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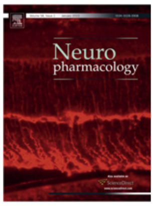

Δ^9-tetrahydrocannabinol is a full agonist at CB1 receptors on GABA neuron axon terminals in the hippocampus

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ABSTRACT

Marijuana impairs learning and memory through actions of its psychoactive constituent, delta-9-tetrahydrocannabinol (Δ^9-THC), in the hippocampus, through activation of cannabinoid CB1 receptors (CB1R). CB1Rs are found on glutamate and GABA neuron axon terminals in the hippocampus where they control neurotransmitter release. Previous studies suggest that Δ^9-THC is a partial agonist of CB1Rs on glutamate axon terminals in the hippocampus, whereas its effects on GABA terminals have not been described. Using whole-cell electrophysiology in brain slices from C57BL6/J mice, we examined Δ^9-THC effects on synaptic GABA IPSCs and postsynaptic GABA currents elicited by laser-induced photo-uncaging (photolysis) of α-carboxy-2-nitrobenzyl (CNB) caged GABA. Despite robust inhibition of synaptic IPSCs in wildtype mice by the full synthetic agonist WIN55,212-2, using a Tween-80 and DMSO vehicle, Δ^9-THC had no effects on IPSCs in this, or in a low concentration of another vehicle, randomly-methylated β-cyclodextrin (RAMEB, 0.023%). However, IPSCs were inhibited by Δ^9-THC in 0.1% RAMEB, but not in neurons from CB1R knockout mice. Whereas Δ^9-THC did not affect photolysis-evoked GABA currents, these responses were prolonged by a GABA uptake inhibitor. Concentration-response curves revealed that the maximal effects of Δ^9-THC and WIN55,212-2 were similar, indicating that Δ^9-THC is a full agonist at CB1Rs on GABA axon terminals. These results suggest that Δ^9-THC inhibits GABA release, but does not directly alter $GABA_A$ receptors or GABA uptake in the hippocampus. Furthermore, full agonist effects of Δ^9-THC on IPSCs likely result from a much higher expression of CB1Rs on GABA versus glutamate axon terminals in the hippocampus.

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1. Introduction

Marijuana (*Cannabis sativa*) is a psychoactive plant that is widely used throughout the world. The marijuana plant contains approximately 70 cannabinoid compounds (Burns and Ineck, 2006). However, Δ^9-THC is thought to be the main psychoactive cannabinoid responsible for mood alterations, feelings of euphoria, and cognitive impairments that are hallmarks of its effects in humans (Gaoni and Mechoulam, 1964). Δ^9-THC exerts its effects on cellular processes by activating cannabinoid CB1 and CB2 receptors that are members of the G-protein coupled receptor family (Pertwee, 1997). However, compared to CB2Rs, CB1Rs are highly expressed in brain tissue, and mediate most of the central actions of marijuana in humans (Huestis et al., 2001). The discovery of the cannabinoid receptors (Matsuda et al., 1990; Munro et al., 1993), and identification of endogenous ligands (Devane et al., 1992; Mechoulam et al., 1995; Stella et al., 1997) demonstrated the existence of a brain endocannabinoid system.

An important cognitive effect of marijuana in humans is the impairment of memory through the disruption of information encoding and recall of newly acquired information (Abel, 1971a,b; Ranganathan and D'Souza, 2006; Wilson et al., 1994). In addition, working memory is necessary for normal performance in many cognitive tasks in humans and animals, and it relies upon intact hippocampal function (Hampson and Deadwyler, 1998; Ranganathan and D'Souza, 2006). In animals, spatial working memory is profoundly impaired by Δ^9-THC given systemically, injected directly into the hippocampus (Lichtman et al., 1995), or by exposure to marijuana smoke (Niyuhire et al., 2007), and this appears to be mediated exclusively by CB1Rs in the hippocampus (Varvel and Lichtman, 2002; Wise et al., 2009). In addition to

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Abbreviations: aCSF, artificial cerebrospinal fluid; APV, D-(−)-2-amino-5-phosphonopentanoic acid; $CB1^{+/+}$, wildtype C57BL6/J mice; $CB1^{-/-}$, C57BL6/J mice lacking the gene for the CB1 receptor; CB1R, cannabinoid type 1 receptor; CNB, of α-carboxy-2-nitrobenzyl; Δ^9-THC, delta-9-tetrahydrocannabinol; DIC, differential interference contrast microscopy; DNQX, 6,7-dinitroquinoxaline-2,3-dione; Nd:YAG, neodymium-doped yttrium aluminum garnet; LTD, long-term depression; LTP, long-term potentiation; RAMEB, randomly-methylated β-cyclodextrin; WIN55212-2, (*R*)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate.

