

## RESEARCH PAPER

# Evaluation of the potential of the phytocannabinoids, cannabidivarin (CBDV) and $\Delta^9$ -tetrahydrocannabivarin (THCV), to produce CB<sub>1</sub> receptor inverse agonism symptoms of nausea in rats

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CB<sub>1</sub> receptor inverse agonism; cannabidivarin; tetrahydrocannabivarin; CB<sub>1</sub> receptor antagonism; nausea; depression; anhedonia; rimonabant; THC

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## BACKGROUND AND PURPOSE

The cannabinoid 1 (CB<sub>1</sub>) receptor inverse agonists/antagonists, rimonabant (SR141716, SR) and AM251, produce nausea and potentiate toxin-induced nausea by inverse agonism (rather than antagonism) of the CB<sub>1</sub> receptor. Here, we evaluated two phytocannabinoids, cannabidivarin (CBDV) and  $\Delta^9$ -tetrahydrocannabivarin (THCV), for their ability to produce these behavioural effect characteristics of CB<sub>1</sub> receptor inverse agonism in rats.

## EXPERIMENTAL APPROACH

In experiment 1, we investigated the potential of THCV and CBDV to produce conditioned gaping (measure of nausea-induced behaviour) in the same manner as SR and AM251. In experiment 2, we investigated the potential of THCV and CBDV to enhance conditioned gaping produced by a toxin in the same manner as CB<sub>1</sub> receptor inverse agonists.

## KEY RESULTS

SR (10 and 20 mg·kg<sup>-1</sup>) and AM251 (10 mg·kg<sup>-1</sup>) produced conditioned gaping; however, THCV (10 or 20 mg·kg<sup>-1</sup>) and CBDV (10 or 200 mg·kg<sup>-1</sup>) did not. At a subthreshold dose for producing nausea, SR (2.5 mg·kg<sup>-1</sup>) enhanced lithium chloride (LiCl)-induced conditioned gaping, whereas  $\Delta^9$ -tetrahydrocannabinol (THC, 2.5 and 10 mg·kg<sup>-1</sup>), THCV (2.5 or 10 mg·kg<sup>-1</sup>) and CBDV (2.5 or 200 mg·kg<sup>-1</sup>) did not; in fact, THC (2.5 and 10 mg·kg<sup>-1</sup>), THCV (10 mg·kg<sup>-1</sup>) and CBDV (200 mg·kg<sup>-1</sup>) suppressed LiCl-induced conditioned gaping, suggesting anti-nausea potential.

## CONCLUSIONS AND IMPLICATIONS

The pattern of findings indicates that neither THCV nor CBDV produced a behavioural profile characteristic of CB<sub>1</sub> receptor inverse agonists. As well, these compounds may have therapeutic potential in reducing nausea.

## Abbreviations

CB<sub>1</sub>, cannabinoid 1; CBD, cannabidiol; CBDV, cannabidivarin; LiCl, lithium chloride; LSD, least significant difference; SAL, saline; SR, SR141716; THC,  $\Delta^9$ -tetrahydrocannabinol; THCV,  $\Delta^9$ -tetrahydrocannabivarin; TR, taste reactivity; TRP, transient receptor potential; TRPA1, transient receptor potential channels of the ankyrin type-1; VEH, vehicle

## Introduction

Side effects of the cannabinoid 1 (CB<sub>1</sub>) receptor inverse agonist/antagonist, rimonabant (SR141716; SR), in humans include nausea and depression (Despres, 2009; de Mattos Viana *et al.*, 2009). The nausea produced by SR and AM251 (another CB<sub>1</sub> inverse agonist/antagonist) is the result of inverse agonism at the CB<sub>1</sub> receptor (Sink *et al.*, 2008) because equivalent doses of AM251, but not the neutral CB<sub>1</sub> receptor antagonists AM4113 (Sink *et al.*, 2008), AM6527 or AM6545 (Cluny *et al.*, 2010), produce lithium chloride (LiCl)-induced conditioned gaping reactions, a measure of nausea-induced behaviour in rats. Only drugs that produce emesis in species capable of vomiting produce conditioned gaping reactions and anti-emetic agents prevent the establishment of these nausea-induced gaping reactions in rats (see Parker *et al.*, 2008; 2009 for a review).

