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RESEARCH PAPER

Effect of low doses of cannabidiolic acid and ondansetron on LiCl-induced conditioned gaping (a model of nausea-induced behaviour) in rats

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BACKGROUND AND PURPOSE

To determine the minimally effective dose of cannabidiolic acid (CBDA) that effectively reduces lithium chloride (LiCl)-induced conditioned gaping reactions (nausea-induced behaviour) in rats and to determine if these low systemic doses of CBDA (5–0.1 μg·kg^{-1}) relative to those of CBD could potentiate the anti-nausea effects of the classic 5-hydroxytryptamine 3 (5-HT$_3$) receptor antagonist, ondansetron (OND).

EXPERIMENTAL APPROACH

We investigated the efficacy of low doses of CBDA to suppress acute nausea, assessed by the establishment of conditioned gaping to a LiCl-paired flavour in rats. The potential of threshold and subthreshold doses of CBDA to enhance the reduction of nausea-induced conditioned gaping by OND were then determined.

KEY RESULTS

CBDA (at doses as low as 0.5 μg·kg^{-1}) suppressed nausea-induced conditioned gaping to a flavour. A low dose of OND (1.0 μg·kg^{-1}) alone reduced nausea-induced conditioned gaping, but when it was combined with a subthreshold dose of CBDA (0.1 μg·kg^{-1}) there was an enhancement in the suppression of LiCl-induced conditioned gaping.

CONCLUSIONS AND IMPLICATIONS

CBDA potently reduced conditioned gaping in rats, even at low doses and enhanced the anti-nausea effect of a low dose of OND. These findings suggest that combining low doses of CBDA and OND will more effectively treat acute nausea in chemotherapy patients.

Abbreviations

5-HT$_{1A}$, 5-hydroxytryptamine 1A; 5-HT$_3$, 5-hydroxytryptamine 3; 8-OH-DPAT, 8-hydroxy-2-dipropylaminotetralin; AN, anticipatory nausea; CBD, cannabidiol; CBDA, cannabidiolic acid; CTA, conditioned taste avoidance; DRN, dorsal raphe nucleus; LiCl, lithium chloride; OND, ondansetron; SAL, saline; THC, Δ^9-tetrahydrocannabinol; TR, taste reactivity; TRP, transient receptor potential; VEH, vehicle

Introduction

Background

The cannabis plant is a source of at least 70 phytocannabinoids, of which only one is psychoactive, Δ^9-tetrahydrocannabinol (THC). The remaining phytocannabinoids are not psychoactive and therefore, may have potential therapeutic effects without producing intoxication. There is convincing evidence that one of these non-psychoactive phytocannabinoids, cannabidiol (CBD), is effective in suppressing nausea and vomiting. CBD can reduce toxin-induced vomiting in *Suncus murinus* (house musk shrew) produced by nicotine, cisplatin or lithium chloride (LiCl, Kwiatkowska *et al*., 2004; Parker *et al*., 2004; Rock *et al*., 2011; Rock *et al*., 2012). CBD also reduces the establishment of conditioned gaping reactions in the taste reactivity (TR) test (Grill and Norgren, 1978) elicited by a LiCl-paired flavour, a model of nausea-induced behaviour in rats (see Parker and Limebeer, 2008 for review). Unlike conditioned taste avoidance (CTA), which can be produced by rewarding drugs as well as 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). As well, in a rodent model of anticipatory nausea (AN), in which chemotherapy patients experience nausea when simply returning to the treatment-paired context, CBD (unlike traditional anti-emetics) effectively suppresses the expression of conditioned gaping elicited by LiCl-paired contextual cues (Rock *et al*., 2008). Both the anti-emetic effect and the anti-nausea effect of CBD appear to be mediated by its action as an indirect 5-hydroxytryptamine 1A (5-HT_{1A}) receptor agonist. Indeed, central administration of CBD directly to the dorsal raphe nucleus (DRN), the site of somatodendritic 5-HT_{1A} autoreceptors, suppressed the establishment of LiCl-induced gaping reactions, and this effect was reversed by systemic administration of the highly selective 5-HT_{1A} receptor antagonist, WAY100635 (Rock *et al*., 2012). These results suggest that CBD reduces nausea by reducing the release of nausea-inducing 5-HT in forebrain regions, most likely the visceral insular cortex (Tuerke *et al*., 2012).

