BJP British Journal of Pharmacology

DOI:10.1111/bph.12316
www.brjpharmacol.org

RESEARCH PAPER

Tetrahydrocannabinolic acid reduces nausea-induced conditioned gaping in rats and vomiting in *Suncus murinus*

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Keywords
THCA; THC; conditioned gaping; vomiting; SR141716; CB_1 receptor

Received
27 February 2013
Revised
5 July 2013
Accepted
19 July 2013

BACKGROUND AND PURPOSE

We evaluated the anti-emetic and anti-nausea properties of the acid precursor of Δ^9-tetrahydrocannabinol (THC), tetrahydrocannabinolic acid (THCA), and determined its mechanism of action in these animal models.

EXPERIMENTAL APPROACH

We investigated the effect of THCA on lithium chloride- (LiCl) induced conditioned gaping (nausea-induced behaviour) to a flavour, and context (a model of anticipatory nausea) in rats, and on LiCl-induced vomiting in *Suncus murinus*. Furthermore, we investigated THCA's ability to induce hypothermia and suppress locomotion [rodent tasks to assess cannabinoid$_1$ (CB_1) receptor agonist-like activity], and measured plasma and brain THCA and THC levels. We also determined whether THCA's effect could be blocked by pretreatment with SR141716 (SR, a CB_1 receptor antagonist).

KEY RESULTS

In rats, THCA (0.05 and/or 0.5 mg·kg^{-1}) suppressed LiCl-induced conditioned gaping to a flavour and context; the latter effect blocked by the CB_1 receptor antagonist, SR, but not by the 5-hydroxytryptamine-1A receptor antagonist, WAY100635. In *S. murinus*, THCA (0.05 and 0.5 mg·kg^{-1}) reduced LiCl-induced vomiting, an effect that was reversed with SR. A comparatively low dose of THC (0.05 mg·kg^{-1}) did not suppress conditioned gaping to a LiCl-paired flavour or context. THCA did not induce hypothermia or reduce locomotion, indicating non-CB_1 agonist-like effects. THCA, but not THC was detected in plasma samples.

CONCLUSIONS AND IMPLICATIONS

THCA potently reduced conditioned gaping in rats and vomiting in *S. murinus*, effects that were blocked by SR. These data suggest that THCA may be a more potent alternative to THC in the treatment of nausea and vomiting.

Abbreviations

5-HT_{1A}, 5-hydroxytryptamine-1A; AN, anticipatory nausea; CB_1, cannabinoid 1; CB_2, cannabinoid 2; CBD, cannabidiol; CBDA, cannabidiolic acid; LiCl, lithium chloride; LSD, least significant difference; SAL, saline; SR, SR141716; THC, Δ^9-tetrahydrocannabinol; THCA, tetrahydrocannabinolic acid; TR, taste reactivity; VEH, vehicle; WAY, WAY100635

Introduction

The cannabis plant is a source of at least 70 phytocannabinoids, including the psychoactive component Δ^9-tetrahydrocannabinol (THC). THC is effective in interfering with nausea and vomiting in human cancer patients (see Cotter, 2009 for review). Comparisons of oral THC with the common anti-emetic agents of the time showed that THC was at least as effective (Frytak *et al.*, 1979; Carey *et al.*, 1983; Ungerleider *et al.*, 1984; Crawford and Buckman, 1986;

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Cunningham *et al.*, 1988; Tramer *et al.*, 2001) if not more effective (Orr *et al.*, 1980; Orr and McKernan, 1981) at reducing nausea and vomiting in human patients.

In animal models, THC reduces vomiting in ferrets (Van Sickle *et al.*, 2001) and has been shown to suppress acute vomiting induced by cisplatin (Darmani, 2001b), SR141716 (SR), a cannabinoid 1 (CB_1) receptor antagonist (Darmani, 2001a), radiation (Darmani *et al.*, 2007) and 5-hydroxytryptophan (an indirect 5-HT receptor agonist, Darmani and Johnson, 2004) in *Cryptotis parva* (least shrews), a well-established animal model for assessing vomiting (see Darmani, 1998). In another animal model for assessing vomiting, *Suncus murinus* (house musk shrew), THC reduces cisplatin- (Kwiatkowska *et al.*, 2004), LiCl- (Parker *et al.*, 2004) and motion-induced vomiting (Cluny *et al.*, 2008). Additionally, the administration of THC prior to reintroduction to a context previously associated with illness suppressed the expression of conditioned retching in shrews (Parker and Kemp, 2001; Parker *et al.*, 2006).

