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Posttraining activation of CB1 cannabinoid receptors in the CA1 region of the dorsal hippocampus impairs object recognition long-term memory

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ABSTRACT

Evidence indicates that brain endocannabinoids are involved in memory processing. However, the participation of CB1 and CB2 cannabinoid receptors in recognition memory has not been yet conclusively determined. Therefore, we evaluated the effect of the posttraining activation of hippocampal cannabinoid receptors on the consolidation of object recognition memory. Rats with infusion cannulae stereotaxically aimed to the CA1 region of the dorsal hippocampus were trained in an object recognition learning task involving exposure to two different stimulus objects. Memory retention was assessed at different times after training. In the test sessions, one of the objects presented during training was replaced by a novel one. When infused in the CA1 region immediately after training, the non-selective cannabinoid receptor agonist WIN-55,212-2 and the endocannabinoid membrane transporter inhibitor VDM-11 blocked long-term memory retention in a dose-dependent manner without affecting short-term memory, exploratory behavior, anxiety state or the functionality of the hippocampus. The amnesic effect of WIN-55,212-2 and VDM-11 was not due to state-dependency and was completely reversed by co-infusion of the CB1 receptor antagonist AM-251 and mimicked by the CB1 receptor agonist ACEA but not by the CB2 receptor agonists JWH-015 and palmitoylethanolamide. Our data indicate that activation of hippocampal CB1 receptors early after training hampers consolidation of object recognition memory.

1. Introduction

During the last decade it has become evident that endogenous ligands for cannabinoid receptors, or endocannabinoids, are released in an activity-dependent manner in areas of the brain crucial for memory processing, such as the hippocampus, the amygdala and the pre-frontal cortex (Chiu & Castillo, 2007; Hashimotodani, Ohno-Shosaku, & Kano, 2007; Lovinger, 2007). In fact, impairment of cognition and memory is perhaps the most pervasive alteration induced by acute exposure to Δ9-tetrahydrocannabinol (Δ9-THC) or to synthetic cannabinoids (DiForti, Lappin, & Murray, 2007; Kalant, 2004; Ranganathan & D'Souza, 2007; Sullivan, 2000).

So far, two subtypes of cannabinoid receptors, namely CB1 (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990) and CB2 receptors (Munro, Thomas, & Abu-Shaar, 1993) have been identified in mammals. CB1 receptors are conspicuously expressed in the peripheral and central nervous systems, particularly in the hippocampal formation, while CB2 receptors are present in non-neuronal tissues, mainly in cells of the immune system. Therefore, it is assumed that virtually all neuropsychological actions of endocannabinoids are controlled by CB1 receptors. However, as pointed recently (Thiemann, Fletcher, Ledent, Molleman, & Hasenöhrl, 2007), most experiments supporting this hypothesis have been carried out using behavioral paradigms involving either appetitive motivation or high emotional arousal, such as the radial maze, the Morris water maze, one-trial inhibitory avoidance and fear conditioning (de Oliveira Alvares et al., 2005, 2006; Lichtman, 2000; Marsicano et al., 2002; Varvel & Lichtman, 2002) but the role of the endocannabinoid system in recognition memory processing has not been yet examined in detail. The few reports on the participation of this system on recognition memory published so far have employed mutant mice (Bilkei-Gorzo et al., 2005; Maccarrone et al., 2002; Reibaud et al., 1999), systemic administration of cannabinoids (Terranova et al., 1996) or a combination of these two experimental approaches (Bura, Castane, Ledent, Valverde, & Maldonado, 2007). Nonetheless, experiments with mutant animals do not allow to distinguish whether CB1 receptors are involved in acquisition, consolidation or retrieval and the systemic administration of cannabinoids can induce non-specific behavioral and physiolog-

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ical effects very difficult to control for. A better understanding of the neuronal circuitry involved in endocannabinoid signaling during the different phases of memory processing requires local drug administration into certain brain structures at very specific pre- or posttraining times.

