RESEARCH PAPER

Cannabidiolic acid prevents vomiting in *Suncus murinus* and nausea-induced behaviour in rats by enhancing 5-HT$_{1A}$ receptor activation

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BACKGROUND AND PURPOSE

To evaluate the ability of cannabidiolic acid (CBDA) to reduce nausea and vomiting and enhance 5-HT$_{1A}$ receptor activation in animal models.

EXPERIMENTAL APPROACH

We investigated the effect of CBDA on (i) lithium chloride (LiCl)-induced conditioned gaping to a flavour (nausea-induced behaviour) or a context (model of anticipatory nausea) in rats; (ii) saccharin palatability in rats; (iii) motion-, LiCl- or cisplatin-induced vomiting in house musk shrews (*Suncus murinus*); and (iv) rat brainstem 5-HT$_{1A}$ receptor activation by 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and mouse whole brain CB$_1$ receptor activation by CP55940, using [^{35}S]GTPγS-binding assays.

KEY RESULTS

In shrews, CBDA (0.1 and/or 0.5 mg·kg^{-1} i.p.) reduced toxin- and motion-induced vomiting, and increased the onset latency of the first motion-induced emetic episode. In rats, CBDA (0.01 and 0.1 mg·kg^{-1} i.p.) suppressed LiCl- and context-induced conditioned gaping, effects that were blocked by the 5-HT$_{1A}$ receptor antagonist, WAY100635 (0.1 mg·kg^{-1} i.p.), and, at 0.01 mg·kg^{-1} i.p., enhanced saccharin palatability. CBDA-induced suppression of LiCl-induced conditioned gaping was unaffected by the CB$_1$ receptor antagonist, SR141716A (1 mg·kg^{-1} i.p.). *In vitro*, CBDA (0.1–100 nM) increased the E_{max} of 8-OH-DPAT.

CONCLUSIONS AND IMPLICATIONS

Compared with cannabidiol, CBDA displays significantly greater potency at inhibiting vomiting in shrews and nausea in rats, and at enhancing 5-HT$_{1A}$ receptor activation, an action that accounts for its ability to attenuate conditioned gaping in rats. Consequently, CBDA shows promise as a treatment for nausea and vomiting, including anticipatory nausea for which no specific therapy is currently available.

Introduction

The cannabis plant is a natural source of at least 70 compounds known collectively as phytocannabinoids, and there is convincing evidence that one of these, cannabidiol (CBD; Figure 1), can suppress nausea and vomiting. CBD can reduce 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; 2012), although not by motion (Cluny *et al.*, 2008). It can also reduce the establishment of conditioned gaping reactions (Grill and Norgren, 1978a,b) 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, which can be produced by rewarding drugs as well as by emetic drugs, conditioned gaping reactions are only produced in rats by drugs that induce vomiting in emetic species, such as shrews (Parker, 2003; Parker *et al.*, 2008). In addition, in a rodent model of anticipatory nausea evident in chemotherapy patients 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).

It has also been found in a phase II clinical trial that Sativex, a medicine that contains the phytocannabinoids, Δ^9-tetrahydrocannabinol (Δ^9-THC) and CBD, was both effective in reducing the incidence of chemotherapy-induced nausea and vomiting, and well tolerated by patients (Duran *et al.*, 2010). It should be noted, however, that the log dose–response curves for the anti-emetic effects produced by CBD in house musk shrews are biphasic. Thus, CBD suppresses acute cisplatin-induced vomiting at 5 mg·kg^{-1}, but potentiates it at 40 mg·kg^{-1} (Kwiatkowska *et al.*, 2004). Similarly, acute vomiting elicited by LiCl is suppressed by low doses of CBD (5–10 mg·kg^{-1}), whereas higher doses (20–40 mg·kg^{-1}) of this phytocannabinoid act to facilitate LiCl-induced vomiting, rather than to reduce its expression (Parker *et al.*, 2004). This narrow range of CBD efficacy may limit its clinical use as an anti-emetic.

Several findings that we recently made (Rock *et al.*, 2012) support the hypothesis that CBD attenuates toxin-induced vomiting in shrews and signs of nausea in rats through indirect agonism of 5-HT$_{1A}$ receptors located in the brainstem. First, each of these effects of CBD can be prevented by the administration of a selective 5-HT$_{1A}$ receptor antagonist, either WAY100135 or WAY100635. Second, CBD displays significant potency at enhancing the ability of the selective 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT, to stimulate [^{35}S]GTPγS binding to rat brainstem membranes. Third, when co-administered with 8-OH-DPAT, CBD suppresses LiCl-induced signs of nausea in rats in an apparently synergistic manner. It is also noteworthy that a number of other *in vivo* effects of CBD 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 Guimarães, 2008; Zanelati *et al.*, 2010; Gomes *et al.*, 2011; Maione *et al.*, 2011). As to the ability of CBD to reduce signs of toxin-induced nausea in rats, we have postulated that this results from CBD-induced enhancement of the activation of somatodendritic 5-HT$_{1A}$ receptors in the dorsal raphe nucleus, as activation of these receptors is expected to reduce the release of nausea-inducing 5-HT in forebrain regions (Rock *et al.*, 2012).

We have shown previously that CBD can also target CB$_1$ receptors with significant potency. For example, in experiments performed with mouse whole brain membranes, we found that CBD (1 μM) can antagonize the cannabinoid receptor agonist, CP55940, in the [^{35}S]GTPγS-binding assay with an apparent K_B of 79 nM. This value is close to the concentration (100 nM) at which CBD increases the maximal effect (E_{max}) of 8-OH-DPAT in rat brainstem membranes (Rock *et al.*, 2012), but significantly below the K_i value of CBD (4.9 μM) for its displacement of CP55940 from specific binding sites in mouse whole brain membranes (Thomas *et al.*, 2007). In view of the ability of CBD to interact with CB$_1$ receptors, it is also noteworthy that its ability to suppress vomiting in house musk shrews is not blocked by the selective cannabinoid CB$_1$ receptor antagonist, SR141716A (Parker *et al.*, 2004).

CBD is formed in cannabis from an acidic precursor, cannabidiolic acid (CBDA; Potter *et al.*, 2008), the isolation from cannabis and structure of which was reported in 1965 (Mechoulam and Gaoni, 1965). In harvested cannabis, CBDA (Figure 1) gradually loses its carboxyl group to form CBD, a process that can be greatly accelerated by heating or burning cannabis, as happens when it is smoked. Currently, much more is known about the pharmacological actions of CBD, which are numerous (reviewed in Pertwee, 2008; Fernández-Ruiz *et al.*, 2012), than about those of CBDA. There is already evidence, however, that CBDA shares the ability of CBD to activate the transient receptor potential (TRP) cation channels, TRPV1 and TRPA1, and to antagonize TRPM8 (De Petrocellis *et al.*, 2008; 2011). Importantly, however, CBDA produces these effects with significantly less potency than
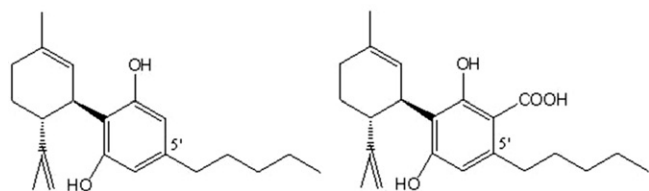
Figure 1
Structures of CBD and CBDA.

CBD. Indeed, the concentration ranges at which CBD targets all of these cation channels, except TRPV1, overlap with the concentration (100 nM) at which it increases the E_{max} of 8-OH-DPAT in rat brainstem membranes (Rock *et al.*, 2012). At 1 μM, both CBD and CBDA have also been shown to produce a significant downward shift in log concentration–response curve of the GPR55 agonist, L-α-lysophosphatidylinositol, for its stimulation of ERK1/2 phosphorylation in human GPR55-transfected HEK293 cells (Anavi-Goffer *et al.*, 2012). Furthermore, CBDA has been found to affect the contractility of gastrointestinal tissue of house musk shrews *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 is a selective inhibitor of COX-2, an enzyme expressed by cells undergoing inflammation; more recently, however, Ruhaak *et al.* (2011) found that CBDA did not inhibit this enzyme, prompting a need for further research.

The present investigation sought to determine whether CBDA can (i) inhibit LiCl-induced conditioned gaping to a flavour and to a context in rats in a 5-HT$_{1A}$ receptor-dependent manner; (ii) prevent toxin-induced vomiting in house musk shrews; and (iii) enhance activation of 5-HT$_{1A}$ receptors in rat brainstem membranes. We also investigated whether CBDA can suppress motion-induced vomiting in shrews. Our overall objective was to explore the possibility that CBDA might inhibit vomiting and conditioned gaping and enhance 5-HT$_{1A}$ receptor activation with greater potency or selectivity, and/or over a wider dose range, than CBD. Some of the results described in this paper have been presented to the International Cannabinoid Research Society (Bolognini *et al.*, 2012).