Using the taste reactivity (TR) test, THC (0.5 $mg \cdot kg^{-1}$) has also been shown to interfere with the establishment and expression of conditioned gaping in rats produced by cyclophosphamide, a commonly used chemotherapy drug (Limebeer and Parker, 1999) and LiCl (Parker and Mechoulam, 2003; Parker *et al.*, 2003). Unlike conditioned taste avoidance, which can be produced by both rewarding drugs and emetic drugs, conditioned gaping reactions are produced only by drugs that induce vomiting in emetic species, such as shrews (Parker, 2003; Parker *et al.*, 2008). In a rodent model of anticipatory nausea (AN), THC (0.5 $mg \cdot kg^{-1}$) also reduces contextually elicited conditioned gaping in rats (Limebeer *et al.*, 2006). The THC-induced suppression of nausea-induced behaviours and vomiting can be reversed by administration of the CB_1 receptor antagonists SR or AM251, so it seems that the anti-emetic and anti-nausea effects of THC are mediated by the CB_1 receptor (Darmani, 2001b; Parker *et al.*, 2004; Darmani and Johnson, 2004; Darmani *et al.*, 2007; Cluny *et al.*, 2008).

THC is formed in cannabis from an acidic precursor, tetrahydrocannabinolic acid (THCA, Gaoni and Mechoulam, 1964). In the fresh plant, THCA is decarboxylated to THC by heating or burning. Interestingly, no psychotomimetic activity was observed with THCA administration to rhesus monkeys (doses ≤ 5 $mg \cdot kg^{-1}$, i.v.), mice (doses ≤ 20 $mg \cdot kg^{-1}$, i.p.) and dogs (doses ≤ 7 $mg \cdot kg^{-1}$; Grunfeld and Edery, 1969), perhaps making THCA a more desirable treatment than THC because it is devoid of psychoactive activity. No *in vivo* studies to date have evaluated THCA's mechanism of action, however *in vitro*, THCA's ability to inhibit the TNF-α levels in culture supernatants from U937 macrophages was not blocked by administration of the CB_1 receptor antagonist AM281, or the cannabinoid 2 receptor (CB_2) receptor antagonist AM630 (Verhoeckx *et al.*, 2006). In addition, binding assays indicate that THCA is not active at the CB_1 receptor (Ahmed *et al.*, 2008).

The present investigation sought to explore whether THCA (0.5 and/or 0.05 $mg \cdot kg^{-1}$) could (i) inhibit LiCl-induced conditioned gaping to a flavour in rats; (ii) reduce LiCl-induced conditioned gaping to a context in rats, and whether this effect was CB_1 or 5-hydroxytryptamine-1A ($5\text{-}HT_{1A}$) receptor dependent; and (iii) reduce LiCl-induced vomiting in *S. murinus*, and whether this effect was CB_1 receptor mediated. Plasma and brain samples from the AN study were also analysed for levels of THCA, as well as THC. Additionally, to determine if an equally low dose of THC could reduce nausea and AN in these models, we also added a low dose THC group (0.05 $mg \cdot kg^{-1}$) to each of our rat studies. To further assess THCA's CB_1 receptor mechanism of action, we also examined whether THCA (0.5 $mg \cdot kg^{-1}$) exhibited any CB_1 agonist-like behavioural activity, as defined in rodents by their characteristic action on a tetrad of tasks developed by Martin *et al.* (1991), including the hypothermia and locomotor activity assays.

Methods

Experimental procedures

Effect of THCA on LiCl*-induced conditioned gaping to a flavour in rats.* All rats were surgically implanted with an intraoral cannula under isofluorane anaesthesia according to the procedures described by Limebeer *et al.* (2010). Following recovery from surgery (at least 3 days), the rats received an adaptation trial in which they were placed in the TR chamber with their cannula attached to an infusion pump (Model KDS100, KD Scientific, Holliston, MA, USA) for fluid delivery. The TR chambers were made of clear Plexiglas (22.5 × 26 × 20 cm) that sat on a table with a clear glass top. A mirror beneath the chamber on a 45° angle facilitated viewing of the ventral surface of the rat to observe orofacial responses. Water was infused into their intraoral cannulae for 2 min at the rate of 1 $mL \cdot min^{-1}$. On the day following the adaptation trial, the rats received a conditioning trial in which they were administered a pretreatment injection of THCA (0.05, 0.5 $mg \cdot kg^{-1}$), THC (0.5 $mg \cdot kg^{-1}$) or VEH [ethanol/Cremophor/saline (SAL), 1:1:18]. Thirty minutes after the pretreatment injection, the rats were individually placed in the chamber and intraorally infused with 0.1% saccharin solution for 2 min at the rate of 1 $mL \cdot min^{-1}$ while the orofacial responses were video recorded from a mirror at a 45° angle beneath the chambers, with the feed from the video camera (Sony DCR-HC48, Henry's Cameras, Waterloo, ON, Canada) into a computer via FireWire connection. Immediately after the saccharin infusion, all rats were injected with 20 $mL \cdot kg^{-1}$ of 0.15 M LiCl and returned to their home cage. The groups were as follows: VEH (n = 8), 0.05 $mg \cdot kg^{-1}$ THCA (n = 8), 0.5 $mg \cdot kg^{-1}$ THCA (n = 7), 0.05 $mg \cdot kg^{-1}$ THC (n = 8). Seventy-two hours later, rats were tested drug free. Rats were again intraorally infused with 0.1% saccharin solution for 2 min at the rate of 1 $mL \cdot min^{-1}$ while the orofacial responses were video recorded. Rats were then returned to their home cages. The videotapes were later scored (at 1/2 speed) by an observer blind to the experimental conditions using 'The Observer' (Noldus Information Technology Inc., Leesburg, VA, USA) for the behaviour of gaping (large openings of the mouth and jaw, with lower incisors exposed). Conditioned taste avoidance was assessed in a single bottle test. Rats were water-restricted at 4:00 PM. The following morning, a single bottle containing 0.1% saccharin was placed on the cage at 9:00 AM. Measures of saccharin consumption were taken at 30, 120 and 360 min. The number of gapes during the TR test trial was entered into a