Object recognition (OR) memory confers the ability to discriminate between novel and familiar entities. Neuropsychological assessment of amnesic patients and experiments in laboratory animals suggest that the functional integrity of the temporal lobe, including the hippocampal formation, is essential for processing this type of information (Clark, Zola, & Squire, 2000; Ennaceur & Delacour, 1988; Logothetis & Scheinberg, 1996; Riesenhuber & Poggio, 2002). In agreement with these findings, recent evidence indicates that consolidation of OR long-term memory (LTM) requires protein synthesis and mTOR activation in the CA1 region of the hippocampus (Miskyw et al., 2007; Rossato et al., 2007). Here we assess the effect of the intra-hippocampal infusion of different synthetic cannabinoids and endocannabinoid membrane transporter inhibitors in OR LTM memory consolidation.

2. Materials and methods

2.1. Surgery and drugs infusion procedure

Naive male Wistar rats (3-month-old 250–280 g) raised in our own facilities or bought at FEPPS (Fundação Estadual de Produção e Pesquisa em Saúde do Rio Grande do Sul, Porto Alegre, Brazil) were used. The animals were housed 5 to a cage and kept with free access to food and water under a 12/12 light/dark cycle, with light onset at 7:00 AM. The temperature of the animal's room was maintained at 22–24 °C. To implant them with indwelling cannulae, rats were deeply anesthetized with thiopental (i.p., 30–50 mg/kg) and 27-gauge cannulae stereotaxically aimed to the CA1 region of the dorsal hippocampus, in accordance with coordinates (A −4.0, L ±3.0, V 1.8) taken from the atlas of Paxinos and Watson (1986). Animals were allowed to recover from surgery for 4 days before submitting them to any other procedure. At the time of drug delivery, 30-gauge infusion cannulae were tightly fitted into the guides. Infusions (0.8 μl/side) were carried out over 60 s and the cannulae were left in place for 60 additional seconds to minimize backflow. The placement of the cannulae was verified postmortem: 2–4 h after the last behavioral test, 0.8 μl of a 4% methylene-blue solution were infused as described above and the extension of the dye 30 min thereafter was taken as an indication of the presumable diffusion of the vehicle or drug previously given to each animal. Only data from animals with correct cannulae implants were analyzed. All procedures were conducted in accordance with the 'Principles of laboratory animal care' (NIH publication No. 85-23, revised 1996). Every effort was made to reduce the number of animals used and to minimize their suffering.

2.2. Object recognition paradigm

The object recognition task was conducted in an open field arena (50 × 50 × 50 cm) built of polyvinyl chloride plastic, plywood and transparent acrylic as described (Miskyw et al., 2007; Rossato et al., 2007). Before training, animals were habituated to the experimental arena by allowing them to freely explore it during 20 min per day for 4 days in the absence of any other behaviorally relevant stimulus. The stimulus objects were made of metal, glass or glazed ceramic. There were several copies of each object, which were used interchangeably. Glued to the base of each object was a round piece of Velcro, which was used to fix the object to the arena's floor. The role (familiar or novel) as well as the relative position of the 2 stimulus objects were counterbalanced and randomly permuted for each experimental animal. The open field arena and the stimulus objects were cleaned thoroughly between trials to ensure the absence of olfactory cues. Exploration was defined as sniffing or touching the stimulus object with the nose and/or forepaws. Sitting on or turning around the objects was not considered exploratory behavior. A video camera was positioned over the arena and the rats' behavior was recorded using a video tracking and analysis system for later evaluation. The experiments were performed by an observer blind to the treatment condition of the animals.

For training, rats were placed in the open field containing two different objects and left to freely explore them for 5 min. The test session was performed 180 min (to analyze short-term memory) or 24 h after training (to evaluate long-term memory retention). In the test sessions one of the familiar objects was randomly replaced by a novel one, and rats were reintroduced into the open field for five additional minutes. The compounds to be tested were bilaterally infused into the CA1 region of the dorsal hippocampus (0.8 μl/side) immediately after training. Data were analyzed using one-sample Student's t-test.

