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Evaluation of prevalent phytocannabinoids in the acetic acid model of visceral nociception

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ABSTRACT

Considerable preclinical research has demonstrated the efficacy of Δ^9-tetrahydrocannabinol (Δ^9-THC), the primary psychoactive constituent of *Cannabis sativa*, in a wide variety of animal models of pain, but few studies have examined other phytocannabinoids. Indeed, other plant-derived cannabinoids, including cannabidiol (CBD), cannabinol (CBN), and cannabichromene (CBC) elicit antinociceptive effects in some assays. In contrast, tetrahydrocannabivarin (THCV), another component of cannabis, antagonizes the pharmacological effects of Δ^9-THC. These results suggest that various constituents of this plant may interact in a complex manner to modulate pain. The primary purpose of the present study was to assess the antinociceptive effects of these other prevalent phytocannabinoids in the acetic acid stretching test, a rodent visceral pain model. Of the cannabinoid compounds tested, Δ^9-THC and CBN bound to the CB_1 receptor and produced antinociceptive effects. The CB_1 receptor antagonist, rimonabant, but not the CB_2 receptor antagonist, SR144528, blocked the antinociceptive effects of both compounds. Although THCV bound to the CB_1 receptor with similar affinity as Δ^9-THC, it had no effects when administered alone, but antagonized the antinociceptive effects of Δ^9-THC when both drugs were given in combination. Importantly, the antinociceptive effects of Δ^9-THC and CBN occurred at lower doses than those necessary to produce locomotor suppression, suggesting motor dysfunction did not account for the decreases in acetic acid-induced abdominal stretching. These data raise the intriguing possibility that other constituents of cannabis can be used to modify the pharmacological effects of Δ^9-THC by either eliciting antinociceptive effects (i.e., CBN) or antagonizing (i.e., THCV) the actions of Δ^9-THC.

1. Introduction

Cannabis has been used for thousands of years as a therapeutic agent for pain relief, as well as for recreational purposes. Delta-9-Tetrahydrocannabinol (Δ^9-THC) is the most prevalent and well characterized constituent of the approximately 70 cannabinoids identified in cannabis (Elsohly and Slade, 2005), and largely accounts for the psychoactive properties of this plant. Δ^9-THC produces antinociceptive effects in a wide range of preclinical assays of pain, including tail-flick, hotplate, inflammatory, cancer, neuropathic, and visceral nociceptive models (Martin et al., 1984; Formukong et al., 1988; Burstein et al., 1988; Compton et al., 1991; Varvel et al., 2005). Visceral pain (e.g., myocardial ischemia, upper gastrointestinal dyspepsia, irritable bowel syndrome, and dysmenorrhea) is one of the most common forms of pain. Importantly, both cannabinoid receptors are expressed in the viscera (Matsuda et al., 1990; Bouaboula et al., 1993; Munro et al., 1993; Galiegue et al., 1995; Wright et al., 2005). Intraperitoneal administration of acetic acid or various other chemicals causes distension of the hollow walled muscular organs and the release of prostaglandins and inflammatory cytokines that induce abdominal stretching. Δ^9-THC has been well established to produce antinociceptive effects in the acetic acid (Sofia et al., 1975), and phenyl-p-quinone (PPQ) (Welch et al., 1995; Haller et al., 2006) models of visceral nociception.

Other prevalent phytocannabinoids that are structurally similar to Δ^9-THC include cannabinol (CBN), cannabidiol (CBD), cannabichromene (CBC), and tetrahydrocannabivarin (THCV). CBD has been demonstrated to have anti-edema effects (Lodzki et al., 2003; Costa et al., 2004) and potentiate the antinociceptive effects of Δ^9-THC (Varvel et al., 2006; Hayakawa et al., 2008). However, orally administered CBD was inactive in the acetic acid stretching model and CBN was only effective at high concentrations (Sofia et al., 1975; Welburn et al., 1976; Sanders et al., 1979). In addition, neither CBC nor THCV has been characterized in visceral pain models. Interestingly, THCV has been shown to act as a competitive cannabinoid receptor antagonist (Thomas et al., 2005). The primary goal of the present study was to compare the antinociceptive effects of

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Δ^9-THC to other prevalent phytocannabinoids, including CBC, CBD, CBN, and THCV, in the acetic acid stretching model.