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disruption of spatial working memory, non-spatial operant learning and memory is also disrupted by Δ^9-THC (Hampson and Deadwyler, 1998; Heyser et al., 1993). In support of the central role of the hippocampus in mediating the cognitive effects of marijuana, this structure contains a dense population of CB1Rs that mediate the effects of exogenous and endogenous cannabinoids (Herkenham et al., 1990; Katona et al., 1999), and a recent study has provided strong evidence for the involvement of CB1Rs on GABA axon terminals in the effects of Δ^9-THC on spatial memory in mice (Puighermanal et al., 2009).

At the cellular level, cannabinoids presynaptically inhibit the release of both GABA and glutamate in the hippocampus and throughout the brain (Gerdeman and Lovinger, 2001; Hoffman et al., 2003; Hoffman and Lupica, 2000, 2001; Levenes et al., 1998; Robbe et al., 2001; Wilson and Nicoll, 2002). Furthermore, it is likely that this is the primary means through which cannabinoids alter hippocampal neuronal network activity (Hajos et al., 2000; Robbe et al., 2006). Another mechanism that may be involved in the disruption of memory by cannabinoids is the inhibition of forms of synaptic plasticity, such as long-term potentiation and depression (LTP, LTD), that are proposed cellular correlates of learning and memory (Bliss and Collingridge, 1993; Lynch, 2004). Acutely, synthetic cannabinoids, endocannabinoids, and Δ^9-THC block LTP in the CA1 region of the hippocampus in vitro (Misner and Sullivan, 1999; Nowicky et al., 1987; Stella et al., 1997), and long-term exposure to Δ^9-THC in vivo can block LTP in vitro during withdrawal, despite the absence of detectable tissue levels of Δ^9-THC at the time of LTP induction (Hoffman et al., 2007; Fan et al., 2010).

Although ample evidence implicates the hippocampus as a site for the actions of Δ^9-THC in the disruption of memory, surprisingly little is known of its physiological actions on specific intact neural pathways in the CNS. Although some studies have described the effects of Δ^9-THC in adult hippocampal brain slices (Foy et al., 1982; Nowicky et al., 1987), these were conducted prior to identification of CB1Rs, and the development of antagonists and CB1R knockout animals. More recent functional studies with Δ^9-THC in vitro have utilized immature hippocampal neurons maintained in culture to demonstrate that this phytocannabinoid can act as an agonist, partial agonist or antagonist at CB1Rs coupled to the inhibition of glutamate release (Kelley and Thayer, 2004; Roloff and Thayer, 2009; Shen and Thayer, 1999; Straiker and Mackie, 2005). However, these studies have not examined the effects of Δ^9-THC in mature hippocampal circuits, nor have they directly examined its effects on GABAergic neurotransmission, despite the much higher density of CB1Rs on these axon terminals compared to glutamate terminals (Kawamura et al., 2006; Marsicano and Lutz, 1999). In addition to the paucity of information of Δ^9-THC effects on GABA release in the hippocampus, it has also been proposed to inhibit the uptake of GABA and other neurotransmitters (Banerjee et al., 1975; Coull et al., 1997; Maneuf et al., 1996). Furthermore, our limited understanding of the effects of Δ^9-THC in specific CNS circuits might be due to its high lipophilicity and poor solubility in aqueous media (Banerjee et al., 1975; Jarho et al., 1998).

In an attempt to identify the specific sites at which Δ^9-THC acts to alter hippocampal function, we have examined its effects on GABA release in mature hippocampal slices obtained from CB1$^{+/+}$ and CB1$^{-/-}$ mice, and compared these actions to those of a full synthetic agonist. Additionally, since Δ^9-THC proved to be highly insoluble for in vitro use, we describe procedures permitting is solubilization.

2. Methods

2.1. Animals

Animal protocols were approved by the Animal Care and Use Committee of the NIDA Intramural Research Program, and were conducted in strict accordance with NIH guidelines to minimize the number of animals used in these studies. Wildtype (WT, CB1$^{+/+}$) and CB1 receptor knockout (KO, CB1$^{-/-}$) littermate C57BL6\J mice (4–10 weeks) were obtained from the NIDA Intramural Research Program transgenic facility colony. These animals were descendants of 3 heterozygous (CB1$^{+/-}$) breeding pairs, donated by Dr. Andreas Zimmer and the National Institute of Mental Health (Bethesda, MD, USA). Genotyping was performed by Charles River Laboratories (Raleigh, NC, USA). Several of the observations made in mice were confirmed using a smaller number of wildtype male Sprague–Dawley rats (4–6 weeks of age), obtained from Charles River Laboratories (Raleigh, NC, USA).