Methods

Animals for in vivo experiments
Animal procedures complied with the Canadian Council on Animal Care and the National Institutes of Health guidelines or the UK Animals Act (Scientific Procedures) 1986. The protocols were approved by the Institutional Animal Care Committee, which is accredited by the Canadian Council on Animal Care or by the University of Bradford animal ethics committee. Toxin-induced emesis experiments were performed with male (36.5–45.8 g) and female (18.9–29.3 g) house musk shrews (*S. murinus*), aged 87–815 days at the time of testing, that had been bred and raised at the University of Guelph. They were single housed in cages in a colony room at an ambient temperature of 22°C on a 10/14 h light-dark schedule (lights off at 19:00 h). Shrews were tested during their light cycle. Motion-induced emesis experiments were performed with male (60–80 g) house musk shrews (*S. murinus*) that had been bred and raised at the University of Bradford colony. They were housed up to three to a cage at 22°C on a 10.5/13.5 h light-dark schedule with lights off at 19:00 h (on at 08:30 h). 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).

Naïve male Sprague–Dawley rats, obtained from Charles River Laboratories (St Constant, Quebec), were used for assessment of anti-nausea-like behaviour. They were single housed in shoebox cages, subjected to an ambient temperature of 21°C and a 12/12 h light-dark schedule (lights off at 08:00 h), and maintained on food and water *ad libitum*. Their body weights ranged from 264 to 430 g on the day of conditioning.

Drugs and materials for in vivo experiments
Samples of CBDA extracted from cannabis were provided by GW Pharmaceuticals (Porton Down, Wiltshire, UK), dimethyl sulfoxide (DMSO) by Sigma-Aldrich (Poole, Dorset, UK), and ethanol, Cremophor, LiCl, cisplatin and WAY100635 by Sigma (St Louis, MO, USA). In both the LiCl- and cisplatin-induced emesis experiments, performed in Guelph with shrews, and the LiCl-induced gaping experiments, performed in Guelph with rats, CBDA was prepared in a vehicle (VEH) consisting of a 1:1:18 mixture of ethanol, Cremophor and saline (SAL) and was administered i.p. in a volume of 2 mL·kg^{-1}. This VEH and injection volume were also used for SR141716A, which was administered to rats i.p. at a dose (1 mg·kg^{-1}) expected to reverse the effects of CB$_1$ agonists (e.g. Vlachou *et al.*, 2003), without potentiating the aversive effects of LiCl (Parker *et al.*, 2003). In the motion-induced emesis experiments, performed in Bradford with shrews, CBDA was prepared in a VEH of 2% DMSO in distilled water and administered i.p. in a volume of 10 mL·kg^{-1}. 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 (see Parker *et al.*, 2004) and in a volume of 20 mL·kg^{-1} (127.2 mg·kg^{-1}) to rats. Cisplatin was prepared as a 1 mg·mL^{-1} solution in SAL and was administered to shrews i.p., in a volume of 20 mL·kg^{-1} (20 mg·kg^{-1}). WAY100635 (0.1 mg·mL^{-1}) was prepared in SAL and administered to rats i.p in a volume of 1 mL·kg^{-1} (0.1 mg·kg^{-1}).

In vivo procedures
Effect of CBDA on LiCl- or cisplatin-induced vomiting in house musk shrews. Shrews were transferred from the colony room to an empty cage in the experimental room that contained four meal worms. After 15 min they were injected with CBDA or VEH. In experiment A, they received an injection of either CBDA, at a dose of 0.05 mg·kg^{-1} ($n = 8$), 0.1 mg·kg^{-1} ($n = 8$), 0.5 mg·kg^{-1} ($n = 6$), or 5 mg·kg^{-1} ($n = 8$), or VEH ($n = 6$), followed 45 min later by an injection of LiCl (390 mg·kg^{-1}). In experiment B, shrews were injected with 0.5 mg·kg^{-1} CBDA ($n = 8$), a dose found to be effective in experiment A, or with VEH ($n = 8$), 45 min before receiving an injection of cisplatin (20 mg·kg^{-1}). Shrews were then immediately put individually into an observation chamber for 45 min in the LiCl experiment or for 70 min in the cisplatin experiment. The Plexiglas observation chambers (22.5 × 26 × 20 cm) were placed on a table with a clear glass top. A mirror beneath the chamber facilitated viewing of the ventral surface of each shrew and hence of all vomiting episodes. The frequency of these episodes was counted by an observer blind to the experimental conditions.

Effect of CBDA on LiCl-induced conditioned gaping to a flavour in rats. All rats were surgically implanted with an intraoral cannula under isoflurane anaesthesia as described by Lime-

beer *et al.* (2010). Following recovery from surgery (at least 3 days), each rat was subjected to an adaptation trial for which it was placed in the taste reactivity chamber with its cannula attached to an infusion pump (Model KDS100; KD Scientific, Holliston, MA, USA) for fluid delivery. The taste reactivity chambers were made of clear Plexiglas ($22.5 \times 26 \times 20$ cm) and placed on a table with a clear glass top. A mirror beneath each chamber facilitated viewing of the ventral surface of the rat and hence of any orofacial responses. Water was infused into the intraoral cannula of each rat for 2 min at a rate of 1 mL·min^{-1}. On the day following this adaptation trial, the rats were subjected to a conditioning trial in which they received i.p. pretreatment injections of VEH ($n = 12$) or of one of four doses of CBDA (0.01 mg·kg^{-1}, $n = 8$; 0.1 mg·kg^{-1}, $n = 12$; 0.5 mg·kg^{-1}, $n = 7$; 5.0 mg·kg^{-1}, $n = 8$). Forty-five min after the pretreatment injection, each rat was individually placed in the taste reactivity chamber and intraorally infused with 0.1% saccharin solution for 2 min at a rate of 1 mL·min^{-1} while any orofacial responses were observed using a mirror located beneath the chambers. These responses were monitored using a 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.

Seventy two hr later, all the rats were tested drug-free. They were again intraorally infused with 0.1% saccharin solution at a rate of 1 mL·min^{-1} over a 2 min period, during which their orofacial responses were video recorded. Rats were then returned to their home cages. The videotapes were later scored (at ½ speed) by an observer blind to the experimental conditions using 'The Observer' (Noldus Information Technology, Inc., Leesburg, VA, USA) for the behaviours of gaping (large openings of the mouth and jaw, with lower incisors exposed) and of tongue protrusions.

Conditioned taste avoidance was assessed in a single bottle test. Rats were water restricted at 16:00 h. The following morning, a single bottle containing 0.1% saccharin was placed in each cage at 09:00 h. Saccharin consumption was measured 30, 120 and 360 min later.

Effect of WAY100635 on CBDA-induced suppression of LiCl-induced conditioned gaping to a flavour in rats. The rats were treated exactly as in the LiCl-induced conditioned gaping experiments described in the previous section, except that they received an injection of either WAY100635 or SAL 15 min prior to the pretreatment injection of CBDA (0.1 mg·kg^{-1}) or VEH. Rats were subjected to one or other of the following treatments ($n = 12$): SAL-VEH, SAL-CBDA, WAY100635-VEH and WAY100635-CBDA.

Effect of SR141716A on CBDA-induced suppression of LiCl-induced conditioned gaping to a flavour in rats. The rats were treated exactly as in the experiment with WAY100635 described in the previous section, except that they received an injection of either SR141716A (1 mg·kg^{-1}) or VEH 15 min prior to the pretreatment injection of CBDA (0.1 mg·kg^{-1}) or VEH. Rats were subjected to one or other of the following treatments: SAL-VEH ($n = 12$), SAL-CBDA ($n = 12$), SR141716A-VEH ($n = 6$) or SR141716A-CBDA ($n = 6$).

Effect of WAY100635 on the expression of CBDA-induced suppression of LiCl-induced conditioned gaping to a context in rats. A distinctive context was created by exposing rats to location, visual and tactile cues different from those to which they were being subjected in their home cage environment. This was achieved by placing a 'contextual' conditioning chamber in a dark room next to a 25 W light source. This chamber was made of black opaque Plexiglas, but was in all other respects identical to the one used in the LiCl-induced conditioned gaping experiments. The rats underwent four conditioning trials, during which the contextual chamber was paired with 127 mg·kg^{-1} LiCl. In every conditioning trial, each rat was injected with LiCl and immediately placed in the distinctive context for a 30 min period. This procedure was repeated four times, with a 72 h interval between each conditioning trial. For the test trial, rats were randomly assigned to one of four treatment groups ($n = 10$): SAL-CBDA, WAY100635-CBDA, SAL-VEH, WAY100635-VEH. CBDA or VEH was administered 15 min after SAL or WAY100635. Forty-five min later, rats were individually placed in the contextual chamber for a period of 5 min during which their orofacial responses were video recorded using a mirror located beneath the chamber.

Effect of CBDA on motion-induced emesis in shrews. The induction and quantification of the emetic response to motion have been described previously (Cluny *et al.*, 2008). Briefly, six linked transparent compartments [100 mm (width) × 150 mm (length) × 150 mm (height)] are placed on a track that moves horizontally at a frequency of 1 Hz and amplitude of 40 mm. This motion induces emetic episodes, each of which consists of a bout of retching that involves strong repeated abdominal contractions accompanied by wide opening of the mouth and, initially, also by the passage of matter from the upper gastrointestinal tract. In this investigation, shrews ($n = 6$–18) were administered CBDA (0.02, 0.1 or 0.5 mg·kg^{-1}) or VEH i.p. They were then immediately placed individually in a compartment of the shaker and monitored over a 45 min period for any signs of emesis and for any other overt behavioural changes. Motion was then applied to the compartments for 10 min during which the number of emetic episodes and the latency of onset of the first of these episodes were noted. If no emetic episodes were observed, the latency of onset was recorded as 600 s. Animals were observed for a further 2 min before being returned to their housing cage.

