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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.

Keywords

phytocannabinoids; cannabis; rimonabant; visceral pain; cannabinoid receptor; antinociception

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Contributors

L. Booker conducted the bulk of the behavioral studies, data analysis, and contributed to the writing of the manuscript. S.P. Naidu contributed to the design of some of these studies. R.K. Razdan and A. Mahadevan synthesized phytocannabinoids used in these studies. A.H. Lichtman oversaw the study, contributed to the experimental design, and contributing to the writing of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of Interest

None of the authors report any conflict of interest that could have influenced, or be perceived to influence, this work.

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 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, Indiana) 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 × 7 × 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 × 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 (50mM Tris-HCl, 1 mM Tris-EDTA, 3mM $MgCl_2$, 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 uM) 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 uM.

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 hr, 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 Figure 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 (Figure 2A). Administration of either rimonabant or SR144528 alone did not significantly affect abdominal stretching behavior (Figure 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 Figure 3A, CBN produced a significant reduction in acetic acid-induced abdominal stretching, [$F(6, 35) = 9.5$, $p < 0.001$]. According to post hoc analysis, CBN produced significant antinociceptive effects at 50 mg/kg ($p < 0.01$), but not at 20 mg/kg ($p = 0.27$). In contrast, high doses of CBC or CBD did not produce antinociceptive effects in this assay. CBN (50 mg/kg) failed to inhibit locomotor activity when administered 60 min prior to recording spontaneous activity (Table 1). As shown in Figure 3B, the antinociceptive effects CBN (50 mg/kg) were blocked by rimonabant, but not by SR144528 [$F(3, 24) = 17.5$, $p < 0.001$]. While THCV (50 mg/kg) administered alone had no affect on the frequency of stretching behavior (Figure 4), it blocked the antinociceptive effects of Δ^9-THC, [$F(3, 26) = 9.52$, $p < 0.001$].

The binding affinity of each of the phytocannabinoids tested to displace [^{3}H]-CP55,940 are summarized in Table 2. Δ^9-THC and THCV bound to the CB_1 receptor with equal affinity (*K*i values= 47.7 ± 4.6 nM and 46.3 ± 6.0 nM, respectively) as illustrated by the similarity of their displacement curves (Figure 5). CBN also displaced [^{3}H]-CP55,940 binding (129.3 ± 12.9 nM), but its affinity was 2–3 fold lower than the affinity of Δ^9-THC at the CB_1 receptor. Neither CBD nor CBC showed any affinity for the CB_1 receptor (*Ki* values > 10,000 nM).

4. Discussion

Marijuana has been overlooked as an analgesic compound, in part, due to its psychoactive properties, which are primarily caused by the actions of Δ^9-THC. However, other constituents of marijuana may have analgesic properties with minimal psychoactive effects compared to Δ^9-THC. The results of the present study demonstrate that while Δ^9-THC and CBN elicited

antinociception in the acetic acid abdominal stretching model, other phytocannabinoids (i.e., CBD, CBC, and THCV) did not affect abdominal stretching when given alone, and THCV actually inhibited the antinociceptive effects of Δ^9-THC. Additionally, the present study determined that the antinociceptive effects of Δ^9-THC and CBN were mediated through a CB_1 receptor mechanism of action.

The results obtained in the present study are consistent with the view that Δ^9-THC is the major phytocannabinoid present in marijuana that produces antinociception in the acetic acid abdominal stretching test. Previous studies reported that Δ^9-THC dose-dependently suppressed abdominal stretching in the *p*-phenylquinone test (Dewey et al., 1972; Sanders et al., 1979), formic acid test (Welburn et al., 1976; Sanders et al., 1979), and acetic acid writhing test (Sofia et al., 1975) in mice, with ED_{50} values ranging between 1.2 and 4.2 mg/kg. In agreement with earlier work, Δ^9-THC dose-dependently reduced abdominal stretching, though we used the s.c. route of administration and the earlier work administered the drug via gavage. The finding that rimonabant completely blocked the antinociceptive effects of Δ^9-THC indicates a CB_1 receptor mechanism of action.