2.2. Brain slice preparation

Hippocampal brain slices were prepared as previously described (Hoffman and Lupica, 2000). Briefly, animals were killed by cervical dislocation followed by decapitation. The brains were rapidly removed and immersed in cold (4 °C), oxygenated high-sucrose, low-Ca^{2+}-containing artificial cerebrospinal fluid (aCSF) of the following composition (mM): NaCl, 87; KCl, 2.5; $MgCl_2$, 7; $CaCl_2$, 0.5; NaH_2PO_4, 1.25; glucose, 25; sucrose 75; $NaHCO_3$, 25. Coronal slices were then cut at 280 μm thickness using a vibrating tissue slicer (VT1000S, Leica Instruments, Germany). Hemi-sectioned brain slices containing the hippocampus were then incubated in a solution composed of 50% high-sucrose and 50% normal aCSF of the following composition (mM): NaCl, 126; KCl, 3.0; $MgCl_2$, 1.5; $CaCl_2$, 2.4; NaH_2PO_4, 1.2; glucose, 11.0; $NaHCO_3$, 26, saturated with 95% O_2 and 5% CO_2, at room temperature for ≥90 min before recordings. Individual brain slices were placed into a low-volume (~300 μL) recording chamber integrated into the fixed stage of a differential interference (DIC) contrast microscope (Olympus America, Center Valley, PA, USA), and submerged in normal aCSF of a fixed volume (~15 mL) that was recirculated at 2 mL/min using a peristaltic pump. This solution was continuously bubbled with 95% O_2 and 5% CO_2, and maintained at 30–32 °C using a solution heater (TC-324B, Warner Instruments, Hamden, CT).

2.3. Electrophysiology

Whole-cell electrophysiological recordings were performed using an Axopatch 200B amplifier (Axon Instruments, Foster CA) and electrodes pulled from borosilicate glass (1.5 mm O.D., 0.86 mm I.D., Sutter Instruments, Burlingame, CA). Electrodes were filled with a solution containing (mM): D-gluconic acid, 125.0; HEPES, 10.0; EGTA, 1.0; $CaCl_2$, 0.1; KCl, 10.0; Mg^{2+}-ATP, 1.0; Na^+-GTP, 0.2, or $CsCH_3SO_3$, 100; CsCl, 60; EGTA 0.2; HEPES, 10; $MgCl_2$, 2.0; Mg^{2+}-ATP, 1.0; Na^+-GTP, 0.3. All intracellular solutions also contained the quaternary lidocaine derivative, QX-314 (Sigma, St. Louis, MO; 1 mg/ml), to block action potentials only in the recorded cells. The internal solutions were adjusted to pH 7.2–7.4 using CsOH. Series resistance was monitored with a −10 mV voltage step (200 ms), initiated every 30 s. Series resistance measurements and the synaptic and photolysis-evoked current amplitudes were all plotted versus time on the same graph to determine whether the observed changes in these currents were associated with altered cellular access resistance. Only cells maintaining stable series resistance (<10% change over the duration of the recording) were included in analyses. Data were directly acquired to a personal computer using an A/D board (Instrutech ITC-18, Bellmore, NY) and Windows-based software (WinWCP, courtesy of Dr. John Dempster, University of Strathclyde, Glasgow, UK; http://spider.science.strath.ac.uk/sipbs/software_ses.htm).

GABAergic currents were measured in hippocampal CA1 pyramidal neurons identified under visual control using differential interference contrast (DIC) video-microscopy and infrared illumination. To measure evoked IPSCs, CA1 pyramidal neurons were voltage clamped at −20 mV when using the gluconic acid intracellular solution (outward currents), and at −70 mV when the $CsCH_3SO_3$ intracellular solution (inward currents) was used. IPSCs were evoked using a custom-built bipolar formvar-insulated nickel–chromium stimulating electrode placed near stratum pyramidale. Synaptic IPSCs and photolysis-evoked $GABA_A$-mediated Cl^- currents were pharmacologically isolated using the glutamate receptor antagonists D-(−)-2-amino-5-phosphonopentanoic acid (APV, 40 μM) to block NMDA receptors, and 6,7-dinitroquinoxaline-2,3-dione (DNQX, 10 μM) to block AMPA/kainate receptors. Synaptic IPSCs were evoked once per minute and alternated with photolysis-evoked postsynaptic GABA currents throughout the duration of the recordings. Photolysis was performed using a solid state, pulsed Nd:YAG laser (Minilite I, Continuum, Santa Clara, CA, USA). The laser output beam was channeled to a 40× water immersion microscope objective using a 400 μm diameter fiber optic light guide. This arrangement yielded a circular illumination area, approximately 25 μm in diameter. This spot was focused upon the pyramidal neuron soma to uncage α-carboxy-2-nitrobenzyl (CNB)-caged GABA (Invitrogen, Carlsbad, CA, USA). Once whole-cell access was obtained, the objective was focused upon the pyramidal neuron and the laser output was adjusted to yield a postsynaptic response that was similar in amplitude to a 50% of maximum electrically-evoked synaptic response. The settings of the laser and the electrical stimulator were then left undisturbed throughout the remainder of the experiment.

2.4. Drugs

WIN55,212-2 and AM251 were purchased from Tocris-Cookson (Ballwin, MO, USA). DNQX, APV, picrotoxin and randomly-methylated β–cyclodextrin (RAMEB)