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RESEARCH PAPER

Cannabidiol fails to reverse hypothermia or locomotor suppression induced by Δ9-tetrahydrocannabinol in Sprague-Dawley rats

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BACKGROUND AND PURPOSE

Growing evidence shows cannabidiol (CBD) modulates some of the effects of Δ9-tetrahydrocannabinol (THC). CBD is a constituent of some strains of recreational cannabis but its content is highly variable. High CBD strains may have less memory-impairing effects than low-CBD strains and CBD can reverse behavioural effects of THC in monkeys. CBD/THC interactions in rodents are more complicated as CBD can attenuate or exacerbate the effects of THC. This study was undertaken to determine if CBD could reverse hypothermia or hypolocomotor effects caused by THC in rats.

EXPERIMENTAL APPROACHES

Male Sprague-Dawley rats were prepared with radiotelemetry devices and then given doses of THC (10–30 mg·kg^{-1}, i.p.) with or without CBD. Experiments determined the effect of simultaneous or 30 min pretreatment with CBD in a 1:1 ratio with THC, as well as the effect of CBD in a 3:1 ratio. Additional experiments determined the effects of pretreatment with the cannabinoid CB$_1$ receptor antagonist SR141716 (rimonabant).

KEY RESULTS

CBD did not attentuate THC-induced hypothermia or hypolocomotion but instead exaggerated these effects in some conditions. The antagonist SR141716 blocked hypolocomotor effects of THC for the first hour after injection and the hypothermia for 6 h; thus validating the pharmacological model.

CONCLUSIONS AND IMPLICATIONS

There is no evidence from this study that elevated CBD content in cannabis could provide protection from the physiological effects of THC, in rats.

Abbreviations

CBD, cannabidiol; SR141716, 5-(4-chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide; THC, Δ9tetrahydrocannabinol

Tables of Links

TARGETS
GPCR
CB$_1$ receptor

LIGANDS
CBD, cannabidiol
SR141716, rimonabant
THC, Δ9tetrahydrocannabinol

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 (Alexander *et al.*, 2013).

Introduction

Cannabidiol (CBD) is a constituent of some strains of recreational cannabis plant material; the content of CBD-enriched strains is, however, highly variable in the market (Morgan *et al.*, 2010; Burgdorf *et al.*, 2011). CBD has been viewed as an inactive constituent of cannabis, for example, it produces minimal disruption of behavioural tasks in humans, monkeys or rodents (Belgrave *et al.*, 1979; Lichtman *et al.*, 1995; Winsauer *et al.*, 1999). There is growing evidence, particularly in human and non-human primates, that CBD may modulate the effects of the primary psychoactive cannabis constituent, Δ^9-tetrahydrocannabinol (THC). CBD may function as an allosteric modulator (antagonist) at cannabinoid CB$_1$ and CB$_2$ receptor subtypes (Turkanis and Karler, 1986; Petitet *et al.*, 1998; Mechoulam *et al.*, 2007), which provides a potential pharmacological mechanism for any *in vivo* effects observed. A recent review argues, however, that CBD influence on THC-related effects may be mediated through non-CB$_1$ receptor mechanisms (McPartland *et al.*, 2015).

The evidence that CBD can attenuate or modify the effects of THC in humans is several fold. A combined cannabinoid oral/mucosal spray Sativex (it delivers a 1:1 ratio of CBD : THC) has been approved in Canada for spasticity associated with multiple sclerosis following many clinical trials (see Oreja-Guevara, 2012). The mechanism of the therapeutic effect of CBD is not well established, but the addition of CBD to the THC may permit higher doses of THC to be administered without significant 'adverse effects', including subjective 'high' (Robson, 2011; Schoedel *et al.*, 2011) and cognitive/behavioural impairment (Wade *et al.*, 2004). Furthermore, a mixed CBD/THC cannabis extract improved EEG mis-match negativity activation whereas THC did not (Juckel *et al.*, 2007) but CBD/THC- and THC-only treatment both reduced P300 amplitude (Roser *et al.*, 2008). In a different vein, Morgan and colleagues showed that smoking CBD-enriched marijuana did not cause deficits of immediate and delayed prose recall caused by CBD-poor cannabis (Morgan *et al.*, 2010) and users habitually exposed to CBD-containing cannabis may have relatively preserved recognition memory versus CBD-poor cannabis users (Morgan *et al.*, 2012). The limits of human field studies (varying CBD/THC dose, no control of individuals who select CBD-rich vs. CBD-poor cannabis) and human laboratory studies (limited dosing ranges of CBD vs THC) have motivated animal studies to investigate how CBD modulates the effects of THC. Finally, a recent study from this laboratory found that CBD could ameliorate or reverse some motor coordination and object-spatial memory effects of THC in macaque monkeys when CBD and THC were administered in equal amount (Wright *et al.*, 2013b). This is important given that CBD : THC ratios in street cannabis do not typically exceed 1:1 (Morgan *et al.*, 2010; Burgdorf *et al.*, 2011).

The available evidence on interactive effects of CBD and THC in rodent models presents a more complicated picture. While CBD can reverse conditioned place aversion produced by 10 mg·kg^{-1} THC in rats (Vann *et al.*, 2008), CBD may potentiate the anxiogenic and locomotor suppressant effects of THC in rats treated chronically (Klein *et al.*, 2011). In addition CBD/THC interactions may depend on the pretreatment offset (see Zuardi *et al.*, 2012). When CBD is administered 30 min (or up to 24 h) prior to THC in rats or mice, a potentiation can be observed whereas co-administration results in blockade or amelioration of THC effects. The picture may be complicated even further by a suggestion that CBD/THC ratios on the order of 8 are necessary for antagonistic properties and only 1.8 for potentiation of THC-related effects in rodents (Zuardi *et al.*, 1984).

