Cannabidiol Indirectly Activates 5-HT_{1A} Somatodendritic Autoreceptors to Attenuate Vomiting and Nausea

by

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ABSTRACT

CANNABIDIOL INDIRECTLY ACTIVATES 5-HT_{1A} SOMATODENDRITIC AUTORECEPTORS TO ATTENUATE VOMITING AND NAUSEA

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Cannabidiol (CBD), a non-psychoactive cannabinoid found in cannabis suppresses vomiting in shrews (*Suncus murinus*, Parker *et al.*, 2004), and conditioned gaping in rats (a selective measure of nausea-like behaviour, Parker *et al.*, 2002). CBD's anti-emetic/anti-nausea mechanism of action is unknown. However, evidence suggests that CBD may act as a somatodendritic 5-hydroxytryptamine 1A (5-HT_{1A}) autoreceptor agonist in the dorsal raphe nucleus (DRN), because the anxiolytic (Campos and Guimaraes, 2008a) and neuroprotectant (Mishima *et al.*, 2005) properties of CBD are 5-HT_{1A}-mediated. Therefore, here we investigated if administration of 5-HT_{1A} receptor antagonists, (WAY100135 or WAY100635) would block CBD's anti-emetic/anti-nausea-like effects.

Systemic administration of WAY100135 prevented the anti-emetic effect of CBD in shrews, and WAY100135 and WAY100635 attenuated the anti-nausea-like effect of CBD in rats. The effect of CBD on conditioned gaping reactions was most likely the result of its action on somatodendritic 5-HT_{1A} receptors in the DRN, because the anti-nausea-like action of systemic CBD was reversed by intra-DRN administration of

WAY100635. As well, when administered into the DRN, CBD suppressed conditioned gaping, an effect that was blocked by systemic WAY100635. *In vitro* studies revealed that CBD enhanced the ability of 8-OH-DPAT to stimulate [^{35}S]GTPγS binding and *in vivo* studies revealed that systemic subthreshold doses of combined CBD and 8-OH-DPAT synergistically suppressed conditioned gaping. These results suggest that CBD produces its anti-emetic/anti-nausea-like effects by indirect receptor agonism of DRN somatodendritic 5-HT_{1A} autoreceptors.

CBD's mechanism of action was explored further, by examining its interaction with cannabigerol (CBG), another cannabinoid, which acts *in vitro* as a 5-HT_{1A} receptor antagonist (Cascio *et al.*, 2010). CBG blocked the systemic CBD-, and 8-OH-DPAT-induced suppression of gaping in rats, as well as the systemic CBD-induced suppression of vomiting in shrews. Therefore, CBG and CBD may be in opposition at the 5-HT_{1A} receptor.

These findings shed light on the mechanism of action of non-psychoactive cannabinoids in the cannabis plant, and their effect on nausea and vomiting. These results suggest CBD alone may be an effective treatment in reducing nausea and vomiting.

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CHAPTER 1

General Introduction

Emesis, more commonly known as vomiting, is thought to be a protective mechanism used to eliminate ingested poisonous or toxic compounds from the body and has been defined as the expulsion of gastrointestinal substance through the mouth. Vomiting can be objectively observed by counting the number of vomiting episodes. Nausea, on the other hand, is a rather subjective experience, often thought to be "an unpleasant, but not painful, sensation associated with a heightened awareness of the upper gut, cold sweating and the expectation that vomiting is imminent" (Andrews *et al.*, 1988). Nausea does not necessarily always result in vomiting. Due to its subjectivity, little research has focused on the neurobiology of nausea, despite it being a distressing symptom associated with many ailments.

Chemotherapy-induced nausea and vomiting

Nausea and vomiting are common side effects associated with anti-cancer drug therapies, and these symptoms are often identified as the most distressing ones experienced by cancer patients undergoing chemotherapy treatment (Griffin *et al.*, 1996; Jordan *et al.*, 2005). Chemotherapy-induced nausea and vomiting can be classified into three categories based on the time of onset: 1) acute onset, occurring within 24 h of the initial chemotherapy administration; 2) delayed onset, occurring 24 h to several days after the initial treatment; and 3) anticipatory nausea and vomiting (ANV), a classically conditioned response to aspects of the hospital context that become associated with the chemotherapy-induced illness (Markman, 2002; Jordan *et al.*, 2005). These distressing

symptoms reported by chemotherapy patients incited a search for effective anti-emetic treatments.

Before the advent of anti-emetic therapy, as many as 20% of patients discontinued their chemotherapy treatments due to the negative impact of nausea and vomiting on their daily activities (Stewart, 1990; Jordan *et al.*, 2005). With improved anti-emetics, vomiting has become somewhat less problematical to cancer patients, now making nausea the most distressing side effect of chemotherapy (Griffin *et al.*, 1996; de Boer-Dennert *et al.*, 1997). Although anti-emetic regimens are quite effective in reducing acute vomiting (Jordan *et al.*, 2005), with possible complete control of emesis within the first 24 h of the initial chemotherapy treatment, 55% to as many as 76% of cancer patients will still experience delayed nausea and/or vomiting during the initial five to seven days after treatment (Griffin *et al.*, 1996; Gralla *et al.*, 1999; Hickok *et al.*, 2003). Therefore, although anti-emetics are effective in reducing acute vomiting, they are less effective in reducing the delayed nausea and vomiting (Rudd and Naylor, 1996; Rudd *et al.*, 1996) and ANV (Hickok *et al.*, 2003). This highlights the importance for improved management of acute and delayed nausea and vomiting so that anticipatory nausea and vomiting will not develop.

While the symptoms of nausea and vomiting can be experienced in combination, often with nausea preceding vomiting, one-quarter of chemotherapy patients report experiencing nausea alone (Griffin *et al.*, 1996). Nausea is typically more distressing to patients as it can be continuously present, while emesis typically occurs in discrete occurrences (Andrews and Horn, 2006). Nausea is reported by 73% of patients as the most commonly occurring symptom, with 50% of patients ranking it within the top five

severe symptoms associated with their treatment (Griffin *et al.*, 1996). Patients also report experiencing nausea with a greater incidence than vomiting (Richardson *et al.*, 1988; Foubert and Vaessen, 2005).

The severity of nausea and vomiting may vary, depending on the chemotherapy drugs administered and patient characteristics (Bartlett and Koczwara, 2002; Markman, 2002). The chemotherapy drug itself is the most predictive factor in the development of chemotherapy-induced nausea and vomiting. Some chemotherapy agents, such as cisplatin, are considered highly emetogenic as they cause nausea and vomiting in more than 90% of patients (Lohr, 2008). Patients receiving cisplatin report significantly worse acute and delayed nausea and vomiting (Griffin *et al.*, 1996; Markman, 2002). Sixty to 90% of patients on cisplatin will experience delayed emesis if no anti-emetic treatments are provided (Jordan *et al.*, 2005). Thus, each patient may experience varying degrees of chemotherapy-induced illness, with the experience of nausea being a more difficult symptom to manage than vomiting.

Animal Models of Nausea and Vomiting

Emesis research in general has been greatly restricted, as only a few species such as cats, dogs, ferrets and shrews vomit in response to toxins. *Suncus murinus*, the house musk shrew, vomits in response to toxins such as nicotine (Matsuki *et al.*, 1988; Matsuki *et al.*, 1990; Torii *et al.*, 1991; Smith *et al.*, 2001; Nakayama *et al.*, 2005; Parker *et al.*, 2009), cisplatin (Matsuki *et al.*, 1988; Matsuki *et al.*, 1990; Torii *et al.*, 1991; Sam *et al.*, 2003; Lau *et al.*, 2005; Parker *et al.*, 2009) and lithium chloride (LiCl, Smith *et al.*, 2001; Parker *et al.*, 2004) and is therefore a practical laboratory species to use for vomiting

research. This species also vomits in response to motion stimulus, making it a useful model for studying motion sickness as well (Ueno *et al.*, 1988; Torii *et al.*, 1991; Okada *et al.*, 1994; Javid and Naylor, 2006; Cluny *et al.*, 2008)

Due to the subjective nature of nausea, animal models have been developed to empirically investigate this phenomenon. Traditionally, rats have been used in assessing the nausea-inducing properties of drugs. Typically, to assess a flavour-nausea association, subsequent consumption of a solution that has been previously paired with illness is measured (Garcia and Koelling, 1966), denoted as conditioned taste avoidance. Alternatively, the taste reactivity test (TRT, Grill and Norgren, 1978b) was developed to measure behavioural displays to unpleasant flavours. Although rats are incapable of vomiting, they do exhibit conditioned disgust reactions, which include gaping, chin rubbing and paw treading in response to the delivery into their oral cavity of a flavour that has been previously paired with illness (Grill and Norgren, 1978b). These disgust reactions are indicators of conditioned taste aversion in rats. The most sensitive and reliable conditioned disgust reaction is that of gaping, described as a wide, triangular opening of the mouth and jaw, exposing the incisors (Breslin *et al.*, 1992; Parker, 2003).

The TRT may be a more direct measure of taste aversion as it eliminates the approach and consummatory phases of responding. It is also a selective measure of taste aversion because almost any psychoactive drug that has been tested has the potential of producing conditioned taste avoidance, but only drugs with emetic properties produce conditioned disgust reactions in the TRT. When a drug that animals will self-administer, such as amphetamine, is paired with a flavoured solution, subsequent taste avoidance can result, indicating a drug's ability to simultaneously exert both negative and positive

properties (Parker, 1998). While rewarding drugs do produce taste avoidance, they do not produce conditioned disgust reactions in the TRT (Parker, 1995). Thus, sucrose paired with a rewarding drug results in taste avoidance, but does not produce conditioned aversion reactions (Parker, 1995). In contrast, nausea-inducing treatments, such as LiCl, cyclophosphamide, high doses of nicotine, apomorphine, rotation and naloxone precipitated withdrawal, all produce conditioned disgust reactions including gaping, chin rubbing and paw treading, when paired with sucrose (Parker and Mechoulam, 2003). Each of these treatments produces vomiting in species capable of vomiting. In fact, Travers and Norgren (1986) suggest that the muscular movements involved in the gaping response mimic those seen in species capable of vomiting. Since these conditioned gaping reactions are only elicited by exposure to flavours paired with emetic treatments and not by those paired with drugs with rewarding properties, these conditioned reactions seem to be a representation of conditioned nausea-like behaviour in rats (Parker and Mechoulam, 2003). If this is the case, then emetic drugs produce a taste aversion, presumably because the taste elicits conditioned nausea-like effects (Parker, 1998). Rats display characteristic gaping reactions (which may reflect nausea) when exposed to a flavoured solution (see, Parker *et al.*, 2003; Limebeer *et al.*, 2006) previously paired with LiCl-induced nausea.

If conditioned gaping is a selective measure of nausea-like behaviour in rats, then drugs that reduce nausea, should also suppress the gaping response, without interfering with conditioned taste avoidance (the non-selective measure). Ondansetron (OND; Zofran), a classic 5-hydroxytryptamine-3 (5-HT_3) receptor antagonist, shown to suppress vomiting in *S. murinus* (Sam *et al.*, 2003; Kwiatkowska *et al.*, 2004; Lau *et al.*, 2005),

effectively reduced LiCl-induced conditioned gaping, without modifying unconditioned gaping reactions to bitter quinine or taste avoidance in a consumption test (Limebeer and Parker, 2000). These results indicate that the effect was specific to the conditioned gaping response, presumably by interfering with the LiCl-induced nausea. Subsequently, it was shown that pretreatment with the 5-hydroxytryptamine- 1A (5-HT_{1A}) autoreceptor agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) reduced LiCl-induced conditioned gaping without modulating conditioned taste avoidance (Limebeer and Parker, 2003). Therefore, conditioned gaping is a selective measure of nausea-like behaviour in the rat that can be suppressed by classic anti-emetic drugs, without interfering with conditioned taste avoidance, as assessed by consumption test.

Not only are flavour cues capable of eliciting conditioned gaping reactions when paired with LiCl- induced nausea in rats, but recently Limebeer *et al.* (2006; 2008) have demonstrated that re-exposure to LiCl-paired contextual cues can also elicit conditioned gaping reactions in rats. In addition, the house musk shrew also displays conditioned retching reactions when re-introduced to a context previously paired with LiCl (Parker and Kemp, 2001; Parker *et al.*, 2006). This paradigm resembles that reported to produce ANV in chemotherapy patients, as re-exposure to the contextual cues of the hospital alone, induces nausea and vomiting.

Neurobiology of Nausea and Vomiting

Significant advances have been made in understanding the neural systems involved in emesis, but little is still known about the system for nausea. This vomiting

system does seem to be distinct from that implicated in nausea (Andrews and Horn, 2006).

The vomiting reflex is coordinated by structures located within the brainstem, as even decerebrate animals are capable of vomiting (Miller and Leslie, 1994). The dorsal vagal complex (DVC) of the medulla—including area postrema (AP), nucleus of the solitary tract (NTS), and the dorsal motor nucleus of the vagus (DMN)—is considered the emetic centre of the brain (Leslie, 1985; Hornby, 2001). The DVC is thought to integrate incoming signals and coordinate system outputs. Emetic stimuli activate the DVC via two pathways. 1) The DVC is activated through chemicals in the bloodstream, which then stimulates neurons in the AP and NTS through a leaky blood-brain barrier (Miller and Leslie, 1994). 2) The NTS is activated through afferent inputs from the vagus and splanchnic nerves, which carry sensory inputs from the gastrointestinal tract (Hornby, 2001). This system is thought to involve both central and peripheral mechanisms with regard to chemotherapy-induced nausea and vomiting.

In *S. murinus*, cisplatin treatment induces Fos expression in the DMN, AP, and NTS—all hindbrain areas associated with emesis—up to 48 h (De Jonghe and Horn, 2009). This group has also identified 24 to 48 h dose-dependent increases in Fos expression in the NTS and AP in rats treated with cisplatin (Horn *et al.*, 2007). These results indicate that similar neural systems (the DVC) are activated in the house musk shrew and rat, in response to the emetic agent cisplatin.

While the brainstem is crucial in integrating emetic signals, the experience of nausea requires the projection of signals rostrally from the brainstem to higher brain

regions (Grill and Norgren, 1978a; Grill and Norgren, 1978b; Andrews and Horn, 2006). Therefore, it seems that the experience of nausea is likely to be forebrain mediated.

Serotonin (5-hydroxytryptamine; 5-HT) and Nausea and Vomiting

Serotonin (5-hydroxytryptamine; 5-HT) is a neurotransmitter, acting mainly at G-protein coupled receptors, that has been implicated in many processes such as sleep, appetite, mood, aggression, pain, anxiety, and emesis. Drugs that act on 5-HT receptors have been shown to be effective in a number of psychiatric disorders such as: depression, schizophrenia, anxiety disorders, and eating disorders. 5-HT exerts its effects on a variety of processes. Separate specialized receptors have been classified into types one to seven. Many subtypes have also been established within these families, but even greater diversity within the system is likely to exist and yet to be discovered.

Role of 5-HT$_3$ receptors

5-HT$_3$ receptors belong to the ligand-gated ion channel receptor superfamily and are located in high densities in the AP, NTS and on the afferent terminals of the vagus nerve, implicating their involvement in modulating the emetic response (see Hsu, 2010). In addition, 5-HT$_3$ receptors are also highly expressed in the hippocampus and amygdala (see Hannon and Hoyer, 2008).

Cisplatin and other toxins produce emesis by inducing 5-HT release from enterochromaffin cells located in the small intestine (Andrews *et al.*, 1988; Hsu, 2010) . Urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of serotonin, increases in patients from two to eight h after cisplatin infusion (Cubeddu *et*

al., 1990; Cubeddu and Hoffmann, 1993). It is important to note that it is not vomiting itself that triggers the release of 5-HT from the enterochromaffin cells, because cisplatin induces similar increases in urine 5-HT regardless of the intensity or presence/absence of vomiting (Cubeddu and Hoffmann, 1993; Cubeddu, 1996). This released 5-HT then acts upon 5-HT_3 receptors located on the terminals of intestinal (Horn *et al.*, 2004) vagal afferent fibers (Andrews *et al.*, 1988; Hsu, 2010). 5-HT_3 receptor antagonists such as OND may reduce emesis by blocking the binding of 5-HT to the 5-HT_3 receptors, centrally in the DVC, and/or peripherally via the vagus nerve of the gastrointestinal tract (Naylor and Rudd, 1992).

With the use of 5-HT_3 receptor antagonists such as OND, control of chemotherapy-induced emesis in humans has been more successful than the suppression of nausea (Morrow *et al.*, 1995; Morrow *et al.*, 1998; Gralla *et al.*, 1999), with the most profound anti-emetic response being seen on the first day of chemotherapy and declining in effectiveness thereafter (Cubeddu *et al.*, 1990; Fox *et al.*, 1993). OND is not effective in modulating the cisplatin-induced increase in urinary 5-hydroxyindoleacetic acid (5-HIAA, the principal 5-HT metabolite) that is detected in the acute phase of vomiting (Cubeddu *et al.*, 1990). Therefore, it seems that OND does not exert its action by reducing the release of 5-HT peripherally, if 5-HIAA is a reliable measure of 5-HT release (Cubeddu, 1992). OND may instead have its effect centrally in the NTS and AP.

Although 5-HT_3 receptor antagonists do seem to be effective in managing acute nausea and vomiting after chemotherapy, they are less helpful with delayed nausea and emesis (Morrow *et al.*, 1995; Hsu, 2010). It is thought that cisplatin-induced delayed nausea and vomiting may reflect a mechanism other than 5-HT_3, as 5-HT_3 receptor

antagonists are largely ineffective, and after 8 h post cisplatin infusion, there are no increases in urinary excretion of 5-HIAA over that obtained prior to infusion (Cubeddu *et al.*, 1990; Cubeddu, 1996). In fact, it has been proposed that the terminology *acute* and *delayed* be replaced with *5-HT$_3$-dependent* and *5-HT$_3$-independent*, respectively (Cubeddu, 1996).