Δ^9-THC binds to and activates both CB_1 (Matsuda et al., 1990) and CB_2 (Gerard et al., 1991) cannabinoid receptors, both of which are coupled to Gi/o proteins (for review see (Howlett et al., 2002). CB_1 receptors are located extensively throughout the central nervous system (CNS) (Matsuda et al., 1990; Munro et al., 1993; Zimmer et al., 1999), and are believed to mediate marijuana's psychomimetic effects. CB_2 receptors are expressed predominately in cells of the immune and hematopoietic systems (Munro et al., 1993) though CB_2 receptor messenger RNA and protein are expressed in microglia (Carlisle et al., 2002; Nunez et al., 2004) and brainstem neurons (Van Sickle et al., 2005). Consequently, a secondary goal of this study was to determine whether phytocannabinoids produce their antinociceptive effects through a cannabinoid receptor mechanism of action. Accordingly, we examined the involvement of CB_1 and CB_2 receptors using rimonabant and SR144528, selective antagonists for these respective receptors. Because cannabinoids elicit antinociceptive effects as well as motor suppressive effects, in the final set of experiments, we evaluated each active drug for hypomotility.

2. Materials and methods

2.1. Subjects

The subjects consisted of male ICR mice (Harlan Laboratories, Indianapolis, IN) weighing 20–25 g. The mice were housed in stainless steel cages in groups of five in a temperature-controlled vivarium on a 12-h light/dark cycle. Food and water were available *ad libitum*. All animal studies were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University in accordance with the *National Institute of Health Guide for the Care and Use of Laboratory Animals.*

2.2. Drugs

Δ^9-THC, CBD, and CBN were obtained from the National Institute on Drug Abuse (Bethesda, MD, USA). SR141716 (rimonabant) and SR144528, respective antagonists for CB_1 and CB_2 receptors, were obtained from NIDA (Bethesda, MD), and Δ^8-tetrahydrocannabivarin (O-4395; THCV), cannabichromene (O-4950, CBC) were synthesized by Organix Inc (Woburn, MA). In all experiments, drugs were dissolved in a 1:1 mixture of absolute ethanol and alkamuls-620 (Aventis, Strasbourg, France) and diluted with saline to a final ratio of 1:1:18 (ethanol/alkamuls/saline). All injections were given in a volume of 10 μl/g body weight.

2.3. Acetic acid stretching

The acetic acid stretching test (Koster et al., 1959) was employed to evaluate visceral nociception. A total of 6–10 naive mice were used per condition in each experiment. For each desired concentration analyzed, subjects were given a subcutaneous (s.c.) injection of drug or vehicle 60 min before an intraperitoneal (i.p.) injection of 0.6% acetic acid. In studies examining the cannabinoid receptor mechanism of action, rimonabant (3 mg/kg), SR144528 (3 mg/kg), or vehicle was administered through the i.p. route of administration 10 min before the agonist or vehicle. All injections were given in a volume of 10 μl/g body weight. After administration of the acetic acid, the subjects were placed in clear cages (11 in. × 7 in. × 5 in.) and scored for abdominal stretches during a 20 min observation period. Stretching was defined as body contortions, belly pressing, and extension of the hind limbs from which visceral nociception was inferred.

2.4. Motor impairment

In an effort to assess motor impairment, subjects were pretreated 60 min (6–8 mice per group) with a subcutaneous (s.c.) injection of Δ^9-THC (1–50 mg/kg). Each mouse was then placed in a clear Plexiglas box (17.5 in. × 8.5 in) situated in a sound attenuating chamber for 20 min. Locomotor activity was recorded using a Fire-i digital camera software (Unibrain Inc, San Ramon, CA) web camera that was located above the activity box and behavior was analyzed using the ANY-maze Software (Stoelting, Wood Dale, IL).

2.5. Cannabinoid receptor binding

Radioligand binding was performed following the method of (Devane et al., 1988) and modified by (Compton et al., 1993). In brief, binding was initiated by the addition of 75 μg whole rat brain protein to silanized tubes containing [^{3}H]-CP-55,940, a potent synthetic cannabinoid analog, (139.6 Ci/mM NEN, DuPont, Boston, MA) and sufficient volume of buffer A (50 mM Tris–HCl, 1 mM Tris–EDTA, 3 mM $MgCl_2$, and 5 mg/ml fatty acid-free BSA, pH 7.4) to bring the total volume up to 0.5 ml. Unlabelled (cold) CP-55,940 (1 μM) was used to assess non-specific binding. CP-55,940 was suspended without evaporation, in buffer A from 1 mg/ml ethanolic stock, as were all cannabinoid constituents. After adding tissue, the reaction mixture was incubated at 30 °C for 60 min. Saturation experiments were conducted with 8 concentrations of [^{3}H]-CP-55,940 ranging from 30 nM to 10 μM.