one-way ANOVA. The amount of saccharin consumed during the conditioned taste avoidance test for each group was entered into a 4 × 3 mixed factors one-way ANOVA. For all analyses, *P*-values of <0.05 were taken as significant.

Assessment of CB_1 agonist-like behavioural activity. To assess whether THCA induced hypothermia, in a separate group of rats, rectal temperatures were monitored using a rectal digital thermometer (Model # 2483, Life Brand; Shoppers Drug Mart Inc., Guelph, ON, Canada). Rats were placed on a towel and mildly restrained with the tail lifted. The probe tip was covered with petroleum jelly and inserted 5 cm into the rat's rectum and held in place for 30 s. A baseline temperature was established, consisting of the average of two baseline measures, taken 15 min apart. Rats were then pretreated with either VEH or THCA (0.5 mg·kg^{-1}, n = 5/group) and temperatures were taken at 30, 45, 60, 75 and 90 min after pretreatment. The temperatures recorded for each group were entered into a 2 × 6 mixed factors one-way ANOVA. *P*-values of <0.05 were taken as significant.

To assess whether THCA reduced spontaneous motor activity, a separate group of rats were pretreated with either VEH or THCA (0.5 mg·kg^{-1}, n = 8/group) 30 min prior to being placed in a novel activity chamber. The activity chamber was constructed of white Plexiglass with the dimensions of 60 cm × 25 cm × 25 cm illuminated with a red light. A video camera mounted on an extension pole captured the activity of the rat, which was sent to a computer for analysis of distance (cm) travelled using the Ethovision software program (Noldus Information Technology Inc., Leesburg, VA, USA). The distance (cm) travelled in the locomotor activity test was entered into an independent-samples *t*-test. *P*-values of <0.05 were taken as significant.

Wiley and Martin (2003) have, however, indicated that because other drug classes (such as dopamine antagonists) are also capable of producing cannabinoid-like effects on some of the tetrad tasks, CB_1 agonist activity should be verified by blocking the behavioral effects using a selective CB_1 antagonist/inverse agonist such as SR (Rinaldi-Carmona *et al.*, 1994). Therefore, we went on to further investigate THCA's effect on LiCl-induced conditioned gaping to a context, as well as the mechanism of action, by examining whether SR or WAY100635 (WAY) could reverse THCA's effect.

Effect of SR or WAY pretreatment with THCA on LiCl*-induced conditioned gaping to a context in rats.* The distinctive context utilized for conditioning used location, visual, and tactile cues different from those in the home cage environment. The room was dark with two 25 Watt lights beside the conditioning chamber. The conditioning apparatus was identical to that used in the LiCl-induced conditioned gaping experiments, except that the chamber was made of black opaque Plexiglas. The rats received four conditioning trials, during which the contextual chamber was paired with 127 mg·kg^{-1} LiCl. In each conditioning trial, each rat was injected with LiCl and immediately placed in the distinctive context for a 30 min period. This procedure was followed for a total of four conditioning trials, with 72 h between each trial. On the test trial, rats were given a pretreatment of SR (2.5 mg·kg^{-1}), WAY (0.1 mg·kg^{-1}), or VEH to investigate THCA's mechanism of action. Fifteen minutes later, rats received a second pretreatment of VEH, THCA (0.05 mg·kg^{-1}) or THC (0.05 mg·kg^{-1}). Thirty minutes later, rats were taken individually to the conditioning context, given a SAL injection (20 mL·kg^{-1}) and placed in the chamber for 5 min while their orofacial responses were video-recorded from a mirror beneath the chamber. The groups were as follows: VEH-VEH (n = 7), VEH-THCA (n = 8), SR-THCA (n = 8), SR-VEH (n = 8), WAY-THCA (n = 7), WAY-VEH (n = 7), VEH-THC (n = 8). The number of gapes during the test trial was analysed using a one-way ANOVA. *P*-values of <0.05 were taken as significant.