This study was therefore designed to determine if CBD attenuates, potentiates or extends the duration of hypothermia and hypomotility produced by acute THC in rats. A radiotelemetry system, shown effective at distinguishing dose-dependent effects of even closely related stimulant drugs (Crean *et al.*, 2006; 2007; Taffe *et al.*, 2006), was selected to minimize any potential anxiogenic or anxiolytic effects of THC which might interact with rectal sampling, as well as providing a full time course of effects. The outcomes of simultaneous CBD/THC injection were compared with those of giving CBD 30 min before THC to determine if this altered the qualitative effects of CBD/THC combinations. Further investigation examined whether a 3:1 CBD : THC ratio altered the effects of CBD, compared with a 1:1 ratio. A final study determined the effect of the cannabinoid CB$_1$ receptor antagonist SR141716 (rimonabant) on the thermoregulatory response to THC to serve as a positive control condition to demonstrate mechanistic specificity.

Methods

Animals

All animal care and experimental procedures were conducted under protocols approved by the Institutional Care and Use Committee of The Scripps Research Institute (Protocol #08–

0053-2,3). All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010). A total of 13 animals were used in the experiments described here. Male Sprague-Dawley (Harlan; Livermore, CA, USA) rats were housed in humidity- and temperature-controlled (23 ± 2°C) conditions, on 12:12 hour light : dark cycles. Animals entered the laboratory at approximately 10 weeks of age. Group 1 rats ($n = 5$) were 14 weeks old and weighed 362–405 g at the start of this study. Group 2 rats ($n = 8$) were 18 weeks old and weighed 400–459 g at the start of this study. Animals had *ad libitum* access to food and water in their home cages.

Radiotelemetry

Rats were anaesthetized with an isoflurane/oxygen vapour mixture (isoflurane 5% induction, 1–3% maintenance) and sterile radiotelemetry transmitters (Data Sciences International, St. Paul, MN, USA; TA-F40) were implanted in the abdominal cavity through an incision along the abdominal midline posterior to the xyphoid space. Absorbable sutures were used to close the abdominal muscle incision and the skin incision was closed with tissue adhesive. A minimum of 7 days was allowed for surgical recovery prior to starting an experiment. For the first 3 days of the recovery period, an antibiotic (cephazolin; 0.4 g·mL^{-1}; 2.0 mL·kg^{-1}, s.c.) and an analgesic (flunixin; 2.5 mg·mL^{-1}; 2.0 mL·kg^{-1}, s.c.) were administered daily.

Animals were evaluated in clean standard plastic homecages (thin layer of bedding) in a dark testing room, separate from the vivarium, during the (vivarium) dark cycle. Radiotelemetry transmissions were collected via telemetry receiver plates (Data Sciences International; RPC-1) placed under the cages as described in prior investigations (Wright *et al.*, 2012; Aarde *et al.*, 2013; Miller *et al.*, 2013). Test sessions started with a 5–10 minute interval to ensure data collection, followed by i.p. injection of drug. The ambient temperature for the studies was 23.5 ± 1°C.

Studies

The order of group 1 studies was (i) simultaneous 30 mg·kg^{-1} CBD and 30 mg·kg^{-1} THC; (ii) 30-min pretreatment of 30 mg·kg^{-1} CBD followed by 30 mg·kg^{-1} THC; (iii) 30 min pretreatment of 60 mg·kg^{-1} CBD followed by 20 mg·kg^{-1} THC. A single 10 mg·kg^{-1} THC challenge was inserted between studies 2 and 3. Treatment conditions (including vehicle and solo-drug challenges) were randomized within the studies, with active THC doses administered no more frequently than a 7 day interval. All challenge studies were completed during a 16 week interval. Group 2 had a prior history of five acute exposures to THC (one including 4 mg·kg^{-1} SR141716, i.p. pretreatment) via inhalation in sessions lasting 10–30 min, administered no more frequently than a 7 day interval.

Data analysis

The body temperature and activity rate (counts per minute) were collected on a 5 min schedule but are expressed as hourly averages for statistical analysis of the group 1 study and 30 min averages for the group 2 study. In the case of the pretreatment studies, the data are expressed relative to the

second injection time. Any missing values were interpolated from the values before and after the lost time point. Data were analysed with two way ANOVA including repeated measures factors for the treatment condition and the time post-injection. Any significant main effects were followed with *post hoc* analysis using Tukey's correction for all comparisons. All analysis used Prism 6 for Windows (v. 6.02; GraphPad Software, Inc, San Diego, CA, USA).

Materials

THC was administered i.p. in doses of 10, 20 and 30 mg·kg^{-1}, CBD was administered i.p. in doses of 30 and 60 mg·kg^{-1} and SR141716 (rimonabant) was administered i.p. in a dose of 4 mg·kg^{-1}. The dose selection for THC was based on assessment of published data and pilot studies showing this to be the effective range for consistent effects. For injection, CBD, SR141716 or THC was suspended in a vehicle of 95% ethanol, Cremophor EL and saline in a 1:1:8 ratio. The THC was provided by the US National Institute on Drug Abuse and the CBD and SR141716 were purchased from Cayman Chemical (Ann Arbor, MI, USA).

Results

Simultaneous administration, 1:1 CBD-THC ratio

Temperature. Body temperature was reduced by THC (30 mg·kg^{-1}, i.p.) and the simultaneous injection of CBD (30 mg·kg^{-1}, i.p.) enhanced the magnitude of hypothermia (Figure 1). The ANOVA confirmed significant main effects of time after injection [$F_{(3, 36)} = 44.60$; $P < 0.0001$], drug treatment condition [$F_{(2, 12)} = 21.63$; $P < 0.0001$] and the interaction of factors [$F_{(6, 36)} = 8.15$; $P < 0.0001$] on temperature. The *post hoc* test confirmed that temperature was significantly lower than the respective vehicle treatment following CBD-THC (all 4 h) and Veh-THC (2–4 h after injection). There was also a significant difference between CBD-THC and Veh-THC conditions in the third hour after injection.