Binding was terminated by the addition of 2 ml ice-cold buffer B (50 mM Tris–HCl, and 1 mg/ml BSA, pH 7.4), and vacuum filtration (Millipore, Bedford, MA) through pretreated (>4 h, 0.1% solution of PEI, pH 7.4) GF/C glass-fiber filters (2.4 cm, Baxter, McGaw Park, IL). The reaction tubes were then rinsed once with 2 ml and twice with 4 ml of ice-cold buffer B. Before radioactivity was quantified by liquid scintillation spectrometry, the filters were incubated in 4 ml Budget-Solve (RPI Corp., Mount Prospect, IL) scintillation fluid, and shaken for 60 min. All assay conditions were conducted in triplicate, and the results reflect three independent experiments.

2.6. Statistical analysis

The total number of abdominal stretches was tabulated for each subject and ED_{50} values were calculated using least squares linear regression. Data were analyzed using one-way ANOVA. Post hoc analyses were conducted with the Tukey test or Dunnett's test for dose–response experiments. All differences were considered significant at $p < 0.05$. The K_i values for the binding assay were generated from the Radlig Ligand program from the Kell software package version 6 for Windows (Biosoft, Milltown, NJ).

3. Results

As shown in Fig. 1, Δ^9-THC dose-dependently suppressed abdominal stretching, with an ED_{50} value of 1.1 mg/kg (95% confidence interval 0.8–1.6 mg/kg). This drug was considerably less potent in decreasing locomotor activity than in producing antinociception. Its ED_{50} value in suppressing locomotor activity was 7.7 mg/kg (95% confidence interval 4.2–14.3 mg/kg) (see Table 1). Δ^9-THC was 8.5 (95% confidence interval: 3.4–20.6) fold more potent in eliciting antinociception than in decreasing locomotor activity. Based on these results, we employed 3 mg/kg Δ^9-THC to evaluate the underlying receptor mechanism of action, as this dose did not significantly interfere with locomotor activity after a 60 min pretreatment time compared to vehicle (Table 1). Rimonabant, but not SR144528, significantly blocked Δ^9-THC's antinociceptive effects [$F(3, 22) = 37.1$, $p < 0.0001$], indicating a CB_1 receptor mechanism of action (Fig. 2A). Administration of either rimonabant or SR144528 alone did not significantly affect abdominal stretching behavior (Fig. 2B).

The question of whether other major, naturally occurring marijuana constituents also possess antinociceptive properties was addressed by administering vehicle, CBC, CBD, CBN, or THCV, 1 h before the administration (i.p.) of acetic acid. As shown in

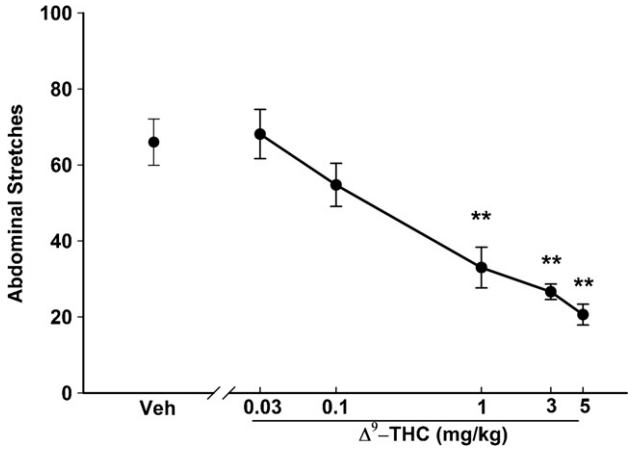

Fig. 1. Subcutaneous administration of Δ^9-THC reduced abdominal stretching in a dose-dependent manner; ED_{50} (95% confidence interval) value = 1.1 mg/kg (0.8–1.6). Each data point represents 6–8 mice. $^{**}p < 0.01$ compared with vehicle. Data reflect the mean ± SEM number of abdominal stretches during the 20 min observation period.