Analysis of *in vivo* data

Values have been expressed as means and variability as SEM. In the house musk shrew studies, the effect of CBDA on emesis was analysed using an independent *t*-test or one-way ANOVA followed by a Bonferroni or Dunnett's multiple comparison *post hoc* test. In the rat studies, the number of gapes or hedonic reactions was analysed using a one-way ANOVA and planned comparison tests. Statistical comparisons of the amounts of saccharin consumed during conditioned taste avoidance tests by different groups of rats were made by using either a 5×3 mixed factors ANOVA, or in the experiments with WAY100635, a 4×3 mixed factors ANOVA. For all analyses, *P*-values <0.05 were considered significant.

Animals for in vitro experiments

Brainstem tissue was obtained from adult male Lister hooded rats, ranging from 7 to 9 weeks of age, and purchased from Harlan UK, Ltd. (Blackthorn, UK). Mouse whole brain membranes were obtained from adult male MF1 mice purchased from Harlan UK, Ltd. All animal care and experimental procedures complied with the UK Animals (Scientific Procedures) Act, 1986, and associated guidelines for the use of experimental animals.

Drugs and materials for in vitro experiments

CBDA, extracted from *Cannabis sativa*, was provided by GW Pharmaceuticals. 8-OH-DPAT HBr and CP55940 were supplied by Tocris (Bristol, UK). [^{35}S]GTPγS (1250 Ci·mmol^{-1}), [^{3}H]8-OH-DPAT (135.2 Ci·mmol^{-1}) and [^{3}H]CP555940 (160 Ci·mmol^{-1}) were obtained from PerkinElmer Life Sciences, Inc. (Boston, MA, USA), GTPγS and adenosine deaminase from Roche Diagnostic (Indianapolis, IN, USA), and GDP and DMSO from Sigma-Aldrich.

In vitro procedures

Preparation of cell membranes from rat brainstem and mouse whole brain. Rat brainstem tissue was homogenized in ice-cold Choi lysis buffer (Tris–HCl 20 mM, sucrose 0.32 M, EDTA 0.2 mM, EGTA 0.5 mM, pH 7.5) containing Roche© protease inhibitor cocktail (1:40 v/v; Roche Diagnostics, Mannheim, Germany) and PMSF (1 mM). The homogenate was centrifuged at 13 500× *g* for 15 min and the resulting pellet was kept at –80°C for at least 2 h. The pellet was then resuspended in a buffer (Tris–HCl 50 mM; EDTA 1.0 mM; MgCl$_2$ 3.0 mM; pH 7.4), homogenized and stored at –80°C (Rock *et al.*, 2012). Mouse whole brain membranes were prepared as described by Thomas *et al.* (2004).

[^{35}S]GTPγS-binding assays. These assays were carried out with rat brainstem or mouse whole brain membranes (100 and 5 µg protein per well respectively), GTPγS-binding buffer (50 mM Tris–HCl; 50 mM Tris-Base; 5 mM MgCl$_2$; 1 mM EDTA; 100 mM NaCl; 1 mM dithiothreitol; 0.1% BSA), 0.1 nM [^{35}S]GTPγS and 30 µM GDP, in a final volume of 500 µL (Thomas *et al.*, 2005). Membranes were pre-incubated for 30 min at 30°C with 0.5 U·mL^{-1} adenosine deaminase (200 U·mL^{-1}) to remove any endogenous adenosine. Binding was initiated by the addition of [^{35}S]GTPγS. Non-specific binding was measured in the presence of 30 µM GTPγS. Assays were performed at 30°C for 60 min. The reaction was terminated by the addition of ice-cold Tris-binding buffer and vacuum filtration using a 24-well sampling manifold (Brandel Cell Harvester; Brandel, Inc., Gaithersburg, MD, USA) and Whatman GF/B glass fibre filters that had been soaked in wash buffer at 4°C for 24 h. Each reaction tube was washed three times with a 4 mL aliquot of buffer. The filters were oven dried for 60 min and then placed in 5 mL of scintillation fluid (Ultima Gold XR; PerkinElmer, Buckinghamshire, UK). Radioactivity was quantified by liquid scintillation spectrometry.

Radioligand displacement assays. Assays were carried out with [^{3}H]8-OH-DPAT or [^{3}H]CP55940 and Tris-binding buffer (50 mM Tris–HCl, 50 mM Tris-Base, 0.1% BSA; pH 7.4), total assay volume of 500 µL, using the filtration procedure described previously by Ross *et al.* (1999). Binding was initiated by the addition of rat brainstem membranes (500 µg protein per well), for experiments with [^{3}H]8-OH-DPAT, or of mouse whole brain membranes (33 µg protein per well), for experiments with [^{3}H]CP55940. All assays were performed at 37°C for 60 min before termination by addition of ice-cold Tris-binding buffer and vacuum filtration using a 24-well sampling manifold (Brandel Cell Harvester) and Brandel GF/B filters that had been soaked in wash buffer at 4°C for at least 24 h. Each reaction well was washed six times with a 1.2 mL aliquot of Tris-binding buffer. The filters were oven dried for 60 min and then placed in 5 mL of scintillation fluid (Ultima Gold XR). Radioactivity was quantified by liquid scintillation spectrometry. Specific binding was defined as the difference between the binding that occurred in the presence and absence of 1 µM unlabelled 8-OH-DPAT or CP55940. The concentration of [^{3}H]8-OH-DPAT and [^{3}H]CP55940 used in these displacement assays was 0.7 nM. Compounds under investigation were stored at –20°C as stock solutions of 10 mM in DMSO, the VEH concentration in all assay wells being 0.1% DMSO. The binding parameters for [^{3}H]CP55940, determined by fitting data from saturation-binding experiments to a one-site saturation plot using GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA), were 2336 fmol·mg^{-1} protein (B_{max}) and 2.31 nM (K_d) (Thomas *et al.*, 2004).

Analysis of in vitro data

Values have been expressed as means and variability as SEM or as 95% confidence limits. The concentration of 8-OH-DPAT or CBDA that produced a 50% displacement of [^{3}H]8-OH-DPAT or [^{3}H]CP55940 from specific binding sites (IC$_{50}$ values) was calculated using GraphPad Prism and the corresponding K_i value of CP55940 was calculated using the equation of Cheng and Prusoff (1973). Values for EC$_{50}$, E_{max} and SEM or 95% confidence limits of these values have been calculated by non-linear regression analysis using the equation for a sigmoidal dose–response curve (GraphPad Prism). The apparent dissociation constant (K_B) value of CBDA for its antagonism of CP55940 in the [^{35}S]GTPγS-binding assay has been calculated by Schild analysis (Graph Pad Prism). Mean values were compared with zero by column statistics analysis using the Wilcoxon signed rank test (GraphPad Prism 5.0). *P*-values <0.05 were considered significant.

Results

CBDA reduces LiCl- and cisplatin-induced emesis in shrews

CBDA reduced LiCl-induced emesis at doses of 0.1 and 0.5 mg·kg^{-1}, and cisplatin-induced emesis at a dose of 0.5 mg·kg^{-1}. A one-way ANOVA of the number of vomiting episodes elicited by LiCl among the pretreatment groups revealed a significant effect of group, F(4, 31) = 7.4; *P* < 0.001 (Figure 2A). Subsequent Bonferroni *post hoc* comparison tests revealed that shrews pretreated with 0.5 mg·kg^{-1} (*P* < 0.01) or 0.1 mg·kg^{-1} (*P* < 0.05) CBDA vomited less frequently than those pretreated with VEH. Similarly, as shown in Figure 2B,

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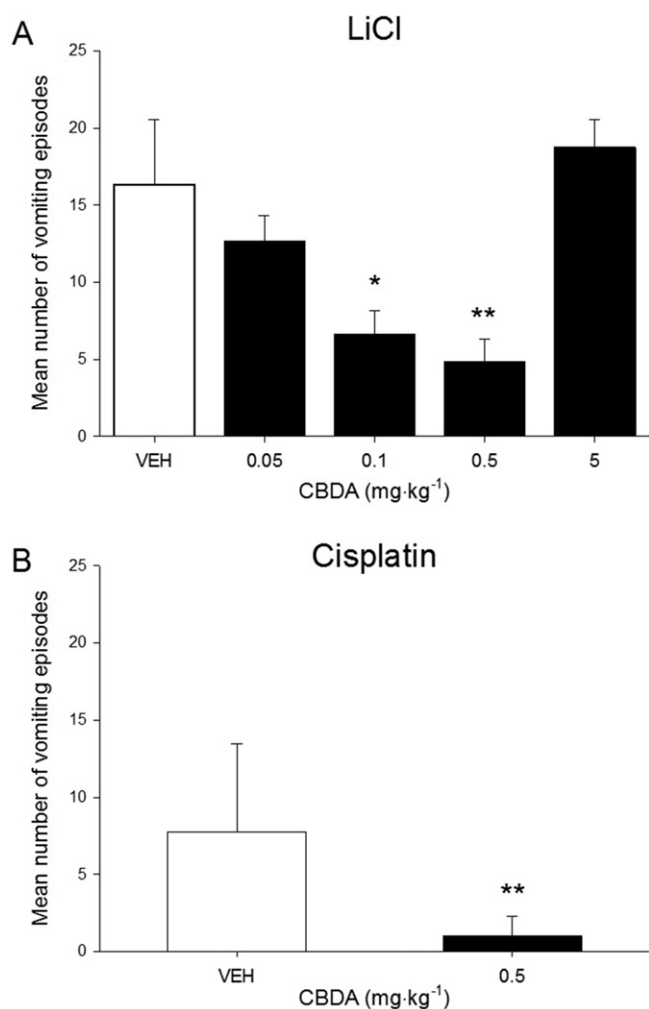
Figure 2

Effect of CBDA (0.05, 0.1, 0.5 or 5.0 mg·kg^{-1}) or VEH administered i.p. 45 min prior to toxin administration. The number of emetic episodes in shrews treated with LiCl (A) or cisplatin (B) was measured. Each bar represents the mean ± SEM ($n = 6$–8). The asterisks indicate a significant difference from the VEH-treated control animals (*$P < 0.05$; **$P < 0.01$, one-way ANOVA).

an independent t-test indicated that shrews given cisplatin and pretreated with CBDA vomited significantly less than VEH-treated controls, $F_{(1,13)} = 9.2$, $P < 0.01$. When administered by themselves, neither CBDA nor VEH produced emesis during the 45 min pretreatment period before toxin administration (data not shown).

