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Differential role of the hippocampal endocannabinoid system in the memory consolidation and retrieval mechanisms

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Abstract

CB1 cannabinoid receptors are abundantly expressed in the brain, with large concentrations present in the hippocampus, a brain structure essential for memory processing. In the present study, we have investigated the possible modulatory role of the endocannabinoid system in the dorsal hippocampus upon the different phases of memory processing of an aversive task. AM251, a selective antagonist of CB1 receptors, and anandamide, an endogenous agonist of cannabinoid receptors, were bilaterally infused into the dorsal hippocampus of male Wistar rats either before training, immediately after training, or before test in the step-down inhibitory avoidance (IA) task. Results showed that pre-training infusion of CB1 drugs did not influence the acquisition of the task. In contrast, post-training infusion of the CB1 antagonist disrupted while the antagonist facilitated memory consolidation of IA. The post-training results demonstrate that memory consolidation depends on the integrity of the endocannabinoid system in the CA1 region of the dorsal hippocampus. While we still have no direct proof of endocannabinoids released there after an aversive task such as IA, these results suggests that (a) AM251 acts blocking the binding of endogenously released cannabinoids and (b) exogenously supplemented anandamide may be adding its contribution to the action of the endogenously released pool. Considering our data and the higher density of CB1 receptors present in the GABAergic interneurons, we propose them as the putative target of the endocannabinoid modulation of memory, a hypothesis that needs to be proven. In addition, pre-test infusion of the CB1 receptor antagonist facilitated while infusion of the agonist did not affect memory retrieval of IA. The completely opposite action of the same drug upon memory at the post-training (consolidation) and pre-test (recall) contexts suggests that some durable change took place in the CA1 region during the consolidation process that modified the logical attributes of the pharmacological response, i.e., the drug response changed from memory disruption to memory facilitation. A similar phenomenon was previously described by us in the M4 cholinergic muscarinic subsystem in the hippocampus for the same task (Diehl, F., Fürstenau, L. O., Sanchez, G., Camboim, C., de Oliveira Alvares, L., Lanziotti, V. B., et al. (2007). Facilitatory effect of the intra-hippocampal pretest administration of MT3 in the inhibitory avoidance task. *Behavioral Brain Research*, *177*(2), 227–231), but the biological nature of such change in the local neural circuitry remains to be investigated.

Keywords: Memory; Anandamide; AM251; CB1 receptors; Hippocampus; Step-down inhibitory avoidance

1. Introduction

In recent years, neurobiologists have increasingly turned their attention towards the endocannabinoid system as an important brain modulatory system able to influence a whole plethora of physiological functions. Examples are pain, appetite control, motor functions, and learning and

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memory (Ameri, 1999; Iversen, 2000). CB1 cannabinoid receptors are widely expressed throughout the brain, with a high concentration in the basal ganglia, cerebellum, neocortex, and the hippocampus (Herkenham et al., 1991), a brain structure essential for memory processes (Izquierdo & Medina, 1995; Squire, 1992). From the five known endogenous substances that bind to the cannabinoid receptors (Bisogno, Ligresti, & Di Marzo, 2005), the two most studied are anandamide (AEA) and 2-arachidonoyl glycerol, both acting upon CB1 receptors, leading to the inhibition of adenylyl cyclase and reduced Ca^{2+} conductance, especially through N-type VGCCs (Davies et al., 2002; Iversen, 2003.

Endocannabinoids are released from post-synaptic neurons and predominantly target pre-synaptic receptors (Wilson & Nicoll, 2002). In the hippocampus, CB1 receptors are mainly located in GABAergic, inhibitory interneurons (Egertová & Elphick, 2000; Katona et al., 1999; Tsou, Mackie, Sanudo-Pena, & Walker, 1999). Recently, however, three independent groups have reported the presence of pre-synaptic CB1 receptors on hippocampal glutamatergic axon terminals, acting as glutamate release inhibitors (Katona et al., 2006; Kawamura et al., 2006, & Takahashi & Castillo, 2006, but the expression of CB1 receptors in these excitatory synapses is at least 20 times lower than in the inhibitory pre-synaptic sites of this brain structure (Kawamura et al., 2006).