A major goal of the present study was to investigate other prevalent cannabinoid constituents of marijuana in the acetic acid model of visceral nociception. These compounds closely resemble Δ^9-THC structurally and, in some cases, bind to CB_1 receptors. Emphasis has been drawn away from naturally occurring compounds, due to their relative low abundance in marijuana compared to Δ^9-THC (ElSohly et al., 2000). CBN (50 mg/kg) suppressed abdominal stretching through a CB_1 receptor mechanism of action. Sofia et al. (1975) also found that high concentrations of CBN were required to elicit antinociceptive effects after gavage administration. Although the binding affinity of Δ^9-THC is 2–3 fold greater than the bind affinity of CBN, Δ^9-THC is at least fold 50 fold more potent than CBN in producing antinociception. However, the relationship between binding affinity and *in vivo* activity of the cannabinoids is not linear, but takes on a logarithmic function (Compton et al., 1993). For example, CBN was 90–250 fold less potent than Δ^9-THC in eliciting Δ^9-THC-like discriminative cues in pigeons (Järbe et al., 1977). Thus, the fact that higher doses of CBN than Δ^9-THC are required to elicit antinociceptive actions in this visceral pain model is consistent with its low binding affinity for the CB_1 receptor compared to the affinity of Δ^9-THC.

The present study is the first to our knowledge to examine the molecular affinity of CBC for the cannabinoid receptor. However, this phytocannabinoid did not bind to CB_1 receptors and did not produce antinociceptive effects in the acetic acid model of visceral nociception. The lack of antinociceptive efficacy of CBD in the acetic acid stretching model is consistent with previous reports (Sofia et al., 1975; Welburn et al., 1976; Sanders et al., 1979). Also, the poor affinity of CBD to the CB_1 receptor was consistent with previous research (Showalter et al., 1996; Thomas et al., 2007). Although THCV, the propyl homologue of Δ^9-THC had equivalent binding affinity as Δ^9-THC, it failed to elicit antinociceptive effects at doses up to 50 mg/kg. This compound has been reported previously to have competitive antagonist effects with Δ^9-THC at low concentrations, though it elicited agonist activity at high intravenous doses (Pertwee et al., 2007). Indeed, we report that a high dose of THC-V (i.e., 50 mg/kg) significantly antagonized the antinociceptive effects of Δ^9-THC (3 mg/kg), further supporting the notion that THCV is a naturally occurring CB_1 receptor antagonist.

Because cannabinoids are known to elicit hypomotility, which would confound interpretations of the behavioral data in the acetic acid-induced stretching assay, we examined the effects of each active drug on locomotor behavior. Δ^9-THC did not impair mobility at doses less than 50 mg/kg, suggesting that the dose range used in the acetic acid stretching test did not provoke motor disturbances. Additionally, CBN did not affect mobility at the dose which produced

antinociception. Traditionally, cannabinoid agonists have been shown to produce reductions in locomotor activity (Hohmann et al., 2005), which is one of the hallmarks of Δ^9-THC. The increased potency of Δ^9-THC in producing antinociception compared to its potency in producing locomotor suppression may be attributed to the fact that the acetic acid assay is particularly sensitive to antinociceptive agents.

Interestingly, there are considerable *in vitro* and *in vivo* data suggesting that endocannabinoids are produced and released on demand (Patel et al., 2003; Di et al., 2005; Jung et al., 2005; Matias et al., 2006). Walker et al. (1999) showed that the electrical stimulation of the periaqueductal gray (PAG) area as well as formalin injected intradermally into the hind paws elevated ananadamide levels in the PAG, supporting the role that endocannabinoids are released in response to pain sensing pathways. In addition, Hohmann et al. (2005) demonstrated that intracerebral administration of inhibitors of endocannabinoid metabolizing enzymes into the PAG potentiated stress-induced antinociception and led to concomitant release of endocannabinoids within this brain region. However, the results of the present study, showing that neither rimonabant nor SR144528 given alone altered acetic acid-induced stretching, suggest that acetic acid-induced stretching is not under endocannabinoid tone.