Immediately following the AN test, the rats in groups VEH-VEH and VEH-THCA were sacrificed and the trunk blood was collected and prepared and separated (under refrigeration) into clean tubes. Plasma samples was stored at –20°C until they were sent in dry ice to Quotient Bioresearch (Fordham, Cambridgeshire, UK) for analysis of both THCA and THC levels present.

Effect of THCA on LiCl*-induced vomiting in* S. murinus. This experiment evaluated the effect of pretreatment with THCA on vomiting induced by LiCl in *S. murinus*. The shrews were moved into the experimental room from the colony room and given four meal worms in an empty cage 15 min prior to receiving their pretreatment injection. Shrews were injected with THCA (0.05, n = 10 or 0.5 mg·kg^{-1}, n = 8) or VEH (ethanol/Cremophor/SAL, 1:1:18, n = 10), followed 30 min later by an injection of LiCl (390 mg·kg^{-1}). An additional group to assess the CB_1 receptor mediation of THCA's effect was given SR (2.5 mg·kg^{-1}, n = 6) 15 min prior to THCA (0.05 mg·kg^{-1}). They were then immediately placed, individually in the chamber and observed for 45 min. The Plexiglas observation chambers (22.5 × 26 × 20 cm) sat on a table with a clear glass top. A mirror beneath the chamber on a 45° angle facilitated viewing of the ventral surface of the shrew to observe vomiting episodes. An observer blind to experimental conditions counted the frequency of vomiting episodes. Data were expressed as the mean ± SEM of vomiting responses (n = 6–10) and analysed using ANOVA. *P*-values of <0.05 were taken as significant.

Animals

Animal procedures complied with the Canadian Council on Animal Care. The protocols were approved by the Institutional Animal Care Committee, which is accredited by the Canadian Council on Animal Care. The authors also followed the ARRIVE guidelines of the *British Journal of Pharmacology*, for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010). Naïve male Sprague-Dawley rats, weighing between 230 and 311 g on the day of conditioning, obtained from Charles River Laboratories (St Constant, Quebec, Canada) were used for assessment of anti-nausea-like behaviour. They were single-housed (except in the assessment of conditioned gaping to a context, for which they were pair-housed) in shoebox cages in a colony room at an ambient temperature of 21°C with a 12/12 h light-dark schedule (lights off at 8:00 AM) and maintained on food and water *ad libitum*. LiCl-induced emesis experiments were performed with male (36.3–47.4 g) and female (21.6–30.2 g) *S. murinus*, ranging from 98 to 814 days of age at time of test, bred and raised in the University of Guelph colony. They were single-housed in cages in a colony room at an ambient temperature

of 22°C on a 10/14 hlight-dark schedule (lights off at 7:00 PM). Shrews were tested during their light cycle.

Drugs and materials

Samples of THCA and THC, extracted from cannabis, were provided by GW Pharmaceuticals (Porton Down, Wiltshire, UK). Ethanol, Cremophor and LiCl were provided by Sigma (St Louis, MO, USA). THCA and THC were prepared in a vehicle (VEH) consisting of a 1:1:18 mixture of ethanol, Cremophor and SAL and was administered i.p. in a volume of 2 mL·kg^{-1}. The initial dose for THCA (0.5 mg·kg^{-1}) was selected based on the effective doses of THC in our models (Parker and Kemp, 2001; Parker and Mechoulam, 2003; Parker *et al.*, 2003; 2006) and the selection of a lower dose of THCA was based on the finding that CBD's acidic precursor was far more potent than CBD in our models (Bolognini *et al.*, 2013; Rock and Parker, 2013). LiCl was prepared as a 0.15 M solution with sterile water and administered i.p. in a volume of 60 mL·kg^{-1} (390 mg·kg^{-1}) to shrews and in a volume of 20 mL·kg^{-1} (127.2 mg·kg^{-1}) to rats. SR, 2.5 mg·kg^{-1} – a dose that on its own does not potentiate conditioned gaping or vomiting over that of VEH (Rock *et al.*, 2008; Parker *et al.*, 2009) – was prepared in a VEH consisting of a 1:1:18 mixture of ethanol, Tween and SAL and administered i.p. in a volume of 1 mL·kg^{-1} in rats and shrews. WAY (0.1 mg·kg^{-1}, Rock *et al.*, 2012) was mixed in SAL and administered i.p. in a volume of 1 mL·kg^{-1}.

