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Research report

Interaction between hippocampal serotonin and cannabinoid systems in reactivity to spatial and object novelty detection

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HIGHLIGHTS

- Activation or blockade of 5-HT4 receptors impaired spatial memory.
- CB1 receptors activation impaired spatial and novelty, while their blockade facilitated novelty.
- Subthreshold dose of RS67333 restored ACPA response.
- Subthreshold dose of RS23597 differently affect ACPA signaling.
- Effective dose of AM251 blocked the effect of 5-HT4 agents on ACPA responses.

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ABSTRACT

Functional interaction between cannabinoid and serotonin neuronal systems have been reported in different tasks related to memory assessment. The present study investigated the effect of serotonin 5-HT4 agents into the dorsal hippocampus (the CA1 region) on spatial and object novelty detection deficits induced by activation of cannabinoid CB1 receptors (CB1Rs) using arachidonylcyclopropylamide (ACPA) in a non-associative behavioral task designed to forecast the ability of rodents to encode spatial and non-spatial relationships between distinct stimuli. Post-training, intra-CA1 microinjection of 5-HT4 receptor agonist RS67333 or 5-HT4 receptor antagonist RS23597 both at the dose of 0.016 μg/mouse impaired spatial memory, while cannabinoid CB1R antagonist AM251 (0.1 μg/mouse) facilitated object novelty memory. Also, post-training, intraperitoneal administration of CB1R agonist ACPA (0.005-0.05 mg/kg) impaired both memories. However, a subthreshold dose of RS67333 restored ACPA response on both memories. Moreover, a subthreshold dose of RS23597 potentiated ACPA (0.01 mg/kg) and reversed ACPA (0.05 mg/kg) responses on spatial memory, while it potentiated ACPA response at the dose of 0.005 or 0.05 mg/kg on object novelty memory. Furthermore, effective dose of AM251 restored ACPA response at the higher dose. AM251 blocked response induced by combination of RS67333 or RS23597 and the higher dose of ACPA on both memories. Our results highlight that hippocampal 5-HT4 receptors differently affect cannabinoid signaling in spatial and object novelty memories. The inactivation of CB1 receptors blocks the effect of 5-HT4 agents into the CA1 region on memory deficits induced by activation of CB1Rs via ACPA.

1. Introduction

Among the numerous serotonin (5-HT) receptors, the 5-HT4 receptors, appear to be of particular importance in cognitive processes [1–3]. High density of 5-HT4 receptors in the limbic sys-

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tem, mainly on the pyramidal neurons of hippocampus, suggests a potential role in learning and memory [4,5]. These receptors belong to the superfamily of G-protein coupled receptors which are positively coupled to adenylate cyclase (AC) [6] leading to the intracellular accumulation of cAMP. This increase of cAMP observed after the application of 5-HT4 agonists contributes to the neuronal excitability of pyramidal cells of hippocampus by inhibiting potassium channels [7,8]. Behavioral studies have shown that 5-HT4 selective agonists improve social learning in rats [9], enhance accuracy performances in delayed matching tasks in macaques [10] and enhance place and object recognition in young adult rats [11]. Indeed, a pro-cognitive effect for 5-HT4 receptor agonists in several phases of memory has been confirmed [12].

Taken in the light of the existence of a high density of cannabinoid CB1 receptors (CB1Rs) in the hippocampal formation and their role in learning and memory, it is not surprising that hippocampus is strongly modulated by *exo*- and *endo*-cannabinoids [13–15]. The CB1R is a G-protein coupled receptor (Gi/o class G proteins) and is primarily located on presynaptic terminals [16]. CB1R agonists inhibit adenylyl cyclase and subsequently decrease intracellular cAMP levels. Also, activation of CB1Rs mediates a wide range of effects on ion channels, including voltage-dependent calcium and potassium channels [17]. Together, CB1R-mediated intracellular signaling results in reduced cellular excitability and reduced neurotransmitter release [18]. Because CB1Rs are located on both GABAergic and glutamatergic terminals, their activation leads to the suppression of both inhibitory and excitatory synaptic transmission in the brain [19–21]. A number of behavioral procedures including reward-based radial maze tasks, the water maze, contextual fear conditioning, passive avoidance and novel object recognition have shown that CB1R agonists disrupt different stages of spatial and non-spatial memory, namely acquisition, consolidation and retrieval processes [22,23]. Radial maze studies examining spatial memory indicate that administration of Δ^9-THC (the major psychoactive constituent of cannabis) and other CB1R agonists usually impair rodents' spatial working memory abilities [24,25]. Galanopoulos et al. reported that WIN55, 212-2 (0.3 mg/kg) disrupted non-associative learning, different aspects of short- and long-term recognition memory (storage and retrieval) and retention of spatial memory. They proposed that learning deficit induced by WIN55, 212-2 appeared to be CB1R dependent since pretreatment with SR141716A (0.03 mg/kg) prevented the WIN55, 212-2 response [15].