In conclusion, our results show that Δ^9-THC dose-dependently suppressed the frequency of acetic acid induced stretching. Its antinociceptive effects were shown to be mediated through a CB_1 receptor mechanism of action, without any indication of CB_2 receptor involvement. The only other naturally occurring constituent of marijuana evaluated that produced antinociception was CBN, but the required dose (i.e., 50 mg/kg) was substantially higher than the minimal dose of Δ^9-THC that produced antinociception (i.e., 1 mg/kg). CBD, CBC, and THCV failed to produce antinociception in the acetic acid test. Conversely, while THCV given alone did not affect visceral nociception, it antagonized the antinociceptive actions of Δ^9-THC when both drugs were given in combination. Although this pattern of findings raises the provocative possibility that other components of this plant can augment (e.g., CBN) or reduce (e.g., THCV) the antinociception actions of Δ^9-THC, it should be noted that marijuana contains a far lower abundance of CBN (0.24–1.44%) than C^9-THC (4–20%) (ElSohly et al., 2000). Additionally, the percentage of THCV is considerably low and varies in samples of marijuana of different origins (ElSohly and Brenneise, 1988). Thus, these specific constituents would not be expected to play a substantial role in marijuana's pharmacological effects. On the other hand, these results suggest that there is potential to develop medications containing various concentrations of specific phytocannabinoids to optimize therapeutic effects (e.g., antinociception) and minimize psychomimetic effects. In sum, the results of the present study further support the notion that Δ^9-THC is the predominant constituent of marijuana that is responsible for eliciting antinociceptive effects and indicate that CB_1 receptors play a predominant role in mediating these effects.

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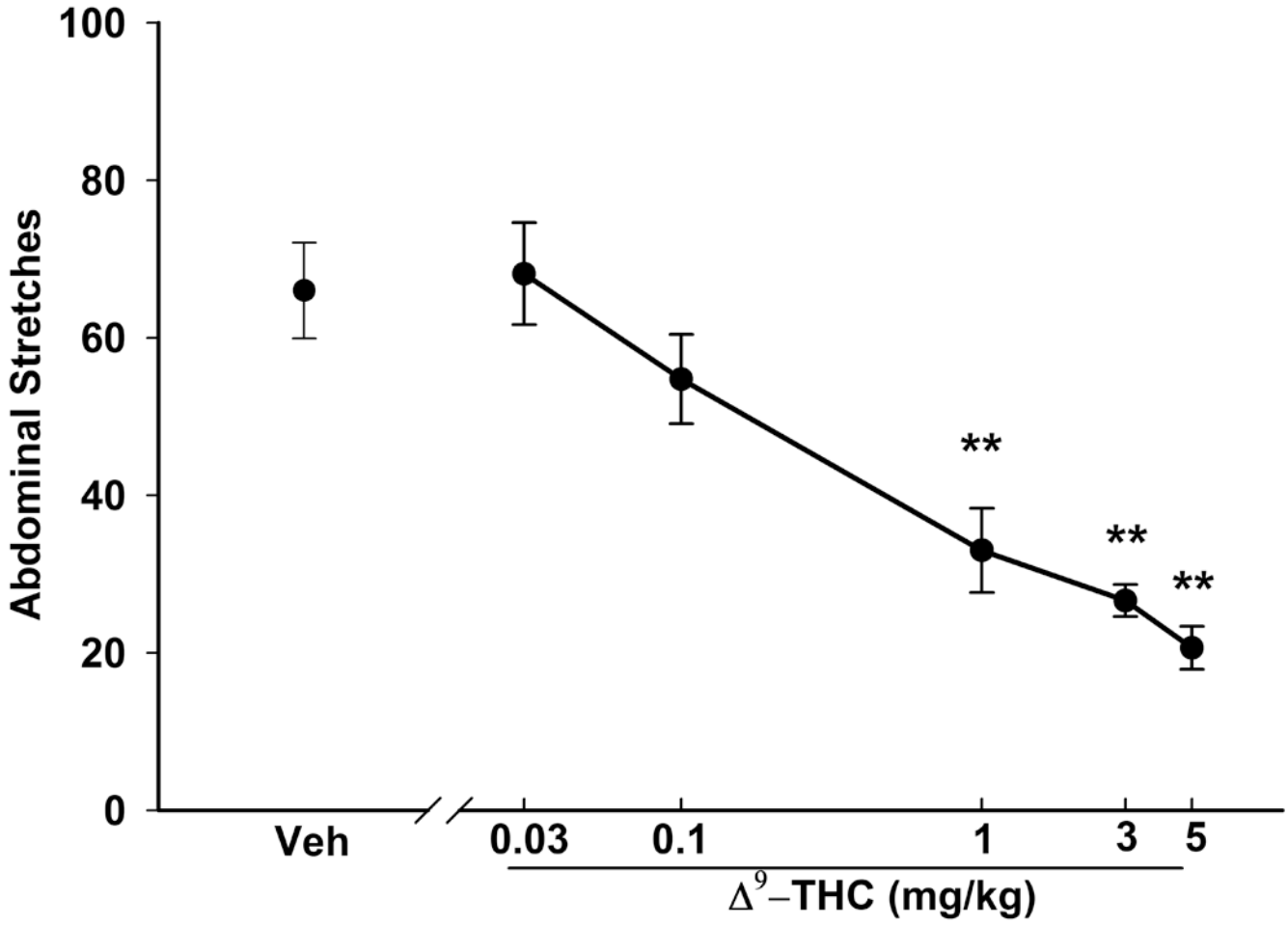