Results

THCA suppresses LiCl*-induced conditioned gaping to a flavour in rats*

We investigated whether THCA could attenuate LiCl-induced gaping (Figure 1A). A one-way ANOVA revealed a significant effect of pretreatment, $F(3, 27) = 4.4$; $P < 0.02$. Least significant difference (LSD) *post hoc* comparisons revealed that both 0.05 and 0.5 mg·kg^{-1} THCA significantly (*P*-values < 0.05) reduced LiCl-induced gaping relative to the VEH-pretreated controls. Group THC (0.05 mg·kg^{-1}) did not differ from the VEH-pretreated controls ($P > 0.05$). It is unlikely that THCA attenuated conditioned gaping in rats through a direct effect on learning because none of the gape-attenuating doses of THCA (0.05 and 0.5 mg·kg^{-1}) or the low dose of THC (0.05 mg·kg^{-1}) interfered with LiCl-induced conditioned taste avoidance. The mean amounts of saccharin consumed during the conditioned taste avoidance test at 30, 120 and 360 min by groups of rats treated with VEH, THCA (0.05 or 0.5 mg·kg^{-1}) or THC (0.05 mg·kg^{-1}) are presented in Figure 1B. A 4 × 3 mixed factors ANOVA revealed only a significant effect of time of test, $F(2,54) = 309.1$, $P < 0.001$.

THCA does not induce the CB1-agonist-like effects of hypothermia or reduced locomotor activity

We investigated whether THCA (0.5 mg·kg^{-1}) could induce hypothermia, as a measure of CB_1-agonist-like activity. The core temperatures measured at baseline, 30, 45, 60, 75 and 90 min after pretreatment are presented in Figure 2. A 2 × 6 mixed factors ANOVA revealed no significant main effects or interaction (*P*s > 0.05).

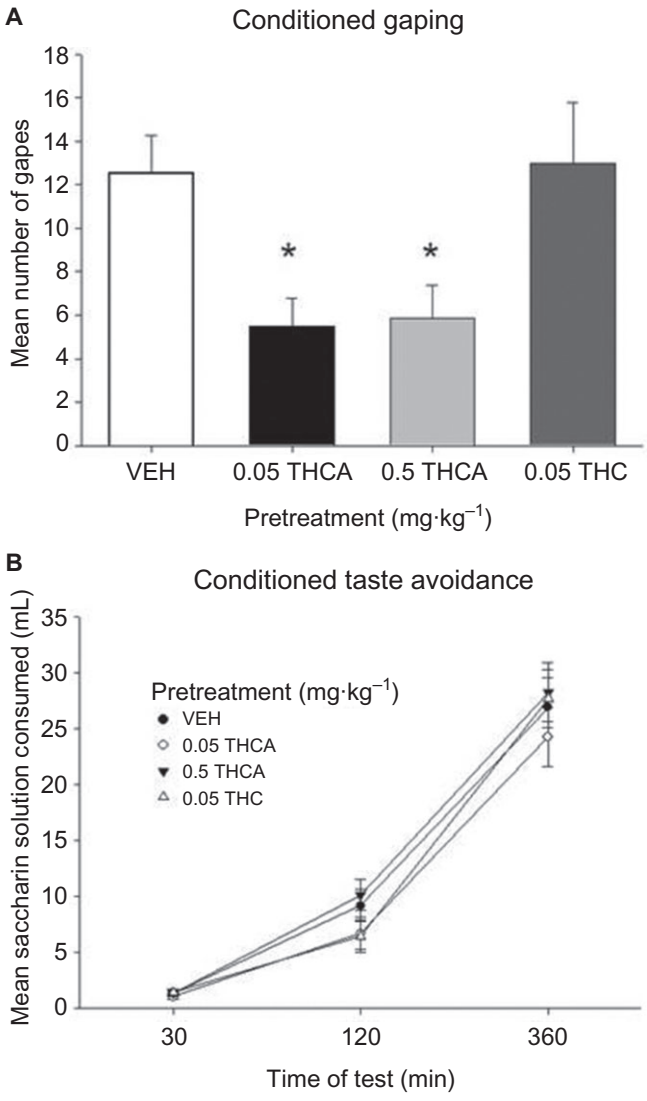


Figure 1

Effect of THCA (0.05, 0.5 mg·kg^{-1}), THC (0.05 mg·kg^{-1}) or VEH (n = 7–8) administered i.p. 30 min prior to LiCl. The mean number of conditioned gaping responses elicited by the LiCl-paired saccharin solution was measured during the test trial (A). Each bar represents the mean ± SEM (n = 7–8). The asterisks indicate a significant difference from the VEH-treated control animals (*$P < 0.05$). The mean cumulative amount of saccharin solution consumed (mL ± SEM) during a one-bottle consumption test was measured at 30, 120 and 360 min after introduction of the bottle to fluid-restricted rats (B).