The conditioned gaping model has been shown to detect the nauseating side effect of several compounds, including selective serotonin reuptake inhibitors (Limebeer *et al.*, 2009; Tuerke *et al.*, 2009), PDE-4 inhibitors (Rock *et al.*, 2009) and CB<sub>1</sub> receptor inverse agonists (McLaughlin *et al.*, 2005; Sink *et al.*, 2008). As well as producing nausea on their own, the CB<sub>1</sub> receptor inverse agonists (at subthreshold doses that do not produce nausea on their own), SR (Parker and Mechoulam, 2003; Parker *et al.*, 2003) and AM251 (Limebeer *et al.*, 2010), potentiate the nausea produced by the toxin LiCl. This potentiation of nausea is the result of CB<sub>1</sub> receptor inverse agonism because neither the neutral antagonist, AM6545 (peripherally restricted; Tam *et al.*, 2010) nor AM6527 (which crosses the blood–brain barrier; Sink *et al.*, 2009), produced potentiation of LiCl-induced nausea in the gaping model (Limebeer *et al.*, 2010). Interestingly, Chambers *et al.* (2007) also reported that AM251, but not the neutral CB<sub>1</sub> receptor antagonist AM4113, also potentiated morphine-6-glucuronide toxin-induced vomiting (at a dose that did not produce vomiting on its own) in the ferret. These findings suggest that it is the CB<sub>1</sub> receptor *inverse agonist* effects rather than their CB<sub>1</sub> receptor *antagonist* effects that mediate the nausea produced as a side effect of these compounds.

Phytocannabinoids have recently become candidates for therapeutic applications; however, their usefulness is limited if they exhibit CB<sub>1</sub> receptor inverse agonist activity. Much research on phytocannabinoids has concentrated mostly on the psychoactive compound,  $\Delta^9$ -tetrahydrocannabinol (THC), and the primary non-psychoactive cannabinoid, cannabidiol (CBD), found in marijuana (e.g. Mechoulam *et al.*, 2002). Low doses (0.5 mg·kg<sup>-1</sup> THC and 1–5 mg·kg<sup>-1</sup> CBD) of both of these compounds have been shown to suppress LiCl-induced conditioned gaping in the rodent model of conditioned nausea (Limebeer and Parker, 1999; Parker *et al.*, 2002; 2003; Rock *et al.*, 2008; 2012).

The effects of the phytocannabinoids cannabidivarin (CBDV) and tetrahydrocannabivarin (THCV), although identified in the 1970s (Merkus, 1971), have only recently been investigated. Under acidic conditions, CBDV isomerizes into THCV, and in the plant, CBDV is the precursor of THCV (Deiana *et al.*, 2012). Higher levels of CBDV and THCV are more common in *Cannabis indica* than in *Cannabis sativa* (Hillig and Mahlberg, 2004).

Recent *in vivo* work has highlighted CBDV's anti-inflammatory effects in mice (Tubaro *et al.*, 2010). Most recently, *in vivo*, Hill *et al.* (2012) demonstrated CBDV's effectiveness as an anti-convulsant ( $\geq 50$ –100 mg·kg<sup>-1</sup>) in various animal seizure models, suggesting possible anti-epilepsy effects, but little else is understood about the behavioural effects of CBDV. CBDV has also been shown to act as an agonist at human transient receptor potential (TRP) channels of the ankyrin type-1 (TRPA1), TRP channels of vanilloid type-1 and type-2 agonist, but as a TRP channel of melastatin type-8 antagonist (De Petrocellis *et al.*, 2011; 2012). These varied effects warrant further investigations into CBDV's mechanism of action.