Figure 1.

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.

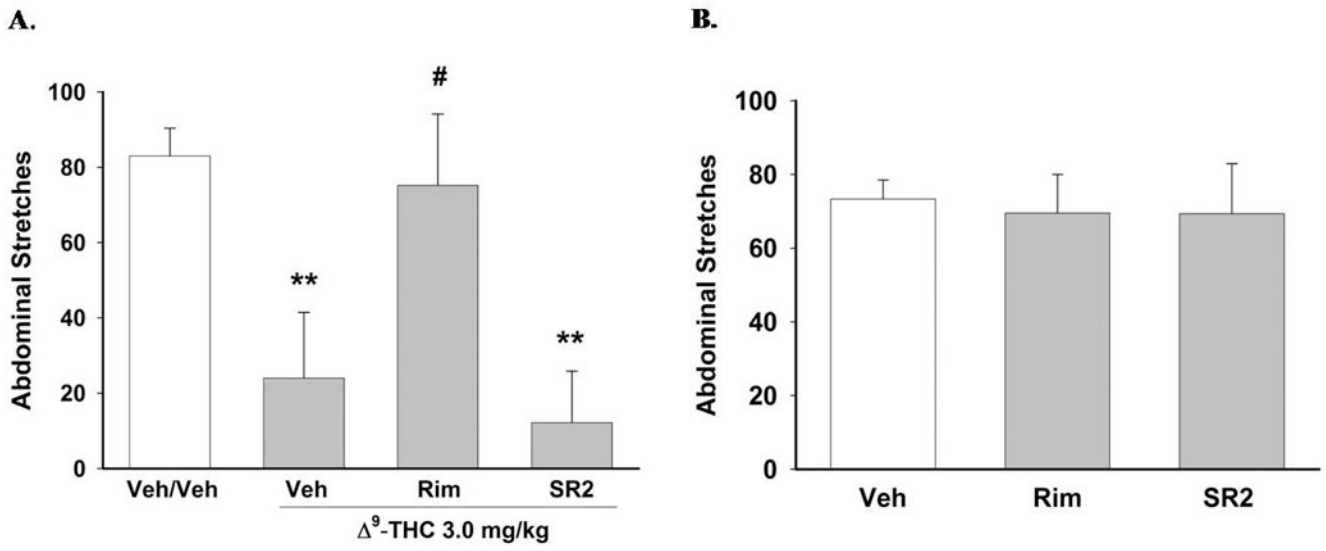

Figure 2.

The antinociceptive effects of Δ^9- THC in the acetic acid model of visceral nociception are mediated through a CB_1 receptor mechanism of action. **A)** The CB_1 receptor antagonist, rimonabant (Rim; 3.0 mg/kg, i.p.), but the CB_2 receptor antagonist, SR144528 (SR2; 3.0 mg/kg, i.p.), blocked the antinociceptive effects of Δ^9- THC (3 mg/kg, s.c.). # indicates significant difference from Vehicle (Veh)/Δ^9-THC control $p < 0.01$; ** indicates significant difference from Vehicle/Vehicle control $p < 0.01$. **B)** Neither rimonabant nor SR144528 given alone affected acetic acid-induced abdominal stretching. N = 6–8 mice/group. Data reflect the mean ± SEM number of abdominal stretches.