Activity. THC also reduced activity, but there was no additional effect of CBD on hypolocomotion. The ANOVA confirmed significant main effects of hours after injection [$F_{(3, 36)} = 23.98$; $P < 0.0001$], drug treatment condition [$F_{(2, 12)} = 10.78$; $P < 0.005$], but not of the interaction of factors [$F_{(6, 36)} = 0.92$; $P = 0.50$], on activity. The *post hoc* test confirmed that activity was significantly lower than the corresponding vehicle condition after CBD-THC (all at 4 h) and after Veh-THC (hours 2–3).

Thirty minute pretreatment, 1:1 CBD-THC ratio

Temperature. The administration of CBD (30 mg·kg^{-1}, i.p.) 30 min before THC (30 mg·kg^{-1}, i.p.) also enhanced the magnitude of hypothermia (Figure 2). The ANOVA confirmed main effects of time after injection [$F_{(3, 12)} = 21.29$; $P < 0.0001$], drug treatment condition [$F_{(3, 12)} = 7.15$; $P < 0.01$] and the interaction of factors [$F_{(9, 36)} = 8.39$; $P < 0.0001$] on temperature. The *post hoc* test confirmed that temperature was significantly lower than both Veh-Veh and CBD-Veh
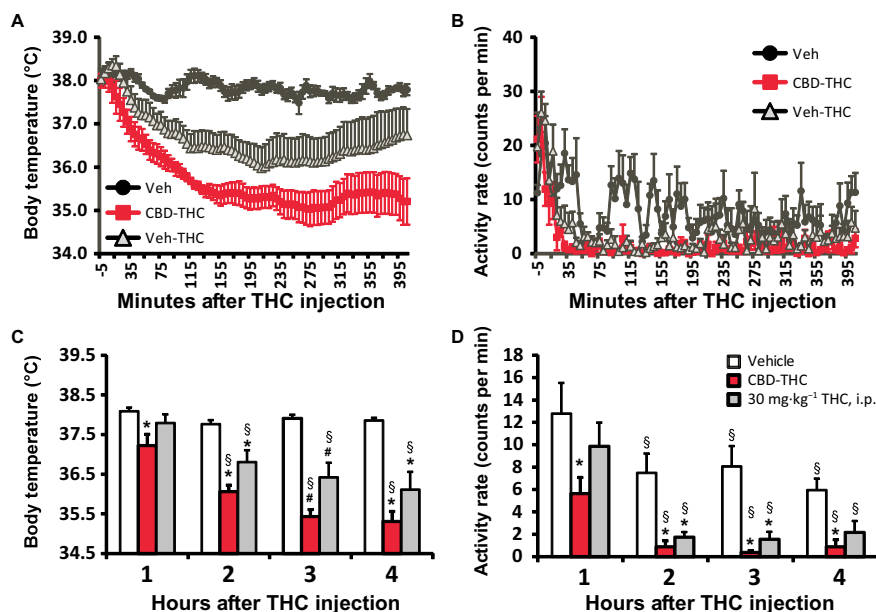
Figure 1

Telemetered body temperature (A and C) and activity rate (B and D) after treatment with 30 mg·kg⁻¹ THC with 30 mg·kg⁻¹ CBD or the vehicle, i.p., administered simultaneously. A vehicle-only control condition (Veh) is also shown. A and B display the data as collected (5 min intervals) and (C and D) depict the hourly averages used for analysis. Values shown are means ± SEM ($n = 5$). *$P < 0.05$, significantly different from Veh (only); #$P < 0.05$, significantly different from both other conditions; §$P < 0.05$, significantly different from the first hour (within treatment condition).

treatment following CBD-THC (2–4 h) and Veh-THC (3–4 h after injection); the Veh-THC condition also differed significantly from the Veh-Veh condition in the second hour after injection. There was also a significant difference between CBD-THC and Veh-THC conditions in the fourth hour after injection. *Post hoc* analysis within treatment condition confirmed that body temperature was significantly lower than the first hour following treatment with CBD-THC (2–4 h after injection) and with Veh-THC (hours 2–4). Significant differences between hours 3–4 and hour 2 were confirmed for the CBD-THC condition as well.

Activity. The hypolocomotor effect of THC was not affected by the administration of CBD 30 min before (Figure 2). The statistical analysis confirmed that there were significant main effects of time after injection [$F(3, 12) = 32.26; P < 0.0001$] and drug treatment condition [$F(3, 12) = 4.61; P < 0.05$], but not of the interaction of factors [$F(9, 36) = 1.83; P = 0.10$], on activity. The *post hoc* test confirmed that activity was significantly lower than the first hour after treatment with CBD-THC (2–4 h after injection) and after Veh-THC (hours 3–4).

Thirty minute pretreatment, 3:1 CBD-THC ratio

Temperature. A lower dose of THC (20 mg·kg⁻¹, i.p.) still reduced body temperature but a threefold higher dose of CBD (60 mg·kg⁻¹, i.p.) had no effect on this response when administered 30 min before THC (Figure 3). The ANOVA confirmed main effects of time after injection [$F(3, 12) = 22.38; P < 0.0001$] and the interaction of factors [$F(9, 36) = 10.31; P < 0.0001$], but not drug treatment condition [$F(3, 12) = 2.14;$

$P = 0.15$], on temperature. The *post hoc* test confirmed that temperature was significantly lower than the respective vehicle condition temperature following CBD-THC (2–4 h after injection) and Veh-THC (1, 3–4 h after injection). Temperature was lower than both Veh-Veh and CBD-Veh conditions 3–4 h after CBD-THC or Veh-THC. There were no significant differences between CBD-THC and Veh-THC conditions at any time after injection. The *post hoc* test also confirmed that temperature was lower compared with the first hour after Veh (2–4 h), CBD-Veh (second hour), Veh-THC (3–4 h, which also differed from 2 h). Temperature following CBD-THC was significantly different between all hourly observations, except for the 1 versus 2 h comparison.