THCA (0.5 mg·kg^{-1}) also did not alter locomotor activity, relative to VEH-pretreated controls during the 15 min activity test (Figure 3). A one-way ANOVA revealed no significant differences in distance moved, $t(14) = -0.8$, $P > 0.05$.

THCA suppresses LiCl*-induced conditioned gaping to a context in rats: a CB_1 receptor mechanism of action*

THCA (0.05 mg·kg^{-1}) interfered with the nausea-inducing effects of the context previously paired with LiCl, and SR, but

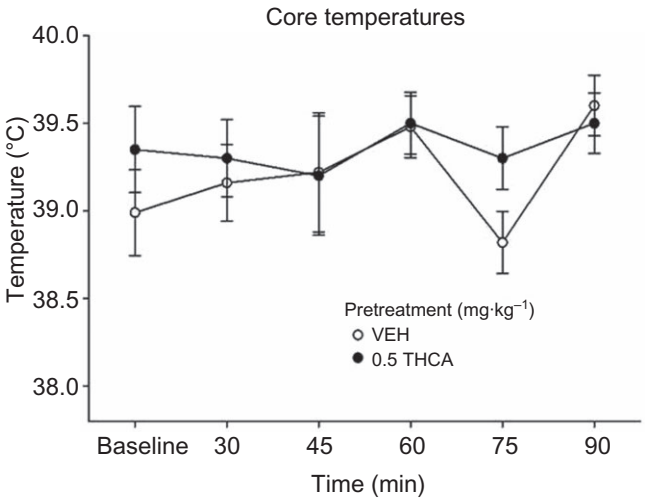


Figure 2

Effect THCA (0.5 mg·kg^{-1}) or VEH (n = 5 per group) administered i.p. on core temperatures, relative to baseline, measured at 30, 45, 60, 75 and 90 min after pretreatment.

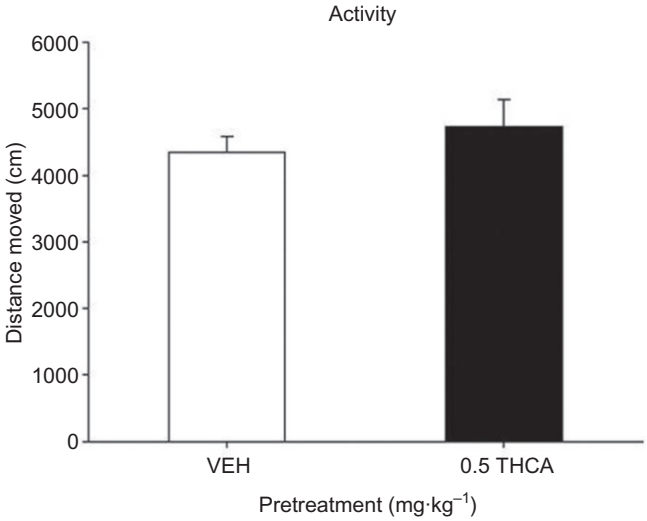


Figure 3

Effect of THCA (0.5 mg·kg^{-1}) or VEH (n = 8 per group) administered i.p. 30 min prior to placement in the activity chamber. The distance moved (cm) was measured for 15 min. Each bar represents the mean ± SEM.

not WAY, blocked this effect. The mean number of gapes during the test trial is presented in Figure 4. A one-way ANOVA revealed a main effect of pretreatment group, $F(6, 46) = 4.7$, $P = 0.001$. LSD *post hoc* comparisons revealed that pretreatment group VEH-THCA gaped significantly less than VEH controls ($P = 0.001$). The suppressed gaping by THCA was reversed in Group SR-THCA ($P < 0.001$), but not in group WAY-THCA. Groups VEH-THC, SR-THCA, WAY-VEH, SR-VEH did not differ from VEH-VEH-pretreated controls ($P > 0.05$).