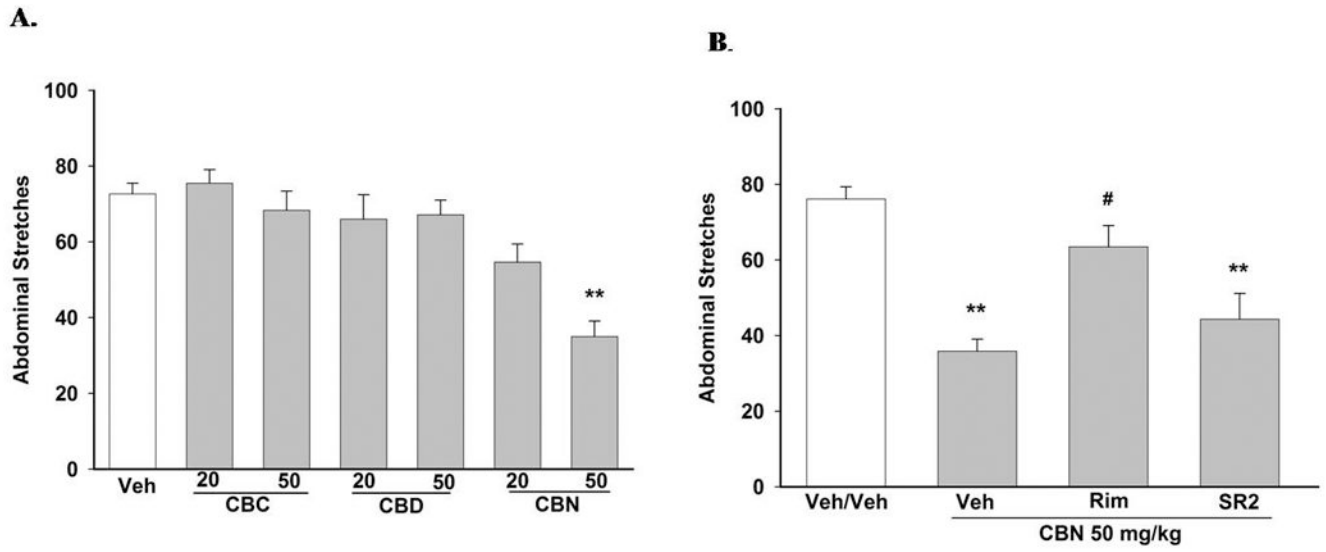

Figure 3.

Evaluation of prevalent marijuana constituents in the acetic acid abdominal stretching model. **A**) The marijuana constituents, CBC and CBD, did not produce antinociceptive effects. However, CBN (50 mg/kg) significantly suppressed the stretching response compared to vehicle)Veh), $^{**}p < 0.01$ vs. Vehicle. **B**) The CB_1 receptor antagonist, rimonabant (Rim), but not by the CB_2 receptor antagonist, SR144528 (SR2), significantly blocked the antinociceptive effects of CBN (50 mg/kg). $^{**}p < 0.01$ vs. Veh/Veh, and $^{\#}p < 0.01$ vs. Veh/CBN. N = 6–10 mice/group. Data reflect the mean ± SEM abdominal stretches.

