic receptors [13] to promote synaptic plasticity (for review see [14]). Imunohistochemistrical studies have also shown that muscarinic receptors that are located on the neural cells in the BLA may be associated with the creation of memory consolidation [15]. Considering that the blockade of muscarinic receptors has been reported to impair the BLA-based memory [12], it seems that these receptors play a crucial role in inducing long-term potentiation (LTP; [16]) and memory formation [17]. In addition, muscarinic M1 receptors regulate the release of other neurotransmitters from presynaptic terminals in the BLA [18] which may affect the function of this site. Using passive avoidance learning or contextual fear conditioning, Vazdarjanova and McGaugh [19] reported that the actiovation of the BLA cholinergic system modulated memory consolidation. This hypothesis is also supported by Boccia et al. [20] who suggested that there is a neuromodulatory cholinergic system within the BLA in fear learning and fear extinction.

The endocannabinoid system, which consists of endocannabinoids and cannabinoid receptors, modulates cognitive performance and emotional processes [21]. N-Arachidonylethanolamine (Anandamide) and 2-arachidonoylglycerol (2-AG), which are known as endocannabinoids, have been detected in different brain regions and nuclei of humans and rats [22,23]. It is well known that endocannabinoids inhibit the release of several neurotransmitters such as acetylcholine [24] and glutamate [25] via triggering the signaling pathways of pre-synaptic CB1 receptors. Arachidonylcyclopropylamide (ACPA) as an analog of anandamide also selectively binds to CB1 receptors to activate neuronal endocannabinoid system in a selective and potent manner [26]. In the BLA, high distribution of CB1 receptors was detected in the axon terminal membrane of the presynaptic side of various neurotransmitter synapses [27–29]. It should be considered that endocannabinoids may be involved in modulating emotional and non-emotional memory stages including acquisition, consolidation and retrieval [30]. The results of animal studies have shown that the activation of the amygdala CB1 receptors may affect memory consolidation [31,32]. In view of the fact that pre-synaptic CB1 receptors are expressed on cholinergic nerve terminal to inhibit acetylcholine release [33], a functional interaction between endocannabinoid and cholinergic systems has been suggested in the hippocampus [34], the BLA [35] and the medial septum [36]. Considering that cannabinoid CB1 [37] and muscarinic cholinergic [38] receptors are well known to mediate synaptic plasticity which is necessary for learning and memory processes, the present study aimed to investigate the involvement of BLA CB1 receptors in the effect of scopolamine, as a non-selective potent antagonist of muscarinic receptors, on memory consolidation. Therefore, the present study tries to critically evaluate the interactions between cannabinoid and cholinergic muscarinic systems on the BLA-based memory formation in a passive avoidance memory task.

2. Materials and methods

2.1. Animals

Male adult Wistar rats (Razi Institute, Tehran, Iran) weighing 200 ± 20 g at the time of surgery were used in the present study. The animals were kept four per plastic cage in a room under controlled temperature ($22 \pm 2^{\circ}$ C) and a 12:12 h light/dark cycle (lights on at 07:00 a.m.) and had free access to food and water. All experiments were conducted during the light portion of the cycle between 9:00 a.m. and 1:00 p.m. Each experimental group consisted of seven animals and each animal was used only once. All procedures were performed in accordance with institutional guideline for animal care and use. The Research and Ethics Committee of Tehran University of Medical Sciences, School of Advanced Technologies in Medicine approved the experimental protocol.

2.2. Surgical and infusions procedures

After induction of anesthesia with ketamine (100 mg/kg, i.p.) and xylazine (5 mg/kg i.p.), the animal were placed in the stereotaxic instrument (Stolting, USA) and two steel guide cannulae made of 22 gauge stainless steel tubing were implanted bilaterally 1 mm above the basolateral nucleus of the amygdala (BLA) according to the atlas of Paxinos and Watson [39]. Coordinates for the BLA were anterior-posterior (AP): −2.5 mm (Fig. 1); lateral: ±4.8 mm; and vertical: 8.3 mm. The cannulae were fixed on the skull surface with a small screw and dentistry cement. To prevent clogging, stainless steel stylets (27 gauge) were also inserted into the guide cannulae until the animals were given the drug injection. The rats were allowed to spend 1 week of recovery period before being submitted to behavioral testing.

For bilateral microinjections of the drugs into the BLA (intra-BLA), each stylet was removed from the guide cannula and replaced by 27-gauge injection needle (1 mm below the tip of the guide cannulae) attached with a polyethylene tube to a 1-μl Hamilton syringe. The BLA was injected with a 0.3 μl per each side (0.6 μl/rat) solution over a 60-s period. In order for the drug to completely release in the tissue spaces, the injection needle was kept at the injection site for 60 s before removal.

2.3. Drugs

The drugs used in the present study included scopolamine (Sigma, USA), ACPA (Arachidonylcyclopropylamide) and AM251 (N-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide) (Tocris, Bristol, UK). Scopolamine was dissolved in sterile 0.9% saline immediately