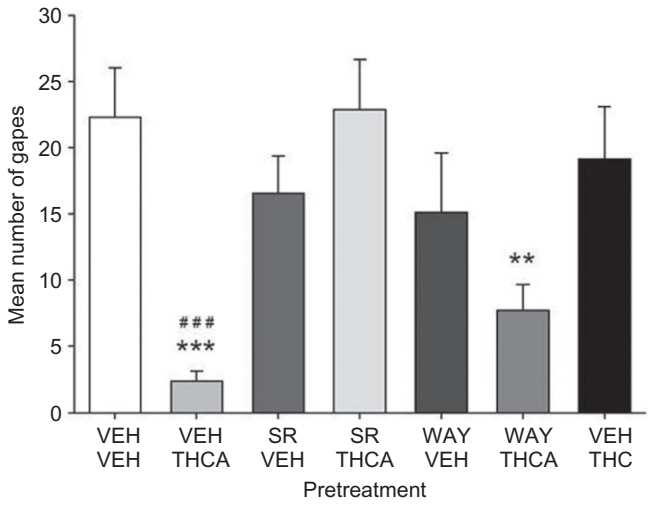


Figure 4

Effect of VEH, SR (2.5 mg·kg^{-1}, i.p.) or WAY (0.1 mg·kg^{-1}, i.p.) on the THCA- (0.05 mg·kg^{-1}, i.p.) induced suppression on the expression of gaping to a context previously paired with LiCl (a model of AN). An additional group was administered VEH-THC (0.05 mg·kg^{-1}, i.p.). Each bar represents the mean number of conditioned gaping responses ± SEM (n = 7–8). The asterisks indicate a significant difference from the VEH-VEH-treated control animals (**$P < 0.025$, ***$P = 0.001$). The pound symbols represent a significant difference from the VEH-0.05 THCA group ($^{###}P < 0.001$).

Analysis of plasma THCA levels

In the VEH-THCA-pretreated rats, THCA, but not THC, was detected in the plasma (mean = 16.1 ng·mL^{-1}, SD = 10.3), ranging in concentration from 3.92–32.5 ng·mL^{-1}. In the VEH-VEH-pretreated controls, neither THCA nor THC was detected in plasma samples. These results indicate that THCA's effects on AN are unlikely to be due to *in vivo* conversion of THCA to THC.

THCA reduces LiCl-*induced vomiting in* S. murinus*: a* CB_1 *receptor-mediated effect*

We finally determined whether THCA could also reduce LiCl-induced emesis in shrews, and whether this effect was CB_1 receptor mediated. We found that THCA does indeed inhibit LiCl-induced emesis. In Figure 5, a one-way ANOVA revealed a significant difference among groups, $F(3,29) = 18.2$, $P < 0.001$; Bonferroni *post hoc* tests revealed that those shrews given LiCl after pretreatment with both doses of THCA vomited significantly less than VEH-pretreated controls (Ps < 0.001) The suppressive effect on emesis appeared to be CB_1 receptor mediated because shrews in the pretreatment group SR-0.05 THCA vomited significantly more than those in group 0.05 THCA ($P = 0.007$). When administered by themselves, neither THCA, VEH nor SR produced emesis during the 45 min pretreatment period before toxin administration (data not shown).

Discussion

THCA (0.5 and 0.05 mg·kg^{-1}) reduced LiCl-induced conditioned gaping to a flavour and THCA (0.05 mg·kg^{-1}) also

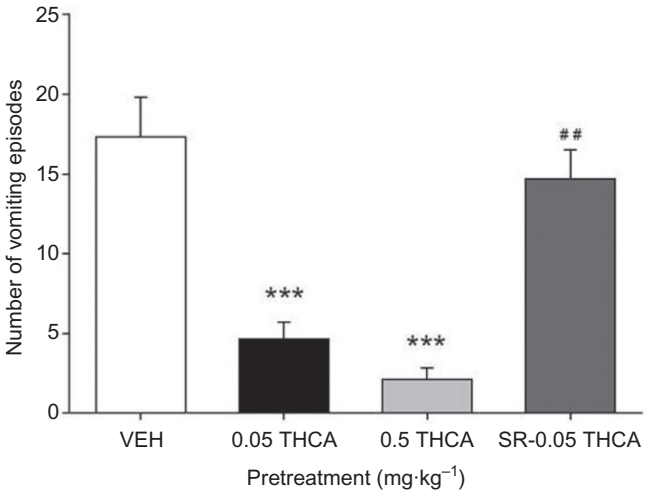


Figure 5

Effect of THCA (0.05, 0.5 mg·kg^{-1}) or VEH administered i.p. 30 min prior to LiCl administration. An additional group was also given SR (2.5 mg·kg^{-1}, i.p.) 15 min prior to THCA (0.05 mg·kg^{-1}). The number of emetic episodes in shrews treated with LiCl was measured. Each bar represents the mean ± SEM (n = 6–10). The asterisk indicates a significant difference from the VEH-treated control animals (***P < 0.001). The pound symbols represent a significant difference from the 0.05 THCA group ($^{\#\#}P$ < 0.01).

reduced conditioned gaping to a context previously paired with LiCl. SR reversed this latter effect (while having no effect on its own), but WAY did not, indicating that THCA's suppressive effects on contextually elicited conditioned gaping may be CB_1 receptor mediated. Furthermore, THCA (0.5 and 0.05 mg·kg^{-1}) was also effective in reducing LiCl-induced vomiting in shrews. The suppressive effect of 0.05 mg·kg^{-1} THCA was also blocked by SR administration, lending further support to a CB_1 receptor-mediated mechanism of action.