CBDA reduces LiCl-induced conditioned gaping to a flavour in rats

Planned comparison tests showed that CBDA significantly reduced LiCl-induced gaping relative to VEH-pretreated controls at doses of 0.01 and 0.1 mg·kg^{-1} ($P < 0.02$), although not at 0.5 or 5 mg·kg^{-1} (Figure 3A), a one-way ANOVA indicating a significant effect of dose, $F_{(4, 42)} = 4.8$; $P = 0.003$. The mean number of tongue protrusions elicited during the conditioning trial by 0.1% saccharin in groups of rats pretreated with
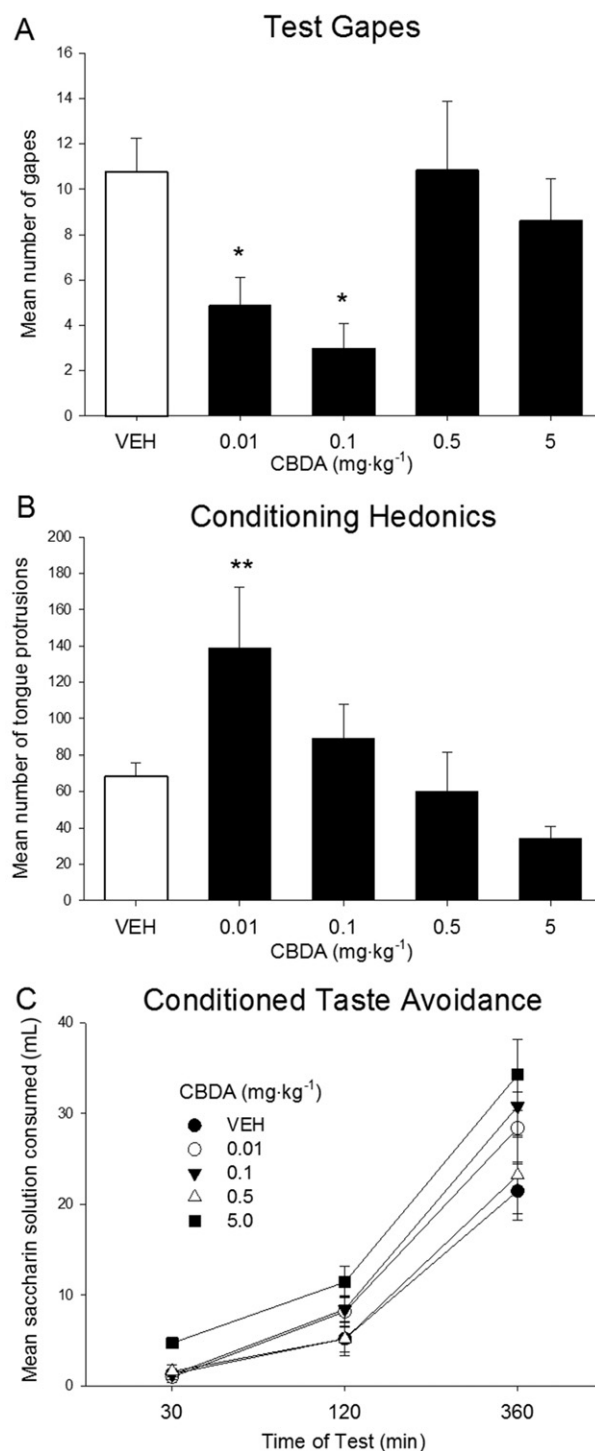
Figure 3

Effect of CBDA (0.01, 0.1, 0.5, 5.0 mg·kg^{-1}) or VEH administered i.p. to rats 45 min prior to LiCl. The number of conditioned gaping responses was measured during the test trial (A). The number of tongue protrusions was measured during the conditioning trial (B). Each bar represents the mean ± SEM ($n = 7$–12). 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 (C). The asterisks indicate a significant difference from the VEH-treated control animals (*$P < 0.02$; **$P < 0.01$; one-way ANOVA).

various doses of CBDA is presented in Figure 3B. A one-way ANOVA of the tongue protrusion data revealed a significant effect of dose, $F(4, 46) = 3.8$; $P = 0.01$. Planned comparisons indicated that at a dose of 0.01 mg·kg^{-1}, CBDA significantly enhanced saccharin hedonics (tongue protrusions) relative to VEH controls as well as to groups pretreated with 0.5 or 5.0 mg·kg^{-1} CBD ($P < 0.02$).

It is unlikely that CBDA attenuated conditioned gaping in rats through a direct effect on learning, because neither of the gape-attenuating doses of CBDA (0.01 and 0.1 mg·kg^{-1}) interfered with LiCl-induced conditioned taste avoidance, a behavioural effect that is not dependent on a nauseating treatment (e.g. Parker *et al.*, 2008). 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 or with 0.01, 0.1, 0.5 or 5.0 mg·kg^{-1} CBDA are presented in Figure 3C. A 5×3 mixed factors ANOVA revealed a significant effect of both time of test, $F(2,84) = 237.5$; $P < 0.01$, and group, $F(4, 42) = 2.9$; $P = 0.03$. Planned comparison tests revealed that the group of rats receiving 5.0 mg·kg^{-1} CBDA drank significantly more saccharin than the VEH-treated group ($P < 0.02$). No other significant differences between treatments were detected and there was no group by time interaction ($P > 0.05$).

Effect of CBDA on LiCl-induced conditioned gaping to a flavour in rats pretreated with WAY100635 or SR141716A

The ability of CBDA (0.1 mg·kg^{-1}) to suppress LiCl-induced conditioned gaping in rats was abolished by pretreatment with the 5-HT$_{1A}$ antagonist, WAY100635, at a dose of 0.1 mg·kg^{-1} (Figure 4A). A one-way ANOVA revealed a significant effect of pretreatment, $F(3, 44) = 8.9$; $P < 0.001$. Planned comparison tests revealed that the SAL-CBDA group displayed fewer gapes than all other groups. Groups pretreated with WAY100635 prior to CBDA did not display suppressed conditioned gaping reactions relative to SAL-VEH. No differences between any of the groups of rats used for this set of experiments were detected during the taste reactivity conditioning trial.

The mean amount of saccharin consumed during the conditioned taste avoidance test at 30, 120 and 360 min is presented in Figure 4B. A 4×3 mixed factors ANOVA revealed a significant effect of time of test, $F(2,88) = 298.2$; $P < 0.01$, and a group by time interaction, $F(6,88) = 2.5$; $P = 0.03$, but no main effect of group. Individual one-way ANOVAs for each time point indicate that only at the 360 min time point did the VEH-CBDA group drink marginally more saccharin than all the other groups. However, individual one-way ANOVAs for each time point indicate that this apparent difference is not statistically significant ($P = 0.055$) and, indeed, that at none of the time points are there statistically significant differences between any of the groups.