S. murinus retches and vomits when exposed to a toxin such as cisplatin and these reactions are reduced by pretreatment with classic 5-HT$_3$ receptor antagonists (Matsuki *et al.*, 1990; Torii *et al.*, 1991; Sam *et al.*, 2003; Kwiatkowska *et al.*, 2004; Lau *et al.*, 2005; Nakayama *et al.*, 2005). 5-HT$_3$ receptor antagonists attenuate cisplatin-induced Fos expression in the NTS and DMN (hindbrain structures of the emetic pathway) at 6 h in the shrew (De Jonghe and Horn, 2009). 5-HT$_3$ receptor agonists dose-dependently induce emesis in *Cryptotis parva* (the least shrew, Darmani, 1998) and the 5-HT$_3$ receptor antagonist tropisetron prevented cisplatin-induced emesis and retching in *C. parva* (Darmani, 1998).

In the ferret, 5-HT$_3$ receptor antagonists, such as OND, FK1052, GR38032F and MDL72222 have been shown to reduce cisplatin-induced vomiting (Miner *et al.*, 1987; Higgins *et al.*, 1989; Rudd and Naylor, 1994; Nakayama *et al.*, 2005) and injections of 5-HT$_3$ receptor antagonists into the AP have prevented cisplatin-induced emesis (Higgins *et al.*, 1989), suggesting a potential central site of action, as well as a peripheral site of action. Additionally, the selective 5-HT$_3$ receptor agonist 2-methyl-5-HT and cisplatin caused a concentration-dependent increase of 5-HT in the ferret ileum, which was significantly reduced in a concentration-dependent manner by various 5-HT$_3$ receptor antagonists (Endo *et al.*, 1998; Endo *et al.*, 1999). Additionally, in the cat, administration

of zacopride (a 5-HT_3 receptor antagonist) blocked cisplatin-induced emesis when administered iv or icv (Smith *et al.*, 1988). Taken together, these studies suggest a central role for the 5-HT_3 receptor in modulating emesis (delayed and acute) in species capable of this response.

In the non-emetic rat, the selective 5-HT_3 receptor agonist 2-methyl-5-HT caused a concentration-dependent increase of 5-HT in the rat ileum, which was significantly reduced by the selective 5-HT_3 receptor antagonist granisetron (Minami *et al.*, 1995). Behaviourally, OND interferes with LiCl-induced conditioned disgust reactions elicited by a taste, but not with LiCl-induced taste avoidance in rats (Rudd *et al.*, 1998; Limebeer and Parker, 2000). As in the emetic shrew and vomiting, the 5-HT_3 receptor also modulates this nausea-like behaviour in the rat.

In contrast, the role of the 5-HT_3 receptor in ANV is not as clear. The classic anti-emetic OND is not effective in interfering with the expression of conditioned retching in *S. murinus* to a context previously paired with LiCl (Parker *et al.*, 2006). This finding mirrors that in the human literature (Morrow et al., 1998), as OND is effective in preventing acute emesis in the shrew but is not effective in interfering with shrew ANV once it has developed. Additionally, as in the shrew, OND did not interfere with the expression of conditioned gaping elicited by a context previously paired with LiCl (Limebeer *et al.*, 2008; Rock *et al.*, 2008). This finding suggests that OND does not interfere with anticipatory nausea-like behaviour in rats once it has developed, similar to the human reports (Morrow *et al.*, 1998).

Role of 5-HT$_{1A}$ receptors

5-HT$_{1A}$ receptors are coupled to $G_{i/o}$ and are distributed throughout the central nervous system (see Hannon and Hoyer, 2008). Across humans, mice, and rats, high levels of 5-HT$_{1A}$ receptors have been identified in limbic areas such as the hippocampus and frontal cortex, and especially in the raphe nuclei of the brainstem (Verge *et al.*, 1986; el Mestikawy *et al.*, 1991). These 5-HT$_{1A}$ receptors mediate inhibitory responses and serve to self-regulate release of 5-HT (eg. Galligan, 1996). Specifically, 5-HT$_{1A}$ autoreceptors, located on serotonergic cell bodies and dendrites (Sotelo *et al.*, 1990; Riad *et al.*, 2000) in the rat median raphe nucleus (MRN) and dorsal raphe nucleus (DRN), regulate 5-HT release (Verge *et al.*, 1986) at terminal forebrain regions by decreasing the rate of firing of the neurons (see Blier *et al.*, 1998). Activation of 5-HT$_{1A}$ autoreceptors leads to opening of cell membrane potassium channels and hyperpolarization of the cell, which then leads to reduced cell firing (Blier and de Montigny, 1987; Sprouse and Aghajanian, 1987). Serotonin released locally in the raphe nuclei reduces neuronal firing, producing negative feedback regulation of neurotransmitter release (Adell and Artigas, 1991; Artigas *et al.*, 1996). Indeed, release of 5-HT from *in vitro* preparations of rodent brain tissue was inhibited by 5-HT receptor agonists (Chase *et al.*, 1969; Farnebo and Hamberger, 1971) and *in vivo*, 5-HT receptor agonists reduce the electrical activity of 5-HT neurons in the DRN (Aghajanian, 1972). Thus, stimulation of this somatodendritic 5-HT$_{1A}$ autoreceptor results in a decrease in 5-HT availability.

8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), a highly selective and centrally active potent ligand (Arvidsson *et al.*, 1981; Hjorth *et al.*, 1982) preferentially binds to the somatodendritic (Hjorth and Magnusson, 1988) *5*-HT$_{1A}$ autoreceptor

(Middlemiss and Fozard, 1983) and has been used as the standard receptor agonist to assess the behavioural effects of the 5-HT_{1A} receptor. In addition, WAY100135 [N-tert-butyl 3-4-(2-methoxyphenyl) piperazin-1-yl-2-phenylpropanamide dihydrochloride], the 5-HT_{1A} receptor antagonist, has also been utilized to investigate the functions of this receptor, however, it is active at both presynaptic and postsynaptic 5-HT_{1A} receptors (Cliffe *et al.*, 1993; Fletcher *et al.*, 1993). More recently, the highly selective and potent 5-HT_{1A} receptor antagonist WAY100635 has instead been used (Forster *et al.*, 1995).

Systemic 8-OH-DPAT causes a dose-dependent reduction in 5-HT synthesis rate, a decrease in 5-HIAA (Sharp *et al.*, 1989) and inhibits 5-HT neuronal firing in the DRN (Sinton and Fallon, 1988; Sprouse and Aghajanian, 1988; Blier *et al.*, 1989), without interfering with dopamine or noradrenalin (Hjorth *et al.*, 1982), ultimately reducing 5-HT release in the brain (Hjorth *et al.*, 1982; Hjorth and Magnusson, 1988). In an *in vivo* microdialysis study, systemically administered 8-OH-DPAT (0.1 mg/kg) decreased 5-HT release in the rat hippocampus (Routledge *et al.*, 1993), presumably by acting on somatodendritic 5-HT_{1A} autoreceptors in the DRN (Sharp and Hjorth, 1990). As the hippocampus, frontal cortex and hypothalamus receive inputs from the DRN, a reduction in 5-HT release at these sites is likely due to an 8-OH-DPAT-induced reduction of neuron firing (Higgins *et al.*, 1988).

When microinfused into the DRN, 8-OH-DPAT (0.5 μg) reduced 5-HT release in the ventral hippocampus, decreased 5-HT synthesis in the hippocampus and the rest of the brain (Hutson *et al.*, 1989) and also reduced 5-HIAA in the frontal cortex, hippocampus and hypothalamus (Higgins *et al.*, 1988). In electrophysiology studies, 8-

OH-DPAT produced a dose-dependent decrease in neuronal activity of 5-HT cells in the DRN (Arborelius *et al.*, 1994; Corradetti *et al.*, 1996; Verbanac *et al.*, 1996).

Using *in vivo* microdialysis, the 5-HT_{1A} receptor antagonists WAY100135 and WAY100635 effectively blocked the effects of 8-OH-DPAT on extracellular levels of 5-HT in the rat hippocampus (Cliffe *et al.*, 1993; Routledge *et al.*, 1993; Assie and Koek, 1996). When given alone, WAY100135 but not WAY100635 can decrease 5-HT levels, indicating possible partial agonist properties at somatodendritic 5-HT_{1A} receptors (Assie and Koek, 1996). Using *in vivo* extracellular electrophysiological recordings in the rat DRN, WAY100135 and WAY100635 blocked the 8-OH-DPAT-induced inhibition of raphe neuron firing (Fletcher *et al.*, 1993; Forster *et al.*, 1995; Fletcher *et al.*, 1996). In addition, using *in vivo* extracellular electrophysiological recordings in the guinea-pig DRN, 8-OH-DPAT reduced firing of 5-HT DRN neurons and WAY100135 reversed the DPAT-induced inhibition of neuronal firing (Mundey *et al.*, 1994). Similarly, systemic administration of WAY100135 moderately depressed neuronal activity in the DRN of freely moving cats, while WAY100635 increased neuronal activity (Fornal *et al.*, 1996). Additionally, pretreatment with WAY100135 weakly reduced 8-OH-DPAT's action, but WAY100635 completely blocked 8-OH-DPAT's effect, providing further evidence that WAY100635 is a more selective 5-HT_{1A} receptor antagonist (Fornal *et al.*, 1996).

5-HT_{1A} receptor knockout (KO) mice have allowed for further examination of the functional role of these receptors. 5-HT neurons of these 5-HT_{1A} receptor KO mice fire at a higher rate on average (see Berger and Tecott, 2006). When 8-OH-DPAT was systemically administered to 5-HT_{1A} receptor KO mice, there was no suppression of 5-

HT efflux in the DRN, in comparison to wildtype mice (Bortolozzi *et al.*, 2004). These findings suggest that 5-HT_{1A} receptors are important modulators of 5-HT neuron firing.

5-HT receptor agonists (including, but not solely 5-HT_{1A} receptor agonists) produce behavioural effects that coincide with their neurochemical effects. One such effect is the 5-HT-related behavioural syndrome characterized by: hyperactivity, head weaving, flat body posture and forepaw treading (Grahame-Smith, 1971; Green, 1984; Tricklebank *et al.*, 1984). The behavioural effects of 8-OH-DPAT are dose-dependent (Hjorth *et al.*, 1982), such that low doses (0.015-0.06 mg/kg) preferentially activate the somatodendritic autoreceptors, decreasing the metabolism of 5-HT and increasing feeding (Dourish and Hutson, 1985; Dourish *et al.*, 1985a; Dourish *et al.*, 1985b). High doses (0.25-4 mg/kg) on the other hand, activate postsynaptic 5-HT receptors and produce the 5-HT-related behavioural syndrome (Dourish *et al.*, 1985a; Dourish *et al.*, 1985b). Indeed, the hypothermic response of rats to 8-OH-DPAT seems to be a postsynaptic mechanism (O'Connell *et al.*, 1992). WAY100635 and WAY100135 block all aspects of 8-OH-DPAT-induced 5-HT syndrome, but have no intrinsic ability to elicit any components of the 5-HT syndrome (Fletcher *et al.*, 1993; Przegalinski *et al.*, 1994; Forster *et al.*, 1995; Fletcher *et al.*, 1996). Both WAY100635 and WAY100135 had no effect on body temperature itself in the rat, but dose-dependently antagonised 8-OH-DPAT’s hypothermic response (Fletcher *et al.*, 1993; Forster *et al.*, 1995; Fletcher *et al.*, 1996). Similarly, WAY100635 had no effect alone on feeding, but antagonised the hyperphagia induced by 8-OH-DPAT (Fletcher *et al.*, 1996). In the delayed matching to position task in rats, WAY100635 had no effect on cognition alone, but did reverse the 8-OH-DPAT-induced impairments in performance (Fletcher *et al.*, 1996).

Evidence suggests involvement of the somatodendritic 5-HT_{1A} autoreceptor in the regulation of emesis and nausea. In humans, administration of buspirone resulted in a reduction of self-report nausea scores in healthy human patients participating in a nutrient drink test to assess gastric functioning (Chial *et al.*, 2003). In the nutrient drink test, participants consume the maximum tolerated volume of a nutrient drink at the rate of 30 ml/min. Thirty minutes later, symptoms of bloating, fullness, nausea, and pain are assessed on a rating scale. Buspirone selectively suppressed nausea in this test.

5-HT_{1A} autoreceptor agonists such as 8-OH-DPAT, buspirone, and LY228729 have been found to suppress vomiting in emetic species such as pigeons (Wolff and Leander, 1994; Wolff and Leander, 1995; Wolff and Leander, 1997), *S. murinus* (Okada *et al.*, 1994; Javid and Naylor, 2002), cats (Lucot and Crampton, 1987; Lucot and Crampton, 1989; Lucot, 1990) and dogs (Gupta and Sharma, 2002).

In addition, 8-OH-DPAT has been shown to interfere with the establishment and expression of conditioned gaping to a LiCl-paired flavour in rats, but as with OND, 8-OH-DPAT had no effect on conditioned taste avoidance (Limebeer and Parker, 2003). 8-OH-DPAT also interfered with the establishment of fluoxetine-induced conditioned gaping in rats (Limebeer *et al.*, 2009). Finally, 5,7-Dihydroxytryptamine (5,7-DHT)-induced lesions of the MRN and DRN, which depleted forebrain 5-HT, interfered with the establishment of LiCl-induced conditioned gaping, but not conditioned taste avoidance (Limebeer *et al.*, 2004). This finding lends further support to the idea that nausea is forebrain mediated because when forebrain 5-HT was eliminated via 5,7-DHT lesions, so too was conditioned gaping. In addition, these findings also further support the

idea that somatodendritic 5-HT_{1A} autoreceptor agonists, which reduce 5-HT release in forebrain terminal regions, result in the alleviation of toxin-induced nausea and vomiting.

Cannabinoids and Nausea and Vomiting

In the late 1970s and early 1980s, in response to the ineffective management of chemotherapy-induced nausea and vomiting at the time, oncologists began to investigate cannabinoids as a potential therapeutic agent. Evidence indicates that the cannabis plant (*Cannabis sativa*) has been used for centuries as a therapeutic for many ailments (Mechoulam, 2005), including the alleviation of nausea and vomiting. More recently, the mechanisms by which cannabinoids reduce chemotherapy-induced vomiting have been investigated (see Darmani, 2010 for review).

Delta-9-tetrahydrocannabinol (Δ^9-THC)

The psychoactive component in marihuana (Mechoulam *et al.*, 1970), delta-9-tetrahydrocannabinol (Δ^9-THC), identified by Gaoni and Mechoulam (1964a), has been explored for its medicinal efficacy (see Mechoulam and Hanu, 2001 for review). One such potential is its ability to interfere with nausea and vomiting in human cancer patients (see Cotter, 2009 for review). Nabilone (Cesamet) which is a synthetic analogue of Δ^9-THC was liscensed for management of chemotherapy-induced nausea and vomiting. In 1985, Dronabinol (Marinol), a synthetic Δ^9-THC, was also used as an anti-emetic and later as an appetite stimulant (Pertwee, 2009).

Oral Δ^9-THC was shown to significantly reduce nausea and vomiting in cancer patients, 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). Comparisons of oral Δ^9-THC

with the common anti-emetic agents of the time showed that Δ^9-THC was at least as effective at reducing nausea and vomiting (Frytak *et al.*, 1979; Carey *et al.*, 1983; Ungerleider *et al.*, 1984; Crawford and Buckman, 1986; Cunningham *et al.*, 1988; Tramer *et al.*, 2001) and in some cases, Δ^9- THC was shown to be more effective (Orr *et al.*, 1980; Orr and McKernan, 1981).

Only one published clinical trial has directly compared the anti-emetic and anti-nausea effects of cannabinoids with the 5-HT_3 receptor antagonists. Meiri *et al.* (2007) compared dronabinol, OND, or their combination, for their efficacy in delayed chemotherapy-induced nausea and vomiting. The results of the study indicated that dronabinol alone was as effective as OND for total response (vomiting and nausea) and the combined therapies were no more effective than either agent alone. When severity of nausea alone was assessed, dronabinol alone was more effective than OND for mildly to moderately severe emesis produced by chemotherapy treatments, but not for severe emesis treatments. Additionally, in a systematic review of adverse cannabis-related events, of 31 studies involving the use of medicinal cannabis 96.6% of the adverse symptoms reported were not serious, with dizziness being the most reported nonserious adverse event (Wang *et al.*, 2008). These results show that Δ^9-THC is as effective, if not more effective as current anti-emetics and it demonstrates no serious adverse effects.

In animal models, Δ^9-THC reduces vomiting in ferrets (Van Sickle *et al.*, 2001) and has been shown to suppress acute vomiting induced by cisplatin (Darmani, 2001b), rimonabant (SR141716), a cannabinoid receptor 1 (CB_1) antagonist (Darmani, 2001a), radiation (Darmani *et al.*, 2007) and 5-hydroxytryptophan (an indirect 5-HT receptor agonist, Darmani and Johnson, 2004) in least shrews. In *S. murinus*, Δ^9-THC reduces

cisplatin- (Kwiatkowska *et al.*, 2004), LiCl- (Parker *et al.*, 2004), and motion-induced vomiting (Cluny *et al.*, 2008). Nabilone reduced cisplatin-induced emesis in cats (McCarthy and Borison, 1981). Additionally, the administration of Δ^9-THC prior to re-introduction to a context previously associated with illness suppressed the expression of conditioned retching in shrews (Parker and Kemp, 2001; Parker *et al.*, 2006).