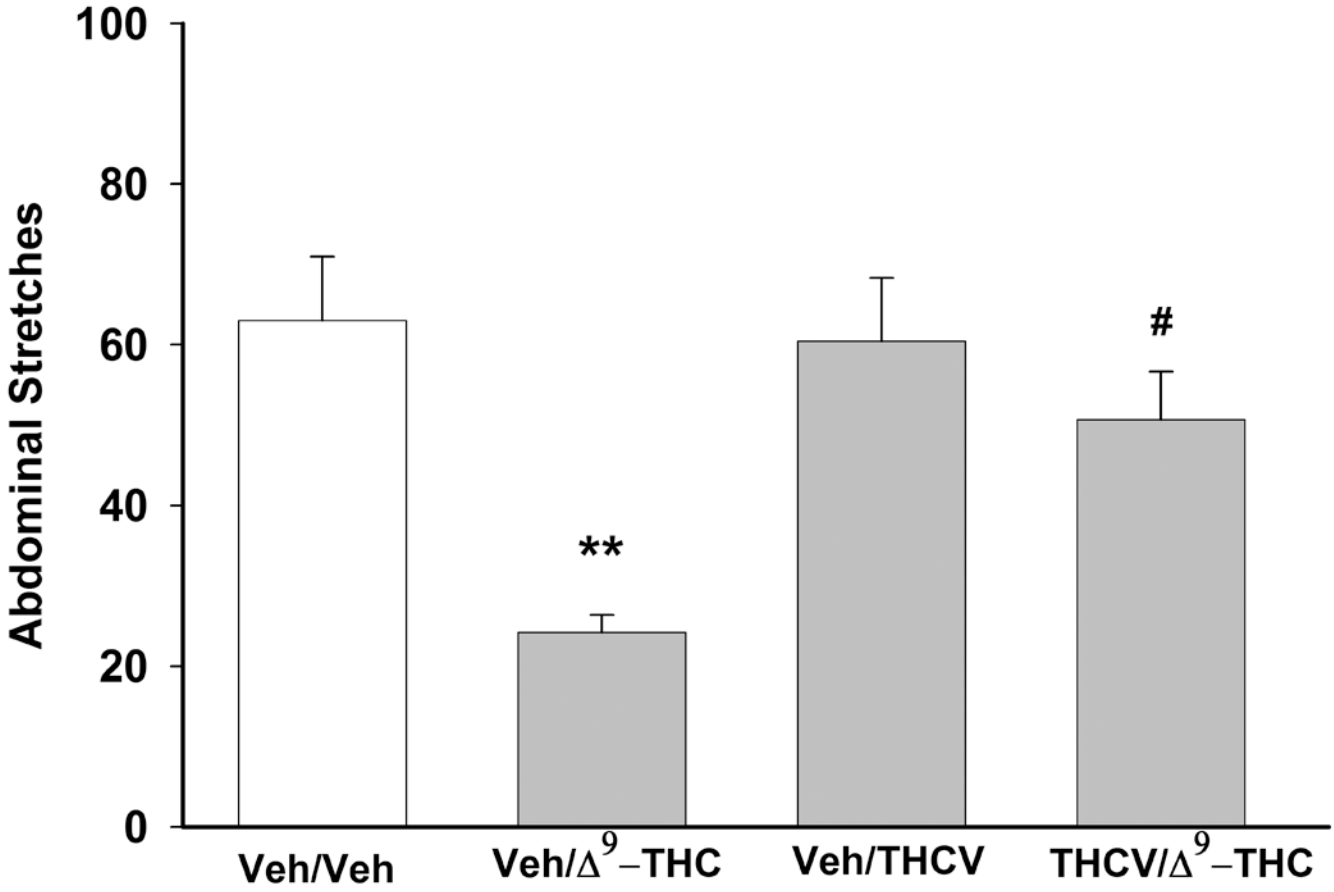

Figure 4.

Delta 8-tetrahydrocannabivarin (THCV, 50 mg/kg, s.c.) had no effects on its own, but blocked the antinociceptive effects of Δ^9-THC (3.0 mg/kg, s.c.). $^{**}p < 0.01$ vs. Veh/Veh group. $^{\#}p < 0.05$ vs. Veh/Δ^9-THC. N = 6–8 mice/group. Data reflect the mean ± SEM abdominal stretches.