Activity. The threefold higher CBD dose likewise did not modulate the reduction of activity caused by 20 mg·kg⁻¹ THC (Figure 3). There was a significant main effect of time after injection [$F(3, 12) = 21.91; P < 0.0001$], but not of drug treatment condition [$F(3, 12) = 1.58; P = 0.25$] or of the interaction of factors [$F(9, 36) = 0.89; P = 0.54$], on activity. *Post hoc* analysis confirmed that activity was lower than the first hour for the fourth hour after Veh, the second and fourth hours after CBD-Veh, the third and fourth hour after Veh-THC and second, third and fourth hours after treatment with CBD-THC.

THC dose comparison

Temperature. A final analysis (Figure 4) compared the effects of administered THC dose by contrasting the vehicle and 30 mg·kg⁻¹ THC dose conditions from the same-dose 30 min pretreatment study (Figure 2) with the 20 mg·kg⁻¹ dose in the
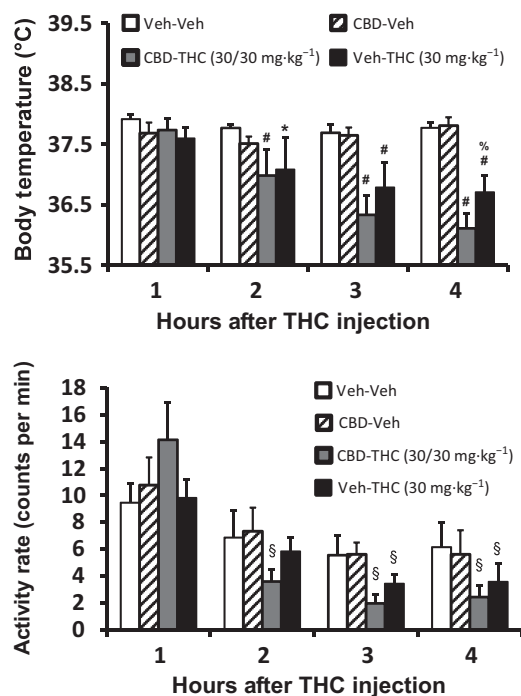
Figure 2

Telemetered body temperature (top) and activity rate (bottom) after treatment with 30 mg·kg⁻¹ THC with and without 30 mg·kg⁻¹ CBD administered 30 min prior. Veh control conditions are also shown. Values shown are means ± SEM ($n = 5$). *$P < 0.05$, significantly different from Veh-Veh (only); #$P < 0.05$, significantly different from Veh-Veh and CBD-Veh; %$P < 0.05$, significantly different between CBD-THC and Veh-THC; §$P < 0.05$, significantly different in activity compared with the first hour (within treatment condition). See text for effects of time on temperature.

Figure 3

Telemetered body temperature (top) and activity rate (bottom) after treatment with 20 mg·kg⁻¹ (lower panels) THC with and without 60 mg·kg⁻¹ CBD administered 30 min prior. Veh control conditions are also shown. Values shown are means ± SEM ($n = 5$). *$P < 0.05$, significantly different from Veh-Veh (only); #$P < 0.05$, significantly different from Veh-Veh and CBD-Veh; &$P < 0.05$, significantly different from CBD-Veh (only); §$P < 0.05$, significantly different in activity compared with the first hour (within treatment condition). See text for effects of time on temperature.

asymmetrical dose study (Figure 3) and a single 10 mg·kg⁻¹ THC challenge conducted in between those studies. Analysis of the temperature data confirmed main effects of time after injection [$F (3, 12) = 9.75; P < 0.005$] and the interaction [$F (9, 36) = 3.50; P < 0.005$], but not of treatment condition, [$F (3, 12) = 1.40; P = 0.29$]. The *post hoc* analysis confirmed that temperature was significantly lower than the vehicle condition in the first hour after injection of 10 mg·kg⁻¹, in the second hour after injection of 10 or 30 mg·kg⁻¹ and in the third and fourth hours after injection of 10, 20 or 30 mg·kg⁻¹ THC. Correspondingly, temperature was lower compared with the first hour after both 20 mg·kg⁻¹ (3–4 h) and 30 mg·kg⁻¹ (2–4 h) THC and compared with the second hour after 20 mg·kg⁻¹ (3–4 h) injection. Significant differences in body temperature were also confirmed between the 10 and 20 mg·kg⁻¹ treatment conditions 1 and 2 h after injection of THC.

Activity. Analysis of the activity data (Figure 4) confirmed a main effect of time after injection [$F (3, 12) = 19.15, P < 0.0001$], but not of treatment condition [$F (3, 12) = 0.36, P = 0.78$] or of dose [$F (9, 36) = 0.49, P = 0.87$]. *Post hoc* analysis confirmed that activity was lower than the first hour for the third and fourth hours after treatment with 20 or 30 mg·kg⁻¹
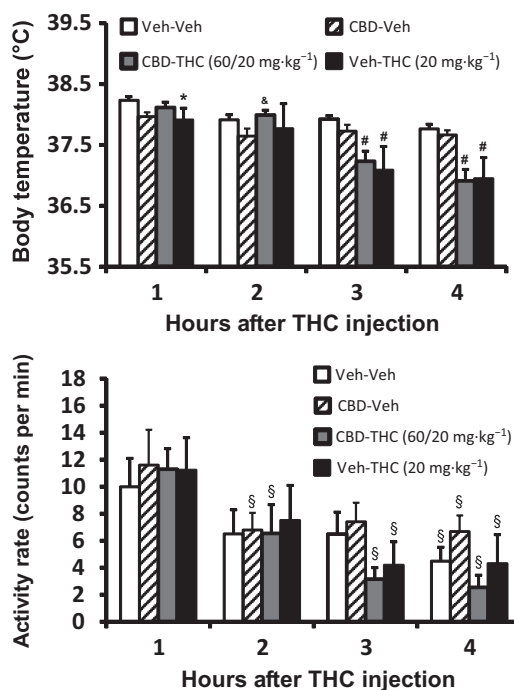
THC but no other comparisons confirmed differential activity.