In contrast to WAY100635, SR141716A (1 mg·kg^{-1}) did not attenuate the suppression of LiCl-induced conditioned gaping produced by CBDA (Figure 5A). A one-way ANOVA revealed a significant effect of pretreatment group, $F(3,32) = 9.4$; $P < 0.001$. Planned comparisons indicated that both the VEH-CBDA and the SR141716A-CBDA groups gaped significantly less than the VEH-VEH-treated controls ($P < 0.002$). No differences between any of the groups of rats used in these
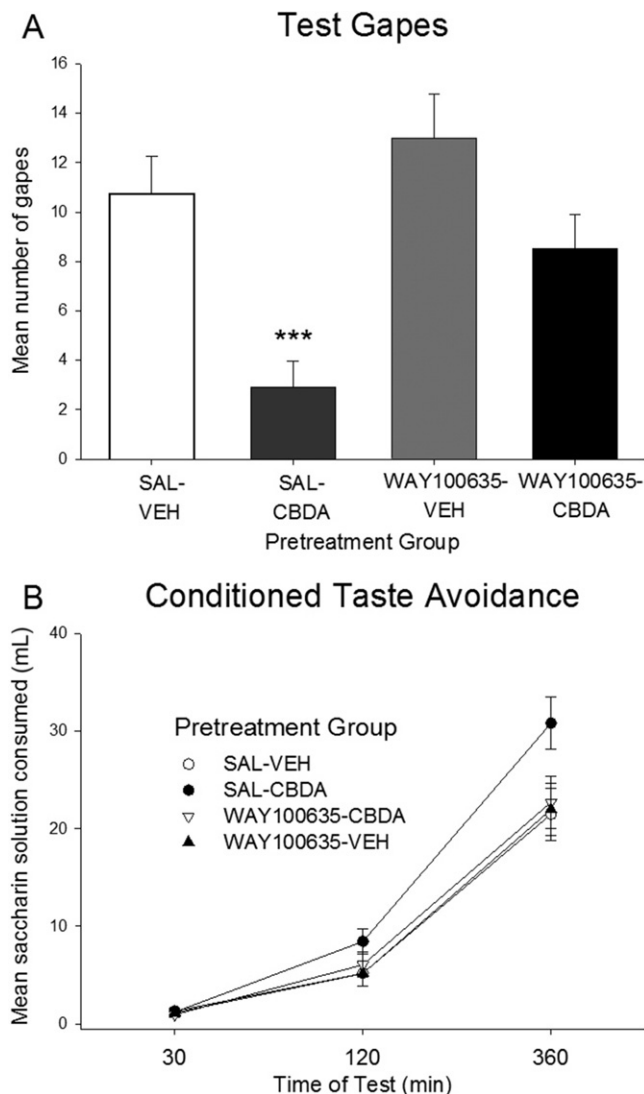
Figure 4

Effect of WAY100635 (0.1 mg·kg^{-1}) or SAL administered i.p. 15 min prior to CBDA (0.1 mg·kg^{-1} i.p.) or VEH in LiCl-treated rats. The number of conditioned gaping responses was measured during the test trial (A). Each bar represents the mean number of conditioned gaping responses ± SEM ($n = 12$). 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 (B). Individual one-way ANOVAs for each time point indicate that there is no significant difference between any of the four pretreatment groups ($P > 0.05$). The asterisks indicate a significant difference from the SAL-VEH-treated control animals (***$P < 0.001$; one-way ANOVA).
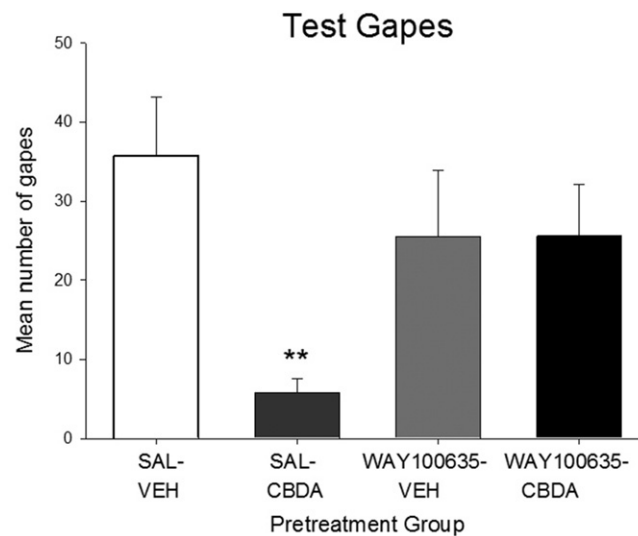
experiments were detected during the taste reactivity conditioning trial. The mean amounts of saccharin consumed during the conditioned taste avoidance test at 30, 120 and 360 min are shown in Figure 5B. A 4×3 mixed factors ANOVA revealed a significant effect of time of test, $F(2,64) = 225.6$; $P < 0.001$, but no significant interaction or pretreatment effect ($P > 0.05$).
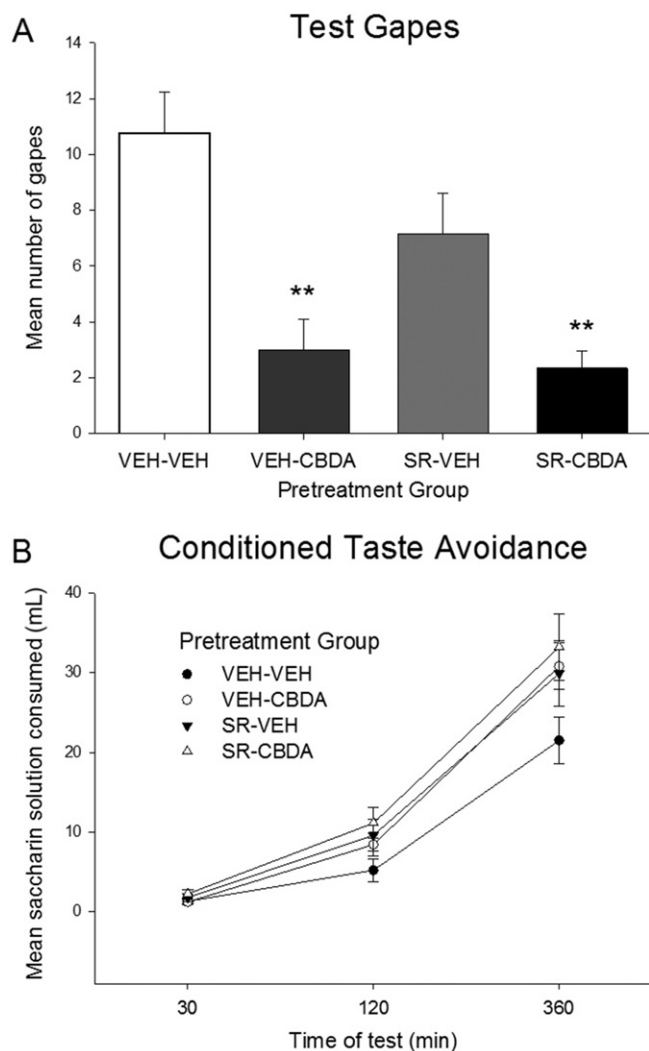
Figure 5
Effect of SR141716A (SR; 1 mg·kg^{-1}) or VEH administered i.p. 15 min prior to CBDA (0.1 mg·kg^{-1} i.p.) or VEH in LiCl-treated rats. The number of conditioned gaping responses was measured during the test trial (A). Each bar represents the mean number of these responses ± SEM (n = 6–12). 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 (B). The asterisks indicate a significant difference from the VEH-treated control animals (**P < 0.002; one-way ANOVA).

Figure 6
Effect of WAY100635 (0.1 mg·kg^{-1}) or SAL administered i.p. on the ability of CBDA (0.1 mg·kg^{-1} i.p.) to suppress the expression of gaping in rats to a context previously paired with LiCl (a model of anticipatory nausea). Each bar represents the mean number of conditioned gaping responses ± SEM (n = 10). The asterisks indicate a significant difference from the SAL-VEH-treated control animals (**P < 0.01; one-way ANOVA).

WAY100635 prevents the expression of CBDA-induced suppression of context-induced conditioned gaping (anticipatory nausea) in rats

CBDA (0.1 mg·kg^{-1}) interfered with the nausea-inducing effects of the context previously paired with LiCl, and this effect was blocked by pretreatment with the 5-HT$_{1A}$ antagonist WAY100635. The mean number of gapes during the test trial is presented in Figure 6. A one-way ANOVA revealed a significant effect of pretreatment, $F_{(3, 36)}$ = 3.7; P = 0.02. Planned comparison tests revealed that the SAL-CBDA group displayed fewer gapes than all other groups. Groups pretreated with WAY100635 prior to CBDA did not display suppressed conditioned gaping reactions relative to SAL-VEH.

CBDA reduces emesis in shrews induced by motion

CBDA (0.1 and 0.5 mg·kg^{-1}) can both reduce the number of emetic episodes and increase the latency of onset of emesis in shrews in response to motion (Figure 7A,B). This it does in a dose-dependent manner. Thus, there is a significant difference between treatment groups in the number of motion-induced emetic episodes, $F_{(3,32)}$ = 8.677; P = 0.0002. Dunnett's *post hoc* analysis revealed that CBDA (0.1 mg·kg^{-1}, P < 0.01; 0.5 mg·kg^{-1}, P < 0.001) reduced the number of motion-induced emetic episodes compared with VEH-treated controls (Figure 7A). ANOVA also revealed a significant difference in the latency of onset of emesis between treatment groups, $F_{(3,32)}$ = 8.530; P = 0.0003. *Post hoc* analysis showed that CBDA (0.1 mg·kg^{-1}, P < 0.05; 0.5 mg·kg^{-1}, P < 0.001) increased the latency to the onset of the first motion-induced emetic episode compared with VEH-treated controls (Figure 7B). No emetic episodes were observed in the 45 min observation period between the administration of CBDA or VEH and the initiation of the motion stimulus.