CBD is formed in cannabis from an acidic precursor, cannabidiolic acid (CBDA, Potter *et al*., 2008). The structure of CBDA and its isolation from cannabis was first described by Mechoulam and Gaoni (1965). CBDA gradually loses its carboxyl group to form CBD when harvested cannabis is heated, as when it is smoked. The literature is replete with the numerous pharmacological actions of CBD (reviewed in Pertwee, 2008), but very little is known about those of CBDA. Recently, we evaluated the potential of CBDA to reduce nausea and vomiting in our models and found that it is about 100 times more potent than CBD in reducing LiCl-induced emesis in the *S. murinus* and LiCl-induced conditioned gaping in rats (Bolognini *et al*., 2013). Like CBD, CBDA's suppression of nausea and vomiting was reversed by pretreatment with the 5-HT_{1A} receptor antagonist, WAY100635.

Objectives

The experiments presented here determined the minimally effective dose of CBDA that would reduce LiCl-induced conditioned gaping reactions. In our previous report, doses of 0.01 and 0.1 mg·kg^{-1}, but not 0.5 or 5 mg·kg^{-1}, effectively suppressed the nausea-induced behaviour of conditioned gaping in rats, without affecting CTA learning; however, the threshold dose for suppressing these nausea-induced responses was not determined. Here, we assessed low doses of CBDA (5, 1, 0.5 and 0.1 μg·kg^{-1}) to interfere with the acute nausea produced by LiCl in the conditioned gaping model in rats. As well, we sought to determine if a subthreshold dose of CBDA would facilitate the anti-nausea effects of low doses of the 5-HT_3 receptor antagonist, ondansetron (OND, e.g. Limebeer and Parker, 2000), a classic anti-emetic drug that is commonly used in chemotherapy treatment.

Methods

Experimental procedures

Experiment 1: Determination of threshold dose of CBDA to suppress LiCl-induced conditioned gaping. All rats were surgically implanted with an intraoral cannula under isofluorane anaesthesia, according to the procedures described by Limebeer *et al*. (2009). The intraoral cannula, implanted to deliver fluids to the rat's oral cavity, consisted of a length of Intramedic plastic tubing with an inner diameter of 0.86 mm and an outer diameter of 1.27 mm with two circular elastic discs placed over the tubing at the back of the neck.

Three days after the surgery, the rats received a TR 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 × 26.0 × 20.0 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·min^{-1}.

On the day following the TR adaptation trial, the rats received a TR conditioning trial in which they were administered a pretreatment injection of CBDA (0.1, 0.5, 1, or 5 μg·kg^{-1}) or vehicle (VEH; ethanol/cremophore/SAL, 1:1:18). Forty-five minutes after the pretreatment injection, the rats were individually placed in the TR chamber and intraorally infused with 0.1% saccharin solution for 2 min at the rate of 1 mL·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) fire-wired into a computer. Immediately after the saccharin infusion, all rats were injected with 20 mL·kg^{-1} of 0.15 M LiCl and returned to their home cage. The groups (with random assignment) were as follows: VEH (n = 12), 0.1 μg·kg^{-1} CBDA (n = 8), 0.5 μg·kg^{-1} CBDA (n = 8), 1 μg·kg^{-1} CBDA (n = 8), and 5 μg·kg^{-1} CBDA (n = 8).

Seventy-two hours following the TR conditioning trial the rats were given the TR test trial, drug-free. Rats were again intraorally infused with 0.1% saccharin solution for 2 min at the rate of 1 mL·min^{-1} while the orofacial responses were video recorded. Rats were then returned to their home cages. At 16:00 h on the day of the TR test trial, the rats were water restricted (water bottles removed from cage). Eighteen hours

later on the following morning, they were given the CTA test. Each rat was presented with a single graduated tube containing 0.1% saccharin solution and consumption measures were taken at 30, 120 and 360 min.

The video tapes of the TR conditioning trial and the TR test trial were later scored (at one-half speed) by an observer blind to the experimental conditions using 'The Observer' (Noldus Information Technology Inc., Leesburg, VA, USA) for the number of occurrences of gaping (large openings of the mouth and jaw, with lower incisors exposed) and tongue protrusions (extensions of the tongue, both forward and lateral, from the mouth as a measure of hedonic reactions).