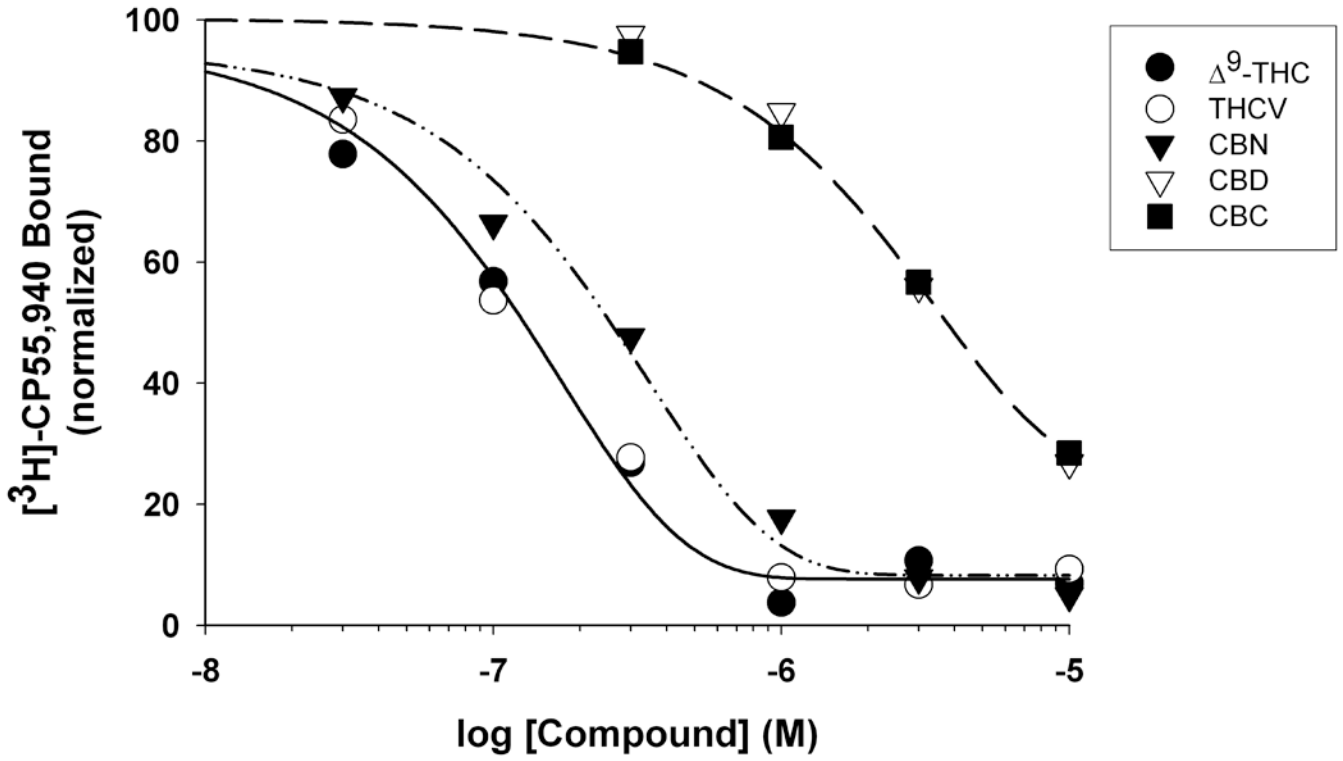

Figure 5.

Activity of prevalent phytocannabinoids at rat cannabinoid receptor type 1. The affinity of Δ^9-THC was determined for rat CB_1 receptor (filled circles/solid line), THCV (open circle/solid line), CBN (filled triangle/dash-dotted line), CBD (open triangle/dashed line), and CBC (filled square/dashed line). Details for competition binding experiments are described in the methods section. The points on the graph represent the mean ± SEM of three independent experiments with duplicate wells on each plate. The data were normalized to the signal in the absence of unlabeled competitor (defined as 100%) and in the presence of excess unlabeled CP55,940 (defined as 0%).

Table 1

Evaluation of Δ^9-THC in spontaneous locomotor activity behavior. Mice were given a s.c. injection of various concentrations of Δ^9-THC and evaluated 60 min later for locomotor activityfor a total of 20 min. Data are represented as the mean ± SEM percentage of time spent immobile or total distance traveled, n=6 mice per group.

	% Time Immobile	Total Distance Traveled (m)
Δ^9-THC (mg/kg)		
Vehicle	21.1 ± 4.2	46.54 ± 5.66
1.0	11.2 ± 2.2	41.79 ± 2.63
3.0	21.5 ± 5.0	43.59 ± 4.70
10.0	37.6 ± 9.2	45.86 ± 7.76
50.0	49.0 ± 6.8*	35.34 ± 3.61
CBN (mg/kg)		
50.0	17.6 ± 2.9	39.02 ± 7.75

* $p < 0.05$, versus vehicle-treated mice.

Table 2

K_i-values for displacement of [^{3}H]-CP-55,940 from mouse whole brain. Δ^9-THC, CBN, and THCV displaced [^{3}H]-CP-55,940 in the nanomolar range. However, CBD and CBC lacked affinity for the receptor. N = 3 brains per drug.

Constituent	***K_i (nM)***	***±S.E.***
Δ^9- THC	47.7	4.6
Cannabinol (CBN)	129.3	12.9
THCV (O-4395)	46.3	6.0
Cannabidiol (CBD)	> 10,000	
CBC (O-4950)	> 10,000	