CBDA enhances the ability of a 5-HT$_{1A}$ receptor agonist to stimulate [^{35}S]GTPγS binding to rat brainstem membranes

As shown in Figure 8 and Table 1, CBDA produced significant increases in the E_{max} of 8-OH-DPAT at concentrations of 0.1, 1.0, 10 and 100 nM, although not at 0.01 nM or 1 μM. These
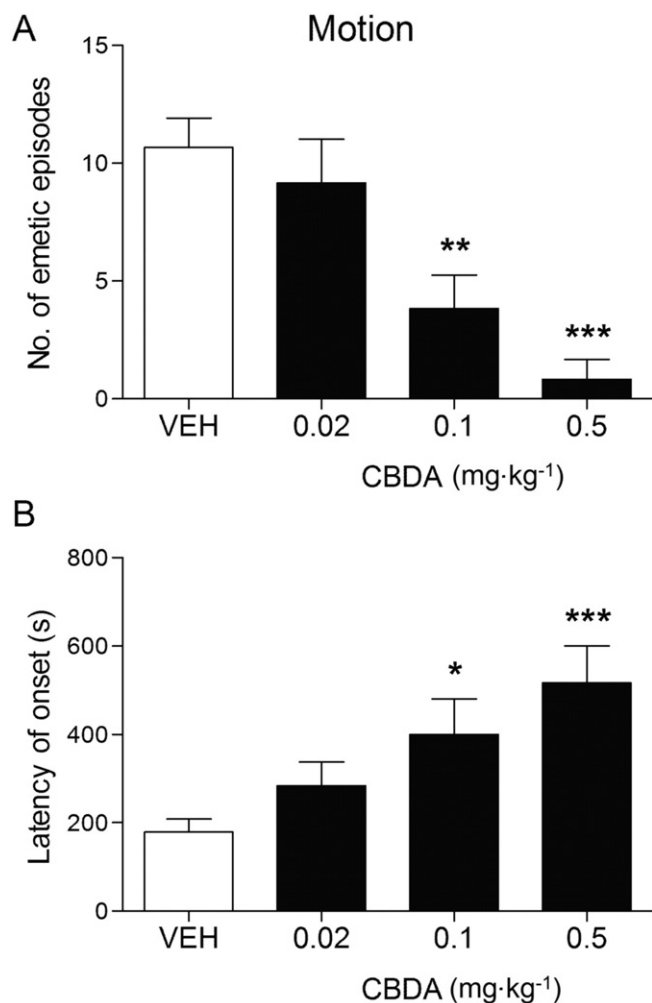
Figure 7

Effect of CBDA (0.02, 0.1, 0.5 mg·kg^{-1}) or VEH administered i.p. to shrews 45 min prior to the application of motion. The number of emetic episodes (A) and latency of onset to the first emetic episode (B) induced by a 10 min horizontal motion stimulus (frequency: 1 Hz; amplitude: 40 mm) was measured in shrews. Each bar represents the mean ± SEM, $n = 5–15$. The asterisks indicate a significant difference from the VEH-treated control animals analysed using one-way ANOVA followed by Dunnett's *post hoc* test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

increases in E_{max} were accompanied by increases in the mean EC$_{50}$ values of CBDA. However, none of these increases were statistically significant ($P > 0.05$; Table 1). By itself, CBDA did not produce any significant stimulation of [^{35}S]GTPγS binding to rat brainstem membranes at concentrations ranging from 0.01 nM to 1 μM ($P > 0.05$; $n = 6$). Its mean E_{max} in this assay, with 95% confidence limits shown in parentheses, was found to be 5.7% (–0.9 and 12.3%). CBDA did not share the ability of 8-OH-DPAT to displace [^{3}H]8-OH-DPAT from rat brainstem membranes at concentrations in the nanomolar range. Thus, CBDA concentrations of 0.1, 1, 10, 100 and 1000 nM did not cause mean values for % displacement of [^{3}H]8-OH-DPAT to rise significantly above zero (column statistics analysis; $n = 8$). The IC$_{50}$ and E_{max} values of 8-OH-DPAT for its displacement of

[^{3}H]8-OH-DPAT with 95% confidence limits shown in parentheses were 4.8 nM (3.2 and 7.5 nM; $n = 8$) and 102.1% (95.9 and 108.3%) respectively.

CBDA can block cannabinoid CB$_1$ receptors

CBDA displaced [^{3}H]CP55940 from specific binding sites on mouse whole brain membranes and antagonized stimulation of [^{35}S]GTPγS binding to these membranes induced by the selective cannabinoid receptor agonist, CP55940 (Figure 9A,B). These mean K_i and K_B values, with their 95% confidence limits shown in parentheses, are 0.9 μM (0.7 and 1.3 μM) and 1.8 μM (0.6 and 5.5 μM) respectively. CBDA neither stimulated nor inhibited [^{35}S]GTPγS binding to mouse whole brain membranes when administered by itself at concentrations ranging from 1 nM to 10 μM ($n = 10$). CBDA concentrations of 1, 10, 100, 1000 and 10 000 nM did not alter [^{35}S]GTPγS binding to a value that was significantly different from zero (column statistics analysis; $P > 0.05$), and the mean E_{max} of CBDA in this assay was not significantly greater than zero (Figure 9C; $P > 0.05$).

Discussion

The results we obtained in this investigation revealed a number of pharmacological similarities between CBDA and CBD. Thus, as we found previously for CBD (Rock *et al.*, 2008; 2012), CBDA appears (i) to suppress LiCl- and cisplatin-induced vomiting in shrews; (ii) to reduce the establishment of LiCl-induced conditioned gaping elicited by a flavour and the expression of LiCl-induced conditioned gaping elicited by a context (anticipatory nausea) in rats; (iii) to enhance the ability of 8-OH-DPAT to stimulate [^{35}S]GTPγS binding to rat brainstem membranes without displaying any detectable activity in this assay in the absence of 8-OH-DPAT; and (iv) to display a bell-shaped log dose–response curve for the production of the second and third of these effects. We also found that like the inhibitory effect of CBD on LiCl-induced conditioned gaping in rats (Rock *et al.*, 2012), the production of such inhibition by CBDA could be abolished by the 5-HT$_{1A}$ receptor-selective antagonist, WAY100635. In contrast, however, CBDA-induced suppression of LiCl-induced conditioned gaping in rats was not attenuated by the CB$_1$ receptor inverse agonist/antagonist SR141716A, suggesting that this effect is not CB$_1$ mediated. Finally, again as found previously for CBD (Rock *et al.*, 2012), concentrations of CBDA that enhanced 8-OH-DPAT-induced stimulation of [^{35}S]GTPγS binding to rat brainstem membranes failed to displace [^{3}H]8-OH-DPAT from such membranes. Taken together, these findings support the hypothesis that, as we have suggested previously for CBD (Rock *et al.*, 2012), CBDA inhibits nausea-induced behaviour in rats by somehow enhancing the activation of 5-HT$_{1A}$ receptors. It would be interesting to investigate the possibility that these are somatodendritic 5-HT$_{1A}$ autoreceptors that are known to be located within the dorsal raphe nucleus and to reduce the firing of 5-HT afferents when activated, for example, by establishing whether CBDA shares the ability of CBD to inhibit nausea-induced behaviour in rats when injected directly into that region of the brain (Rock *et al.*, 2012).
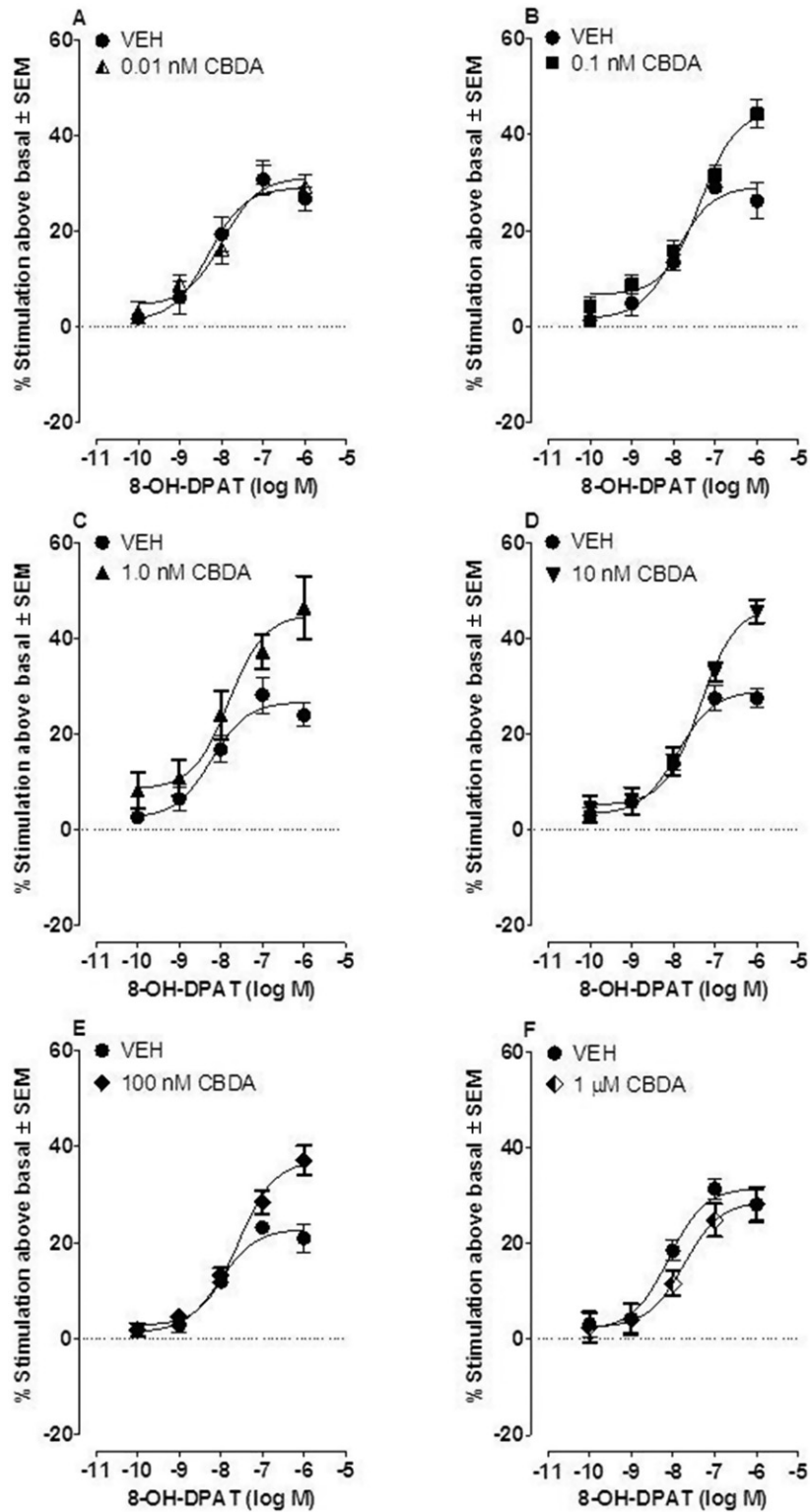
Figure 8