Experiment 2: Effect of combined CBDA and OND on LiCl-induced conditioned gaping. The rats were treated as in Experiment 1, except as specified. On the day following the TR adaptation trial, the rats received a TR conditioning trial in which they were administered a pretreatment injection of CBDA (0.5 $\mu g \cdot kg^{-1}$ in Experiment A or 0.1 $\mu g \cdot kg^{-1}$ in Experiment B) or VEH (ethanol/Cremophore/SAL, 1:1:18). Fifteen minutes after the first pretreatment injection, the rats then received an additional pretreatment injection of OND (10 $\mu g \cdot kg^{-1}$ in Experiment 2A or 1 $\mu g \cdot kg^{-1}$ in Experiment 2B) or SAL. Thirty minutes after the second pretreatment, the rats were conditioned as in Experiment 1. The groups were as follows for Experiment 2A (n = 8 per group): VEH–SAL, VEH–10 OND, 0.5 CBDA–SAL, 0.5 CBDA–10 OND. The groups were as follows for Experiment 2B: VEH–SAL (n = 8), VEH–1 OND (n = 6), 0.1 CBDA–SAL (n = 8), 0.1 CBDA–1 OND (n = 6). The rats were given the 2 min TR test as in Experiment 1, 72 h following the conditioning trial. On the following day, while water deprived for 18 h, they received the 6 h single bottle CTA test as in Experiment1.

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 consulted with the ARRIVE guidelines of the *British Journal of Pharmacology*, for reporting experiments involving animals. Male Sprague-Dawley rats (Experiment 1, n = 44; Experiment 2, n = 60), weighing between 252 and 321 g on the day of conditioning, obtained from Charles River Laboratories (St Constant, Quebec) were used in the experiments. They were single-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 08:00 h) and maintained on food and water *ad libitum*.

Drugs and materials

Samples of CBDA extracted from cannabis were provided by GW Pharmaceuticals (Porton Down, Wiltshire, UK). Ethanol, Cremophor, LiCl and OND were purchased from Sigma (St Louis, MO, USA). CBDA was prepared in a VEH consisting of a 1:1:18 mixture of ethanol, Cremophor and saline (SAL) and was administered intraperitoneally (i.p.) in a volume of 2 $mL \cdot kg^{-1}$ (in Experiment 1) or 1 $mL \cdot kg^{-1}$ (in Experiment 2). LiCl was prepared as a 0.15 M solution with sterile water and administered i.p. in a volume of 20 $mL \cdot kg^{-1}$ (127.2 $mg \cdot kg^{-1}$). OND was prepared in SAL and administered at a volume of 1 $mL \cdot kg^{-1}$, i.p.

Statistical methods

In each experiment, the number of tongue protrusions during the TR conditioning trial and the number of gapes during the TR test trial were each entered into separate one-way ANOVAs, with subsequent planned comparisons. The amount of saccharin consumed during the CTA test for each group was entered into a mixed factors ANOVA [group × time of measurement (30, 120, 360 h)]. For all analyses, P-values of <0.05 were taken as significant.

Results

Experiment 1: Determination of threshold dose of CBDA to suppress LiCl-induced conditioned gaping

CBDA interfered with the establishment of LiCl-induced conditioned gaping reactions at doses as low as 0.5 $\mu g \cdot kg^{-1}$. The mean number of gapes displayed during the drug-free TR test trial for each group is presented in Figure 1. The one-way ANOVA of gaping reactions on the TR test trial revealed a significant effect of dose, $F(4, 39) = 89.6$; $P < 0.001$. Planned comparison tests revealed that, at doses of 5, 1 and 0.5 $\mu g \cdot kg^{-1}$, CBDA significantly ($P < 0.03$) reduced LiCl-induced gaping relative to the VEH-pretreated controls. The dose of 0.1 $\mu g \cdot kg^{-1}$ CBDA was ineffective. The mean number of tongue protrusions elicited during the conditioning trial by 0.1% saccharin in groups of rats pretreated with various doses of CBDA is presented in Figure 2. A one-way ANOVA of the tongue protrusion data revealed no significant effect of dose, $P > 0.05$. None of the doses of CBDA interfered with LiCl-induced CTA, a behavioural effect that is not dependent on a nausea-inducing treatment (e.g. Parker *et al.*, 2008). The