2.3. One-trial, step-down inhibitory avoidance

Rats were trained in a one-trial, step-down inhibitory avoidance task as previously described (Bevilaqua, da Silva, Medina, Izquierdo, & Cammarota, 2005). Briefly, the training apparatus was a 50 × 25 × 25 cm plexiglass box with a 5 cm-high, 8 cm-wide and 25 cm long platform on the left end of a series of bronze bars which made up the floor of the box. For training, animals were gently placed on the platform facing the left rear corner of the training box. When they stepped down and placed their four paws on the grid, received a 2 s, 0.5 mA scrambled footshock. Memory retention was evaluated in a non-reinforced test session carried out at 24 h after training. Data were analyzed using ANOVA.

2.4. Training in the spatial version of the MWM

The water maze was a black circular pool (200 cm in diameter) conceptually divided in four equal imaginary quadrants for the purpose of data analysis. The temperature of the water was 21–23 °C. Two centimeter beneath the surface of the water and hidden from the rat's view was a black circular platform (12 cm in diameter). It had a rough surface, which allowed the rat to climb onto it easily once its presence was detected. The swimming path was recorded using a video camera mounted above the center of the pool and analyzed using a video tracking and analysis system. The water maze was located in a well-lit white room with several posters and other distal visual stimuli hanging on the walls to provide spatial cues. A curtain separated the water maze room from the room where the computer setup was installed and where the animals were temporarily housed during the behavioral sessions. Training was carried out during five successive days. On each day rats received eight consecutive training trials during which the hidden platform was kept in a constant location. A different starting location was used on each trial, which consisted of a swim followed by a 30-s platform sit. Any rat that did not find the platform within 60 s was guided to it by the experimenter. The intertrial interval (ITI) was 30-s. During the ITI, rats were carefully dried with a towel by the experimenter. Data were analyzed using repeated-measures two-way ANOVA.

2.5. Open field and plus maze

To analyze exploratory and locomotor activities, the animals were placed on the left rear quadrant of a 50 × 50 × 39 cm open field with black plywood walls and a brown floor divided into 12 equal imaginary squares. The number of line crossings and the number of rearings were measured over 5 min and taken as an indicator of locomotor and exploratory activities, respectively. To evaluate anxiety state, rats were exposed to an elevated plus maze as detailed in Pellow, Chopin, File, and Briley (1985). The total number of entries into the four arms, the number of entries and the time spent into the open arms were recorded over a 5 min session. Data were analyzed using ANOVA.

2.6. Drugs

WIN-55,212-2, ACEA, AM-251, VDM-11, JWH-015 and palmitoylethanolamide were purchased from Sigma–Aldrich (St Louis, MO, USA). All drugs were dissolved in saline containing 0.1% DMSO (pH 7.2) and were infused at room temperature. The doses utilized were determined based on pilot studies and on previous results showing the effect of different cannabinoids on memory and hippocampal plasticity.

3. Results

To determine the role of hippocampal cannabinoid receptors on recognition memory, adult male Wistar rats were trained in an OR task involving exploration of two different objects (Miskyw et al., 2007; Rossato et al., 2007). Immediately after that, they received bilateral intra-CA1 infusions of WIN-55,212-2, a non-selective cannabinoid receptor agonist (1–10 nmol/side; Hohmann, Tsou, & Walker, 1999; Pop, 1999; Showalter, Compton, Martin, & Abood, 1996), VDM-11 (1–100 pmol/side; D'Argenio et al., 2006; de Lago et al., 2004), a cannabinomimetic that selectively inhibits endocannabinoid cellular uptake, or vehicle (VEH; 0.1% DMSO in saline). LTM retention was evaluated 24 h later. During the LTM retention test session animals were exposed for 5 min to one of the objects presented in the training session together with a novel object. Rats that received VEH or low doses of WIN-55,212-2 (1 and 2.5 nmol/side) and VDM-11 (1 and 10 pmol/side) explored the novel object significantly longer than the familiar one (Fig. 1; $t_{(8)} = 4.95$, $p < .01$ for VEH; $t_{(8)} = 3.50$, $p < .01$ for 1 nmol/side WIN-55,212-2 and $t_{(8)} = 2.36$, $p < .05$ for 2.5 nmol/side WIN-55,212-2 in one-sam-