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Cannabidiol-Δ^9-tetrahydrocannabinol interactions on acute pain and locomotor activity

Stevie C. Britch[a], Jenny L. Wiley[b], Zhihao Yu[c], Brian H. Clowers[c], Rebecca M. Craft[a,*]

[a] Department of Psychology, Washington State University, Pullman, WA, 99164-4820, USA
[b] RTI International, Research Triangle Park, NC, 27709-2194, USA
[c] Department of Chemistry, Washington State University, Pullman, WA, 99164-4630, USA

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ABSTRACT

Background: Previous studies suggest that cannabidiol (CBD) may potentiate or antagonize Δ^9-tetrahydrocannabinol's (THC) effects. The current study examined sex differences in CBD modulation of THC-induced antinociception, hypolocomotion, and metabolism.

Methods: In Experiment 1, CBD (0, 10 or 30 mg/kg) was administered 15 min before THC (0, 1.8, 3.2, 5.6 or 10 mg/kg), and rats were tested for antinociception and locomotion 15–360 min post-THC injection. In Experiments 2 and 3, CBD (30 mg/kg) was administered 13 h or 15 min before THC (1.8 mg/kg); rats were tested for antinociception and locomotion 30–480 min post-THC injection (Experiment 2), or serum samples were taken 30–360 min post-THC injection to examine CBD modulation of THC metabolism (Experiment 3).

Results: In Experiment 1, CBD alone produced no antinociceptive effects, while enhancing THC-induced paw pressure but not tail withdrawal antinociception 4–6 h post-THC injection. CBD alone increased locomotor activity at 6 h post-injection, but enhanced THC-induced hypolocomotion 4–6 h post-THC injection, at lower THC doses. There were no sex differences in CBD-THC interactions. In Experiments 2 and 3, CBD did not significantly enhance THC's effects when CBD was administered 13 h or 15 min before THC; however, CBD inhibited THC metabolism, and this effect was greater in females than males.

Conclusions: These results suggest that CBD may enhance THC's antinociceptive and hypolocomotive effects, primarily prolonging THC's duration of action; however, these effects were small and inconsistent across experiments. CBD inhibition of THC metabolism as well other mechanisms likely contribute to CBD-THC interactions on behavior.

1. Introduction

Cannabis has been used to treat pain for centuries (reviewed in Aggarwal et al., 2008). The main psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol (THC), produces antinociception in studies of acute pain in animals (Smith et al., 1998; Tseng and Craft, 2001; Varvel et al., 2005) and in humans (Greenwald and Stitzer, 2000). Cannabinoids such as THC also produce antinociception against chronic pain in animals (Cox and Welch, 2004; Mao et al., 2000; Schley et al., 2006) and in humans (reviewed in Lynch and Ware, 2015). Studies on the pain-relieving effects of cannabidiol (CBD), another cannabis constituent that may have therapeutic potential, are limited. CBD did not produce antinociception using acute pain tests in mice (Booker et al., 2009; Sanders et al., 1979; Sofia et al., 1975; Varvel et al., 2006) or rats (Sofia et al., 1975), although it did prevent the development of allodynia in paclitaxel-treated mice (Ward et al., 2014). However, CBD and THC have been shown to synergize on several outcomes, including inhibition of glioblastoma cell proliferation, *in vitro* (Marcu et al., 2010), and THC-induced place preference (Klein et al., 2011), hypolocomotion (Fernandes et al., 1974; Hayakawa et al., 2008) and antinociception (Karniol and Carlini, 1973) in male rodents. In humans, a 1:1 CBD-THC combination produced greater pain relief than THC alone (Johnson et al., 2010).

Several studies have shown that THC has greater effects in females than males. For instance, female rats given THC show greater catalepsy (Craft et al., 2012; Tseng and Craft, 2001), anxiety-like behavior (Harte-Hargrove and Dow-Edwards, 2012), hypothermia (Wiley et al., 2007), hypolocomotion (Craft et al., 2012; Tseng and Craft, 2001), and antinociception (e.g., Romero et al., 2002; Tseng and Craft, 2001) compared to males given the same dose of THC. Although several studies have found sex differences in THC's behavioral effects, to our knowledge no previous studies have compared CBD alone or CBD-THC

* Corresponding author.
E-mail address: craft@wsu.edu (R.M. Craft).

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interactions on behavior in males and females of any species.

One potential mechanism underlying CBD-THC interactions is CBD-induced inhibition of THC metabolism. THC is metabolized primarily by the liver enzyme cytochrome P450 3A (Watanabe et al., 2007) into the active metabolite 11-OH-THC and inactive metabolite THC−COOH (Wall et al., 1983), but also may be converted into cannabinol (CBN), a less potent psychoactive (Perez-Reyes et al., 1973) and antinociceptive (Sofia et al., 1975; Sanders et al., 1979; Booker et al., 2009) cannabinoid in comparison to THC. Female rats produce more 11-OH-THC than males do (Narimatsu et al., 1991; Wiley and Burston, 2014), and this appears to contribute to greater THC-induced behavioral effects in female compared to male rats (Tseng et al., 2004). CBD has been shown to inhibit metabolism of THC by inactivating cytochrome P450 3A (Bornheim and Grillo, 1998). For example, in an *in vitro* assay, CBD reduced THC metabolism by 60% (Jaeger et al., 1996). Further, when CBD was given to mice 15–60 min prior to THC administration, increased THC levels were found in the brain (Jones and Pertwee, 1972; Reid and Bornheim 2001).