Using the TRT, Δ^9-THC has also been shown to interfere with the establishment and expression of conditioned gaping in rats produced by cyclophosphamide, a commonly used chemotherapy drug (Limebeer and Parker, 1999) and LiCl (Parker and Mechoulam, 2003; Parker *et al.*, 2003). The Δ^9-THC-induced suppression of nausea-like behaviours and vomiting can be reversed by administration of the CB_1 receptor antagonists rimonabant or AM251, so it seems that the anti-emetic and anti-nausea-like effects of Δ^9-THC are mediated by the CB_1 receptor (Darmani, 2001b; Darmani and Johnson, 2004; Parker *et al.*, 2004; Darmani *et al.*, 2007; Cluny *et al.*, 2008).

Interestingly, when combined, low doses (those that were ineffective alone) of Δ^9-THC and OND completely abolished cisplatin-induced vomiting *S. murinus (Kwiatkowska et al., 2004)*. Cannabinoids have also been shown to reduce emetogenic effect of 5-HT_3 receptor agonists, and rimonabant was able to block this effect (Darmani and Johnson, 2004). Therefore, cannabinoids may be acting at CB_1 receptors located presynaptically on serotonergic neurons (Haring *et al.*, 2007), possibly inhibiting 5-HT release (Schlicker and Kathmann, 2001; Howlett *et al.*, 2002; Darmani and Johnson, 2004), and this may be how cannabinoids such as Δ^9-THC exert their anti-emetic effect.

The Endogenous Cannabinoid (EC) system

The classic cannabinoid receptors, CB_1 and CB_2, were identified 25 years after the identification of Δ^9-THC. CB_1 receptors are abundantly expressed throughout the nervous system, while CB_2 receptors are predominantly found in immune tissues, such as the spleen and leukocytes, but these receptors have also been identified within the central nervous system. Cannabinoid receptors are co-expressed with 5-HT_3 receptors (Hermann *et al.*, 2002) in brain areas such as the hippocampus and cortex (Hermann *et al.*, 2002). There is recent evidence of CB_1 receptors located in the DRN, with more than 20% of DRN 5-HT neurons expressing CB_1 (Haring *et al.*, 2007). As well, in the DVC, along with the 5-HT_3 receptors, CB_1 receptors are also located there (Himmi *et al.*, 1996; Himmi *et al.*, 1998) and Δ^9-THC along with other cannabinoids has been shown to inhibit currents through human 5-HT_3 receptors (Fan, 1995; Barann *et al.*, 2002).

The discovery of the endogenous cannabinoid, or endocannabinoid (EC) receptors inspired the search for their endogenous ligand. The first EC was identified as an unsaturated fatty acid ethanolamide of arachidonic acid, named anandamide (AEA, Devane *et al.*, 1992) followed by 2-arachidonoylglycerol (2-AG, Mechoulam *et al.*, 1995). AEA and 2-AG are produced on demand and rapidly degraded by fatty acid amide hydrolase (FAAH, Deutsch and Chin, 1993) and monoacylglycerol-lipase (MAGL, Dinh *et al.*, 2002), respectively. Once released, these ECs bind to metabotropic CB_1 receptors located on pre-synaptic axon terminals, resulting in neurotransmitter release inhibition and then undergo inactivation via uptake and enzymatic hydrolysis postsynaptically (Deutsch and Chin, 1993; Di Marzo *et al.*, 1994). CB_1 receptors have been located in GABAergic, dopaminergic, adrenergic, glutamatergic, cholinergic and

serotonergic neurons (see Haring *et al.*, 2007). As evidence indicates that cannabinoid receptor agonists such as Δ^9-THC interfere with vomiting and conditioned gaping, this suggests an involvement of the EC system in the regulation of nausea and vomiting (see Sanger, 2007 for review).

Anandamide (AEA)

AEA reduced vomiting in the least shrew (Darmani, 2002) and in ferrets and this effect was attenuated by AM251 (Van Sickle *et al.*, 2005; Sharkey *et al.*, 2007). The action of AEA can be prolonged by inhibiting its degradation, through the use of URB597, an FAAH enzyme inhibitor, that can increase basal levels of AEA in the rat brain (Fegley *et al.*, 2005). URB597 attenuated cisplatin- and nicotine-induced vomiting in *S. murinus* and the combination of URB597 with AEA suppressed vomiting produced by cisplatin (Parker *et al.*, 2009). This effect of URB597 on nicotine-induced vomiting was reversed by rimonabant. Additionally in rats, URB597 administration has been shown to reduce the establishment of conditioned gaping elicited by a LiCl-paired saccharin solution (Cross-Mellor *et al.*, 2007). URB597 also interferes with the establishment and the expression of conditioned gaping to a LiCl-paired context and this effect seems to be CB_1 mediated, as pretreatment with rimonabant reversed the URB597-induced suppression of gaping (Rock *et al.*, 2008). Thus, prolonging the action of AEA with the FAAH inhibitor URB597 has been shown to interfere with vomiting in shrews and conditioned gaping in rats through a CB_1-dependent mechanism.

2-arachidonoylglycerol (2-AG)

Few studies have investigated the effect of 2-AG on vomiting, but those that have, report both emetic effects in the least shrew that can be reversed by rimonabant (Darmani, 2002) and anti-emetic effects in ferrets that were reversed by both CB_1 (AM251) and CB_2 (AM630) receptor antagonists (Van Sickle *et al.*, 2005). JZL184, a selective MAGL inhibitor (Long *et al.*, 2009a; Long *et al.*, 2009b) suppressed vomiting in the house musk shrew and effectively inhibited MAGL activity in shrew brain tissue (Sticht *et al.*, 2011). Additionally, 2-AG administration suppressed LiCl-induced conditioned gaping in rats (Sticht *et al.*, 2011), further demonstrating that manipulations that elevate 2-AG have an anti-emetic/anti-nausea-like effect. The anti-nausea-like effects of 2-AG may be produced by downstream metabolites of 2-AG, because the suppressive effects of 2-AG on gaping was not reversed by AM251, but was reversed by the cyclooxygenase inhibitor indomethacin.

Cannabigerol (CBG)

Cannabigerol (CBG) is a non-psychoactive component found in cannabis (Gaoni and Mechoulam, 1964b; Mechoulam *et al.*, 1970), but little work has focused on this extract. CBG has been shown to increase fluid drainage from the eye, reducing intraocular pressure, demonstrating its efficacy in the treatment of glaucoma (Colasanti *et al.*, 1984; Colasanti, 1990). CBG also has potential in the treatment of psoriasis as it inhibits keratinocyte proliferation in a concentration-dependent manner (Wilkinson and Williamson, 2007). In addition, CBG may also be useful in the treatment of pain (De

Petrocellis *et al.*, 2008), and it exhibits anti-tumor activity *in vitro* (Ligresti *et al.*, 2006) as well as having antimicrobial activity (Eisohly *et al.*, 1982) and showing potent antibacterial properties (Appendino *et al.*, 2008). In addition, CBG also decreases prostaglandin production in TNF-α stimulated cells, demonstrating an anti-inflammatory effect (Ruhaak *et al.*, 2011). The mechanism of action for CBG is still unclear, but it does elicit a TRPA1-mediated increase in intracellular calcium, as well as activity at TRPV1 and TRPV2 channels (De Petrocellis *et al.*, 2011). In addition, CBG acts as a potent α_2-adrenoceptor agonist, displays affinity for CB_1 and CB_2 receptors, and acts as a 5-HT_{1A} receptor antagonist (Cascio *et al.*, 2010). Clearly more research is needed on this potentially promising phytocannabinoid.

Cannabidiol (CBD)

Another component in cannabis known as Cannabidiol (CBD) was first isolated in 1940 from Mexican marihuana by Roger Adams and from Indian charas by Alexander Todd. It was not until 1963 that Mechoulam isolated CBD from Lebanese hashish and established its structure (Mechoulam and Hanus, 2002). This non-psychoactive component in cannabis (Mechoulam *et al.*, 1970) received little attention in the research community until the last decade where there has been a great increase in publications. This increase in interest was mainly stimulated by the elucidation of its anti-inflammatory, anti-oxidative and neuroprotective effects (see Zuardi, 2008 for review) along with its lack of psychoactivity in comparison to Δ^9-THC. CBD has also been shown to have an effect on anxiety and rheumatoid arthritis (see Mechoulam *et al.*, 2007 for review), cancer (Ligresti *et al.*, 2006), schizophrenia, (Zuardi *et al.*, 2006) epilepsy,

(Cunha *et al.*, 1980; Consroe *et al.*, 1981) depression, (Ren *et al.*, 2009; El-Alfy *et al.*, 2010; Zanelati *et al.*, 2010) and addiction (Ren *et al.*, 2009), and is now available as a sublingual spray called Nabidiolex (GW Pharmaceuticals).

Most recently, a phase II clinical trial evaluated an oromucosally administered cannabis-based medicine (Sativex) containing Δ^9-THC and CBD (in a 1:1 ratio), taken in conjunction with standard anti-emetic therapies such as a 5-HT_3 receptor antagonist, to control delayed chemotherapy-induced nausea and vomiting (Duran *et al.*, 2010). Compared with placebo, when Sativex was added to the standard therapy, it was effective in reducing the incidence of delayed nausea and vomiting and was well tolerated by patients. Fifty-seven percent of Sativex patients experienced no delayed nausea compared to 22% in the placebo group. In terms of emesis, 71% of Sativex patients experienced no delayed emesis compared to 22% in the placebo group.

CBD has been shown to exert anti-spasmodic, anxiolytic, anti-rheumatoid, and anti-emetic effects (see Mechoulam *et al.*, 2002 for review) in animal models. In *S. murinus*, CBD produced a biphasic effect, suppressing acute cisplatin-induced vomiting at 5mg/kg and potentiating it at 40 mg/kg (Kwiatkowska *et al.*, 2004). In addition, acute vomiting elicited by LiCl has been suppressed by low doses (5 – 10 mg/kg) of CBD, while higher doses (20 – 40 mg/kg) of CBD were found to facilitate LiCl-induced vomiting, rather than reducing its expression (Parker *et al.*, 2004). Consistent with the low binding affinity of CBD to the CB_1 receptor (Pertwee, 2004; Pertwee, 2008a), the suppression of vomiting was not reversed by the CB_1 receptor antagonist SR141716 (Parker *et al.*, 2004).

Low doses of CBD (5 mg/kg) but not OND, have also been shown to suppress AN in shrews when administered prior to placement in a context previously paired with nausea and vomiting (Parker *et al.*, 2006). These results again demonstrate the superiority of cannabinoids over 5-HT_3 receptor antagonists to suppress ANV.

In rats, CBD (5 mg/kg) has been shown to reduce the establishment of LiCl-induced conditioned gaping to a flavour previously paired with illness as well as its expression (Parker *et al.*, 2002; Parker and Mechoulam, 2003). Since rats exposed to CBD prior to both conditioning and testing also showed suppressed conditioned gaping reactions, this suppressed responding was not merely a state-dependent decrement in responding (Parker *et al.*, 2002). CBD at low doses (1 and 5 mg/kg) also interfered with the expression of conditioned gaping to a context previously paired with LiCl, while a higher dose (10 mg/kg) did not (Rock *et al.*, 2008). As CBD is a non-psychoactive component in marihuana (Mechoulam *et al.*, 1970), these results are promising for its use in the suppression of ANV even though its mechanisms of action remain unclear.

Possible Mechanisms of CBD action

CBD is known to have a very low affinity (in the micromolar range) for the CB_1 and CB_2 receptors (Pertwee, 2004; Pertwee, 2008a), although CBD can antagonize CB_1 and CB_2 receptor agonists, such as Δ^9-THC, at low concentrations (Thomas *et al.*, 2007; Pertwee, 2008a; Pertwee, 2008b). In addition, higher doses of CBD (50 mg/kg) exacerbated the behavioural effects of low doses (1 mg/kg) of Δ^9-THC and increased CB_1 receptor expression (Hayakawa *et al.*, 2008). Very high doses of CBD can raise blood and brain concentrations of Δ^9-THC, indicating that CBD likely inhibits Δ^9-THC

metabolism (Jones and Pertwee, 1972; Bornheim *et al.*, 1995). CBD can also block AEA uptake (Rakhshan *et al.*, 2000), inhibit anandamide amidase (Watanabe *et al.*, 1996) and inhibit its hydrolysis (Bisogno *et al.*, 2001). In addition, CBD also displays inverse agonism at the CB_2 receptor (Thomas *et al.*, 2007).

Several hypotheses concerning the mechanism of action of the therapeutic effects of CBD have been proposed, including: 1) antagonist at the orphan GPR55 receptor, 2) adenosine uptake inhibitor, 3) vanilloid receptor type 1 (VR1) agonist, 4) 5-HT_{1A} autoreceptor agonist. The evidence for each of these hypotheses will be briefly discussed.

With the recent discovery of the orphan G-protein-coupled receptor, GPR55, another possible site of action for CBD has been suggested. Receptor agonists such as Δ^9-THC and HU-210 and receptor antagonists such as rimonabant and AM251 have been shown to activate this receptor in some assays, but not in others (see Pertwee, 2010 for review). This mixed agonism/non-agonism may indicate partial agonism of these compounds at this receptor (Pertwee, 2010). Preliminary evidence has revealed that *in vitro*, CBD may act at GPR55 as a reasonably potent receptor antagonist (Ryberg *et al.*, 2007; Whyte *et al.*, 2009) at a concentration below that at which it displaces agonists for the CB_1 and CB_2 receptors (Zuardi, 2008), but further research is needed to understand the pharmacology of this new receptor.

CBD (5 mg/kg) has been shown to attenuate inflammation in murine collagen-induced arthritis (eg. Malfait *et al.*, 2000). This anti-inflammatory effect of CBD may be mediated by uptake-inhibition of adenosine. ZM 241385, an A_{2A} adenosine receptor antagonist, reverses the CBD-induced anti-inflammatory effects in murine collagen-

induced arthritis (Carrier *et al.*, 2006). Additionally, the potential of CBD to act as a full, but weak agonist of the VR1 receptor, may play a role in its analgesic effects (Bisogno *et al.*, 2001). In a rat model of acute inflammation, CBD-induced anti-hyperalgesia was reversed with capsazepine (a TRPV1 antagonist, Costa *et al.*, 2004) and some of CBD's other effects (anti-convulsive, and anti-rheumatoidarthritus) are similar to those of the VR1 receptor agonist capsaicin (Consroe *et al.*, 1981; Dib and Falchi, 1996; Lorton *et al.*, 2000; Malfait *et al.*, 2000). CBD has also been shown to reduce striatal atrophy following neurochemical lesions (Sagredo *et al.*, 2007); however, these effects were not mediated by its action on adenosine, VR1 receptors or CB_1 receptors.

Considerable recent evidence indicates that the neuroprotective effects of CBD are mediated by its action at 5-HT_{1A} autoreceptors. CBD has been reported to act as a neuroprotective agent, like BAY x 370 (a selective 5-HT_{1A} receptor agonist), by reducing the volume of cerebral infarction in animal models of ischemic injury (eg. Hampson *et al.*, 1998; Berends *et al.*, 2005; Durst *et al.*, 2007). Therefore, it seems that the neuroprotective effects of CBD may be mediated by its action as a 5-HT_{1A} autoreceptor agonist (Hayakawa *et al.*, 2004; Mishima *et al.*, 2005; Hayakawa *et al.*, 2007a). Indeed, Russo (2005) reported that CBD (at 16 µM) displaces the receptor agonist [^{3}H] 8-OH-DPAT from a cloned human 5HT_{1A} receptor in a concentration dependent manner. Furthermore, CBD was shown to act as an agonist at the 5-HT_{1A} receptor, because, like 5-HT, it increased GTP biding to the receptor coupled G protein, G_i, characteristic of a receptor agonist. Finally, the agonist CBD was shown to reduce cAMP production, characteristic of G_i activation.

CBD is a neuroprotective antioxidant (Hampson *et al.*, 1998) reducing the volume of cerebral infarction in animal models of ischemic injury (Hayakawa *et al.*, 2004; Hayakawa *et al.*, 2007a; Hayakawa *et al.*, 2007b). Interestingly, WAY100135 reverses this neuroprotective effect of CBD (Mishima *et al.*, 2005; Hayakawa *et al.*, 2007a), while capsazepine (a TRPV1 antagonist, Mishima *et al.*, 2005), rimonabant and AM630 (Hayakawa *et al.*, 2004; Hayakawa *et al.*, 2007a; Hayakawa *et al.*, 2007b) did not. Furthermore, when injected into the dorsolateral peri-aqueductal gray, the anxiolytic effects of CBD were prevented by WAY100135, but not AM251 (Campos and Guimaraes, 2008a). These findings further implicate the somatodendritic $5HT_{1A}$ autoreceptor as the mechanism by which CBD exerts it neuroprotective and anxiolytic properties. No such study investigating the involvement of the somatodentric $5HT_{1A}$ autoreceptor in the anti-emetic and anti-nausea-like effects of CBD has been conducted.