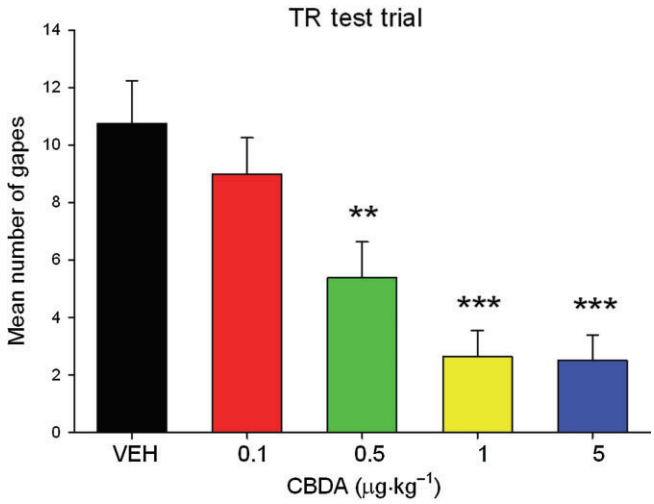


Figure 1

Effect of CBDA (0.1, 0.5, 1 and 5 $\mu g \cdot kg^{-1}$) or VEH (i.p.) administered 45 min prior to LiCl. The number of conditioned gaping responses was measured during the drug-free test trial. Each bar represents the mean ± SEM (n = 8–12). The asterisks indicate a significant difference from the VEH-treated control animals (**$P < 0.03$, ***$P < 0.001$).

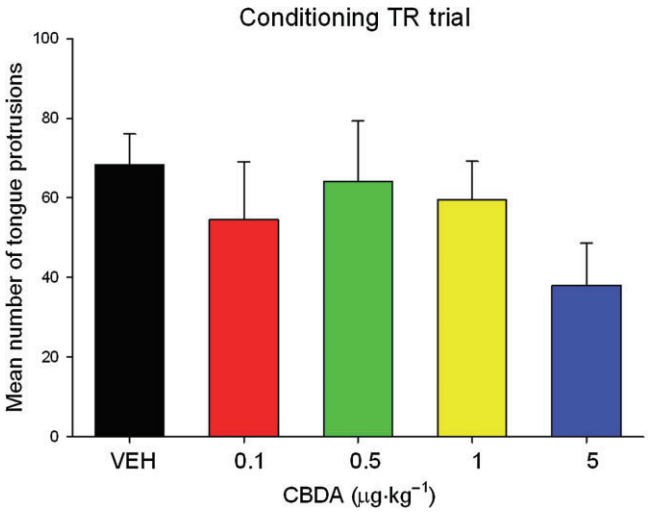


Figure 2

Effect of CBDA (0.1, 0.5, 1 and 5 μg·kg^{-1}) or VEH (i.p.) administered 45 min prior to LiCl. The number of tongue protrusions was measured during the conditioning trial. Each bar represents the mean ± SEM (n = 8–12).

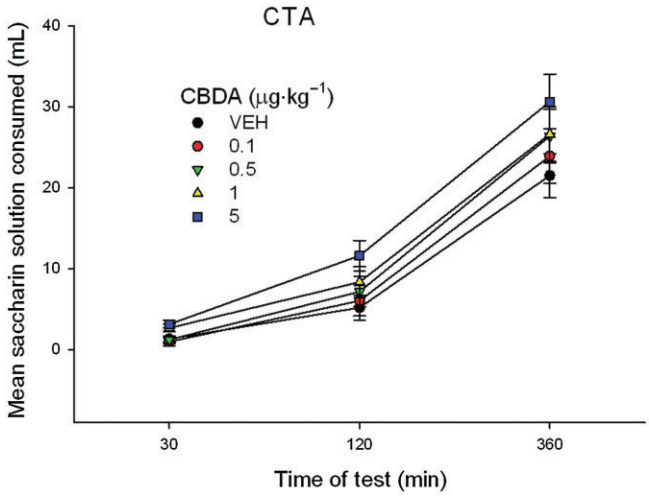


Figure 3

Effect of CBDA (0.1, 0.5, 1, and 5 μg·kg^{-1}) or VEH (i.p.) administered 45 min prior to LiCl. The 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.

mean amounts of saccharin consumed during the CTA test at 30, 120 and 360 min by various groups are presented in Figure 3. A 5 × 3 mixed factors ANOVA revealed only a significant effect of time of test, $F(2,78) = 298.3$, $P < 0.001$.