Several studies have shown that the administration of cannabinoid agonists impairs memory formation (Davies et al., 2002; Hampson & Deadwyler, 1998; Hernandez-Tristan, Arevalo, Canals, & Leret, 2000; Lichtman, Dimen, & Martin, 1995), while, on the other hand, studies with selective CB1 antagonists are less consistent, with some showing memory impairment (Arenos, Musty, & Bucci, 2006), others improvement (Lichtman, 2000; Takahashi, Pamplona, & Fernandes, 2005) and in others no effect at all (Da Silva & Takahashi, 2002; Davies et al., 2002; Hampson & Deadwyler, 1998). Furthermore, the inhibition of anandamide metabolism enhanced the acquisition of the Morris water maze task (Varvel, Wise, Niyuhire, Cravatt, & Lichtman, 2006). This variety of cognitive effects may be attributed to the fact that CB1 receptors are ubiquitously distributed in the CNS, and most of the aforementioned studies have basically employed systemic infusions of cannabinoids (De Oliveira Alvares et al., 2005).

Previously, we have reported a memory deficit in an aversive behavioral task after a direct intrahippocampal infusion of the selective CB1 antagonist AM251 (De Oliveira Alvares et al., 2005) and, consistent with that, the exact same treatment was able to disrupt LTP induction in the CA1 region of the hippocampus (De Oliveira Alvares et al., 2006). The objective of the present study was to perform a wider investigation of the modulatory role of the hippocampal endocannabinoid system in the different phases of memory formation and retrieval processes; in particular, we investigated the effects of AM251 or anandamide administered into the dorsal hippocampus upon the acquisition/learning session (infusing before the training session), the memory consolidation phase (infusing after the training session) or the memory recall (infusing before the test session) in a step-down inhibitory avoidance task in rats.

2. Experimental procedures

2.1. Animals

Three hundred male Wistar rats (age 2–3 months, weight 210–300 g) from our breeding colony were used in the experiments. The animals were housed in plastic home-cages, 4–5 per cage, under a 12-h light/dark cycle and at constant temperature (24±1 °C), with water and food available *ad libitum*; behavioral tests were performed at daytime only. All the experiments were performed in strict accordance to the Brazilian law, to the recommendations of the Brazilian Society for Neurosciences and Behavior (SBNeC) and the International Brain Research Organization (IBRO), and are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (publication No. 85-23, revised 1985).

2.2. Stereotaxic surgery

Rats were deeply anesthetized by an i.p. injection of ketamine/xylazine (75 and 10 mg/Kg, respectively) and bilaterally implanted with 27-gauge guide cannulae aimed at AP −4.2 mm (from bregma), LL ±3.0 mm, DV 1.8 mm, positioned just 1.0 mm above the region of the dorsal hippocampus (according to Paxinos & Watson, 1998). Before the behavioral tests, animals were allowed a recovery period of 5–7 days.

2.3. Drugs and administration

AM251 and anandamide were purchased from Tocris and dissolved in a vehicle solution of 8% DMSO in 0.1 M of a phosphate-buffered saline (PBS) solution. Previous observations showed that this concentration of DMSO does not cause any effect by itself when compared to a PBS-infused group (data not shown). At the time of infusion, a 30-gauge infusion cannulae was fitted into the guide cannulae, its tip protruding 1.0 mm from the cannulae end, and aimed at the pyramidal cell layer of CA1 in the dorsal hippocampus. A volume of 0.5 μl of either AM251 (5.5 ng per side/hemistructure), anandamide (0.17, 1.75, and 17.5 ng per side/hemistructure injected), or vehicle (control group) was mechanically and slowly infused (during 90 s) at the following time points: 15 min before training (experiments 1 and 2), immediately after training (experiments 3 and 4), or 15 min before tests (experiments 5–7). The doses of AM251 were selected based on previous studies of our group (De Oliveira Alvares et al., 2005; De Oliveira Alvares et al., 2006); a dose–response study was performed for the anandamide with doses loosely derived from the literature. The 15 min time lapse proved to be effective for and is well within the window defined by other authors studying similar drugs (such as anandamide) infused either 10 min (Barros et al., 2004) or 30 min (Egashira, Mishima, Iwasaki, & Fujiwara, 2002).

2.4. Step-down inhibitory avoidance

The step-down inhibitory avoidance task was carried out in an automatically operated, brightly illuminated box (Albarsch; 15 W lamp), in which the left extreme of the grid—42.0 × 25.0 cm grid of parallel 0.1 cm caliber bronze bars spaced 1.0 cm apart—was covered with a 7.0 cm wide, 5.0 cm high formica-covered platform. Animals were placed on the platform and the latency to step-down, placing their four paws on the grid, was recorded. In the training session, immediately after stepping down, a 0.5 mA, 3.0 s scrambled footshock was delivered (only in experi-