Present Studies

In the present thesis, we sought to determine the mechanism of action by which CBD exerts its anti-emetic and anti-nausea-like effects. The experiments reported in Chapter 2 are in press in *The British Journal of Pharmacology.* Our hypothesis was that, if CBD is indeed acting as a somatodendritic 5-HT_{1A} autoreceptor agonist, then administration of a 5-HT_{1A} receptor antagonist should block CBD's effect. The first set of experiments in Chapter 2 investigated the ability of systemic administration of a 5-HT_{1A} receptor antagonist, WAY100135, to block the systemic CBD-induced suppression of nicotine-, LiCl-, and cisplatin-induced vomiting in *S. murinus*. Next, we elucidated the potential of systemic administration of the 5-HT_{1A} receptor antagonist WAY100135 and

the more selective and more potent 5-HT_{1A} receptor antagonist WAY100635 to prevent the anti-nausea-like effects (conditioned gaping in rats) of systemic CBD. Additionally, the ability of intra-DRN administration of WAY100635 to block the suppressive effect of systemic CBD on conditioned gaping was assessed. Conversely, the potential of intra-DRN administration of CBD to produce anti-nausea-like effects (and reversal by systemic WAY100635) was assessed. *In vitro* studies evaluated the ability of CBD to directly bind to 5-HT_{1A} receptors. As well, these studies determined CBD's potential to modify the ability of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, to stimulate [^{35}S]GTPγS binding in rat brainstem membranes.

The experiments reported in Chapter 3 appear in *Psychopharmacology,* 215: 505-512. The purpose of these experiments was to examine the interaction of CBD and CBG, two phytocannabinoids present in cannabis, in the regulation of nausea and vomiting. We sought to evaluate the ability of CBG to reverse the anti-emetic and anti-nausea-like effects of CBD. We evaluated the hypothesis that if CBG does indeed act as a 5-HT_{1A} receptor antagonist, and CBD acts as a 5-HT_{1A} receptor agonist, then the anti-emetic and anti-nausea-like effects of CBD should be blocked by CBG. First, we examined the ability of systemic CBG to block the systemic CBD-induced suppression of conditioned gaping. Additionally, we examined the potential of CBG to block the effect of 8-OH-DPAT on conditioned gaping. The interaction of CBG and CBD was further examined in *S. murinus* with systemic administration of CBD and its reversal by systemic CBG.

Cancer patients undergoing chemotherapy still report nausea as a distressing symptom associated with their treatment because it is not adequately managed within the clinic by 5-HT_3 receptor antagonists. Cancer patients often smoke cannabis, to manage

their nausea, exposing themselves to over 60 cannabinoids, including the psychoactive Δ^9-THC, along with CBG and CBD. Interestingly, within cannabis, CBD and CBG may be counteracting each others effects, making it imperative to understand how these cannabinoids modify nausea and vomiting so that effective treatments can be elucidated.

CHAPTER 2

British Journal of Pharmacology, in press.

Cannabidiol, a Non-Psychotropic Component of Cannabis, Attenuates Vomiting and Nausea-like Behaviour via Indirect Agonism of 5-HT_{1A} Somatodendritic Autoreceptors in the Dorsal Raphe Nucleus

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Running Title: CBD, 5-HT_{1A} agonism and nausea

Background and purpose: To evaluate the hypothesis that agonism of somatodentdritic 5-HT_{1A} autoreceptors in the dorsal raphe nucleus (DRN) produces the anti-emetic/anti-nausea-like effects of cannabidiol (CBD), a primary non-psychoactive cannabinoid found in cannabis. *Experimental approach:* The potential of systemic and intra-DRN administration of 5-HT_{1A} receptor antagonists (WAY100135 or WAY100635) to prevent the anti-emetic effect of CBD (in shrews-*Suncus murinus)* and the anti-nausea-like effects of CBD (conditioned gaping in rats) were evaluated. As well, the ability of intra-DRN administration of CBD to produce anti-nausea-like effects (and reversal by systemic WAY100635) was assessed. *In vitro* studies evaluated the potential of CBD to directly target 5-HT_{1A} receptors and to modify the ability of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, to stimulate [^{35}S]GTPγS binding in rat brainstem membranes. *Key results:* CBD suppressed nicotine-, LiCl- and cisplatin (20 mg/kg, but not 40 mg/kg)-induced vomiting in the *S. murinus* and LiCl-induced conditioned gaping in rats. The anti-emetic and anti-nausea-like effects of CBD were suppressed by WAY100135 and the latter by WAY100635. When administered to the DRN: 1) WAY100635 reversed the anti-nausea-like effect of systemic CBD, and 2) CBD suppressed nausea-like effects, an effect that was reversed by systemic WAY100635. CBD also displayed significant potency (in a bell shaped dose-response curve) at enhancing the ability of 8-OH-DPAT to stimulate [^{35}S]GTPγS binding to rat brainstem membranes *in vitro*. Finally, systemically administered subthreshold doses of CBD and 8-OH-DPAT enhanced suppression of LiCl-induced conditioned gaping. *Conclusions and implications:* These results suggest that CBD produces its anti-emetic/anti-nausea-like effects by indirect agonism of the somatodendritic 5-HT_{1A} autoreceptors in the DRN.

Key words: Cannabidiol, serotonin, 5-HT_{1A}, endocannabinoid, nausea, vomiting, shrew, rat, taste reactivity, gaping, conditioned disgust, vomiting, emesis

Abbreviations: 5-HT, serotonin; 5-HT_{1A}, 5-hydroxytryptamine 1A; 5-HT_3, 5-hydroxytryptamine-3; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; ANOVA, Analysis of Variance; BSA, bovine serum albumin; CCAC, Canadian Council on Animal Care; CB_1 and CB_2, cannabinoid receptors; CBD, cannabidiol; Δ^9-THC, ;Δ^9-tetrahydrocannibinol; DRN, dorsal raphe nucleus; ip, intraperitoneally; LiCl, lithium chloride; MRN, median raphe nucleus; SAL, saline; sc, subcutaneously; TR, taste reactivity; VEH, vehicle; WAY100135, (S)-N-tert-butyl- 3-(4-(2-methoxyphenyl)-piperazin-1-yl)- 2-phenylpropanamide; WAY100635, *N*-[2-[4-(2-methoxyphenyl)-1 piperazinyl]ethyl]-*N*-2- pyridinylcyclohexanecarboxamide maleate

Introduction

The cannabis plant has been used for centuries for the suppression of nausea and vomiting (for review, Russo, 2007). Recent research has revealed that among the over 80 cannabinoid compounds found in marihuana, both the intoxicant, Δ^9-tetrahydrocannabinol (Δ^9-THC, e.g. Darmani, 2001b; Van Sickle *et al.*, 2001; Kwiatkowska *et al.*, 2004) and the non-intoxicant, cannabidiol (CBD, Kwiatkowska *et al.*, 2004; Parker *et al.*, 2004), suppress vomiting in animal models. CBD acts in a biphasic manner, such that low doses suppress toxin-induced vomiting, but high doses potentiate (Kwiatkowska *et al.*, 2004; Parker *et al.*, 2004) or have no effect (Darmani *et al.*, 2007) on vomiting. Both Δ^9-THC and CBD also suppress the development of malaise-induced conditioned gaping reactions (Grill and Norgren, 1978a; Grill and Norgren, 1978b) in rats; a model of rat nausea-like behavior (see Parker and Limebeer, 2008 for review). These conditioned gaping reactions in rats are only produced by drugs that also produce emesis in species capable of vomiting and are specifically prevented by pretreatment with anti-emetic drugs, such as the 5-hydroxytryptamine-3 (5-HT_3) receptor antagonist, ondansetron (Limebeer & Parker, 2000). This pattern contrasts with the more typically employed nonselective measure of conditioned taste avoidance (see Parker *et al.*, 2008).

Considerable recent interest has been directed to the therapeutic potential of CBD. It has been shown to protect against cerebral ischemia, inflammation, anxiety (e.g., Mechoulam *et al.*, 2007 for review), and most recently depression (Zanelati *et al.*, 2010) and even addiction (Ren *et al.*, 2009). Several mechanisms of action have been identified for the various physiological effects of CBD (Mechoulam and Hanus, 2002; Pertwee *et*

al., 2002; Mechoulam *et al.*, 2007). Although CBD has very low affinity for both cannabinoid receptors (CB_1 and CB_2, Pertwee, 2004; Pertwee, 2008a) it has recently been shown to display unexpectedly high potency *in vitro* as an antagonist of CB_1 receptor agonists in mouse vas deferens (Pertwee *et al.*, 2002) and brain (Thomas *et al.*, 2007) tissues. Additionally, CBD displays inverse agonism at the human CB_2 receptor (Thomas *et al.*, 2007). CBD has also been reported to enhance adenosine signaling by inhibiting its reuptake; *in vivo* treatment with a low dose of CBD decreased TNF-α production in lipopolysaccharide-treated mice (anti-inflammatory effect), an effect that was reversed with an A_{2A} adenosine-receptor antagonist and abolished in A_{2A} receptor knockout mice (Carrier *et al.*, 2006). CBD also acts as an anti-oxidant, potentially preventing damage in neurological disorders such as cerebral ischemia (Hampson *et al.*, 1998). The anti-arthritic potential of CBD may be the result of diminished IFN –γ release from lymph-node cells in CBD-treated mice; subsequent *in vitro* experiments found that CBD suppressed the collagen-type-II-specific proliferation of lymph-node cells from arthritic mice (Malfait *et al.*, 2000).

Russo and colleagues (2005) recently reported that at a rather high concentration of 16 μM, CBD can bind to and activate human 5-hydroxytryptamine 1A (5-HT_{1A}) receptors. Somatodendritic 5-HT_{1A} autoreceptors in the raphe nuclei regulate the rate of firing of raphe serotonergic afferents and low doses of the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT; a classic 5-HT_{1A} receptor agonist) reduce their firing rate, thereby reducing the release of serotonin (5-HT) in terminal regions (Verge *et al.*, 1985; Blier and de Montigny, 1987). CBD also reduces the volume of cerebral infarction in animal models of ischemic injury (e.g., Hampson *et al.*, 1998).

This neuroprotective effect of CBD is mediated by 5-HT_{1A} receptors, because it is reversed by the 5-HT_{1A} receptor antagonist, (S)-N-tert-butyl- 3-(4-(2-methoxyphenyl)-piperazin-1-yl)- 2-phenylpropanamide, WAY100135 (Hayakawa *et al.*, 2004; Mishima *et al.*, 2005; Hayakawa *et al.*, 2007a; Hayakawa *et al.*, 2007b). As well, CBD-induced reversal of cognitive and motor function impairments in a mouse model of hepatic encephalopathy are prevented by a 5-HT_{1A} receptor antagonist (Magen *et al.*, 2010). When injected into the dorsolateral peri-aqueductal gray, the anxiolytic effects of CBD are prevented by pretreatment with WAY100135, but not by the CB_1 receptor antagonist/inverse receptor agonist AM251 (Campos and Guimaraes, 2008a). Most recently, CBD has been reported to produce an anti-depressant-like action which is 5-HT_{1A} receptor mediated (Zanelati *et al.*, 2010).

It is only recently that the mechanism of action for CBD's anti-emetic and anti-nausea-like effects has been evaluated (see Parker and Limebeer, 2008). Unlike Δ^9-THC, which produces its effect on emetic behaviours by its action on the CB_1 receptor (Darmani, 2001b; Van Sickle *et al.*, 2001; Parker *et al.*, 2003; Parker *et al.*, 2004), CBD has a very low affinity for the CB_1 receptors (Mechoulam and Hanus, 2002) and its anti-emetic effect is not reversed by pharmacological blockade of these receptors (Parker *et al.*, 2004). Like CBD, low doses of 8-OH-DPAT, attenuate vomiting (Lucot and Crampton, 1989; Okada *et al.*, 1994; Wolff and Leander, 1994; Andrews *et al.*, 1996; Gupta and Sharma, 2002; Javid and Naylor, 2006) and conditioned gaping (Limebeer and Parker, 2003) in animal models. It is likely that the attenuation of nausea-like behaviour by low doses of 8-OH-DPAT is the result of action at somatodendritic 5-HT_{1A} autoreceptors in the dorsal raphe nucleus (DRN) and median raphe nucleus (MRN),

because selective serotonin lesions of these nuclei also attenuate the establishment of lithium chloride- (LiCl) induced conditioned gaping reactions (Limebeer *et al.*, 2004). Data reported in the literature show that 5-HT is able to displace fully [^{3}H]8-OH-DPAT from specific binding sites both in rat cerebral cortex membranes (Carli *et al.*, 1996) and in human 5-HT_{1A}-expressing CHO cell membranes (Newman-Tancredi *et al.*, 1998). These findings suggest that 5-HT and 8-OH-DPAT bind to the same 5-HT_{1A} receptor binding site.

Here we provide evidence that systemic pretreatment with a 5-HT_{1A} receptor antagonist attenuates the anti-emetic and anti-nausea-like effects of CBD. Furthermore, when administered intracranially into the DRN, WAY100635 attenuates the anti-nausea-like effects of systemic CBD in rats. When CBD is administered directly into the DRN, LiCl-induced conditioned gaping reactions are suppressed in rats, an effect that is reversed by systemic WAY100635. *In vitro* data also demonstrates that CBD augments the effect of 8-OH-DPAT in rat brainstem tissues and this effect is confirmed by an enhanced suppressive effect of combined subthreshold systemic doses of CBD and 8-OH-DPAT on LiCl-induced conditioned gaping in rats.

Materials and Methods:

Animals

Animal procedures were according to the Canadian Council on Animal Care (CCAC) and the National Institutes of Health guidelines. The protocols were approved by the Institutional Animal Care Committee, which is accredited by the CCAC. The male

(30-45 g) and female (20-37 g) *S. murinus* (house musk shrews) ranging from 40 days to 180 days of age were bred and raised at the University of Guelph colony. They were single-housed in cages in a colony room at an ambient temperature of 21^0 C on a 14/10 light dark schedule (lights off at 9 PM) as described in Parker *et al.* (2009). Shrews were tested during their light cycle, between 8 AM and 5 PM. The shrews had previous emetic experience with the limitation of a minimum of 3 week recovery between treatments. Because of its toxicity, cisplatin was always administered as the final treatment and shrews were euthanized thereafter.

Naïve male Sprague-Dawley rats, weighing between 275-350 g on the day of conditioning, obtained from Charles River Laboratories (St Constant, Quebec) were used for assessment of anti-nausea-like behavior. They were single-housed in shoebox cages in the colony room at an ambient temperature of 21^0 C with a 12/12 light/dark schedule (lights off at 8 AM) and maintained on *ad-libitum* food and water.

Drugs and Materials

In vivo experiments: When systemically administered, CBD was prepared (2.5 mg/ml or 0.5 mg/ml) in a vehicle (VEH) of ethanol/Cremaphor (Sigma)/saline (SAL; 1:1:18) and administered subcutaneously (sc). 8-OH-DPAT HBr (8-OH-DPAT; Sigma) was prepared in SAL (0.05 mg/ml or 0.005 mg/ml) and administered sc. The 5-HT_{1A} receptor antagonist, WAY100135 (Sigma; 5 mg/ml; Mishma et al, 2005), and the more selective 5-HT_{1A} receptor antagonist (Forster *et al.*, 1995), *N*-[2-[4-(2-methoxyphenyl)-1 piperazinyl]ethyl]-*N*-2- pyridinylcyclohexanecarboxamide maleate (WAY100635;

Sigma; 0.1 mg/ml; Campos and Guimaraes, 2008), were prepared in SAL and administered intraperitoneally (ip). LiCl (Sigma) was prepared in a 0.15 M solution with sterile water and was administered ip at a volume of 60 ml/kg (390 mg/kg) in shrews (see Parker *et al.*, 2004) and 20 ml/kg (127.2 mg/kg) in rats. Nicotine (Sigma) bitartrate salt was prepared as a 2.5 mg/ml solution (expressed as a salt) in SAL and administered sc at a dose of 5 mg/kg (2 ml/kg, Parker *et al.*, 2009). Cisplatin (Sigma) was prepared as a 1 mg/ml solution in SAL and was administered ip at doses of 20 mg/kg (20 ml/kg) and 40 mg/kg (40 ml/kg).

When administered intracranially into the DRN, WAY100635 was prepared in sterile SAL at 21 ng/0.5 µl (Herges and Taylor, 1999) and intracranially microinfused at 0.5 ul/min for 1 min. Intracranial CBD was prepared in 45% 2-hydroxypropyl-β-cyclodextrin (2-HPβCD) at 10 µg/µl and intracranially microinfused into the DRN at 1 µl/min for 1 min (based on Murillo-Rodriguez *et al.*, 2008).

In vitro experiments: CBD and 8-OH-DPAT HBr were supplied by Tocris (Bristol, UK). WAY100635 and fatty acid-free bovine serum albumin (BSA) by Sigma-Aldrich (Poole, Dorset, UK). For the binding experiments, $[^{35}S]GTP\gamma S$ (1250 $Ci \cdot mmol^{-1}$) and $[^{3}H]$8-OH-DPAT (187 $Ci \cdot mmol^{-1}$) were obtained from PerkinElmer Life Sciences Inc (Boston, MA, USA), GTPγS and adenosine deaminase from Roche Diagnostic (Indianapolis, IN, USA) and GDP from Sigma- Aldrich.

In Vivo Procedures

In Vivo Experiments

Effect of systemic injections of 5-HT_{1A} receptor antagonists on CBD-induced suppression of nicotine- LiCl- and cisplatin-induced vomiting in shrews : The shrews were moved into the experimental room from the colony room and given four meal worms in an empty cage 15 min prior to receiving two pretreatment injections. The first pretreatment injection was 1 ml/kg of SAL or WAY100135, followed 15 min later by an injection of VEH (2 or 4 ml/kg) or CBD (2 ml/kg [5 mg/kg] or 4 ml/kg [10 mg/kg], depending upon the emetic treatment). Thirty min later, shrews were given an injection of nicotine (5 mg/kg), LiCl (390 mg/kg) or cisplatin (20 or 40 mg/kg). All shrews were then individually placed immediately into the clear Plexiglas observation chamber (22.5 x 26 x 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 shrew to observe vomiting episodes. The duration of the test was determined by the duration of onset/action of the emetic effect of the drug: nicotine (15 min), LiCl (45 min), or cisplatin (60 min). The frequency of vomiting episodes (expulsion of fluids from the stomach) was counted by an observer blind to experimental conditions. Additionally, to evaluate the potential of the pretreatment drugs to produce vomiting on their own, six groups (n=8/group) of shrews were injected with SAL (60 ml/kg) following pretreatment with SAL or WAY100135 (10 mg/kg) and 15 min later with VEH, 5 mg/kg or 10 mg/kg of CBD. They were observed for 60 min. None of the SAL treated shrews displayed vomiting following the pretreatment injections; therefore, these groups were not included in the overall analyses.