Although our *in vivo* results suggest a CB_1 receptor-mediated mechanism for THCA, the few *in vitro* studies that have been published to date do not seem to support this finding. THCA has been shown to inhibit TNF-α (a pro-inflammatory cytokine), demonstrating its ability to reduce the inflammatory response, albeit through a mechanism independent of CB_1 or CB_2 receptors (Verhoeckx *et al.*, 2006). Binding assays also suggest that THCA is not active at CB_1 receptors (Ahmed *et al.*, 2008). Likewise, N-arachidonoyl-L-serine, a novel endocannabinoid, demonstrated neuroprotective effects, which were blocked *in vivo* by the CB_2 receptor antagonist SR144528, demonstrating a CB_2 mechanism of action (Cohen-Yeshurun *et al.*, 2011; 2013); however, it has been shown to bind very weakly to this receptor *in vitro* (Milman *et al.*, 2006). Therefore, further studies need to be conducted to further elucidate THCA's mechanism of action.

We have previously reported that at a dose as low as 0.5 mg·kg^{-1} ip, THC reduces both the establishment of LiCl-induced conditioned gaping to a flavour (Parker and Mechoulam, 2003; Parker *et al.*, 2003) and the expression of LiCl-induced conditioned gaping to a context (Limebeer *et al.*, 2006). Here, we found that at a lower dose of 0.05 mg·kg^{-1}, THC did not reduce these behaviours reflective of nausea, but at that dose, THCA was effective. Therefore, THCA appears a more potent anti-nausea treatment than THC, even though it is not psychoactive. Additionally, it is unlikely that the anti-nausea effects of THCA in our models could be due to the metabolism of THCA into THC, given that the equivalent dose of THC is ineffective. Furthermore, when plasma samples were analysed, no detectable levels of THC were present, only THCA. This evidence discounts the possibility that the effects were due to conversion of THCA to THC.

Measuring these plasma levels does not, however, rule out the possibility that THCA is modified *in vivo* to produce another metabolite that has CB_1 agonist-like properties. We attempted to assess this possibility by measuring core temperatures and locomotor activity in response to VEH or THCA pretreatments. If the THCA effects seen here were due to its *in vivo* conversion to a metabolite with CB_1 agonist-like properties, then we would expect to see a hypothermic response and reduced activity (characteristic of CB_1 agonist-like activity as measured in the tetrad of tasks). Relative to VEH-pretreated controls, there was no significant change in rectal temperatures or activity as a result of THCA pretreatment. Because we saw no such changes, it seems unlikely that these effects can be explained by THCA's conversion to a metabolite with CB_1 agonist-like properties.

In contrast to LiCl-induced conditioned gaping reactions, THCA and THC pretreatment did not interfere with the establishment of conditioned taste avoidance at any dose tested. This pattern is similar to that evident in previous studies with ondansetron (e.g. Limebeer and Parker, 2000), cannabidiol (CBD; Parker *et al.*, 2002; Rock *et al.*, 2012), cannabidiolic acid (CBDA; Bolognini *et al.*, 2013) and THC (Limebeer and Parker, 1999). Since conditioned taste avoidance is produced by emetic drugs, but also by rewarding drugs, and because anti-emetic drugs do not interfere with taste avoidance, unlike conditioned gaping, we have argued that conditioned taste avoidance is not a selective measure of nausea (see Parker *et al.*, 2008).

THCA may be a more desirable therapeutic treatment for nausea and vomiting than THC because it is both more potent and devoid of psychoactive properties. There is an intriguing parallel between the effects of THCA and the acid precursor of CBD, CBDA, on nausea and vomiting in our models. Our group (Bolognini *et al.*, 2013; Rock and Parker, 2013) has recently also evaluated the potential of CBDA to prevent the establishment of nausea-induced conditioned gaping reactions elicited by a flavour (as a model of acute nausea), to prevent the expression of contextually elicited conditioned gaping reactions (a model of AN) in rats and to prevent vomiting in shrews. Like THCA, CBDA was much more potent in reducing these nausea-induced responses than has been shown with CBD (e.g. Parker *et al.*, 2002; Rock *et al.*, 2008) and these effects like those of CBD (Rock *et al.*, 2012) are 5-HT_{1A} receptor mediated. As both CBDA and THCA are present in the unheated cannabis plant, future work will evaluate potential synergistic effects (potentially by an interaction between CB_1 and 5-HT_{1A} mechanisms) of the two compounds on these behaviours reflective of nausea in rats as well as on vomiting in shrews.

Acknowledgements

This research was supported by grants from GW Pharmaceuticals to L. A. P. and from the Natural Sciences and Engineering Council of Canada (NSERC-92057) to L. A. P. The authors would like to thank Quotient Bioresearch (Fordham, Cambridgeshire, UK) for the analysis of plasma samples. The authors would also like to thank Linda Groocock for management of the shrew colony at the University of Guelph, and Martin Sticht for assistance with these studies.

Conflict of interest

The research performed in Guelph was funded by GW Research Ltd, UK.

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