CB₁ receptor antagonism

Temperature. Treatment of rats with 4 mg·kg⁻¹ SR141716, i.p., 15 min before THC (20 mg·kg⁻¹, i.p.) significantly attenuated the hypothermic response. The ANOVA confirmed main effects of time after injection [$F (12, 84) = 33.24; P < 0.0001$], SR/Veh pretreatment condition [$F (1, 7) = 44.19; P < 0.0005$] and the interaction of factors [$F (12, 84) = 37.99; P < 0.0001$] on temperature. The *post hoc* test confirmed that temperature was significantly different between Veh and SR141716 pretreatment conditions from 90 to 390 min after THC administration. Within the SR141716 pretreatment condition, body temperature was lower than at 30 min (60–270, 330–360 min after THC) and higher than at 90 (270–390 min after THC), 120 (270–290 min after THC), 150 (300–390 min after THC), 180 (300 min after THC) and 210 (300 min after THC) minute time points. In contrast, body temperature following vehicle pretreatment and 20 mg·kg⁻¹ THC, i.p. was significantly lower than the 30 or 60 min time points at all following points, as well as lower than the temperature at 90 (150–390 min),120 (210–390 min) and 150 (270–330 min) minutes after THC.
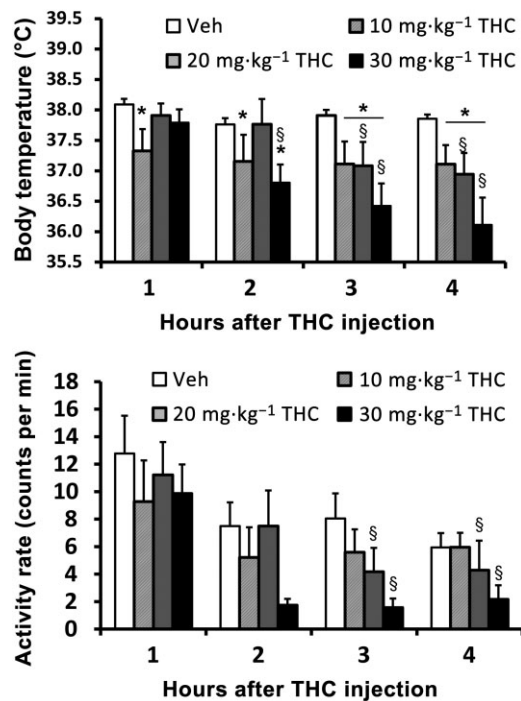
Figure 4

Telemetered body temperature (upper) and activity rate (lower) after treatment with 10 mg·kg⁻¹ THC is compared with data after administration of 20 or 30 mg·kg⁻¹ THC as reported in Figure 2 in the Veh-THC conditions. Values shown are means ± SEM ($n = 5$). *$P < 0.05$, significantly different from Veh (only); §$P < 0.05$, significant differences from the first hour (within treatment condition).

Activity. The ANOVA confirmed main effects of time post-injection [$F (12, 84) = 25.46$; $P < 0.0001$], SR141716 /Veh pretreatment condition [$F (1, 7) = 7.57$; $P = 0.028$] but not the interaction of factors [$F (12, 84) = 1.46$; $P = 0.15$] on activity. *Post hoc* analysis confirmed that activity was only significantly higher in the SR141716 pretreatment condition relative to vehicle pretreatment 60 min after THC injection.

Discussion

This study used a minimally invasive radiotelemetry procedure to determine the effect of CBD on hypothermia and hypolocomotion induced by i.p. THC. The major finding was that CBD failed to attenuate the effects of THC on activity and thermoregulation, and in fact potentiated the effects of THC in some cases. CBD was ineffectual at blocking THC-mediated hypothermia and locomotor suppression when administered either simultaneously or as a pretreatment 30 min before THC injection. The study also found that there was no differential effect when CBD was administered 30 min prior to THC at three times the dose. Finally, CBD had no effect on body temperature or activity when administered in the absence of THC, consistent with many earlier findings of the lack of effect of treatment with CBD alone. The methodological approach was effective as body temperature was

reduced significantly for up to 6 h after 20–30 mg·kg⁻¹ THC, i.p. as predicted for rats (Tulunay *et al.*, 1981; Wiley *et al.*, 2007), and the effect was reversed by pretreatment with the CB₁ receptor antagonist SR141716 (rimonabant) as had been shown in both rats (Nava *et al.*, 2000) and monkeys (Taffe, 2012b). Activity was also suppressed by THC administration, although this effect was only reversed in the first hour by pretreatment with the antagonist. The effects of THC were roughly dose-dependent and reproducible across several challenges in the same animals, which further validate this model.