The number of vomiting episodes elicited by nicotine or LiCl was entered into a one way Analysis of Variance (ANOVA), with subsequent Bonferroni post-hoc comparison tests of significant main effects. The number of vomiting episodes elicited by either 20 or 40 mg/kg of cisplatin was entered into a 2 (SAL or WAY100135) x 3 (VEH, 5 mg/kg CBD or 10 mg/kg CBD) ANOVA. For all analyses significance is defined as $p < 0.05$.

Effect of systemic injections of 5-HT$_{1A}$ receptor antagonists on CBD-induced suppression of LiCl-induced conditioned gaping in rats: The effect of systemic pretreatment with the 5-HT$_{1A}$ receptor antagonists on the anti-nausea-like effects of CBD in rats. All rats were surgically implanted with an intraoral cannula under isoflorane anesthesia according to the procedures described by Limebeer *et al.* (2009). Following recovery from surgery (4 days), the rats received an adaptation trial in which 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. The TR chambers had the same specifications as the shrew observation chambers. Water was infused into their intraoral cannula for 2 min at the rate of 1 ml/min. On the day following the adaptation trial, the rats received a conditioning trial in which they received two pretreatment injections. The first pretreatment injection was 2 ml/kg of SAL, 2 ml/kg of WAY100135 (10 mg/kg) or 1 ml/kg of WAY100635 (0.1 mg/kg). The second pretreatment injection given 15 min later was 2 ml/kg of VEH or CBD (5 mg/kg). The groups were as follows: SAL-VEH (n=13), SAL-CBD (n=9), WAY100135-VEH (n=8), WAY100135-CBD (n=8), WAY100635-VEH (n=7), WAY100635-CBD (n=8). Thirty min after the second

pretreatment injection, the rats were individually placed in the chamber and intraorally infused with 0.1% saccharin solution for 2 min at the rate of 1 ml/min 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 of 0.15 M LiCl and returned to their home cage. The video tapes 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 behavior of gaping (large openings of the mouth and jaw, with lower incisors exposed). The mean number of gaping reactions elicited by the LiCl-paired saccharin solution was entered into a 3 (SAL, WAY100135 or WAY100635) x 2 (VEH or CBD) between groups ANOVA, with subsequent planned comparison tests.

<u>Effect of Intra-DRN WAY100635/Systemic CBD on conditioned gaping in rats</u>: In all experiments, each rat was also permanently implanted unilaterally, entering from either the left or right hemisphere (counterbalanced across rats) with an intracranial cannula directed toward the DRN. Rats were anaesthetized with isofluorane gas and stabilized in the flat skull position (according to Paxinos and Watson, 1986) in the stereotaxic frame. A stainless steel guide cannula (22 G, 8 mm below pedestal; Plastics One, Roanoke, VA) was implanted at an angle of 20° to the vertical so that the tip was located 2 mm dorsal to the DRN. Co-ordinates (relative to interaural zero) were: anterior-posterior (A-P) +1.2 mm, medial-lateral (ML) 0.0 mm, ventral (V) + 5.0 mm. The cannula was secured by 3 stainless steel screws and dental cement. At this time the rat was administered carprofen

(0.1 mg/kg ip; Pfizer, Kirkland QC) as an analgesic and a stainless steel obdurator was inserted in the cannula to maintain patency. All rats were then surgically implanted with an intraoral cannula under isoflorane anesthesia according to the procedures described by Limebeer *et al.* (2010). The rats had at least 5 days recovery before behavioral testing.

Verification of cannula placement into the DRN was determined by histological evaluation of tissue. Rats were injected with 85 mg/kg sodium pentobarbital (Euthansol, Intervet Canada Corp., Kirkland, QC, Canada) and were transcardially perfused with phosphate buffered saline (0.1 M) and 4% formalin. The brains were removed and stored at 4° C in 4% formalin solution for 24-48 hours after which they were placed in a 20% sucrose solution overnight at room temperature. The brains were then sliced in 60 µm sections using a CM1850 Leica cryostat and relevant sections were mounted on glass microscope slides. The tissue was later stained with cresyl violet and examined for accurate injector tip placement using a Leica MZ6 Stereomicroscope with a Leica DFC420 Digital Camera and Leica Application Suite software (Leica Microsystems Inc., Concord, ON, Canada).

Following recovery from surgery, the rats received an adaptation trial in which they were placed in the TR chamber with their cannula attached to the infusion pump for fluid delivery. Water was infused into their intraoral cannula for 2 min at the rate of 1 ml/min. On the day following the adaptation trial, the rats received a conditioning trial in which they received two pretreatment injections. The first pretreatment injection, SAL or WAY100635, was infused into the DRN at 0.5 µl/min for 1 min (with the injector tip protruding 2 mm below the tip of the cannula). The injector remained in place for an additional 1 min. Fifteen min later, the rats received a 2 ml/kg pretreatment injection of

either VEH or CBD (5 mg/kg, sc). Thirty min later, the rats were individually placed in the chamber and intraorally infused with 0.1% saccharin solution for 2 min at the rate of 1 ml/min while the orofacial responses were video recorded. Immediately after the saccharin infusion, all rats were injected with 20 ml/kg of 0.15 M LiCl and returned to their home cage. The final groups (with proper placement) were as follows: SAL-VEH (n=7), SAL-CBD (n=7), WAY100635-VEH (n=9), WAY100635-CBD (n=6). Additionally, rats in group WAY100635-CBD (n=5) with placements outside of the DRN were included in the analysis as a separate group. The mean number of gaping reactions elicited by the LiCl-paired saccharin solution was entered into a one way ANOVA, with subsequent planned comparison tests.

Effect of Intra-DRN CBD/Systemic WAY100635 on conditioned gaping in rats: The rats were treated exactly as those receiving intra-DRN WAY100635, except as indicated. On the conditioning trial, they were injected ip with 1 ml/kg of SAL or WAY100635 (0.1 mg/kg). Fifteen min later, VEH or CBD (0.21 ng) was infused into the DRN at 0.5 μl/min for 1 min (with the injector tip protruding 2 mm below the tip of the cannula). The injector remained in place for one additional min. Immediately after microinfusion, the rats were individually placed into the TR chamber. The final groups (with proper placement) were as follows (DRN drug/Systemic drug): VEH-SAL (n=6), CBD-SAL (n=7), VEH-WAY100635-VEH (n=6), CBD-WAY100635-CBD (n=5). The mean number of gaping reactions elicited by the LiCl-paired saccharin solution during the drug free test was entered into a one way ANOVA, with subsequent planned comparison tests.

Effect of subthreshold doses of systemic injections of CBD and 8-OH-DPAT on LiCl-induced conditioned gaping in rats : In Experiment A, the doses of CBD (2.5 mg/kg) and 8-OH-DPAT (0.05 mg/kg) initially tested were the half optimal doses of each of these compounds previously demonstrated to interfere with conditioned gaping (Parker *et al.*, 2002; Limebeer and Parker, 2003). However, at these doses both CBD and 8-OH-DPAT each suppressed LiCl-induced conditioned gaping on their own; therefore, in Experiment B, lower (subthreshold) doses of each compound (0.5 mg/kg of CBD and 0.005 mg/kg 8-OH-DPAT) were then evaluated.

Twenty-four h following adaptation, the rats received a conditioning trial in which they were administered a pretreatment and a treatment injection. The pretreatment injection was 1 ml/kg of VEH or CBD (2.5 mg/ml in Experiment A and 0.5 mg/ml in Experiment B) followed 15 min later by a treatment injection of 1 ml/kg of SAL or 8-OH-DPAT (0.05 mg/ml in Experiment A or 0.005 mg/ml in Experiment B). This design resulted in the following groups for each of Experiments A and B: VEH-SAL (n=7), CBD-8-OH-DPAT (n=9), CBD-SAL (n=8), and VEH-8-OH-DPAT (n=8). Thirty min after the treatment injection, the rats were individually placed in the chamber and intra-orally infused with a 0.1% saccharin solution for 2 min at a rate of 1 ml/min while their orofacial and somatic responses were video-recorded. Immediately following the saccharin infusion, the rats were injected with 20 ml/kg of 0.15 M LiCl and returned to their home cages. Ninety-six hr following the conditioning trial, the rats individually received a single drug-free test trial in which they were returned to the chamber and intra-orally infused with the 0.1% saccharin solution for 2 min (1 ml/min) while their orofacial and somatic responses were video-recorded. For each of Experiment A and B, the mean

number of gaping reactions during the test trial was entered into a one way ANOVA, with subsequent planned comparison tests.

In Vitro **Procedures**

Membranes preparation

Rat brainstem tissues were 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 phenylmethylsulphonyl fluoride (PMSF; 1 mM). The homogenate was centrifuged at 13500 x *g* for 15 min and the resulting pellet was kept in -80°C for at least 2h. The pellet was then resuspended in TME buffer (50 mM Tris–HCl; EDTA 1.0 mM; MgCl2 3.0 mM; pH 7.4), homogenized and stored at -80°C.

Radioligand displacement assay

The assays were carried out with [^{3}H]-8-OH-DPAT and Tris-binding buffer (50 mM Tris-HCl, 50 mM Tris-Base, 0.1% BSA; pH 7.4), total assay volume 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). All assays were performed at 37 ºC 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; Brandel Inc., Gaitherburg, MD, USA) and Brandel GF/B filters that had been soaked in wash buffer at 4 ºC 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, PerkinElmer). 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. The concentration of $[^3H]$-8-OH-DPAT used in our displacement assays was 0.7 nM. The compounds under investigation were stored at -20°C as stock solutions of 10 mM in DMSO, the vehicle concentration in all assay wells being 0.1% DMSO.

$[^{35}S]GTP\gamma S$ binding assay

The assays were carried out with GTPγS binding buffer (50 mM Tris-HCl, 100 mM NaCl, 3 mM $MgCl_2$, 0.2 mM EGTA and 0.1% BSA fatty acid free; pH 7.4) in the presence of $[^{35}S]GTP\gamma S$ and GDP, in a final volume of 500 µl. Binding was initiated by the addition of $[^{35}S]GTP\gamma S$ to the wells. Nonspecific binding was measured in the presence of 30 µM GTPγS. Rat brainstem membranes were preincubated for 30 min at 30ºC with 0.5 $U{\cdot}ml^{-1}$ adenosine deaminase to remove endogenous adenosine. The drugs were incubated in the assay for 60 min at 30 ºC. The reaction was terminated by a rapid vacuum filtration method using Tris-binding buffer, and the radioactivity was quantified by liquid scintillation spectrometry. In all the $[^{35}S]GTP\gamma S$-binding assays we used 0.1 nM $[^{35}S]GTP\gamma S$, 30 µM GDP and a protein concentration of 100 µg per well.

Dissociation Kinetics

Dissociation kinetic assays were performed with the 5-HT_{1A} receptor agonist [^{3}H]-8-OH-DPAT (0.7 nM) and Tris-binding buffer, total assay volume 500 µl. We used the "isotopic dilution" method to measure the dissociation rate constant for [^{3}H]-8-OH DPAT from brainstem membranes (Christopoulos, 2000; Price *et al.*, 2005). [^{3}H]-8-OH-DPAT (0.7 nM) was incubated with rat brainstem membranes (500 µg protein per well) for 60 min at 25°C. Dissociation was initiated by the addition of 1 µM unlabeled ligand in the presence and absence of test compounds. Dissociation times of 0.5 to 120 min at 25°C were used. To determine the nonspecific binding, experiments were also performed in the presence of a 1 µM concentration of the unlabeled ligand. Binding was terminated by addition of ice-cold wash buffer (50 mM Tris-HCl, 50 mM Tris-base, and 0.1% BSA) followed by vacuum filtration.

In Vitro Data Analysis

Values have been expressed as means and variability as SEM or as 95% confidence limits. The concentrations of the compounds under investigation that produced a 50% displacement of radioligand from specific binding sites (IC_{50} values) were calculated using GraphPad Prism and the corresponding K_i values were calculated using the equation of Cheng and Prusoff (1973). Values for EC_{50}, maximal effect (E_{max}) and SEM or 95% confidence limits of these values have been calculated by nonlinear regression analysis using the equation for a sigmoid concentration-response curve (GraphPad Prism). The dissociation rate constant for [^{3}H]8-OH-DPAT was calculated using a one phase exponential decay equation (GraphPad Software, Onc., San Diego, CA, USA).

Results

CBD-induced suppression of toxin-induced vomiting in shrews was reversed by pretreatment with 5-HT_{1A} receptor antagonist: CBD suppressed nicotine- and LiCl-induced vomiting in *S. murinus,* effects which were reversed by pretreatment with the 5-HT_{1A} receptor antagonist WAY100135. Figure 1 shows the mean number of vomiting episodes elicited by nicotine (top half) and LiCl (bottom half) for each pretreatment group. The pretreatment group effect was significant for both the nicotine-treated shrews, $F(3, 36) = 5.2$; $p < 0.01$ and the LiCl-treated shrews, $F(3, 50) = 5.6$; $p < 0.01$. In each experiment, Group SAL-CBD displayed significantly less vomiting than any other group ($p < 0.05$), which did not differ among themselves.

CBD also suppressed vomiting produced by 20 mg/kg of cisplatin, but not 40 mg/kg of cisplatin, with the former effect prevented by pretreatment with WAY100135. Figure 2 presents the mean number of vomiting episodes elicited by 20 mg/kg of cisplatin (top panels A) and 40 mg/kg cisplatin (bottom panels B) displayed by the shrews pretreated with SAL (left hand sections) or WAY100135 (right hand sections) prior to an injection of VEH, 5 mg/kg CBD or 10 mg/kg CBD. Among shrews administered 20 mg/kg cisplatin, but not 40 mg/kg, the 3 x 2 ANOVA revealed a significant interaction, $F(2, 47) = 4.3$; $p < 0.05$. For the group treated with 20 mg/kg cisplatin, among the SAL pretreated groups, but not the WAY100135 pretreated groups, both groups pretreated with CBD (5 or 10 mg/kg) displayed fewer vomiting episodes ($p < 0.05$) than the VEH pretreated group. CBD did not interfere with vomiting produced by the higher dose of cisplatin (40 mg/kg).

Systemic CBD-induced suppression of LiCl-induced conditioned gaping in rats was reversed by systemic pretreatment with 5-HT_{1A} receptor antagonists: Systemic administration of the 5-HT_{1A} receptor antagonists, WAY100135 and WAY100635, prevented the anti-nausea-like effect of CBD in rats, just as WAY100135 prevented the anti-emetic effects of CBD in shrews. As seen in Figure 3, the 2 x 3 ANOVA revealed a significant interaction, $F(2, 47) = 3.4$; $p < 0.05$, only rats that were pretreated with SAL-CBD displayed a suppression of LiCl-induced conditioned gaping reactions during the drug-free test ($p < 0.05$). Groups pretreated with either receptor antagonist prior to CBD did not display suppressed conditioned gaping reactions.

Intra-DRN WAY100635 reversed the suppressive effect of systemic CBD on conditioned gaping in rats: The accurate injector tip placements (circles) are presented in the Panel A of Figure 4. The tips of the injectors were located in the DRN between 2.04 and 0.84 mm anterior to the interaural line for a total of 29 rats. A total of 20 rats had placements outside the DRN. The rats in group WAY-CBD with inaccurate placements (triangles) were included in the analysis as a separate group (WAY-CBD-OUT; n=5) for comparison with those receiving the receptor antagonist in the DRN. Panel B presents a representative photomicrograph of the tip/track of the injector in the DRN.

When administered intracranially to the DRN, the 5-HT_{1A} receptor antagonist WAY100635 prevented the suppression of LiCl-induced gaping by CBD. As seen in Figure 5, there was a significant effect of pretreatment group, $F(4, 28) = 4.4$; Groups SAL-CBD and WAY-CBD-OUT displayed fewer gaping reactions than all other groups (p's < 0.05).

Intra-DRN administration of CBD-induced suppression of LiCl-induced conditioned gaping, reversal by systemic administration of 5-HT_{1A} receptor antagonist: The injector tip placements (circles) are presented in the Panel A of Figure 6. The tips of the injectors were located in the DRN between 2.04 and 0.84 mm anterior to the interaural line for a total of 24 rats. Only one rat in the CBD-SAL group had his guide cannula placed outside of the DRN and this rat did not show the CBD-induced suppression of gaping, but was not included in the analysis. Panel B presents a representative photomicrograph of the tip/track of the injector in the DRN.

When administered intracranially to the DRN, CBD suppressed LiCl-induced gaping and this effect was blocked by systemic administration of the 5-HT_{1A} receptor antagonist WAY100635. As seen in Figure 7, there was a significant effect of pretreatment group, $F(3,20) = 11.0$; $p < 0.001$; Group CBD-SAL displayed fewer gaping reactions than all other groups (p's < 0.001).