The interval between CBD and THC administration has been suggested to affect CBD-THC interactions (Zuardi et al., 2012), but no systematic investigation of the effects of CBD pretreatment time has been conducted. Thus, the purpose of the present study was three-fold: to examine sex differences in CBD-THC interactions on nociception and locomotion, to determine whether CBD pretreatment time alters the drug interaction, and to determine whether CBD alters THC metabolism similarly in both sexes. In the first experiment, multiple CBD-THC dose combinations were examined using a 15-min interval between CBD and THC injections. Zuardi et al. (2012) estimated that a 1.8:1 CBD:THC ratio is optimal for observing CBD enhancement of THC's effect. Therefore, we examined a wide range of CBD:THC dose ratios that included this dose ratio. Based on the results of the first experiment, the dose combination of CBD 30 mg/kg + THC 1.8 mg/kg was chosen to compare two pretreatment times for CBD, one short (15 min) and one long (13 h), to determine if CBD pretreatment time would alter CBD-THC interactions (Experiment 2). One previous study using a 24-h CBD pretreatment time reported that CBD enhanced THC's effect on corticosterone levels, suggesting that CBD can alter THC's effects even when CBD is administered long before THC (Zuardi et al., 1984). The same dose combination was examined in Experiment 3, to determine the effects of CBD given at the two pretreatment times on THC metabolism.

2. Methods

2.1. Animals

All experiments were completed in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 2011). Gonadally intact, male and female Sprague-Dawley rats aged 60–100 days were used (bred in-house from Harlan stock, Livermore, CA). They were housed in same-sex groups of 2–3, under a 12:12 h light:dark cycle (lights on at 0700 h). The room was maintained at 21 ± 2 °C. Food and water were available *ad libitum* except during testing. Rats were randomly assigned to treatment groups with the exception that same-sex siblings generally were not assigned to the same treatment group.

2.2. Drugs

Drugs were obtained from the National Institute on Drug Abuse (Bethesda, MD). THC and CBD were dissolved in a 1:1:18 ethanol:cremophor:saline solution, which also served as the vehicle. Drugs were administered by i.p. injection.

2.3. Apparatus

Tail withdrawal antinociception was measured using a 2.5-l warm water bath (Precision Scientific Inc., Winchester, VA) set to 50 ± 0.5 °C; latency to withdraw the tail was measured using a hand-held stopwatch with a 15-s cutoff. Paw pressure antinociception was measured using an Analgesy meter (Ugo-Basile, Varese, Italy). The pressure on the paw began at 30 g and increased at a constant rate of 48 g/s to a maximum of 750 g (15-s cutoff). Horizontal locomotor activity was measured in a 20 × 40 × 23 cm clear Plexiglas rodent cage placed within a photobeam apparatus (Opto-Varimex, Columbus Instruments, Columbus, OH) that has 15 photobeams spaced 2.5 cm apart, and the number of photobeam breaks in 10 min was recorded.

2.4. Behavioral procedures

All testing was completed during the light phase (between 0730 and 1700 h). In Experiments 1 and 2, baseline data on the tail withdrawal and paw pressure tests were collected 3 times over 30 min just prior to the first injection. In Experiment 1, rats were given vehicle or CBD (10 or 30 mg/kg) i.p. followed 15 min later by vehicle or THC (1.8, 3.2, 5.6, or 10 mg/kg) i.p. Rats were then tested on tail withdrawal and paw pressure tests at 15, 30, 60, 120, 240 and 360 min post-THC injection. Locomotor activity was tested for 10 min at each time point, after completion of nociceptive testing. In Experiments 2 and 3, rats were given vehicle or CBD 30 mg/kg i.p. either 13 h or 15 min before vehicle or THC 1.8 mg/kg i.p. In Experiment 2 rats were then tested on tail withdrawal and paw pressure tests at 30, 60, 120, 240, 360 and 480 min post-THC injection. Again, locomotor activity was tested for 10 min at each time point, after completion of nociceptive testing. In Experiment 3, trunk blood was collected at 30, 120, or 360 min post-THC injection (separate rats at each time point); blood samples were centrifuged for 20 min at 2000 rpm at −4 °C, and serum samples were stored at −80 °C for later determination of cannabinoid concentrations.

2.5. Determination of estrous cycle

Vaginal lavage samples were collected from all females after completion of behavioral testing. Vaginal cytology was scored as follows: proestrus was identified by a predominance (75% or more of cells in the sample) of nucleated epithelial cells; estrus by dense sheets of cornified epithelial cells; diestrus by scattered, nucleated and cornified epithelial cells, and leukocytes (Freeman, 1994).

2.6. Serum cannabinoid analysis

Quantitation of THC, THC metabolites, CBD and CBN in rat blood was achieved using an ultra performance liquid chromatography system (Waters Acquity I-Class UPLC, Milford, MA, USA) coupled with a quadrupole time of flight mass spectrometer (QTOF, Waters Xevo G2, Manchester, UK). The first step of sample preparation was sample centrifugation at 8000 rpm for 10 min to remove any remaining cells. 185 uL of the resulting supernatant was spiked with 15 uL of solution containing 200 ppb each of the deuterated standards (THC-d3, OH-THC-d3, COOH-THC-d3, CBD-d3 and CBN-d3, Cerilliant, Round Rock, TX). Combined with the high resolution and accurate mass of the QTOF platform, these internal standards can minimize contributions from non-ideal metabolite extraction and instrumental variability and allow for direct quantitation of each targeted analyte. Following the internal standard addition, protein precipitation was promoted by adding 400 uL of cold acetonitrile (ACN) dropwise while vortexing. Immediately the samples were centrifuged at 4000*g* for 10 min at 25 °C. 0.6 mL of 1% ammonium hydroxide was added to the sample and vortexed before solid phase extraction (SPE). A mixed mode SPE cartridge (OAXIS Max 1 cc, Waters, Ireland) was used for cannabinoid isolation. Each SPE cartridge was conditioned with 1 mL of methanol