Neuroscience Research 84 (2014) 60–63

Contents lists available at ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

Rapid Communication

Intrahypothalamic injection of cannabidiol increases the extracellular levels of adenosine in nucleus accumbens in rats

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ARTICLE INFO

Article history:
Received 20 February 2014
Received in revised form 1 April 2014
Accepted 22 April 2014
Available online 4 May 2014

Keywords:
Microdialysis
Adenosine
Hypothalamus
HPLC
Wakefulness

ABSTRACT

Cannabidiol (CBD) is a constituent of *Cannabis sativa* that promotes wakefulness as well as enhances endogenous levels of wake-related neurotransmitters, including dopamine. However, at this date, the effects of CBD on the sleep-inducing molecules, such as adenosine (AD), are unknown. Here, we report that intrahypothalamic injection of CBD (10 μg/1 μL) increases the extracellular levels of AD collected from nucleus accumbens. Furthermore, the pharmacodynamic of this drug shows that effects on the contents of AD last 2 h post-injection. These preliminary findings suggest that CBD promotes the endogenous accumulation of AD.

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Δ^9-Tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD) are two major constituents of *Cannabis sativa* (Mechoulam et al., 1970). Whereas Δ^9-THC is a psychoactive molecule that induces stereotypical behaviors (Adams and Martin, 1996), CBD displays non-psychoactive effects. However, recent evidence has pointed out that this cannabinoid promotes pharmacological effects (Mechoulam et al., 2007). For instance, systemic administrations of CBD modulate the sleep–wake cycle (Monti, 1977; Carlini and Cunha, 1981; Nicholson et al., 2004; Hsiao et al., 2012; Chagas et al., 2013). Moreover, microinjections of CBD either into lateral ventricles or lateral hypothalamus in rats enhance wakefulness (Murillo-Rodríguez et al., 2006, 2008, 2011). The significant increase in alertness caused by this cannabinoid has been linked to the enhancement of wake-related neurotransmitters, including dopamine (DA) collected from nucleus accumbens (AcbC; Murillo-Rodríguez et al., 2006, 2011).

Sleep modulation involves the activity of several endogenous molecules, such as adenosine (AD; Brown et al., 2012). Current evidence shows that extracellular concentrations of AD are enhanced in association with natural or prolonged alertness in multiple brain areas (Porkka-Heiskanen et al., 2000, 2002; Blanco-Centurión et al., 2006; Huang et al., 2011). Since CBD promotes waking and AD is increased during alertness, we tested the hypothesis that this cannabinoid increases extracellular levels of AD in rats. To test this hypothesis, rats received an intrahypothalamic injection of CBD and microdialysis samples were collected from AcbC to analyze extracellular contents of AD. We find that CBD enhances levels of AD during 2 h post-injection.

Male wistar rats ($n = 10$; 250–300 g) were singly housed in polycarbonate cages (48.26 cm × 26.67 cm × 20.32 cm; Harlan Laboratories, Mexico) at constant temperature (21 ± 1 °C), and under a controlled light–dark cycle (lights on: 07:00–19:00 h) with access to Purina Rat Chow (Harlan Laboratories, Mexico) and tap water *ad libitum*. The animal care was followed according to our Institutional Animal Care and Use Committee, the Ethical Use of Animals from the Mexican Institutes of Health Research (DOF. NOM-062-Z00-1999) as well as the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23; revised 1996).

For pharmacological studies, CBD was prepared in vehicle (polyethylene glycol/saline; 5:95, v/v) as previously reported (Murillo-Rodríguez et al., 2006, 2008, 2011). All reagents, materials and compounds were purchased from Sigma–Aldrich (St Louis, MO.

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http://dx.doi.org/10.1016/j.neures.2014.04.006
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USA). Under deep anesthesia (acepromazine [0.75 mg/kg] xylazine [2.5 mg/kg] and ketamine [22 mg/kg, ip]), animals were placed in a stereotaxic apparatus for surgeries which consisted into the implantation of miniature screws placed on the skull. Additionally, it was implanted a microdialysis guide-cannula (IC guide. Bioanalytical Systems [BAS], West Lafayette, IN. USA) placed in the AcbC (coordinates: $A = +1.2$ and $L = 2.0$, $H = -7.0$; with reference to Bregma; Paxinos and Watson, 2005) as well as a cannula placed in the lateral hypothalamus (LH; $A = -3.3$ mm, $L = \pm 1.6$ mm, $H = -8.2$ mm, with reference to Bregma; Paxinos and Watson, 2005). At the end of the implantation procedures, all rats received amoxicillin (100 mg/kg, sc) and they were placed individually in microdialysis bowls for recovery and habituation to the experimental conditions. The entire surgical procedure was followed according to previous reports (Murillo-Rodríguez et al., 2008, 2011).

One week after surgery, the microdialysis stylet was withdrawn from the microdialysis guide-cannula and microdialysis probe (1 mm in length. Polyacrylonitrile, MWCO = 30,000 Daltons; 340 im OD; BAS. West Lafayette, IN, USA) was inserted at 07:00 h. Right after this procedure, artificial cerebrospinal fluid (ACSF: NaCl [147 mM], KCl [3 mM], CaCl [1.2 mM], MgCl [1.0 mM], pH 7.2) was continuously perfused through a minitube (0.65 O.D. mm ID × 0.12 mm; BAS. West Lafayette, IN, USA) attached to a 2.5 mL syringe (BAS, West Lafayette, IN, USA) and it was perfused using a pump (BAS Bee, West Lafayette, IN, USA) at a flow rate of 0.25 μL/min. Microdialysis probe was stabilized before the experiment via perfusing ACSF during a 24 h period as previously reported (Murillo-Rodríguez et al., 2006, 2011). After the stabilization period of the microdialysis probe, treatments were given at 07:00 h trough the cannula implanted in the LH. Experimental trials consisted in microinjection of either vehicle (1 μL; $n = 5$), or CBD (10 μg/1 μL; $n = 5$). Microinjections were done manually at 07:00 h using a 10 μL Hamilton microsyringe (1 μL/min) as previously reported (Murillo-Rodríguez et al., 2006, 2008). Once injections were carried out, animals were placed back into their microdialysis bowls.