Accumulating evidence suggests that there is an interaction between CB1Rs and the 5-HT neuronal system [2,13]. We previously reported the involvement of 5-HT4 receptors in learning deficits induced by activation of CB1Rs in passive avoidance step-down task [26] and fear conditioning apparatus [27]. As we know these tasks are dependent upon the encoding and retrieval of emotionally aversive and inherently stressful training events. While these types of memories are important, they do not reflect the typical day-to-day experiences or memories most commonly affected in human disease. In addition, stress hormone release alone can modulate memory and thus obscure or artificially enhance these types of tasks. To avoid these sorts of confounds, we have utilized tasks testing animals' memory for object location and novel object recognition. These tasks involve exploiting rodents' innate preference for novelty, and are inherently not stressful [28]. Therefore, the purpose of the present study was to investigate whether hippocampal 5-HT4 receptors are involved in spatial and object novelty detection memory consolidation impairments induced by CB1R agonist arachidonylcyclopropylamide (ACPA) in a non-associative task.

2. Material and methods

2.1. Subjects

Male NMRI mice, weighing 30–35 g at the time of surgery, were used. They were kept in plastic cages, with free access to water and food, and maintained under a 12/12 regular light/dark cycle. All experiments were performed during the light phase. Mice for this study were obtained from Institute of cognitive science (ICSS, Tehran, Iran). Animals were handled in accordance with approved institutional animal care procedures and NIH guidelines. Mice were housed in an animal facility different from the room where experiments took place.

2.2. Surgery

Mice were subjected to surgery under anesthesia (pharmacological mixture: ketamine hydrochloride 50 mg/kg plus xylazine 5 mg/kg). They were implanted bilaterally with 22-gauge stainless steel cannulae, aimed at the CA1 region of the hippocampus (AP: −2 mm posterior to the bregma; ML: ± 1.6 from midline; V: −1.5 from to dura). Cannulae coordinates were derived from [29]. Stylets were used to prevent the cannulae from clogging. The tip of the cannulae was left 1 mm above the desired injection site. Mice were allowed to recover for 5–7 days following surgery.

2.3. Drugs and microinjections

ACPA (arachidonylcyclopropylamide; N-(2-cyclopropyl) 5Z, 8Z, 11Z, 14Z eicosatertraenanmide) and AM251 (N-(piperidin-1-yl)5-(4-iodophenyl) 1-(2,4-dichlorophenyl)4-methyl-1H-Pyrazole-3-carboxamide) purchased from (Tocris, Bristol, UK) were used. ACPA was obtained in Tocrisolve™ (a soya oil and water emulsion) and diluted directly into sterile 0.9% saline and at the volume of 10 ml/kg administered intraperitoneally. AM251 was dissolved in dimethylsulphoxide (DMSO; up to 10% v/v) and sterile 0.9% saline and a drop of Tween 80 and was bilaterally injected into the CA1 region in a volume of 0.5 μl/side. 5-HT4 receptor agonist RS67333 and 5-HT4 receptor antagonist RS23597 were dissolved in sterile 0.9% saline and microinjected into the CA1 region.

Microinfusions were delivered to hand restrained, conscious animals. Stylets were withdrawn and 27-gauge injectors were inserted into cannulae. These injectors were connected via polyethylene tubing to 1 μl Hamilton microsyringes. Total volume infused was 0.5 μl in 60 s, into either CA1 region. Injectors were left in place for an additional minute, to allow diffusion of the solutions into the tissue.

2.4. Description of the experimental apparatus

The apparatus used for the study was the same as in previous reports [30,31]. It was a metal circular box, 60 cm in diameter with a 20 cm-high wall. The floor was painted white and divided into 16 identical sectors by black lines. The apparatus was placed into a soundproof room surrounded by a visually uniform environment. The apparatus was illuminated by a red light (80 W) located on the ceiling. A video camera above the field was connected to a video recorder and a monitor. Five objects were simultaneously present in the open field: A cube (a metal-plated parallelepiped, measuring 7 cm × 4 cm × 4 cm with irregular holes distributed on all sides), a cone (a plastic cone on a transparent cylinder base, 8 cm in diameter and 6 cm high), a ladder (a small plastic white ladder, 5 cm wide and 16 cm high, having 10 steps connected to the two parallel arms, 3 cm thick), a cylinder (a black cylinder, 10 cm high and 4 cm in diameter, having a 2 cm in diameter hole on the top), a glass of steel