Effect of CBDA (0.01, 0.1, 1.0, 10, 100, 1000 nM) on 8-OH-DPAT-induced stimulation of [^{35}S]GTPγS binding to rat brainstem membranes. Symbols represent mean values ± SEM (n = 6 or 7). Mean E_{max} and EC$_{50}$ values for 8-OH-DPAT in panels (A)–(F) that were determined in the presence of VEH (DMSO) or CBDA, together with the 95% confidence limits of these values, are listed in Table 1.

Table 1

Effects of various concentrations of CBDA on mean EC_{50} and E_{max} values of 8-OH-DPAT for its stimulation of [^{35}S]GTPγS binding to rat brainstem membranes

Pretreatment	Mean EC_{50} (nM)	95% confidence limits (nM)	Mean E_{max} (%)	95% confidence limits (%)	n
Vehicle	5.0	1.6 and 15.0	29.1	24.6 and 33.7	7
0.01 nM CBDA	10.4	3.6 and 30.1	31.3	26.7 and 35.9	7
Vehicle	11.4	5.0 and 25.9	29.2	25.1 and 33.3	7
0.1 nM CBDA	43.9	21.9 and 88.1	45.1*	39.8 and 50.3	7
Vehicle	6.0	1.8 and 20.3	26.8	22.2 and 31.4	6
1.0 nM CBDA	15.5	4.0 and 59.7	45.1*	36.7 and 53.4	6
Vehicle	12.6	5.3 and 30.0	29.1	25.2 and 33.0	7
10 nM CBDA	43.7	21.1 and 90.2	46.8*	40.9 and 52.8	7
Vehicle	9.5	4.1 and 21.9	22.9	19.9 and 25.8	7
100 nM CBDA	26.3	13.4 and 51.6	37.1*	33.0 and 41.2	7
Vehicle	7.8	3.1 and 19.8	31.7	26.7 and 36.7	6
1000 nM CBDA	18.3	5.0 and 67.4	28.8	22.6 and 35.0	6

*The 95% confidence limits of this mean value do not overlap with those of the mean value in the previous row, indicating it to be significantly greater than the mean value obtained from experiments with vehicle-treated membranes ($P < 0.05$). See also Figure 8.

We also detected some pharmacological differences between CBDA and CBD. First, we found that at doses of 0.1 and 0.5 mg·kg^{-1} i.p., CBDA reduced vomiting in shrews when this was induced by motion. In contrast, we have found previously that CBD does not produce such an effect, at least when it is administered at doses ranging from 0.5 to 40 mg·kg^{-1} i.p. (Cluny et al., 2008). Second, we found that CBDA displays markedly greater potency than CBD (Rock et al., 2012) at enhancing 8-OH-DPAT-induced stimulation of [^{35}S]GTPγS binding to rat brainstem membranes, and that this enhancement was induced over a much wider range of concentrations by CBDA than by CBD. More specifically, whereas this effect was produced by CBD at 100 nM but not at 10 nM or 1 μM (Rock et al., 2012), it was produced by CBDA at concentrations ranging from 0.1 to 100 nM. Third, we found that these CBDA-induced increases in the E_{max} of 8-OH-DPAT were not accompanied by an upward shift in the log concentration–response curve of this 5-HT$_{1A}$ receptor agonist of the sort we found previously to be produced by 100 nM CBD (Rock et al., 2012). Whether there is a concentration of CBDA, for example, somewhere between 0.1 and 100 nM, that does produce an upward shift of this kind remains to be established.

In addition, CBDA differs from CBD in the manner in which it appears to target the cannabinoid CB$_1$ receptor. Thus, in contrast to CBD (Thomas et al., 2007), CBDA seemed to produce a dextral but not a downward shift in the log concentration–response curve of CP55940 in mouse whole brain membranes, and its mean apparent K_B value for this antagonism does not differ significantly from its mean K_i value for the displacement of [^{3}H]CP55940 (Results). CBDA also differs from CBD (Thomas et al., 2007), first, by failing to alter [^{35}S]GTPγS binding to mouse whole brain membranes, at concentrations ranging from 1 nM to 10 μM (Results), and second (Figure 8), by increasing the E_{max} of 8-OH-DPAT in rat

brainstem membranes at a concentration (0.1–100 nM) well below those at which it antagonizes CP55940 in mouse whole brain membranes, as indicated by the apparent K_B value of CBDA for this antagonism (1.8 μM). These data suggest that CBDA does not possess significant activity as either an agonist or an inverse agonist at the CB$_1$ receptor even at a concentration that is 10 000-fold higher than a concentration (0.1 nM) at which it can enhance the activation of 5-HT$_{1A}$ receptors by 8-OH-DPAT.

CBDA undergoes slow non-enzymatic decarboxylation to CBD, a process that can be accelerated by heat (Potter et al., 2008). This prompts a need for further research directed at investigating the extent to which any of the effects we observed following CBDA administration in vivo or in vitro were due partly or wholly to CBD or any other compounds that may have been formed from CBDA by enzymatic or non-enzymatic processes. In the meantime, however, it is noteworthy that because of the differences we detected between these two phytocannabinoids (see previous two paragraphs), it is unlikely that CBD played a major role in the production of the effects we observed after CBDA administration, particularly in those experiments in which CBDA displayed higher potency than CBD.

It will also be important to seek out the mechanism(s) by which CBDA induces its apparent enhancement of 5-HT$_{1A}$ receptor activation. Such research should be directed initially at establishing whether, at concentrations in the submicromolar range, CBDA can interact directly with 5-HT$_{1A}$ receptors to enhance activation of this kind. This could be investigated by carrying out in vitro experiments with 5-HT$_{1A}$-transfected cells that, in contrast to brain tissue, do not express other types of receptor. These experiments could be performed using not only the [^{35}S]GTPγS-binding assay, but also a second in vitro assay in which, for example, the measured response is inhibition of forskolin-stimulated cyclic
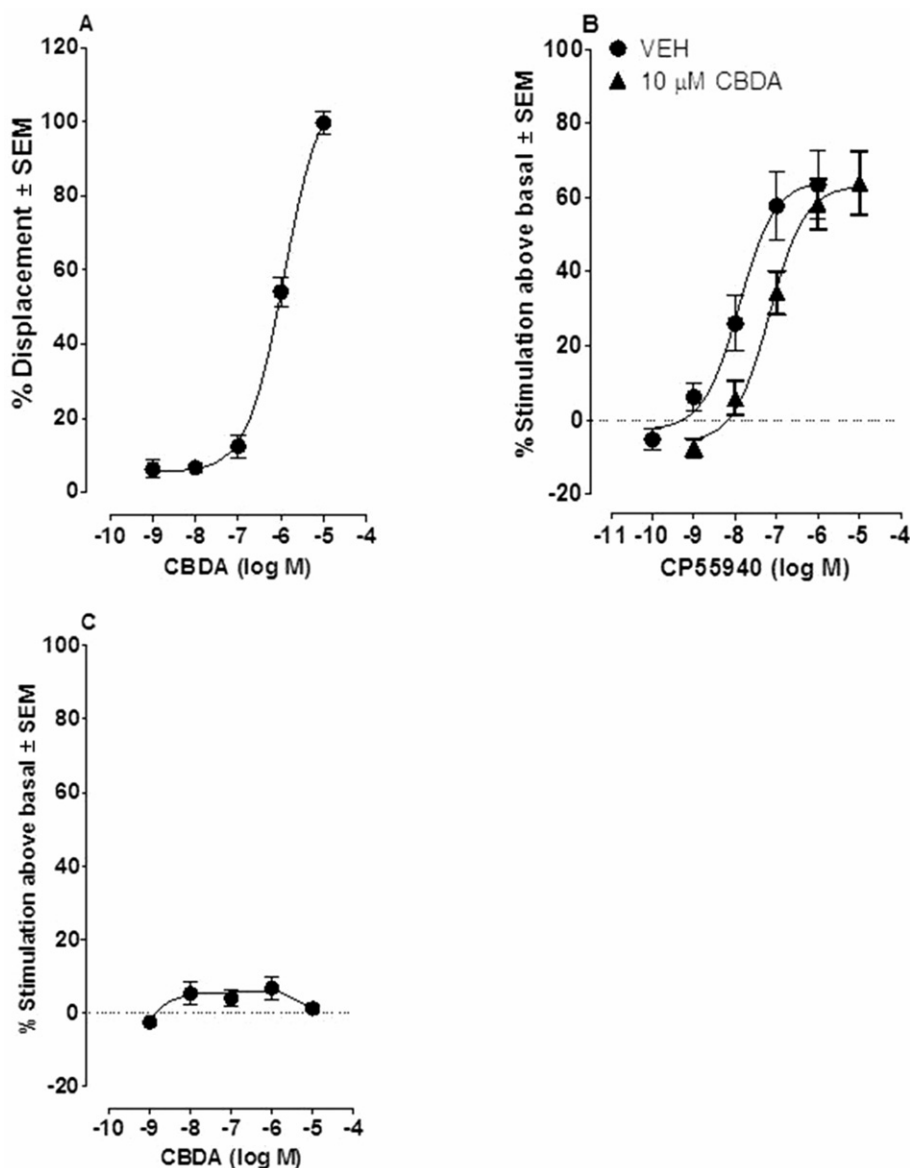
Figure 9