Work with both plant-derived THCV and synthetic THCV (0–4394) has elucidated its behavioural effects and mechanism of action. *In vitro*, THCV has been shown to act as a CB<sub>1</sub> and CB<sub>2</sub> receptor antagonist (Thomas *et al.*, 2005; Pertwee *et al.*, 2007), and also to activate CB<sub>2</sub> receptors (Bolognini *et al.*, 2010). As well, in contrast to SR, THCV (0–4394) does not produce inverse agonist activity in the [<sup>35</sup>S] GTP $\gamma$ S binding assay in mouse whole brain membrane and fails to produce stimulation of [<sup>35</sup>S] GTP $\gamma$ S binding to such membranes (Pertwee *et al.*, 2007). Indeed, THCV shares the ability of AM251 to reduce the food intake and body weight of non-fasted and fasted mice (Riedel *et al.*, 2009), and at a dose of 2.5 mg·kg<sup>-1</sup> (i.p.), THCV has recently been shown not to modify saccharin palatability (O'Brien *et al.*, 2013). If THCV is devoid of the CB<sub>1</sub> receptor inverse agonist properties of SR, which are responsible for nausea (McLaughlin *et al.*, 2005; Sink *et al.*, 2008) and potentially depression (Beyer *et al.*, 2010; Sink *et al.*, 2010), then THCV may be a potential candidate for therapeutic use. Indeed, THCV has been shown in animal models to suppress seizure activity (Hill *et al.*, 2010), reduce inflammation and inflammatory pain (Bolognini *et al.*, 2010), reduce weight due to hypophagia (Riedel *et al.*, 2009) and most recently to even reduce Parkinson's disease symptoms, as well as disease progression (Garcia *et al.*, 2011). In addition, unlike SR (2.5 mg·kg<sup>-1</sup>), THCV (2.5 mg·kg<sup>-1</sup>) did not produce an anxiogenic-like response of avoidance of an open lit area in the light/dark immersion test (O'Brien *et al.*, 2013), an effect consistently observed with CB<sub>1</sub> receptor inverse agonists, but not with neutral CB<sub>1</sub> receptor antagonists such as AM4113 (Sink *et al.*, 2010). Therefore, further examination of potential nauseating side effects of these phytocannabinoids is important to exclude potential *in vivo* CB<sub>1</sub> receptor inverse agonist activity.

If a compound acts as a CB<sub>1</sub> receptor inverse agonist, like SR and AM251, it is expected to (i) produce conditioned gaping reactions to a novel flavour with which it is paired (experiment 1), and (ii) at subthreshold doses for producing nausea on their own, enhance the nauseating effects of another toxin, that is, produce potentiation of LiCl-induced conditioned gaping (experiment 2). If CBDV and THCV do not produce conditioned gaping on their own and do not enhance LiCl-induced conditioned gaping, it is likely that they do not exhibit CB<sub>1</sub> receptor inverse agonist activity. These experiments can also be used to evaluate the potential of the compounds to reduce nausea-induced conditioned gaping in rats, like THC (Limebeer and Parker, 1999), HU-210 (Parker and Mechoulam, 2003; Parker *et al.*, 2003), URB597

(Cross-Mellor *et al.*, 2007), CBD (Parker *et al.*, 2002; Rock *et al.*, 2012) and cannabidiolic acid (Bolognini *et al.*, 2013; Rock and Parker, 2013). Therefore, if a compound reduces nausea, these effects will be detected by the following experiments as well.

## Methods

### Experimental procedures

*Experiment 1: does the compound produce conditioned gaping on its own?* Rats were implanted with intraoral cannulae under isoflurane anaesthesia (as described by Limebeer *et al.*, 2010). Following recovery from surgery (at least 3 days), rats received an adaptation trial. They were placed in the taste reactivity (TR) chamber with their cannula attached to an infusion pump (model KDS100; KD Scientific, Holliston, MA, USA) for fluid delivery, and water was infused into their intraoral cannula for 2 min at a rate of 1 mL·min<sup>-1</sup>. 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 the orofacial responses. On the following day, the rats received a conditioning trial, during which they were intraorally infused with a 0.1% saccharin solution for 2 min at a rate of 1 mL·min<sup>-1</sup>. Immediately after the saccharin infusion, they ( $n = 10$  per group, except AM251 with  $n = 6$ ) were injected with either VEH (1/1/18: ethanol/Cremophor/saline), 10 mg·kg<sup>-1</sup> SR, 20 mg·kg<sup>-1</sup> SR, 10 mg·kg<sup>-1</sup> AM251, 10 mg·kg<sup>-1</sup> THCv, 20 mg·kg<sup>-1</sup> THCv, 10 mg·kg<sup>-1</sup> CBDV or 200 mg·kg<sup>-1</sup> CBDV.

Seventy-two hours after the conditioning trial, rats were returned to the TR chamber for a test and intraorally infused with 0.1% saccharin solution. Their orofacial reactions were video recorded with the feed from the video camera (Sony DCR-HC48; Henry's Cameras, Waterloo, ON, Canada) fire-wired into a computer. The video tapes were later scored (at ½ speed) by a trained 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). The videotapes were scored by two trained raters, resulting in an extremely high inter-rater reliability score ( $r = 0.97$ ).