CBD enhanced the ability of a 5-HT_{1A} receptor agonist to stimulate [^{35}S]GTPγS binding to rat brainstem membranes: There is already evidence that CBD, albeit at the rather high concentration of 16 µM, can directly bind to and activate human 5-HT_{1A} receptors that have been transfected into Chinese hamster ovary cells (Russo *et al.*, 2005). However, the ability of lower concentrations of this cannabinoid to activate 5-HT_{1A} receptors *in vitro* when they are expressed naturally at physiological levels in rat brainstem membranes has not been investigated before. Accordingly, we sought for evidence that CBD can directly target 5-HT_{1A} receptors in rat brainstem when administered *in vitro* at concentrations ranging from 1 nM to 10 µM.

First, we compared the abilities of CBD and the 5-HT_{1A} receptor-selective agonist, 8-OH-DPAT, to displace [^{3}H]8-OH-DPAT from specific binding sites on rat brainstem membranes. These experiments showed that at concentrations of up to 10 μM, CBD does not share the ability of 8-OH-DPAT to induce such displacement (Figure 8). Since 5-HT_{1A} receptors signal through $G_{i/o}$ proteins (Alexander *et al.*, 2008), we also compared the abilities of 8-OH-DPAT and CBD to stimulate [^{35}S]GTPγS binding to rat brainstem membranes in a concentration-related manner. We found that 8-OH-DPAT can indeed induce such stimulation, and also, that this effect could be potently antagonized by the 5-HT_{1A} receptor-selective antagonist, WAY100635 (Figure 9). In contrast, no detectable stimulation was observed in response to any of the concentrations of CBD used (Figure 9). We therefore decided to investigate whether the stimulatory effect of 8-OH-DPAT on [^{35}S]GTPγS binding could be enhanced by CBD. We did this to explore the possibility that 5-HT_{1A} receptor antagonists reduce CBD-induced anti-nausea-like effects, because CBD augments activation of 5-HT_{1A} receptors in the brainstem by endogenously released 5-HT. As shown in Figure 10, we found that at 100 nM, CBD produced an upward shift in the log concentration response curve of 8-OH-DPAT that resulted in a statistically significant increase in the E_{max} but not the EC_{50} of this 5-HT_{1A} receptor-selective agonist. However, CBD did not increase the E_{max} of 8-OH-DPAT at 1, 10 or 31.6 nM or at 1 μM (Figure 10). We also found that 100 nM CBD caused [^{35}S]GTPγS binding to rise significantly above the basal level (zero) in the presence of 10^{-14} and 10^{-12}M 8-OH-DPAT, but not in the presence of 10^{-16}M 8-OH-DPAT (P < or >0.05; 1-sample t-test; n=7). The mean values for percent stimulation of [^{35}S]GTPγS

binding by 100 nM CBD in the presence of 10^{-16}, 10^{-14} and 10^{-12}M 8-OH-DPAT were 3.3 ± 1.5%, 9.5 ± 1.8% and 12.2 ± 2.7%, respectively.

There is good evidence that some compounds that enhance the ability of receptor agonists to activate certain GPCRs do so by targeting allosteric sites on these receptors in a manner that slows the rate at which these receptor agonists dissociate from their receptors (Christopoulos and Kenakin, 2002). Accordingly, since there is also evidence for the presence of an allosteric site on the 5-HT_{1A} receptor (Barrondo and Salles, 2009), we investigated the ability of an 8-OH-DPAT-potentiating concentration of CBD (100 nM) to alter the rate at which [^{3}H]8-OH-DPAT dissociates from specific binding sites in rat brainstem membranes. Our experiments showed that the mean dissociation rates of [^{3}H]8-OH-DPAT in the presence of VEH or of 100 nM CBD were not significantly different. More specifically, these values, with their 95% confidence limits shown in parentheses, were 10.0 min (7.1 and 17.0) and 6.0 min (4.4 and 9.4), respectively (n=6).

CBD and 8-OH-DPAT enhanced suppression of LiCl-induced conditioned gaping in rats: Since the *in vitro* data suggested that at 100 nM CBD potentiated the stimulation of [^{35}S]GTPγS binding of 8-0H-DPAT in rat brainstem tissue, we next evaluated the potential of subthreshold doses of CBD enhance suppression of the anti-nausea-like effect of 8-0H-DPAT. When given half the optimal dose during conditioning (Parker *et al.*, 2002; Limebeer and Parker, 2003), both pretreatments suppressed LiCl-induced conditioned gaping reactions at test. The upper half of Figure 11 presents the mean number of conditioned gaping reactions elicited on the test trial for each group in

Experiment A. There was a significant effect of group $F(3, 28) = 3.9$, $p < 0.025$. All groups displayed fewer gaping reactions than VEH-SAL, with no other significant group differences.

When the doses of CBD and 8-OH-DPAT were reduced such that each was ineffective in suppressing LiCl-induced conditioned gaping alone, the combined effect of these two doses interfered with the establishment of conditioned gaping reactions. The lower half of Figure 11 presents the mean number of conditioned gaping reactions elicited on the test trial for each group in Experiment B. There was a significant pretreatment effect of group, $F(3,26) = 5.9$, $p <. 0.01$. As is apparent, group 0.5 CBD-0.005 8-OH-DPAT gaped significantly ($p < 0.05$) less than all other groups, with no other significant group differences.

Discussion

Consistent with previous work, a low dose of systemically administered CBD suppressed nicotine-, LiCl- and cisplatin-induced vomiting in *S. murinus* (Kwiatkowska *et al.*, 2004; Parker *et al.*, 2004) and LiCl-induced conditioned gaping in rats (Parker *et al.*, 2002). The CBD-induced suppression of vomiting and conditioned gaping was attenuated by pretreatment with 5-HT_{1A} receptor antagonists (WAY100135 and WAY100635). Most interestingly, when WAY100635 was infused directly into the DRN, but not in adjacent structures, the CBD-induced suppression of gaping was also attenuated. The effectiveness of CBD itself to suppress nausea-like behaviors when administered to the DRN and the reversal of this effect by systemic administration of WAY100635 provides additional evidence that the anti-nausea-like action of CBD is

produced by its action on 5-HT_{1A} receptors in the DRN. Since the DRN is a site of the somatodendritic 5-HT_{1A} autoreceptors, stimulation of which reduces the firing rate of 5-HT afferents to terminal regions (Verge *et al.*, 1985; Sotelo *et al.*, 1990), these results suggest that CBD may exert its anti-nausea-like effects by reducing the firing rate of 5-HT afferents to terminal forebrain regions. It is likely that these effects were mediated by CBD-induced augmentation of the action of endogenous 5-HT on the 5-HT_{1A} receptor, because CBD enhanced the action of 8-OH-DPAT in both *in vitro* (% stimulation of [^{35}S]GTPγS binding above basal) and *in vivo* (conditioned gaping) experiments, but did not have a direct agonist action on the 5-HT_{1A} receptor in the brainstem preparation.

Although rats (unlike shrews) are not capable of vomiting, they do exhibit conditioned gaping reactions in response to oral infusion of a flavour that has previously been paired with illness (Grill and Norgren, 1978a; Grill and Norgren, 1978b). These gaping reactions are only produced by drugs that induce vomiting in other emetic species, such as shrews (Parker, 2003; Parker *et al.*, 2008). In fact, Travers and Norgren (1986) suggest that the muscular movements involved in the gaping response mimic those seen in species capable of vomiting. Previously we found that the anti-emetic drug, OND (a 5-HT_3 receptor antagonist), suppressed LiCl-induced conditioned gaping in rats, presumably by reducing the nausea produced by the emetic treatment (Limebeer and Parker, 2000). Consistent with the findings of the present study, the 5-HT_{1A} receptor agonist, 8-OH-DPAT, also interfered with LiCl- (Limebeer and Parker, 2003) and fluoxetine-induced gaping in rats (Limebeer *et al.*, 2009) as well as toxin-induced vomiting in other species (e.g., Lucot and Crampton, 1989; Andrews *et al.*, 1996; Javid and Naylor, 2006). Of most relevance, depletion of forebrain 5-HT induced by 5,7-

Dihydroxytryptamine (5,7-DHT) lesions of the median raphe nucleus (MRN) and the DRN, also prevented LiCl-induced conditioned gaping reactions (Limebeer *et al.*, 2004), that rely on an intact forebrain (Grill and Norgren, 1978a). Forebrain 5-HT may, therefore, be critical for the establishment of these nausea-like behaviours in rats.

The therapeutic effects of CBD on ischemic injury (Hayakawa *et al.*, 2004; Mishima *et al.*, 2005; Hayakawa *et al.*, 2007a), hepatic encephalopathy (Magen *et al.*, 2010), anxiety (Campos and Guimaraes, 2008a; Gomes *et al.*, 2011) and depression (Zanelati *et al.*, 2010) are each attenuated by pretreatment with 5-HT_{1A} receptor antagonists. Here we report that the CBD-induced suppression of vomiting in shrews and conditioned gaping in rats is also reversed by 5-HT_{1A} receptor antagonists. Furthermore, the site of action of the anti-nausea-like effect of CBD appears to be the somatodendritic 5-HT_{1A} autoreceptors in the DRN, which have been reported to reduce the firing rate of 5-HT afferents to terminal forebrain regions (Verge *et al.*, 1985; Sotelo *et al.*, 1990). These results suggest that CBD may exert its anti-nausea-like effects by reducing the release of 5-HT in terminal forebrain regions (as yet to be identified).

Since we found that CBD enhances the ability of the 5-HT_{1A} receptor-selective agonist, 8-OH-DPAT, to stimulate [^{35}S]GTPγS binding to rat brain membranes with significant potency, CBD may suppress LiCl-induced conditioned gaping in rats by augmenting activation of 5-HT_{1A} receptors in the brainstem produced by endogenously released 5-HT. Indeed, this hypothesis was supported by the enhanced suppressive effects of subthreshold doses of 8-OH-DPAT and CBD on suppression of LiCl-induced conditioned gaping in rats. As well, this hypothesis is strengthened further by our finding that the concentration-response curve of CBD for the production of its *in vitro* effect on

[^{35}S]GTPγS binding is bell-shaped, consistent with the 5-HT$_{1A}$ mediated bell-shaped dose-response curves of its effects on emesis (Kwiatkowska *et al.*, 2004), nausea-like-behavior (Rock *et al.*, 2008), ischemic injury (Mishima *et al.*, 2005), anxiety (Campos and Guimaraes, 2008a) and depression (Zanelati *et al.*, 2010).

Our finding that the concentration of CBD (100 nM) that potentiated 8-OH-DPAT in the [^{35}S]GTPγS binding assay performed with rat brainstem membranes did not significantly alter the rate at which [^{3}H]8-OH-DPAT dissociates from specific binding sites in these membranes does not support the hypothesis that 100 nM CBD potentiates 8-OH-DPAT-induced activation of 5-HT$_{1A}$ receptors in an allosteric manner. However, this hypothesis cannot yet be entirely excluded since it is also possible that the action of an agonist at its receptor could be enhanced by compounds that act on an allosteric site to increase the rate at which the agonist binds to its receptor and/or the intensity of receptor signaling that is induced by such binding (Christopoulos and Kenakin, 2002). That CBD might act in this way to potentiate 5-HT$_{1A}$ receptor activation by 8-OH-DPAT, or indeed by 5-HT, merits further investigation. It will also be important to establish whether CBD produces such potentiation by directly targeting 5-HT$_{1A}$ receptors/allosteric sites or by acting on a different target which then somehow augments 5-HT$_{1A}$ receptor activation by 8-OH-DPAT through an indirect mechanism. There is, therefore, a need for further experiments directed at investigating whether CBD can potentiate 8-OH-DPAT in a cell line that expresses only 5-HT$_{1A}$ receptors.

The anti-emetic potential of CBD, not only depends upon its dose, but also upon the nature of the inducing stimulus. In Experiment 3, CBD suppressed vomiting produced by 20 mg/kg cisplatin, but the suppression of vomiting was surmounted by the

greater emetic efficiency of the higher dose of 40 mg/kg of cisplatin, suggesting that CBD may not be effective in reducing nausea produced by highly emetogenic therapies. Furthermore, CBD does not appear to attenuate vomiting produced by activation of the vestibular system by motion (Cluny *et al.*, 2008). This inconsistency may be due to differing neuronal pathways involved in the induction of emesis by these emetogenic stimuli, with the vagal (cisplatin) and blood borne (LiCl and nicotine) activation of the area postrema (e.g., Leslie and Reynolds, 1992) that are not essential to the development of motion sickness.

Interestingly, Yang *et al.* (2010) have reported that CBD acts as an allosteric inhibitor of 5-HT_{3A} receptor mediated currents in *Xenopus laevis* oocytes. Since 5-HT_3 receptor antagonists are highly effective anti-emetic/anti-nausea agents (eg. Kwiatkowska *et al.*, 2004; Limebeer *et al.*, 2009) it is also likely that the allosteric inhibition of the 5-HT_3 receptor may be important in the potential of CBD to regulate nausea and vomiting.

It is not clear which forebrain regions are critical for the effects of CBD on conditioned gaping reactions, but a likely candidate is the insular cortex; the site of convergence of gustatory and interoceptive information (Cechetto and Saper, 1987). Indeed, ablation of the rat insular cortex prevents the establishment of LiCl-induced gaping (Kiefer and Orr, 1992), unlike lesions of the basolateral or central amygdala (Rana and Parker, 2008). Electrical stimulation of the insular cortex produces vomiting in cats (Kaada, 1951) and humans (Catenoix *et al.*, 2008), as well as a sensation of nausea in humans (Penfield and Faulk, 1955). Reversible lesions (lidocaine) of the rat insular cortex interfere with the unconditioned behaviour of lying on belly (see Parker, 1984) produced by LiCl (Contreras *et al.*, 2007). In order to determine the role of 5-HT

availability in the insular cortex on nausea, future studies will examine the potential of 5,7-DHT lesions of the insular cortex to prevent conditioned gaping reactions in rats.

Acknowledgments

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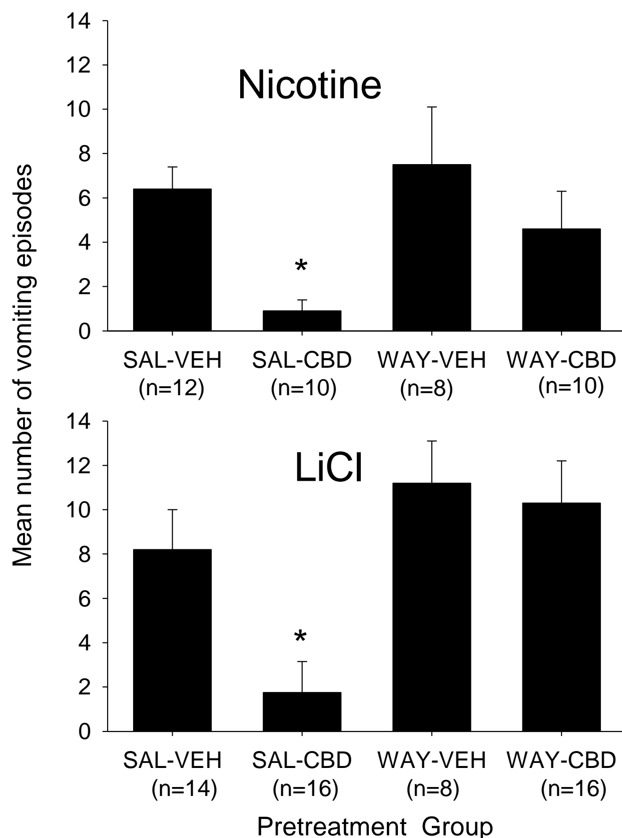


Figure 1. Mean (+ sem) number of vomiting episodes elicited by nicotine (5 mg/kg, sc) and LiCl (390 mg/kg, ip), among shrews pretreated with SAL or WAY100135 (10 mg/kg, ip) and VEH or CBD (5 mg/kg, sc). Asterisks indicate significant difference from all other groups at $p < 0.05$. The number of shrews in each condition is indicated in parentheses below pretreatment group name. The male (M) to female (F) (M:F) ratio within each treatment group for nicotine treated groups is SAL-VEH (7:5), SAL-CBD (6:4), WAY-VEH (4:4), WAY-CBD (6:4) and shrew age at the time of experimentation ranged from 44 d to 178 d with a mean of 96d. The M:F ratio within each treatment group for LiCl treated groups is: SAL-VEH (6:8), SAL-CBD (8:8), WAY-VEH (4:4), WAY-CBD (8:8).