Throughout the experiment, rats were given free access to food and water, and they were housed under a controlled room temperature (21 ± 1 °C), as well as the light–dark cycle (lights on: 07:00–19:00 h). Right after the intrahypothalamic injections, microdialysis samples (10 μL) were collected during the first 20 min of each hour during a total time of 4 h. Once collected, dialysates were stored (−80 °C) for further HPLC analysis. All microdialysis procedures were developed as published previously

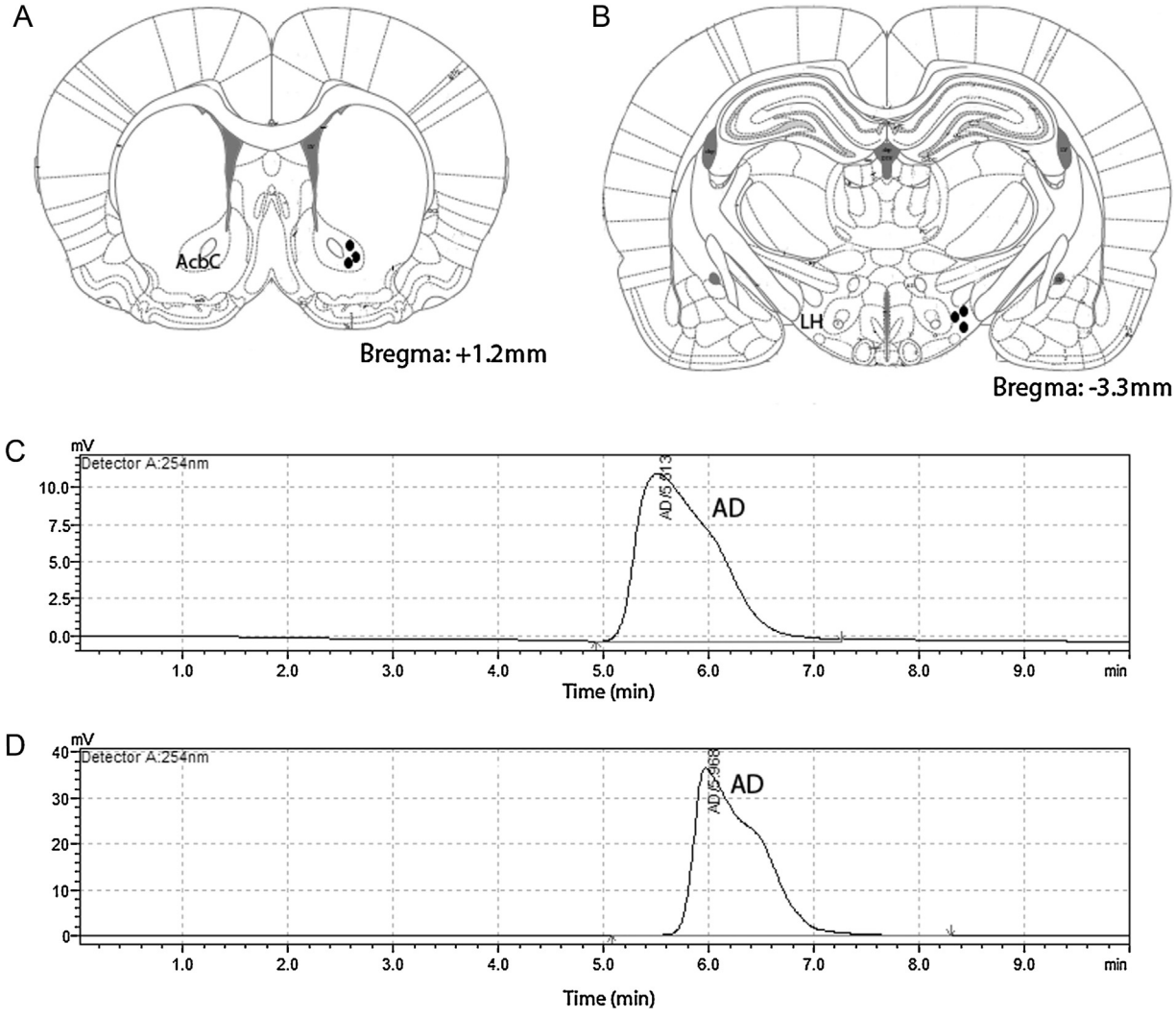

Fig. 1. Schematic representation of the position of cannula in rat brain as well as the chromatographic data obtained from HPLC. *Panel A* shows the position of the microdialysis probe placed into nucleus accumbens whereas *Panel B* displays the position of the cannula into lateral hypothalamus as represented by the black dots. Chromatogram of content of adenosine obtained from microdialysis samples and analyzed by HPLC from a rat that received vehicle (*Panel C*) as well as a CBD-treated animal (*Panel D*). *Abbreviations*: AcbC, nucleus accumbens; AD, adenosine; LH, lateral hypothalamus. Drawings in *Panels A* and *B* were taken from the rat brain atlas of Paxinos and Watson (2005).

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