*Experiment 2: does the compound modify saccharin palatability and/or potentiate (or reduce) the nausea produced by LiCl?* Following recovery from intraoral cannulation surgery, the rats received the adaptation trial and the TR test as described in experiment 1, except as noted. On the day of conditioning, the rats were injected with vehicle (VEH;  $n = 10$ ), 2.5 mg·kg<sup>-1</sup> SR ( $n = 10$ ), 2.5 mg·kg<sup>-1</sup> THC ( $n = 6$ ), 10 mg·kg<sup>-1</sup> THC ( $n = 7$ ), 2.5 mg·kg<sup>-1</sup> THCv ( $n = 10$ ) or 10 mg·kg<sup>-1</sup> THCv ( $n = 6$ ), 2.5 mg·kg<sup>-1</sup> CBDV ( $n = 10$ ) or 200 mg·kg<sup>-1</sup> CBDV ( $n = 13$ ). Thirty minutes later, each rat was intraorally infused with 0.1% saccharin solution, while their orofacial responses were video recorded from the mirror beneath the chamber. Immediately following the 2 min intraoral infusion of saccharin, the rats were injected with 20 mg·kg<sup>-1</sup> of 0.15 M LiCl.

Seventy-two hours later, the rats received a drug-free test trial, during which they received a 2 min intraoral infusion of 0.1% saccharin solution and the frequency of gaping was measured. The videotapes were later scored (at ½ speed) by an observer blinded to the experimental conditions using 'the Observer' for conditioned gaping as well as tongue protrusions (extensions of the tongue, both forward and lateral, from the mouth as a measure of hedonic reactions) during conditioning. As well, to determine other non-specific effects of the drug during conditioning, the conditioning tapes were scored for the duration (s) of bouts of active locomotion (forward propulsion of the rats' body by movement of the forepaws on the floor of the chamber).

### Animals

Animal procedures were according to the Canadian Council on Animal Care (CCAC). The protocol was approved by the Institutional Animal Care Committee, which is accredited by the CCAC. The authors consulted with 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 (experiment 1,  $n = 76$ ; experiment 2,  $n = 73$ ), obtained from Charles River Laboratories (St Constant, QC, Canada), were single-housed in shoebox cages in the colony room at an ambient temperature of 21°C with a 12/12 light/dark schedule (lights off at 0800 hours) and maintained on *ad libitum* food and water. Rats weighed between 236 and 455 g on the day of conditioning.

### Drugs and materials

All cannabinoid compounds were prepared in a VEH of ethanol/Cremophor (Sigma, St Louis, MO, USA)/saline (SAL; 1:1:18) and administered i.p. LiCl (Sigma) was prepared in a 0.15 M solution with sterile water and administered i.p. at a volume of 20 mL·kg<sup>-1</sup> (127.2 mg·kg<sup>-1</sup>) in experiment 2.

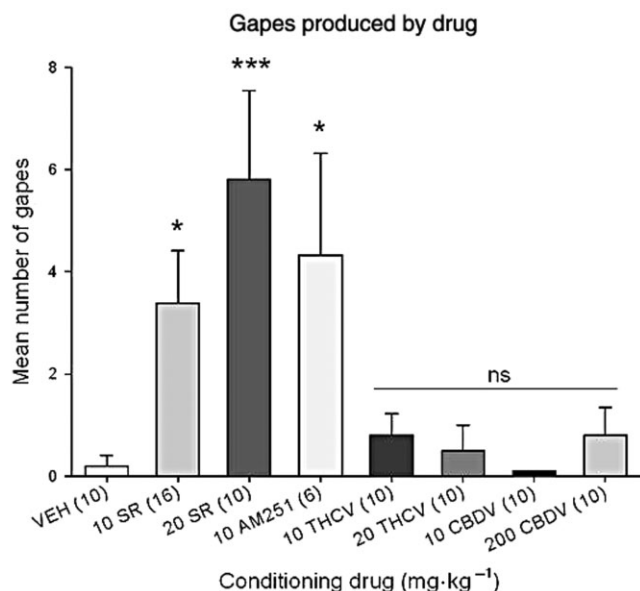
SR was prepared at 2.5, 10 and 20 mg·kg<sup>-1</sup> and administered at a volume of 2 mL·kg<sup>-1</sup>. Two sources of SR were used at the 10 mg·kg<sup>-1</sup> dose ( $n = 8$  per group): one purchased from Sequoia laboratories, GB, and the other provided by B. Whalley's laboratory from Otsuka Pharmaceuticals (Osaka, Japan). A comparison of the gaping produced by these two sources of SR revealed no significant difference in potential to produce conditioned gaping [Sequoia laboratories SR (mean gaping = 3.6); Otsuka SR (mean gaping = 3.8)]; therefore, the data collected with 10 mg·kg<sup>-1</sup> SR were combined. The 20 mg·kg<sup>-1</sup> dose of SR was from the Otsuka supply.