The CBD-mediated enhancement of hypothermia is similar to an earlier result in rats in which equal doses were administered simultaneously (Fernandes *et al.*, 1974). In that study, CBD acted mostly to prolong the duration of hypothermia, whereas here it also changed the magnitude of temperature reduction, at least when administered at a 1:1 dose ratio either simultaneously or at a 15 min offset (Figures 1 and 2). Similarly, when CBD ws given 20 min before an equal dose of THC, it also slightly potentiated behavioural impairment in rats (Klein *et al.*, 2011). There was some evidence for a dose ratio dependency of the enhancement of hypothermia as it was not observed in the experiments using the 3:1 CBD : THC treatment (Figure 3). This may be similar to the clinical observation that cannabis strains of 6% THC/7.5% CBD produced equivalent therapeutic relief in chronic pain in MS patients as did 19% THC/>1% CBD strain without any difference in the amount of cannabis used per day (Brunt *et al.*, 2014). That finding is consistent with a CBD-mediated enhancement of THC effects, rather than a diminution.

The present study also showed that thermoregulatory and activity responses to THC in the rat may not model the expected outcome in humans quite as well as studies in non-human primates. Human studies have shown that CBD : THC ratios in cannabis strains that approach 1:1 may prevent cognitive disruption associated with low CBD strains of approximately equal THC content (Morgan *et al.*, 2010; 2012). We have previously shown that CBD reversed the effect of behaviourally impairing doses of THC when administered at a 1:1 ratio in the macaque monkey and these effects were similar when CBD was injected either simultaneously with THC or with a 20 min pretreatment interval (Wright *et al.*, 2013b). Furthermore, the dose ranges for behavioural impairment in monkeys (Taffe, 2012a; Wright *et al.*, 2013a) overlap with those that produce significant hypothermia (Taffe, 2012b).

The magnitude and duration of hypothermia produced by 20 mg·kg⁻¹ THC i.p. in the two treatment groups was very similar (Figures 1 and 5), demonstrating the consistency of the effect across minor differences in animal age and body weight. The delayed effect of SR141716 on hypothermia induced by 20 mg·kg⁻¹ THC, i.p., in the validation study was unexpected since most thermoregulatory investigations, particularly in mice, appear to demonstrate immediate blockade of THC-induced hypothermia. Nevertheless, SR141716 pretreatment affected a prolactin response to THC at 90, but not 20, min after injection in rats (Fernandez-Ruiz *et al.*, 1997). Similarly, SR141716 attenuated hypothermia at 30, but not 10, min after injection of the CB₁ receptor full agonist WIN 55,212-2 in mice (Son *et al.*, 2010). The rather modest effect of SR141716 on hypolocomotion may be a reflection of the
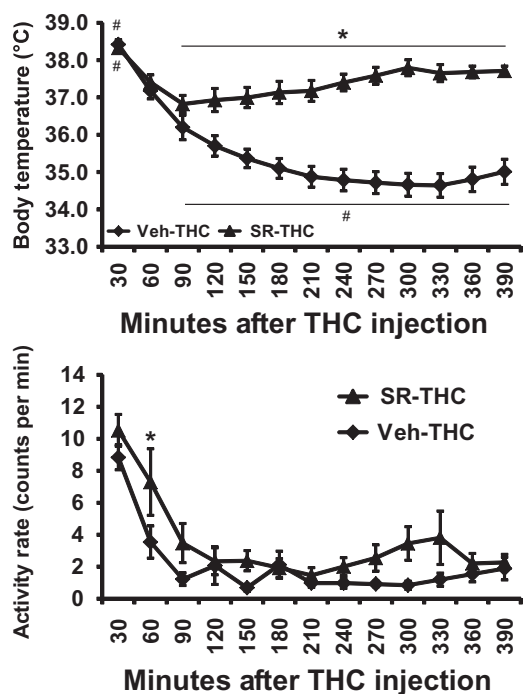
Figure 5

Telemetered body temperature (upper) and activity rate (lower) after treatment with 20 mg·kg⁻¹ THC with 4 mg·kg⁻¹ SR141716 or the vehicle administered, i.p., 15 min prior to THC. Values shown are means ± SEM ($n = 8$). *$P < 0.05$, significant differences between treatment conditions; #$P < 0.05$, significantly different from the 60 min time point (within treatment) for temperature.

the same magnitude of effects of identical THC doses across the repeated measures design, it is possible that minor degrees of tolerance were produced. Given the primary interpretive comparisons of interest for this study, however, it is unclear how minor degrees of tolerance would affect the major conclusions.

In conclusion, this investigation found no evidence that CBD could ameliorate the thermoregulatory or hypolocomotor effects of THC when administered either simultaneously or prior to THC. Increasing the ratio of CBD : THC from 1:1 to 3:1 had no differential effect. As both street marijuana products and the medication Sativex do not exceed a 1:1 CBD : THC ratio, and drugs are administered simultaneously, the present studies have the best construct validity for translational inferences. It cannot be excluded that a longer pretreatment interval or much larger CBD : THC ratio might produce a different result, but it is difficult to establish relevance of such approaches to the actual use of available strains of cannabis (Morgan *et al.*, 2010; Burgdorf *et al.*, 2011). Overall, there was no evidence from this study that elevated CBD content in cannabis would provide protection from the physiological effects of THC.

Acknowledgements

Author contributions

M. A. T. designed the study with significant input from K. M. C. and S. A. V. K. M. C. and S. A. V. performed the research and conducted initial data analysis. M. A. T. conducted statistical analysis of data, created figures and wrote the paper. M. A. T., K. M. C. and S. A. V. revised the final draft and approved of the submitted version of the manuscript.

Conflict of interest

The authors have no competing interests.

fact that the activity measure was not highly sensitive or perhaps because tetrad effects of THC may be maximal at different doses, for example see Marshell *et al.* (2014). In other studies in mice, the first time point for temperature measurement was 30 min or 60 min after a CB_1 receptor agonist was administered (Fox *et al.*, 2001) so it is difficult to definitively establish the time course of effects. Interestingly, when SR141716 was administered 20 min after THC, it appeared to ameliorate hypothermia within 10 min (Nava *et al.*, 2000). Overall, the present results are mostly consistent with the published data available and further experiments with varied SR141716 /THC administration offsets would be required to fully characterize the response within the first 30 min of THC injection. It is possible, for example, that prior exposure to THC in these animals had a conditioning effect or induced some unknown neuroplastic effect that was only effective in the first hour after injection.