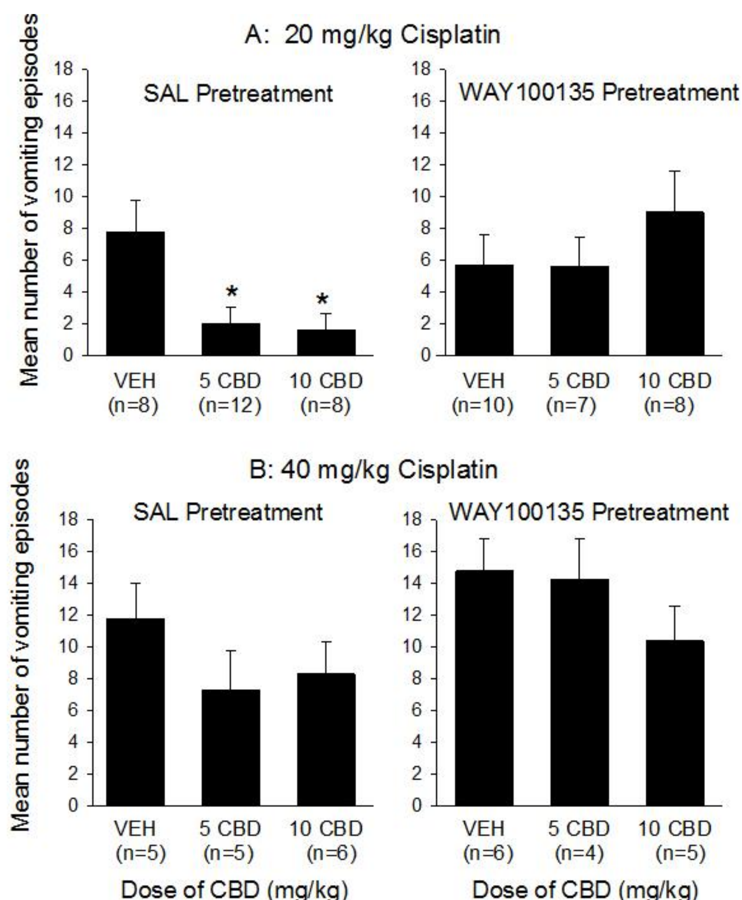


Figure 2. Mean (±sem) number of vomiting episodes elicited by 20 mg/kg cisplatin (Panel A) or 40 mg/kg cisplatin (Panel B) by shrews pretreated with SAL (right hand sections) or WAY100135 (10 mg/kg, ip; left hand sections) prior to the second pretreatment of VEH, 5 mg/kg CBD or 10 mg/kg CBD. Asterisks indicate significant differences at $p < 0.05$. The number of shrews in each group is indicated in parentheses. The M:F ratio within each treatment group for 20 mg/kg cisplatin treated shrews in Panel A is: SAL-VEH (4:4), SAL-5 CBD (6:6), SAL-10 CBD (5:3), WAY100135-VEH (5:5), WAY100135-5 CBD (4:3), WAY100135-10 CBD (3:5). For Panel B the M:F ratio is: SAL-VEH (3:2), SAL-5 CBD (2:3), SAL-10 CBD (3:3), WAY100135-VEH (3:3), WAY100135-5 CBD (2:2), WAY100135-10 CBD (2:3).

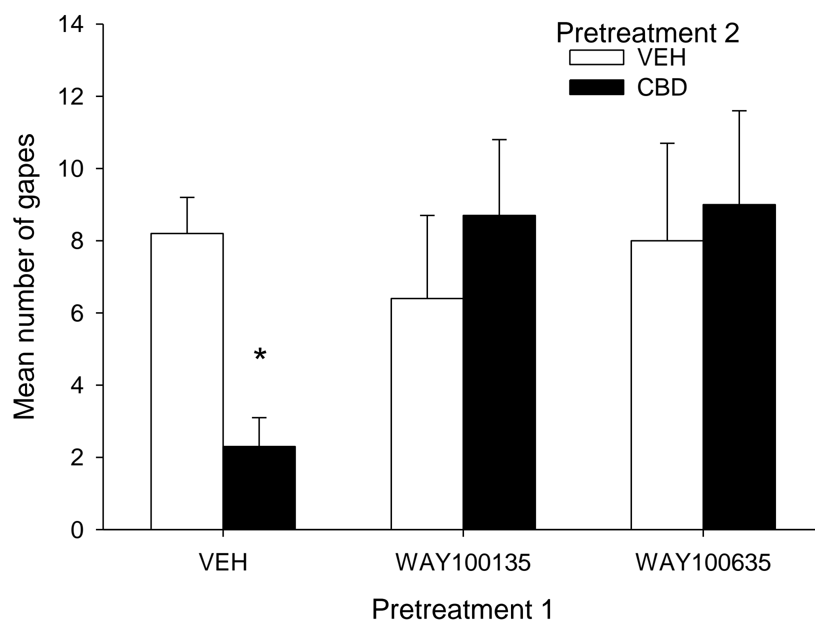


Figure 3. Mean (±sem) number of gapes elicited by LiCl-paired saccharin solution during the drug-free test trial. During conditioning, rats were pretreated with systemic SAL, WAY100135 (10 mg/kg, ip) or WAY100635 (0.1 mg/kg, ip) 15 min prior to systemic VEH or CBD (5 mg/kg, sc). Asterisks indicate significant difference at $p < 0.05$.

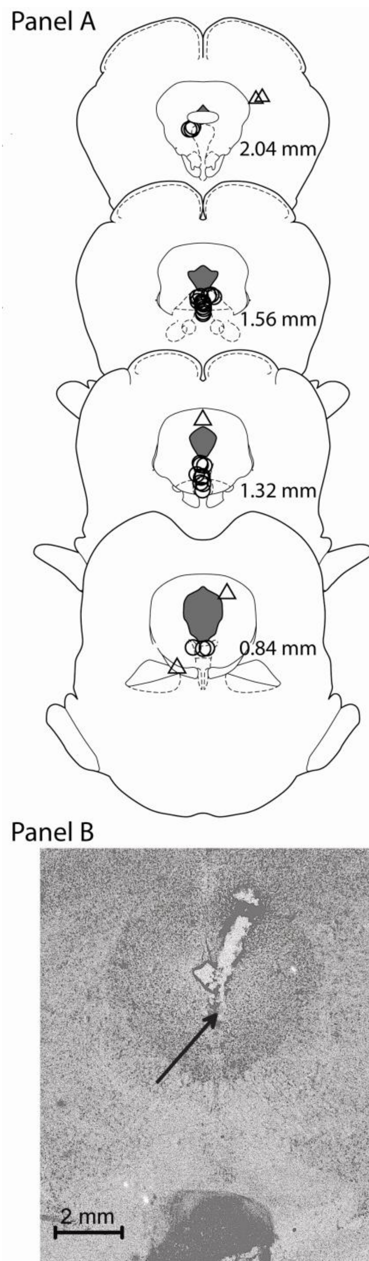


Figure 4. Panel A: Traces of infusion sites in the DRN (circles) and out of the DRN for Group WAY-CBD (triangles) on drawings of coronal sections. Numbers indicate sections relative to interaural zero. Panel B: A representative photomicrograph of the tip/track of the injector in the DRN.

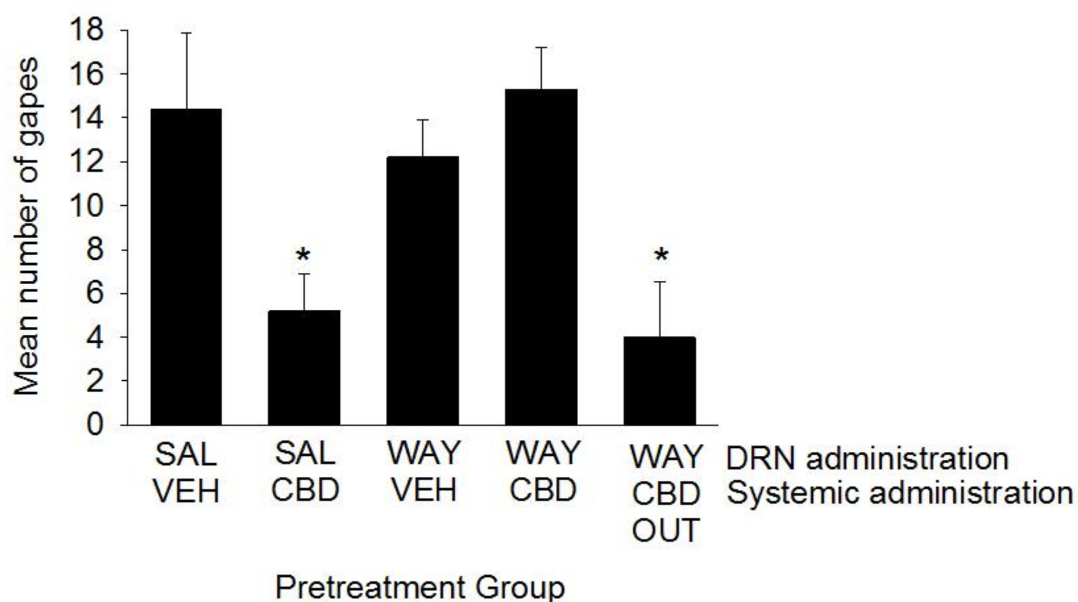


Figure 5. Mean (±sem) number of gapes elicited by LiCl-paired saccharin solution during the drug free test. During conditioning, rats were pretreated with intracranially administered SAL or WAY100635 (21 ng) into the DRN 15 min prior to systemic VEH or CBD (5 mg/kg, sc.) An additional group of rats with the cannula outside of the DRN, intracranially administered WAY and systemically administered CBD is also presented. Asterisks indicate significance at $p < 0.05$.

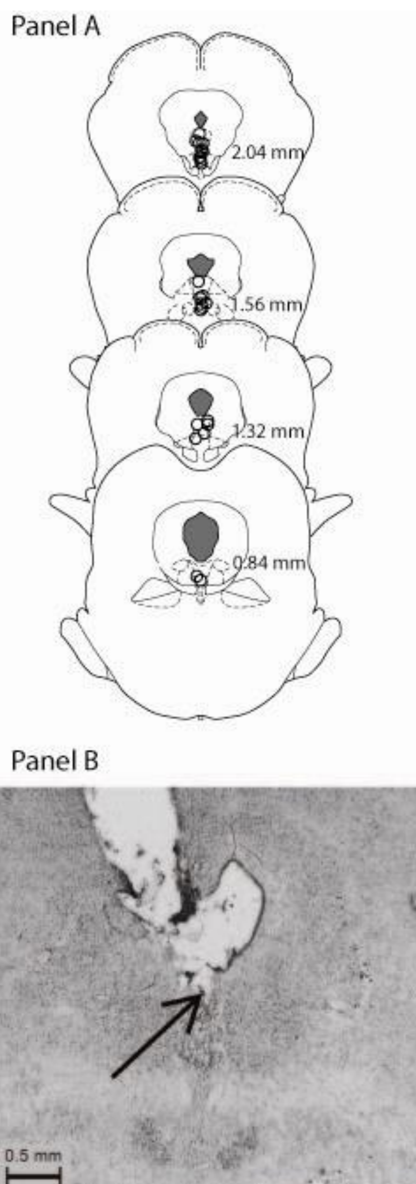


Figure 6. Panel A: Traces of infusion sites in the DRN for all groups (circles). Numbers indicate sections relative to interaural zero. Panel B: A representative photomicrograph of the tip/track of the injector in the DRN.

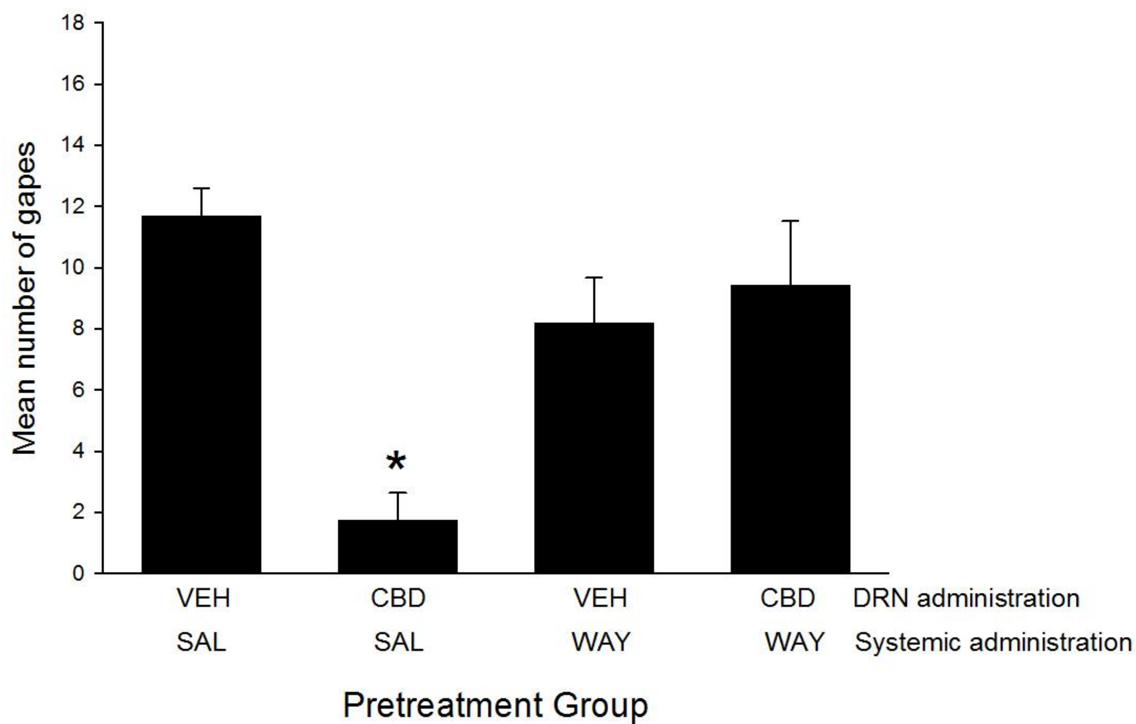


Figure 7. Mean (± sem) number of gapes elicited by LiCl-paired saccharin solution during the drug-free test. During conditioning, rats were pretreated with systemic SAL or WAY100635 (0.1 mg/kg, ip.) 15 min prior to intracranially administered VEH or CBD (10 μg) into the DRN. Asterisks indicate significance at $p < 0.001$.

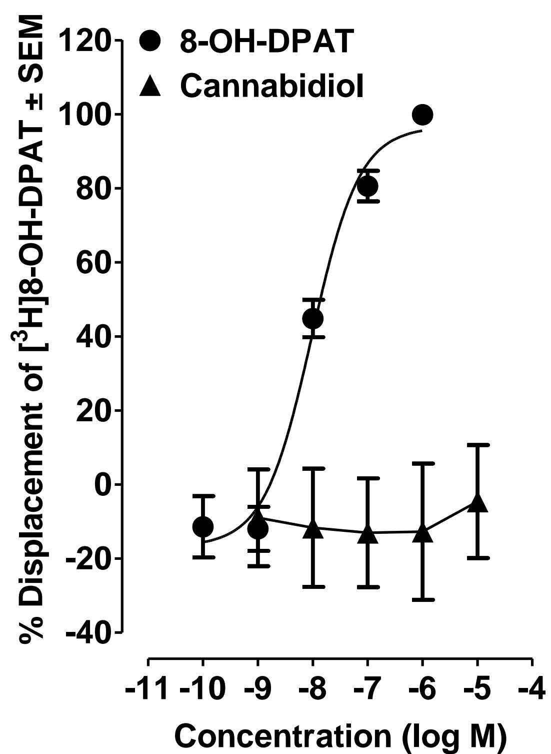


Figure 8. Effects of 8-OH-DPAT and CBD on specific binding of [^{3}H]8-OH-DPAT to rat brainstem membranes (n=6). The IC_{50} and E_{max} values of 8-OH-DPAT for its displacement of [^{3}H]8-OH-DPAT, with 95% confidence limits shown in parentheses, were 9.6 nM (5.6 and 16.3 nM) and 96.6% (86.8 and 106.5%), respectively. Symbols represent mean values ± SEM.