Experiment 2. Effect of combined CBDA and OND on LiCl-induced conditioned gaping

Experiment 2A. In Experiment 2A, we evaluated the potential of doses of 0.5 μg·kg^{-1} CBDA and 10 μg·kg^{-1} OND alone

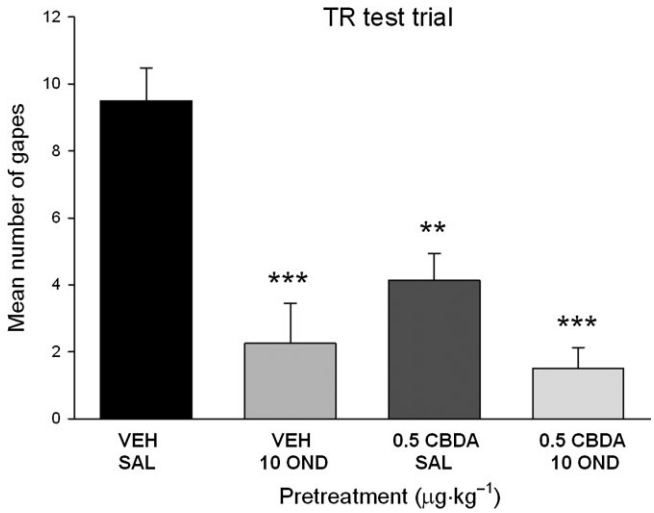
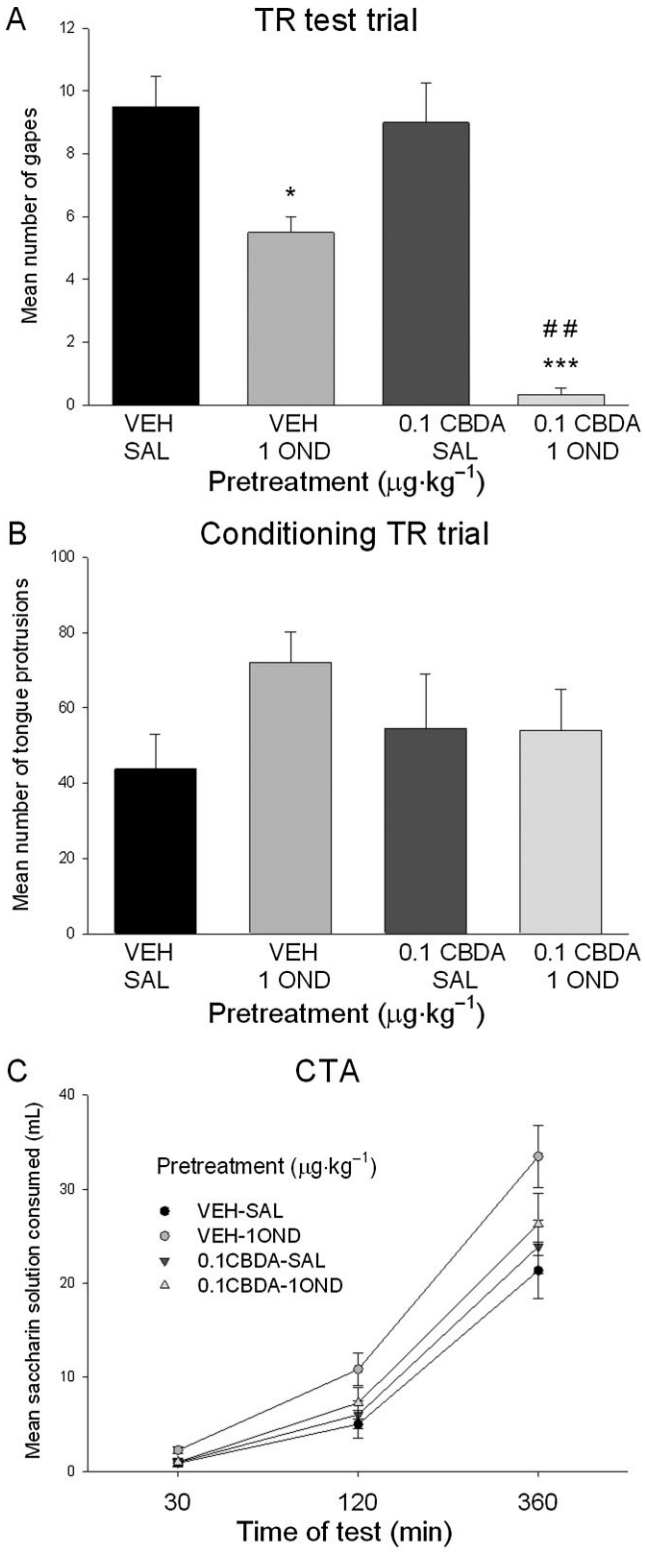