A minor caveat to the study is that it is possible that interactive effects would be observed at a lower dose of THC. The dose range for this study was selected based on published data and pilot studies showing consistent effects on the temperature and activity end points that were selected. Thus, it seems most likely that lower doses would simply make the THC-related effects too variable for interpretation; but the possibility CBD/THC interactions would be detectable at lower doses cannot be definitively excluded. Likewise, although the treatment intervals resulted in approximately

References

Aarde SM, Huang PK, Creehan KM, Dickerson TJ, Taffe MA (2013). The novel recreational drug 3,4-methylenedioxypyrovalerone (MDPV) is a potent psychomotor stimulant: self-administration and locomotor activity in rats. Neuropharmacology 71: 130–140.

Alexander SP, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M et al. (2013). The Concise Guide to PHARMACOLOGY 2013/14: G protein-coupled receptors. Br J Pharmacol 170: 1459–1581.

Belgrave BE, Bird KD, Chesher GB, Jackson DM, Lubbe KE, Starmer GA et al. (1979). The effect of cannabidiol, alone and in combination with ethanol, on human performance. Psychopharmacology (Berl) 64: 243–246.

Brunt TM, van Genugten M, Honer-Snoeken K, van de Velde MJ, Niesink RJ (2014). Therapeutic satisfaction and subjective effects of different strains of pharmaceutical-grade cannabis. J Clin Psychopharmacol 34: 344–349.

Burgdorf JR, Kilmer B, Pacula RL (2011). Heterogeneity in the composition of marijuana seized in California. Drug Alcohol Depend 117: 59–61.

Crean RD, Davis SA, Von Huben SN, Lay CC, Katner SN, Taffe MA (2006). Effects of (+/–)3,4-methylenedioxymethamphetamine, (+/–)3,4-methylenedioxyamphetamine and methamphetamine on temperature and activity in rhesus macaques. Neuroscience 142: 515–525.

Crean RD, Davis SA, Taffe MA (2007). Oral administration of (+/–)3,4-methylenedioxymethamphetamine and (+)methamphetamine alters temperature and activity in rhesus macaques. Pharmacol Biochem Behav 87: 11–19.

Fernandes M, Schabarek A, Coper H, Hill R (1974). Modification of delta9-THC-actions by cannabinol and cannabidiol in the rat. Psychopharmacologia 38: 329–338.

Fernandez-Ruiz JJ, Munoz RM, Romero J, Villanua MA, Makriyannis A, Ramos JA (1997). Time course of the effects of different cannabimimetics on prolactin and gonadotrophin secretion: evidence for the presence of CB1 receptors in hypothalamic structures and their involvement in the effects of cannabimimetics. Biochem Pharmacol 53: 1919–1927.

Fox A, Kesingland A, Gentry C, McNair K, Patel S, Urban L et al. (2001). The role of central and peripheral Cannabinoid1 receptors in the antihyperalgesic activity of cannabinoids in a model of neuropathic pain. Pain 92: 91–100.

Juckel G, Roser P, Nadulski T, Stadelmann AM, Gallinat J (2007). Acute effects of Δ9-tetrahydrocannabinol and standardized cannabis extract on the auditory evoked mismatch negativity. Schizophr Res 97: 109–117.

Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG (2010). Animal research: Reporting in vivo experiments: the ARRIVE guidelines. Br J Pharmacol 160: 1577–1579.

Klein C, Karanges E, Spiro A, Wong A, Spencer J, Huynh T et al. (2011). Cannabidiol potentiates Delta(9)-tetrahydrocannabinol (THC) behavioural effects and alters THC pharmacokinetics during acute and chronic treatment in adolescent rats. Psychopharmacology (Berl) 218: 443–457.

Lichtman AH, Dimen KR, Martin BR (1995). Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. Psychopharmacology (Berl) 119: 282–290.

Marshell R, Kearney-Ramos T, Brents LK, Hyatt WS, Tai S, Prather PL et al. (2014). In vivo effects of synthetic cannabinoids JWH-018 and JWH-073 and phytocannabinoid Delta-THC in mice: inhalation versus intraperitoneal injection. Pharmacol Biochem Behav 124C: 40–47.

McGrath J, Drummond G, McLachlan E, Kilkenny C, Wainwright C (2010). Guidelines for reporting experiments involving animals: the ARRIVE guidelines. Br J Pharmacol 160: 1573–1576.

McPartland JM, Duncan M, Di Marzo V, Pertwee R (2015). Are cannabidiol and Δ⁹-tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. Br J Pharmacol 172: 737–753.

Mechoulam R, Peters M, Murillo-Rodriguez E, Hanus LO (2007). Cannabidiol – recent advances. Chem Biodivers 4: 1678–1692.

Miller ML, Creehan KM, Angrish D, Barlow DJ, Houseknecht KL, Dickerson TJ et al. (2013). Changes in ambient temperature differentially alter the thermoregulatory, cardiac and locomotor stimulant effects of 4-methylmethcathinone (mephedrone). Drug Alcohol Depend 127: 248–253.

Morgan CJ, Schafer G, Freeman TP, Curran HV (2010). Impact of cannabidiol on the acute memory and psychotomimetic effects of smoked cannabis: naturalistic study: naturalistic study [corrected]. Br J Psychiatry 197: 285–290.