Panel (A) Effect of CBDA on specific binding of [^{3}H]CP55940 to mouse whole brain membranes ($n = 6$). Its mean K_i value with the 95% confidence limits of this value shown in parentheses is 0.9 μM (0.7 and 1.3 μM). Panel (B) Effect of 10 μM CBDA on CP55940-induced stimulation of [^{35}S]GTPγS binding to mouse whole brain membranes. The mean EC$_{50}$ and E_{max} values of CP55940 with their 95% confidence limits shown in parentheses are 12.4 nM (4.3 and 35.8 nM; $n = 8$) and 64.5% (52.5 and 76.5%), respectively, in the presence of VEH (DMSO), and 66.6 nM (27.0 and 164 nM; $n = 8$) and 63.3% (53.7 and 72.9%), respectively, in the presence of 10 μM CBDA. The mean apparent K_B value of CBDA for this antagonism with its 95% confidence limits shown in parentheses is 1.8 μM (0.6 and 5.5 μM). Panel (C) Effect of CBDA on [^{35}S]GTPγS binding to mouse whole brain membranes ($n = 16$). None of the five mean values shown are significantly different from zero (column statistics analysis; $P > 0.05$).

AMP production. It would also be of interest to explore the possibility that CBDA affects 5-HT$_{1A}$ receptor activation in brain tissue indirectly, by interacting with one or more other pharmacological targets that are functionally linked to this receptor.

It is important to note that CBDA selectively interfered with the production of LiCl-induced conditioned gaping reactions, but spared LiCl-induced conditioned taste avoidance; therefore, it did not interfere with learning *per se* (see Parker *et al.*, 2008 for review). Furthermore, a low dose of

CBDA (0.01 mg·kg^{-1} i.p.) also enhanced saccharin palatability unconditionally, as indicated by our finding that rats injected with this dose displayed more saccharin-elicited tongue protrusions (hedonic reactions) than controls during conditioning (Figure 3B). This suggests that this dose of CBDA may have a hedonic or an appetite-enhancing effect, as has been found for Δ^9-THC (Jarrett *et al.*, 2005). It would be interesting, therefore, to establish whether low doses of CBDA enhance feeding or modify depression-like behaviours in other paradigms, such as the forced swim test.

The ability of CBDA to reduce both motion- and toxin-induced vomiting is noteworthy, as compounds such as CBD and 5-HT$_3$ receptor antagonists that are effective in reducing toxin-induced vomiting often fail to reduce signs of motion sickness (Stott et al., 1989; Levine et al., 2000; Cluny et al., 2008). This is not the case with CBDA, as it suppressed both toxin- and motion-induced vomiting in shrews. Toxin-induced vomiting involves the area postrema (Horn et al., 2007; De Jonghe and Horn, 2009), a structure that is not essential for motion-induced vomiting (Wilpizeski et al., 1986; Lang et al., 1999), whereas motion-induced vomiting requires a functional vestibular system (Johnson et al., 1962; 1999; Money and Friedberg, 1964; Kennedy et al., 1965; Reason, 1978; Wilpizeski et al., 1986; Cheung et al., 1991) and is thought to result from a sensory mismatch between vestibular, visual and non-vestibular information from sensory inputs. Furthermore, when saccharin is paired with vestibular stimulation, rats display a conditioned gaping reaction (like that induced by LiCl) when re-exposed to the saccharin flavour (Cordick et al., 1999), and vestibular lesions selectively abolish motion-, but not LiCl-induced conditioned gaping reactions (Ossenkopp et al., 2003). These published findings indicate that the neuronal pathways that mediate the inhibitory effect of CBDA on motion-induced vomiting are most probably not the same as those that mediate its inhibitory effect on toxin-induced vomiting. It is also noteworthy that the suppressive effect of 8-OH-DPAT on motion-induced emesis has been found not to be reversed by pretreatment with WAY100635, even at the rather high dose of 1 mg·kg^{-1} i.p. (Javid and Naylor, 2006), suggesting that this suppressive effect is not 5-HT$_{1A}$ receptor mediated. It is possible, therefore, that the suppressive effect of CBDA on motion-induced vomiting is also induced through one or more 5-HT$_{1A}$ receptor-independent mechanisms.

The current first-line therapy for treatment of acute and delayed chemotherapy-induced nausea and vomiting is the combination of 5-HT$_3$ receptor antagonists (e.g. ondansetron), NK$_1$ receptor antagonists (e.g. aprepitant) and dexamethasone (e.g. Poli-Bigelli et al., 2003). Although this therapy is effective in controlling acute and delayed vomiting, it is somewhat less effective in controlling acute and delayed nausea (e.g. de Boer-Dennert et al., 1997; Poli-Bigelli et al., 2003; Meiri et al., 2007). However, in patients who experience anticipatory nausea, there is no specific therapy available (Nesse et al., 1980; Morrow et al., 1998; Ballatori and Roila, 2003; Hickok et al., 2003; Foubert and Vaessen, 2005); indeed, the most commonly prescribed treatment for this side effect is a non-specific anxiolytic (Malik et al., 1995). Anticipatory nausea remains an area of unmet clinical need in cancer therapy. As current anti-emetics, such as the 5-HT$_3$ antagonist ondansetron, are not effective in reducing anticipatory nausea once it develops in rats (Limebeer et al., 2008) and humans (Morrow et al., 1998), CBDA's ability to reduce the expression of anticipatory nausea is important. Other cannabinoids such as CBD (Rock et al., 2008), Δ^9-THC, (Limebeer et al., 2006), and the fatty acid amide hydrolase inhibitor, URB597 (Rock et al., 2008), have also been shown to be effective in reducing anticipatory nausea in rats, highlighting a unique therapeutic potential for cannabinoids.

In conclusion, this investigation has shown for the first time that CBDA can potently suppress signs of nausea in rats

in a 5-HT$_{1A}$ receptor-dependent manner, and that it can increase the ability of the 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT, to stimulate [^{35}S]GTPγS binding to rat brainstem membrane, again with high potency. We also found that CBDA potently inhibits toxin- and motion-induced vomiting in shrews, although any involvement of 5-HT$_{1A}$ receptors in this inhibition has yet to be investigated. The cannabinoid receptor agonist, Marinol (synthetic Δ^9-THC), is currently indicated to treat nausea and vomiting in patients who have failed to respond adequately to conventional anti-emetics (e.g. Meiri et al., 2007). Δ^9-THC (3–10 mg·kg^{-1}) has been shown to reduce vomiting in house musk shrews (Kwiatkowska et al., 2004), whereas doses of CBDA as low as 0.5 mg·kg^{-1} were effective in this study. We found that, in contrast to Δ^9-THC (Pertwee, 2008), CBDA does not seem to activate CB$_1$ receptors in vitro even at the rather high concentration of 10 μM. When taken together, our findings support the hypothesis that CBDA can suppress signs of nausea and vomiting in a CB$_1$ receptor-independent manner, raising the possibility that if used to ameliorate nausea or vomiting in the clinic, it would not trigger any CB$_1$ receptor-mediated tolerability or abuse liability problems. Importantly, these findings suggest that CBDA could be developed as a potent and selective treatment for nausea and vomiting, and in particular for the treatment of anticipatory nausea, a symptom with no specific therapy currently available. Our findings also raise the possibility that compared with CBD (Mishima et al., 2005; Campos and Guimarães, 2008; Zanelati et al., 2010; Gomes et al., 2011; Maione et al., 2011), CBDA may display greater potency, efficacy or selectivity at ameliorating signs of cerebral infarction, pain, anxiety and depression in an apparent 5-HT$_{1A}$ receptor-dependent manner in animal models, and so possibly also in the clinic.

Acknowledgements

Conflict of interest

The research performed in Guelph and Aberdeen was funded by GW Research Ltd, UK. CS and MD are employees of GW Research, Ltd., UK.

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