Figure 4

Effect of CBDA (0.5 μg kg^{-1}) or VEH (i.p.) administered 15 min prior to OND (10 μg·kg^{-1} i.p.) or SAL (i.p.) in LiCl-treated rats. The number of conditioned gaping responses was measured during the test trial. Each bar represents the mean number of conditioned gaping responses ± SEM (n = 8). The asterisks indicate a significant difference from the VEH-treated control animals (**P < 0.003, ***P < 0.001).

and in combination to interfere with LiCl-induced conditioned gaping; however, each of these doses effectively suppressed conditioned gaping on their own. In Figure 4, a one-way ANOVA revealed a significant effect of pretreatment $F(3,28) = 15.0$, $P < 0.001$. Planned comparisons revealed that all treatment groups gaped less than the VEH–SAL pretreatment group, but no other groups differed. As well, neither the tongue protrusion data nor the CTA data revealed group differences (not depicted).

Experiment 2B. As both doses of CBDA and OND significantly reduced conditioned gaping in Experiment 2A, lower doses of both CBDA and OND were evaluated on their own and in combination in Experiment 2B. OND (1 μg·kg^{-1}) alone reduced nausea-induced conditioned gaping, but when it was combined with CBDA (0.1 μg·kg^{-1}) the suppression was enhanced. In Figure 5A, a one-way ANOVA revealed a significant effect of pretreatment, $F(3,28) = 18.9$, $P < 0.001$. Planned comparisons revealed that that those rats pretreated with VEH–1 μg·kg^{-1} OND, but not 0.1 μg·kg^{-1} CBDA–SAL, gaped significantly less than the VEH–SAL pretreatment group (P < 0.05). However, rats in Group 0.1 μg·kg^{-1} CBDA –1 μg·kg^{-1} OND showed significantly less nausea-induced gaping than rats in group VEH –1 μg·kg^{-1} OND (P < 0.01). Figure 5B presents the mean number of tongue protrusions displayed by each group during the conditioning TR trial. A one-way ANOVA of the tongue protrusion data revealed no significant effect of group, $P > 0.05$. The mean amounts of saccharin consumed during the CTA test at 30, 120 and 360 min by groups of rats are presented in Figure 5C. A 4 × 3 mixed factors ANOVA revealed a significant effect of both time of test, $F(2,48) = 225.6$ $P < 0.001$ and group $F(3,24) = 3.7$, $P = 0.03$.



Planned comparison tests revealed that those rats receiving VEH –1 μg·kg^{-1} OND drank significantly more saccharin overall than the VEH-SAL group ($P < 0.005$). No other significant differences were seen between treatments and there was no group by time interaction ($P > 0.05$).

Figure 5

Effect of CBDA (0.1 μg·kg^{-1}) or VEH (i.p.) administered 15 min prior to OND (1 μg·kg^{-1}) or SAL (i.p.) in LiCl-treated rats. The number of conditioned gaping responses was measured during the test trial. In Part A, each bar represents the mean number of conditioned gaping responses ± SEM ($n = 6$–8). In Part B, the number of tongue protrusions was measured during the conditioning trial. Each bar represents the mean ± SEM. In part C, the 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. The asterisks indicate a significant difference from the VEH-treated control animals (*$P <$ 0.05, ***$P < 0.001$). As well, ## indicates that the group given the combination of 0.1 CBDA-1 OND showed less gaping than Group VEH-OND ($P < 0.01$).