Morgan CJ, Gardener C, Schafer G, Swan S, Demarchi C, Freeman TP et al. (2012). Sub-chronic impact of cannabinoids in street cannabis on cognition, psychotic-like symptoms and psychological well-being. Psychol Med 42: 391–400.

Nava F, Carta G, Gessa GL (2000). Permissive role of dopamine D(2) receptors in the hypothermia induced by delta(9)-tetrahydrocannabinol in rats. Pharmacol Biochem Behav 66: 183–187.

Oreja-Guevara C (2012). Clinical efficacy and effectiveness of Sativex, a combined cannabinoid medicine, in multiple sclerosis-related spasticity. Expert Rev Neurother 12: 3–8.

Pawson AJ, Sharman JL, Benson HE, Faccenda E, Alexander SP, Buneman OP et al.; NC-IUPHAR (2014). The IUPHAR/BPS Guide to PHARMACOLOGY: an expert-driven knowledge base of drug targets and their ligands. Nucl. Acids Res. 42 (Database Issue): D1098–1106.

Petitet F, Jeantaud B, Reibaud M, Imperato A, Dubroeucq MC (1998). Complex pharmacology of natural cannabinoids: evidence for partial agonist activity of delta9-tetrahydrocannabinol and antagonist activity of cannabidiol on rat brain cannabinoid receptors. Life Sci 63: PL1–PL6.

Robson P (2011). Abuse potential and psychoactive effects of delta-9-tetrahydrocannabinol and cannabidiol oromucosal spray (Sativex), a new cannabinoid medicine. Expert Opin Drug Saf 10: 675–685.

Roser P, Juckel G, Rentzsch J, Nadulski T, Gallinat J, Stadelmann AM (2008). Effects of acute oral Delta9-tetrahydrocannabinol and standardized cannabis extract on the auditory P300 event-related potential in healthy volunteers. Eur Neuropsychopharmacol 18: 569–577.

Schoedel KA, Chen N, Hilliard A, White L, Stott C, Russo E et al. (2011). A randomized, double-blind, placebo-controlled, crossover study to evaluate the subjective abuse potential and cognitive effects of nabiximols oromucosal spray in subjects with a history of recreational cannabis use. Hum Psychopharmacol 26: 224–236.

Son MH, Kim HD, Chae YN, Kim MK, Shin CY, Ahn GJ et al. (2010). Peripherally acting CB1-receptor antagonist: the relative importance of central and peripheral CB1 receptors in adiposity control. Int J Obes 34: 547–556.

Taffe MA (2012a). Delta(9)tetrahydrocannabinol impairs visuo-spatial associative learning and spatial working memory in rhesus macaques. J Psychopharmacol 26: 1299–1306.

Taffe MA (2012b). Delta9-tetrahydrocannabinol attenuates MDMA-induced hyperthermia in rhesus monkeys. Neuroscience 201: 125–133.

Taffe MA, Lay CC, Von Huben SN, Davis SA, Crean RD, Katner SN (2006). Hyperthermia induced by 3,4-methylenedioxymethamphetamine in unrestrained rhesus monkeys. Drug Alcohol Depend 82: 276–281.

Tulunay FC, Ayhan IH, Portoghese PS, Takemori AE (1981). Antagonism by chlornaltrexamine of some effects of delta 9-tetrahydrocannabinol in rats. Eur J Pharmacol 70: 219–224.

Turkanis SA, Karler R (1986). Cannabidiol-caused depression of spinal motoneuron responses in cats. Pharmacol Biochem Behav 25: 89–94.

Vann RE, Gamage TF, Warner JA, Marshall EM, Taylor NL, Martin BR et al. (2008). Divergent effects of cannabidiol on the discriminative stimulus and place conditioning effects of Delta(9)-tetrahydrocannabinol. Drug Alcohol Depend 94: 191–198.

Wade DT, Makela P, Robson P, House H, Bateman C (2004). Do cannabis-based medicinal extracts have general or specific effects on symptoms in multiple sclerosis? A double-blind, randomized, placebo-controlled study on 160 patients. Mult Scler 10: 434–441.

Wiley JL, O'Connell MM, Tokarz ME, Wright MJ Jr (2007). Pharmacological effects of acute and repeated administration of Delta(9)-tetrahydrocannabinol in adolescent and adult rats. J Pharmacol Exp Ther 320: 1097–1105.

Winsauer PJ, Lambert P, Moerschbaecher JM (1999). Cannabinoid ligands and their effects on learning and performance in rhesus monkeys. Behav Pharmacol 10: 497–511.

Wright MJ Jr, Angrish D, Aarde SM, Barlow DJ, Buczynski MW, Creehan KM et al. (2012). Effect of ambient temperature on the thermoregulatory and locomotor stimulant effects of 4-methylmethcathinone in Wistar and Sprague-Dawley rats. PLoS ONE 7: e44652.

Wright MJ Jr, Vandewater SA, Parsons LH, Taffe MA (2013a). Δ9Tetrahydrocannabinol impairs reversal learning but not extra-dimensional shifts in rhesus macaques. Neuroscience 235: 51–58.

Wright MJ Jr, Vandewater SA, Taffe MA (2013b). Cannabidiol attenuates deficits of visuospatial associative memory induced by Delta(9) tetrahydrocannabinol. Br J Pharmacol 170: 1365–1373.

Zuardi AW, Teixeira NA, Karniol IC (1984). Pharmacological interaction of the effects of delta 9-trans-tetrahydrocannabinol and cannabidiol on serum corticosterone levels in rats. Arch Int Pharmacodyn Ther 269: 12–19.

Zuardi AW, Hallak JE, Crippa JA (2012). Interaction between cannabidiol (CBD) and (9)-tetrahydrocannabinol (THC): influence of administration interval and dose ratio between the cannabinoids. Psychopharmacology (Berl) 219: 247–249.