CBDV (GW Pharmaceuticals, Porton Down, Wiltshire, UK) was prepared at 2.5 and 10 mg·kg<sup>-1</sup> and administered at a volume of 2 mL·kg<sup>-1</sup>. CBDV prepared at 200 mg·kg<sup>-1</sup> and was administered at a volume of 10 mL·kg<sup>-1</sup>. This high dose of CBDV was evaluated, given recent findings that it has anti-epileptic potential at this high dose (Hill *et al.*, 2012). THCv (GW Pharmaceuticals) was prepared at 2.5, 10 and 20 mg·kg<sup>-1</sup> and administered at a volume of 2 mL·kg<sup>-1</sup>. AM251 (Cayman Chemicals, Ann Arbor, MI, USA) was prepared at 10 mg·kg<sup>-1</sup> and administered at a volume of 2 mL·kg<sup>-1</sup>. THC (GW Pharmaceuticals) was prepared at 2.5 and 10 mg·kg<sup>-1</sup> and administered at a volume of 2 mL·kg<sup>-1</sup>.

## Results

### Experiment 1: does the compound produce conditioned gaping on its own?

The CB<sub>1</sub> inverse agonists/antagonists, SR and AM251, produced more conditioned gaping reactions (nausea-like behaviours) than any other group. Figure 1 presents the mean ( $\pm$ SEM) number of gapes displayed by the various groups in experiment 1. The one-way ANOVA revealed a main effect of group [ $F(7, 74) = 4.9; P < 0.001$ ]. Using Fisher's least significant difference (LSD) comparison tests, all doses of the CB<sub>1</sub> receptor inverse agonists/antagonists (SR and AM251) produced significantly more conditioned gaping than any of the other compounds when paired with saccharin solution ( $P$ 's  $< 0.05$ ). As well, none of the other compounds (at any dose tested) significantly differed from VEH controls. Finally, SR produced marginally more gaping at 20 mg·kg<sup>-1</sup> than at 10 mg·kg<sup>-1</sup> ( $P = 0.057$ ). Therefore, at all doses tested, CBDV and THCV did not produce nausea-induced gaping (inverse agonist effect) when compared with VEH, but SR and AM251 both produced the inverse agonist effect of nausea that is not produced by CB<sub>1</sub> receptor neutral antagonists (Sink *et al.*, 2008).



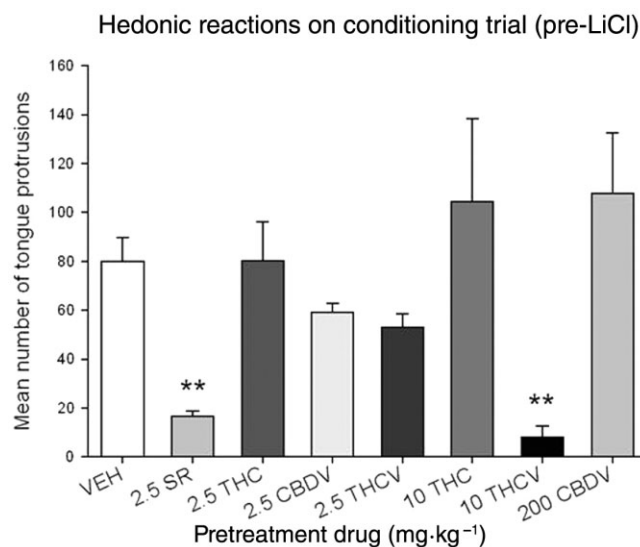
**Figure 1**

Mean number ( $\pm$ SEM) of gapes at test elicited by 0.1% saccharin solution previously paired with each compound during the drug-free test trial. Numbers in parentheses indicate  $n$  per group. \* $P < 0.05$ ; \*\*\* $P < 0.001$  from other groups indicated by line [10 mg·kg<sup>-1</sup> THCv, 20 mg·kg<sup>-1</sup> THCv, 10 mg·kg<sup>-1</sup> CBDV, 200 mg·kg<sup>-1</sup> CBDV and VEH, which did not differ from one another (ns)]. Those conditioned with 10 mg·kg<sup>-1</sup> SR, 20 mg·kg<sup>-1</sup> SR and AM251 did not significantly differ from one another, although there was a trend for 20 mg·kg<sup>-1</sup> SR to display more gaping than 10 mg·kg<sup>-1</sup> SR ( $P = 0.07$ ).

### Experiment 2: does the compound modify saccharin palatability and/or potentiate (or reduce) the nausea produced by LiCl?