Discussion

Low doses of CBDA (as low as 0.5 μg·kg^{-1}) effectively reduced the establishment of LiCl-induced conditioned gaping, suggesting that it is a highly potent treatment for acute nausea. CBDA (0.1 and 0.5 mg·kg^{-1}) also inhibits toxin-induced vomiting in shrews, but not at a dose of 0.05 mg·kg^{-1} (Bolognini *et al.*, 2013). The data collected here demonstrate that the effective doses of CBDA, which attenuate the nausea-induced reaction of conditioned gaping are approximately 1000 times lower than those of CBD at 1–5 mg·kg^{-1} (Parker *et al.*, 2002; Rock *et al.*, 2008; 2011; 2012). As well, at least at the doses evaluated to date, the dose–response curve for the anti-emetic effect of CBDA is not biphasic as has been reported for CBD; that is in the *S. murinus* CBD suppresses acute LiCl- and cisplatin-induced vomiting at 5–10 mg·kg^{-1}, but potentiates it at 20–40 mg·kg^{-1} (Kwiatkowska *et al.*, 2004). This narrow range of CBD efficacy may limit its clinical use as an anti-emetic. In contrast, CBDA is no longer effective at higher doses of 1–5 mg·kg^{-1} (Bolognini *et al.*, 2013), but does not enhance LiCl-induced conditioned gaping or vomiting in *S. murinus* at these higher doses. Therefore, CBDA may be more desirable than CBD as an anti-emetic/anti-nausea agent. It has been found in a phase II clinical trial that Sativex, which contains the phytocannabinoids, THC and CBD, was effective in reducing the incidence of chemotherapy-induced nausea and vomiting, and was well tolerated by patients (Duran *et al.*, 2010). No such study has yet evaluated CBDA in humans.

Several findings that we recently made support the hypothesis that both CBD (Rock *et al.*, 2012) and CBDA (Bolognini *et al.*, 2013) attenuate signs of nausea in rats through indirect agonism of 5-HT$_{1A}$ receptors located in the brainstem. First, the nausea-reducing effects of both of these compounds can be prevented by the administration of a selective 5-HT$_{1A}$ receptor antagonist, WAY100635. Second, both CBD (100 nM) and CBDA (1–100 nM) display significant potency at enhancing the ability of the selective 5-HT$_{1A}$ receptor agonist, 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT), to stimulate [^{35}S]GTPγS binding to rat brainstem membranes. Third, intracranial administration of WAY100635 to the DRN prevented the suppressive effects of CBD on LiCl-induced gaping and intracranial administration of CBD to the DRN suppressed LiCl-induced conditioned gaping on its

own (an effect that was reversed by systemic WAY100635). Therefore, we (Rock *et al.*, 2012) have postulated that the reduction of nausea by CBD (and possibly CBDA; Bolognini *et al.*, 2013) is mediated by its enhancement of the activation of somatodendritic 5-HT_{1A} receptors in the DRN, which reduces the release of 5-HT in forebrain regions (Verge *et al.*, 1985; Blier and de Montigny, 1987). It is also noteworthy, that a number of other *in vivo* effects of CBD, such as its neuroprotective, anxiolytic, and antidepressant-like properties, seem to be 5-HT_{1A} receptor-mediated and that the log dose–response curve of CBD for the production of these effects is bell-shaped (Mishima *et al.*, 2005; Campos and Guimaraes, 2008; Zanelati *et al.*, 2010). To further investigate the 5-HT-CBDA interaction, experiments would need to examine whether intra-DRN administration of CBDA reduces conditioned gaping, and whether there is a subsequent reduction of 5-HT in the forebrain region mediating this response (e.g. visceral insular cortex, Tuerke *et al.*, 2012).

There is also *in vitro* evidence that CBDA activates the transient receptor potential (TRP) cation channels, TRPV1 (EC_{50} = 19.7 ± 3.9 μM) and TRPA1 (EC_{50} = 12.0 ± 8.8 μM), and antagonizes TRPM8 (IC_{50} = 1.6 ± 0.4 μM) (De Petrocellis *et al.*, 2008; De Petrocellis *et al.*, 2011). Furthermore, CBDA has been found to affect the contractility of gastrointestinal tissue of *S. murinus in vitro*, as indicated by its ability, at 10 μM, to reduce both the magnitude of contractions induced by carbachol or by electrical field stimulation and the tension of intestinal segments that had been pre-contracted with potassium chloride (Cluny *et al.*, 2011). In addition, Takeda *et al.* (2008) have reported that CBDA (IC_{50} = 2 μM) is a selective inhibitor of COX-2, an enzyme expressed by cells undergoing inflammation; however, Ruhaak *et al.* (2011) found more recently that CBDA did not inhibit this enzyme, but rather COX-1 (IC_{50} = 4.7 × 10^{-4} M), prompting a need for further research. Because CBDA enhanced the ability of the 5-HT_{1A} agonist 8-0H-DPAT to stimulate [^{35}S]GTPγS binding to rat brainstem membranes at a lower concentrations than other reported *in vitro* effects (1–100 nM) and the 5-HT_{1A} antagonist WAY100635 reversed the *in vivo* anti-nausea and anti-emetic effects of CBDA (Bolognini *et al.*, 2013), it is most likely that the anti-nausea effects reported in the present paper are 5-HT_{1A} mediated.