**Saccharin palatability during conditioning.** Figure 2 presents the mean ( $\pm$ SEM) number of tongue protrusions displayed during the conditioning trial following exposure to the pretreatment injections, prior to receiving LiCl. A one-way ANOVA revealed a significant pretreatment group effect [ $F(7, 62) = 4.3; P = 0.001$ ]. VEH pretreated rats displayed significantly more hedonic reactions than those pretreated with 2.5 mg·kg<sup>-1</sup> SR ( $P < 0.02$ ) or 10 mg·kg<sup>-1</sup> THCv ( $P < 0.02$ ). Those rats pretreated with CBDV (2.5 and 200 mg·kg<sup>-1</sup>), THCv (2.5 mg·kg<sup>-1</sup>) and THC (2.5 and 10 mg·kg<sup>-1</sup>) did not significantly differ from VEH-pretreated controls ( $P$ 's  $> 0.05$ ). The ANOVA for total activity [ $F(7, 62) = 1.4; P = 0.2$ ] was non-significant (data not shown). Therefore, the drugs did not affect overall activity. These results suggest that both 2.5 mg·kg<sup>-1</sup> SR and 10 mg·kg<sup>-1</sup> THCv reduced saccharin palatability, which may reflect an anti-appetite effect of these compounds, both shown to reduce feeding (e.g. Riedel *et al.*, 2009).

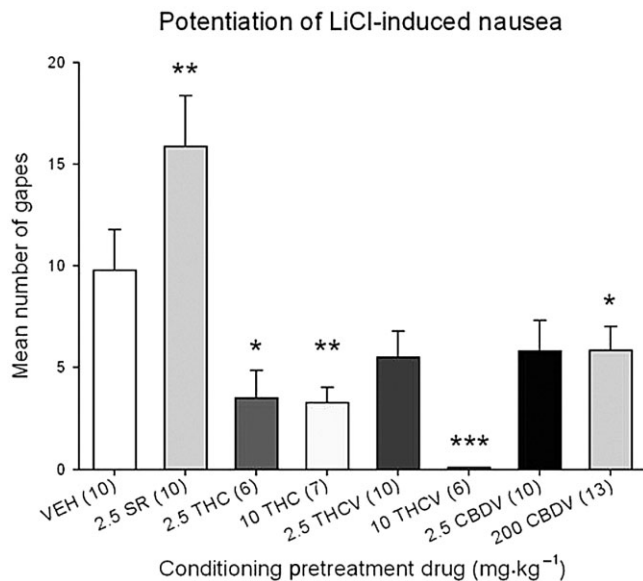
**Potentiation of LiCl-induced conditioned gaping during the test trial.** Figure 3 presents the mean ( $\pm$ SEM) number of conditioned gaping reactions during the drug-free test, 72 h following the conditioning trial. The one-way ANOVA revealed a significant pretreatment group effect [ $F(7, 62) = 8.0; P < 0.001$ ]. Subsequent LSD tests revealed that only those rats pretreated with 2.5 mg·kg<sup>-1</sup> SR displayed potentiated LiCl-induced conditioned gaping reactions relative to VEH-pretreated controls ( $P < 0.025$ ).



**Figure 2**

Mean number ( $\pm$ SEM) of tongue protrusions during conditioning elicited by 0.1% saccharin solution 30 min following pretreatment with VEH, 2.5 mg·kg<sup>-1</sup> SR, 2.5 mg·kg<sup>-1</sup> THC, 10 mg·kg<sup>-1</sup> THC, 2.5 mg·kg<sup>-1</sup> CBDV, 200 mg·kg<sup>-1</sup> CBDV, 2.5 mg·kg<sup>-1</sup> THCv or 10 mg·kg<sup>-1</sup> THCv. Both groups pretreated with 2.5 mg·kg<sup>-1</sup> SR and 10 mg·kg<sup>-1</sup> THCv displayed suppressed (\*\* $P < 0.02$ ) hedonic reactions relative to VEH-pretreated controls.





**Figure 3**

Mean number ( $\pm$ SEM) of gapes elicited by 0.1% saccharin solution previously paired with LiCl during the drug-free TR test trial. Only those pretreated with SR displayed potentiated gaping (\*\* $P < 0.025$  relative to VEH-pretreated controls) during the drug-free test, suggesting that pretreatment during conditioning enhanced the nausea produced by LiCl. In contrast, 10 mg·kg<sup>-1</sup> THCV completely eliminated LiCl-induced gaping (\*\*\* $P < 0.001$  relative to VEH) and 200 mg·kg<sup>-1</sup> CBDV, 2.5 mg·kg<sup>-1</sup> THC (\* $P < 0.05$  relative to VEH) and 10 mg·kg<sup>-1</sup> THC (\*\* $P < 0.01$ ), reduced LiCl-induced gaping during the drug-free test trial, suggesting that pretreatment during conditioning eliminated the nausea produced by LiCl.