When combined with OND (1 μg·kg^{-1}), a dose of CBDA (0.1 μg·kg^{-1}) that was ineffective on its own enhanced the suppressive effect of OND on LiCl-induced acute nausea. Similar results were found with coadministration of subthreshold doses of OND and THC on cisplatin-induced vomiting in shrews (Kwiatkowska *et al.*, 2004). Future work should investigate the potential of CBDA to enhance the effectiveness of OND to suppress vomiting in an emetic species. Because we have shown that the anti-nausea effect of CBDA is reversed by the 5-HT_{1A} antagonist, WAY100635 (Bolognini *et al.*, 2013), it is likely that the combined suppression of serotonin transmission by the action of CBDA on somatodendric 5-HT_{1A} receptors (like CBD, Rock *et al.*, 2012) and blockade of the 5-HT_3 receptors by OND in terminal regions mediating nausea (e.g. visceral insular cortex, Tuerke *et al.*, 2012) may mediate this enhanced anti-nausea effect. Therefore, it is likely that CBDA would also facilitate the action of other 5-HT_3 receptor antagonists, such as tropisetron or palonosetron, to enhance their anti-nausea effects. In addition, action of 5-HT_{1A} agonists, such as 8-OH-DPAT and perhaps even CBD, could also be enhanced by coadministration with CBDA.

In contrast to LiCl-induced conditioned gaping reactions, CBDA pretreatment did not interfere with the establishment of CTA at any dose tested. This pattern is similar to that evident in previous studies with OND (e.g. Limebeer and Parker, 2000), CBD (Parker *et al.*, 2002; Rock *et al.*, 2012), THC (Limebeer and Parker, 1999) and 8-OH-DPAT, a 5-HT_{1A} receptor agonist (Limebeer and Parker, 2003). Because CTA is produced by rewarding drugs as well as by emetic drugs and anti-emetic drugs do not interfere with taste avoidance learning produced by a high dose of LiCl, unlike conditioned gaping, we have argued that CTA is not a selective measure of nausea (see Parker and Limebeer, 2006). However, it should be noted that the very low dose of OND (1 μg·kg^{-1}) not only reduced the establishment of LiCl-induced conditioned gaping, but also taste avoidance.

The finding that low doses of CBDA and OND can be combined to enhance the suppression of acute nausea produced by LiCl is of considerable therapeutic value. These findings suggest that CBDA should be considered as an adjunctive treatment along with OND to better control chemotherapy-induced acute nausea, which is more difficult to control than acute vomiting (de Boer-Dennert *et al.*, 1997; Hickok *et al.*, 2003; Foubert and Vaessen, 2005; Ballatori *et al.*, 2007). In fact, when combined, effective doses of CBDA and OND are much lower than when administered on their own. Interestingly, recent results from Takeda *et al.* (2012) indicate that CBDA (5, 10, 25 μM) inhibits highly aggressive human breast cancer cell migration. Taken together, these results suggest an important dual role for CBDA in cancer treatment, acting not only to reduce the symptoms of nausea and vomiting, but also to actually reduce cancer cell migration, an important factor in cancer metastasis. As well, we have previously shown that unlike OND (e.g. Limebeer *et al.*, 2006; Parker *et al.*, 2006; Rock *et al.*, 2008), both CBD (Parker *et al.*, 2006; Rock *et al.,* 2008) and CBDA (Bolognini *et al.*, 2013) are highly effective in reducing the expression of AN in our animal models. In human chemotherapy patients, AN is not well controlled by OND (Morrow *et al.*, 1998) and there are currently no specific treatments available. Because CBDA has a wider margin of efficacy than CBD, it may be the preferred treatment for the control of both acute and AN.

Acknowledgements

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Conflict of interest

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