In fact, 10 mg·kg<sup>-1</sup> THCV ( $P < 0.001$ ), 200 mg·kg<sup>-1</sup> CBDV ( $P < 0.05$ ) and both doses of THC ( $P$ 's  $< 0.02$ ) produced suppression of LiCl-induced conditioned gaping reactions relative to VEH-pretreated controls, a pattern suggesting an anti-nausea effect of these compounds. In addition, rats pretreated with 2.5 mg·kg<sup>-1</sup> CBDV ( $P = 0.08$ ) and 2.5 mg·kg<sup>-1</sup> THCV ( $P = 0.07$ ) showed marginally attenuated LiCl-induced conditioned gaping reactions relative to VEH-pretreated controls.

## Discussion

The results of experiment 1 revealed that when explicitly paired with a novel saccharin solution, neither CBDV (10 or 200 mg·kg<sup>-1</sup>) nor THCV (10 or 20 mg·kg<sup>-1</sup>) produced the nausea-like profile of conditioned gaping produced by the CB<sub>1</sub> receptor inverse agonists SR and AM251. Previous studies revealed that the nausea produced by both SR and AM251 is mediated by the inverse agonism of the CB<sub>1</sub> receptor because CB<sub>1</sub> receptor neutral antagonists do not produce this side effect (Sink *et al.*, 2008; Cluny *et al.*, 2010). As conditioned gaping in rats is produced only by compounds that produce nausea and emesis in other species (see Parker and Limebeer, 2008), these *in vivo* results support previous *in vitro* findings,

suggesting that THCV acts as a CB<sub>1</sub> receptor neutral antagonist (Pertwee *et al.*, 2007). Neither CBDV nor THCV should produce the symptoms of nausea, an effect that appears to be caused by the CB<sub>1</sub> receptor inverse agonist (and not the CB<sub>1</sub> receptor antagonist) effects of SR and AM251. Therefore, these results suggest that the inverse agonism effect of nausea will not hinder the proposed clinical applications for CBDV and THCV, (i.e. anti-inflammatory and anti-convulsant properties).

Experiment 2 evaluated the potential of CBDV and THCV to potentiate (or reduce) nausea produced by LiCl. At doses subthreshold for producing nausea on their own (2.5 mg·kg<sup>-1</sup>), both SR (Parker and Mechoulam, 2003) and AM251 (Limebeer *et al.*, 2010) pretreatments prior to a saccharin-LiCl pairing potentiate the nausea produced by LiCl, as revealed by potentiated conditioned gaping displayed in the subsequent drug-free test trial 72 h after conditioning. In contrast, CB<sub>1</sub> neutral antagonists, AM6527 or AM6545 do not potentiate LiCl-induced nausea (Limebeer *et al.*, 2010). Experiment 2 revealed that only the group pretreated with 2.5 mg·kg<sup>-1</sup> SR showed potentiated LiCl-induced conditioned gaping relative to all other groups. In fact, all other phytocannabinoids tested appeared to produce an anti-nausea effect, at least at the higher doses. Rats pretreated with THC, CBDV or THC during conditioning showed suppressed gaping during the subsequent drug-free test, relative to those pretreated with VEH. This suppression of conditioned gaping by pretreatment with THC, CBDV and THC indicates that these compounds exhibit anti-nausea potential. The low dose effects of THC shown here are consistent with those previously reported (Pertwee *et al.*, 2007). At a low dose (2.5 mg·kg<sup>-1</sup>), THC acts as a CB<sub>1</sub> receptor antagonist (Pertwee *et al.*, 2007) and does not enhance LiCl-induced nausea evidenced as potentiation of conditioned gaping (like AM4113), suggesting that it is a neutral antagonist.

The potential of SR, CBDV, THCV and THC to modify saccharin palatability (the hedonic reaction of tongue protrusions) was assessed during the conditioning trial of experiment 2. We have previously shown that doses of SR and AM251 that are subthreshold for producing nausea (2.5 mg·kg<sup>-1</sup>) reduce sucrose or saccharin palatability (Jarrett *et al.*, 2007; Limebeer *et al.*, 2010) and at a low dose of 0.5 mg·kg<sup>-1</sup> THC enhances sucrose palatability in the TR test (Jarrett *et al.*, 2005). Here, we show that 30 min following injection of both 2.5 mg·kg<sup>-1</sup> SR and 10 mg·kg<sup>-1</sup> THCV (but not 2.5 mg·kg<sup>-1</sup> THCV, or 2.5 or 200 mg·kg<sup>-1</sup> CBDV) saccharin palatability was suppressed. These results may reflect the potential of THC, like SR, to modify appetite for sweet tastes (Riedel *et al.*, 2009).

Interestingly, rats pretreated with 10 mg·kg<sup>-1</sup> THCV showed *no* conditioned gaping reactions during intraoral infusion of saccharin that had been previously paired with LiCl, suggesting that it completely blocked LiCl-induced nausea, an effect evident with drugs that are anti-nausea agents such as ondansetron (Limebeer and Parker, 2000), palonosetron (Limebeer *et al.*, 2009) and 8-hydroxy-2-(dipropylamino)tetralin (Limebeer and Parker, 2003). Given that the same dose of THCV also suppressed saccharin palatability during conditioning, it is unlikely that the suppression of nausea was mediated by CB<sub>1</sub> receptor agonism (e.g.

Pertwee *et al.*, 2007). Further experiments are warranted to determine the mechanism of action of the anti-nausea effects of this phytocannabinoid.

In these studies, CBDV was found to be devoid of inverse agonist properties (unlike SR and AM251), not to modify hedonics, and to reduce conditioned gaping (at a high dose). These actions of CBDV are not consistent with CB<sub>1</sub> receptor antagonism because CBDV does not potentiate conditioned gaping, nor does CBDV act like a CB<sub>1</sub> receptor agonist (like 0.5 mg·kg<sup>-1</sup> THC) to enhance hedonics (Jarrett *et al.*, 2005). CBDV reduced LiCl-induced conditioned gaping as is observed with THC and HU-210 (Parker *et al.*, 2003), but this suppression of gaping was not accompanied by enhanced hedonic reactions during conditioning, suggesting that it may not be due to a CB<sub>1</sub> mechanism of action. Alternatively, CBDV could also be acting at the TRP channel receptors in this model, as De Petrocellis *et al.* (2011; 2012) have shown that CBDV may act at these receptors. Sharkey *et al.* (2007) have indeed shown that the dual TRPV<sub>1</sub>/CB<sub>1</sub> agonist, arvanil, has anti-emetic effects in ferrets that are mediated by activation of both the TRPV<sub>1</sub> and the CB<sub>1</sub> receptor. Further experiments are warranted to elucidate the mechanism of action of CBDV's anti-nausea effect.

The finding that higher doses of THC (2.5 and 10 mg·kg<sup>-1</sup>) reduced LiCl-induced conditioned gaping extend previous findings that a low dose (0.5 mg·kg<sup>-1</sup>) of THC interferes with cyclophosphamide- (Limebeer and Parker, 1999) and LiCl-induced conditioned gaping reactions (Parker and Mechoulam, 2003; Parker *et al.*, 2003). These previous experiments also demonstrated that the THC-induced suppression of conditioned gaping can be reversed by SR, indicating a CB<sub>1</sub> receptor mechanism of action for THC's anti-nausea effects (Parker and Mechoulam, 2003; Parker *et al.*, 2003). These findings are also consistent with the human literature. In cancer patients undergoing chemotherapy treatment, administration of oral THC significantly reduces nausea and vomiting, in comparison to placebo controls (Sallan *et al.*, 1975; Chang *et al.*, 1979; Frytak *et al.*, 1979; Orr *et al.*, 1980; Sweet *et al.*, 1981).

In conclusion, at all doses tested, unlike SR and AM251, both CBDV and THCV neither (i) produced conditioned gaping on their own when explicitly paired with saccharin solution nor (ii) potentiated LiCl-induced conditioned gaping. In fact, both compounds produced an anti-nausea-like effect in experiment 2; that is, CBDV partially reduced LiCl-induced nausea, and at the highest dose tested, THCV blocked LiCl-induced nausea. Taken together, these results suggest that neither THCV nor CBDV acts as a CB<sub>1</sub> receptor inverse agonist. Therefore, it seems that these two phytocannabinoids may be promising therapeutics, with anti-nausea properties, devoid of symptoms associated with CB<sub>1</sub> receptor inverse agonism.

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## Conflict of interest

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