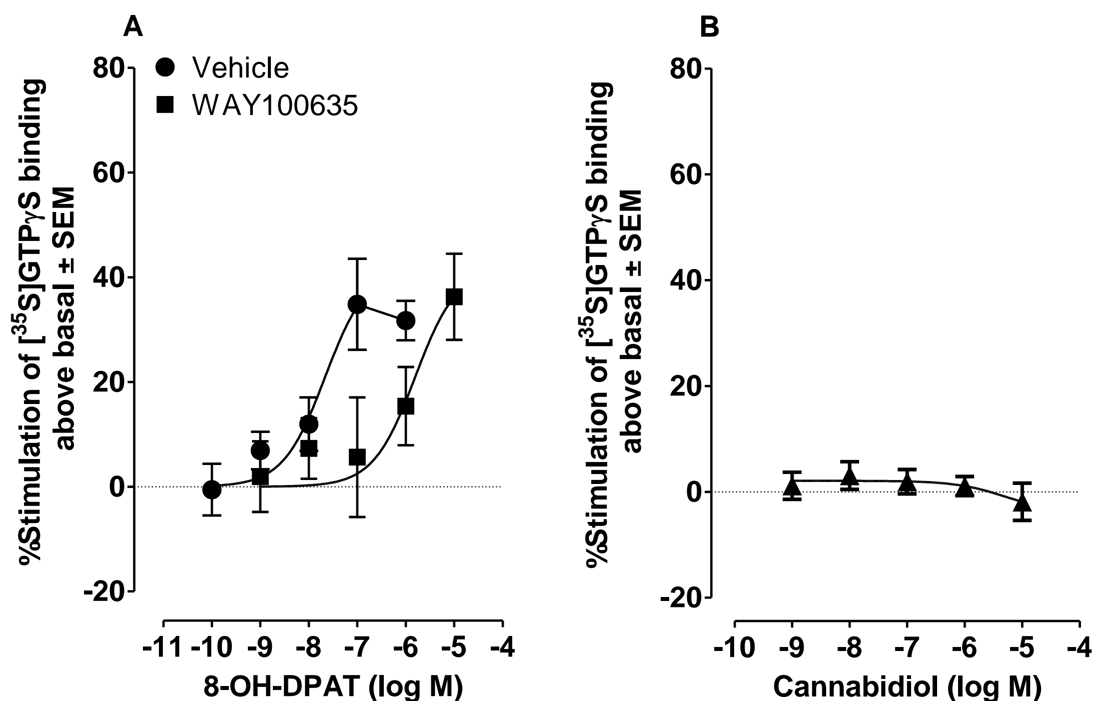
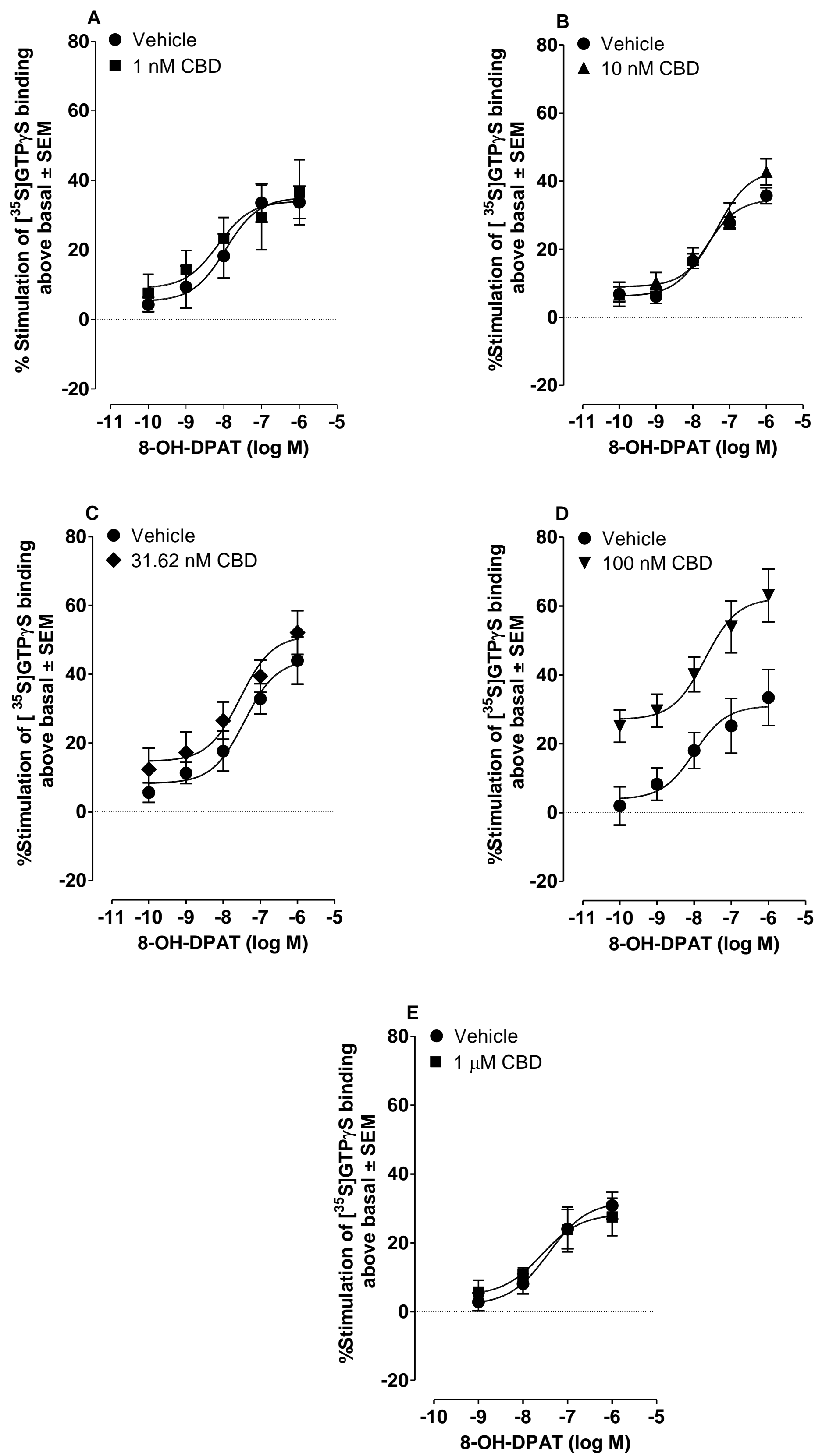


Figure 9. Effects of (A) 8-OH-DPAT in the presence of DMSO (Vehicle; n=7) or 100 nM WAY100635 (n=7) and (B) CBD (n=4) on $[^{35}S]GTP\gamma S$ binding to rat brainstem membranes. Mean EC_{50} values for 8-OH-DPAT, with 95% confidence limits shown in parentheses were 21.7 nM (7.6 and 61.9 nM), in the presence of VEH and 1565 nM (390 and 6277 nM) in the presence of WAY100635. Symbols represent mean values ± SEM.

Figure 10. Effect of 8-OH-DPAT on [^{35}S]GTPγS binding to rat brainstem membranes in the presence of DMSO (VEH) or CBD. Mean E_{max} values for 8-OH-DPAT in panels A, B, C, D and E with 95% confidence limits shown in parentheses were 35.1% (26.6 and 43.5%; n=8), 34.8% (30.3 and 39.3%; n=8), 44.3% (33.4 and 55.1%; n=6), 32.4% (22.8 and 41.9%; n=10) and 32.0% (22.9 and 41.1%; n=9), respectively, in the presence of VEH and 34.0% (22.2 and 45.9%; n=8), 43.3% (35.5 and 51.0%; n=6), 51.3% (39.3 and 63.3%; n=6), 62.3% (51.0 and 73.6%; n=10) and 28.4% (18.5 and 38.3%; n=9), respectively, in the presence of 1nM, 10 nM, 31.6 nM, 100 nM or 1 µM CBD. Corresponding mean EC_{50} values for 8-OH-DPAT were 11.2 nM (1.9 and 66.7 nM), 22.0 nM (9.5 and 50.9 nM), 37.3 nM (8.1 and 172.7 nM), 12.7 nM (1.2 and 139 nM) and 37.4 nM (6.3 and 222 nM), respectively, in the presence of VEH and 7.1 nM (0.4 and 126.3 nM), 45.7 nM (14.1 and 149 nM), 28.6 nM (40.5 and 180.1 nM), 19.7 nM (2.8 and 139 nM) and 26.2 nM (2.1 and 332 nM), respectively, in the presence of 1nM, 10 nM, 31.6 nM, 100 nM or 1 µM CBD. Symbols represent mean values ± SEM.


A
Vehicle
1 nM CBD
% Stimulation of [35S]GTPγS binding above basal ± SEM
8-OH-DPAT (log M)
B
Vehicle
10 nM CBD
%Stimulation of [35S]GTPγS binding above basal ± SEM
8-OH-DPAT (log M)
C
Vehicle
31.62 nM CBD
%Stimulation of [35S]GTPγS binding above basal ± SEM
8-OH-DPAT (log M)
D
Vehicle
100 nM CBD
%Stimulation of [35S]GTPγS binding above basal ± SEM
8-OH-DPAT (log M)
E
Vehicle
1 μM CBD
%Stimulation of [35S]GTPγS binding above basal ± SEM
8-OH-DPAT (log M)

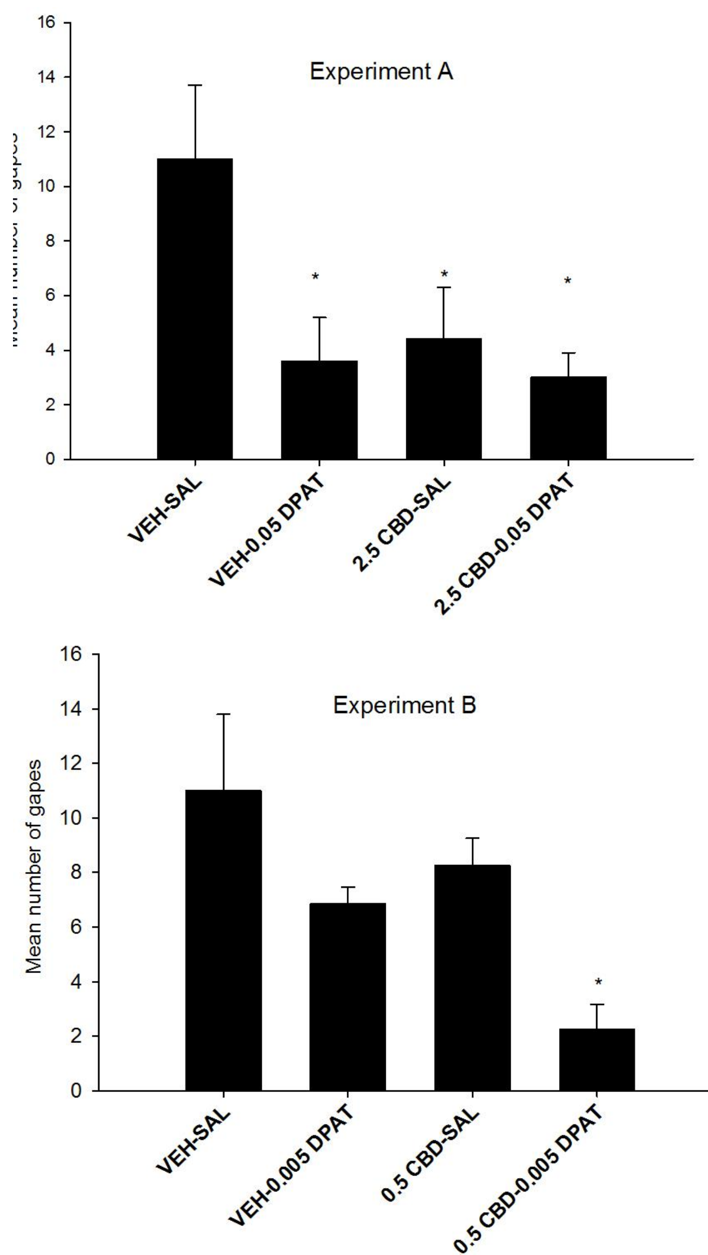


Figure 11. Mean (±sem) number of gapes elicited by LiCl-paired saccharin solution during the drug-free test trial in Experiments A and B. During conditioning, rats were pretreated with systemic CBD (20.5 mg/kg in Experiment A or 00.5 mg/kg in Experiment B) 15 min prior to SAL or 8-OH-DPAT (0.05 mg/kg in Experiment A or 0.005 mg/kg in Experiment B). Thirty min later, all rats were conditioned with 0.1% saccharin, followed immediately by LiCl. Asterisks indicate significant difference at $p < 0.05$.

CHAPTER 3

Psychopharmacology, 2011, 215: 505-512.

Interaction between Non-Psychotropic Cannabinoids in Marihuana: Effect of Cannabigerol (CBG) on the Anti-Nausea or Anti-Emetic Effects of Cannabidiol (CBD) in Rats and Shrews

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Rationale: The interaction between two non-psychotropic cannabinoids, cannabidiol (CBD) and cannabigerol (CBG), which have been reported to act as a 5-hydroxytryptamine 1A (5-HT_{1A}) receptor agonist and antagonist, respectively, was evaluated. *Objective:* To evaluate the potential of CBG to reverse the anti-nausea-like, anti-emetic effects of CBD. *Materials and methods:* In Experiment 1, rats were pretreated with CBG (VEH, 1, 5 and 10 mg/kg, ip), 15 min prior to being treated with CBD (Experiment 1a: VEH or 5 mg/kg, ip) or 8-OH-DPAT (Experiment 1b: VEH or 0.01 mg/kg, sc). Thirty min later, all rats received a pairing of 0.1% saccharin solution and LiCl (20 ml/kg of 0.15 M, ip). Seventy-two hr later, the rats received a drug-free Taste Reactivity (TR) test with saccharin to evaluate the effects of the treatments on the establishment of conditioned gaping reactions (a model of nausea). As well, conditioned saccharin avoidance was measured. In Experiment 2, *Suncus murinus* were injected with CBG (5 mg/kg, ip) or VEH 15 min prior to CBD (5 mg/kg) or VEH and 30 min later were injected with LiCl (60 ml/kg of 0.15 M, ip) and the number of vomiting episodes was measured. *Results:* CBD (5 mg/kg) suppressed conditioned gaping in rats and vomiting in shrews, which were reversed by pretreatment with all doses of CBG evaluated. CBG also prevented the anti-nausea-like effects of 8-OH-DPAT. *Conclusions:* Interactions between moderate doses of CBG and CBD may oppose one another at the 5-HT_{1A} receptor in the regulation of nausea and vomiting.

Key words: Nausea, vomiting, cannabinoids, cannabigerol, cannabidiol, serotonin, 5-HT_{1A}, phytocannabinoid

Introduction

Considerable evidence suggests that cannabinoids are effective in the treatment of nausea and vomiting (see Abrahamov *et al.*, 1995; Tramer *et al.*, 2001; Parker *et al.*, 2005; Parker and Limebeer, 2008). The primary psychoactive cannabinoid found in marihuana, Δ^9-tetrahydrocannabinol (Δ^9-THC, Gaoni and Mechoulam, 1964a), has been shown to suppress nausea and vomiting in humans (eg., Sallan *et al.*, 1975; Orr *et al.*, 1980; see Tramer *et al.*, 2001) and vomiting in other animals (eg. for review see Parker *et al.*, 2005; Parker and Limebeer, 2008). As well, Δ^9-THC interferes with the establishment and expression of conditioned gaping reactions (a measure of nausea-like behaviour) in the Taste Reactivity (TR) test (Grill and Norgren, 1978b) in rats (Limebeer and Parker, 1999; Parker and Mechoulam, 2003; Parker *et al.*, 2004). The effects of Δ^9-THC and other synthetic psychoactive cannabinoids (eg. HU-210, WIN 55, 212-2, CP 55,940) on both nausea (Parker *et al.*, 2003) and vomiting (Darmani, 2001b; Van Sickle *et al.*, 2001; Kwiatkowska *et al.*, 2004; Parker *et al.*, 2004) are mediated by their action on the Cannabinoid 1 (CB_1) receptor, because these effects are reversed by pretreatment with the CB_1 receptor antagonist/inverse agonist, rimonabant.

Another prominent cannabinoid found in marihuana is cannabidiol (CBD) which is non-psychotropic (Mechoulam, 1970; see also Mechoulam *et al.*, 2007). CBD has a wide range of therapeutic effects (see Pertwee, 2004; Mechoulam *et al.*, 2007 for reviews) including the suppression of nausea-like behaviour and vomiting (see Parker and Limebeer, 2008). Low doses (but not high doses of 20 mg/kg or greater, Kwiatkowska *et al.*, 2004; Parker *et al.*, 2004; Darmani *et al.*, 2007) of CBD interfere with conditioned gaping reactions in rats (Parker *et al.*, 2002; Parker *et al.*, 2003; Rock *et*

al., 2008), as well as vomiting (Kwiatkowska *et al.*, 2004; Parker *et al.*, 2004) and conditioned retching (Parker *et al.*, 2006) in shrews. This suppression of nausea-like behaviour and vomiting is not mediated by action at the CB_1 or CB_2 receptors (see Mechoulam *et al.*, 2002; Parker *et al.*, 2004). Russo *et al.* (2005) showed that micromolar concentration of CBD displaces $[^3H]$8-OH-DPAT (a 5-HT_{1A} receptor agonist) from cloned human 5-HT_{1A} receptors *in vitro*, increases GTP binding to the receptor coupled G protein, G_i, and reduces cAMP production, all characteristic of a receptor agonist. These findings suggest that CBD may act as a 5-HT_{1A} receptor agonist. Interestingly, the neuroprotective effects of CBD are reversed by WAY100135, a 5-HT_{1A} receptor antagonist (Mishima *et al.*, 2005) and not by rimonabant (Hayakawa *et al.*, 2004). Recent experiments by Rock *et al.* (2011) found that WAY100135 suppresses the ability of CBD to reduce nicotine, cisplatin, and lithium-induced vomiting in shrews and (as well as the more selective receptor antagonist WAY100635) to interfere with the establishment of LiCl-induced conditioned gaping in rats. This is consistent with earlier work that very low doses (0.001-0.01 mg/kg) of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, suppressed LiCl-induce gaping in rats (Limebeer and Parker, 2003) and vomiting in cats (Lucot and Crampton, 1989). Furthermore, Rock *et al*. (2011) found that when delivered directly to the dorsal raphe nucleus (DRN), CBD prevented LiCl-induced conditioned gaping reactions in rats and WAY100635 delivered to the DRN reversed the effects of systemic CBD. Thus, it appears that CBD may exert its anti-emetic and anti-nausea effects by agonism of the 5-HT_{1A} somatodendritic autoreceptors in the raphe.

Another non-psychoactive cannabinoid found in marihuana is cannabigerol (CBG, Gaoni and Mechoulam, 1964b; Mechoulam, 1970). CBG has shown potential for

the treatment of glaucoma (Colasanti *et al.*, 1984; Colasanti, 1990), psoriasis (Wilkinson and Williamson, 2007), and pain (De Petrocellis *et al.*, 2008). CBG also shows antitumor activity *in vitro* (Ligresti *et al.*, 2006) and displays antibacterial properties (Eisohly *et al.*, 1982), making it a potential candidate for the treatment of antibiotic resistant bacteria (Appendino *et al.*, 2008). Recently, Maor et al. (2005) reported that the synthetic dimethylheptyl homolog of cannabigerol (CBG-DMH) displays hypotensive potential. The mechanism of action of CBG is still under investigation. Preliminary evidence suggests that CBG may act as a moderately potent 5-HT_{1A} receptor antagonist (Cascio *et al.*, 2010), as well as having other effects. If CBG indeed acts as a 5-HT_{1A} receptor antagonist, it may block the anti-emetic, anti-nausea-like effects of CBD

It is of therapeutic interest to investigate the role of cannabinoids in the regulation of nausea and vomiting, as well as the effects of their interactions on such regulation. The following experiments evaluated the potential of CBD and CBG to regulate nausea-like behaviour in rats and vomiting in the *Suncus murinus*. If the hypothesis is correct that CBG acts *in vivo* as a 5-HT_{1A} receptor antagonist, then the anti-nausea-like and anti-emetic effects of CBD should be blocked by pretreatment with CBG. As well, the potential of CBG to reverse the anti-nausea-like effect of the classic 5-HT_{1A} receptor antagonist, 8-OH-DPAT (Limebeer and Parker, 2003) was also assessed.

Materials and Methods:

Animals

Animal procedures were according to the Canadian Council on Animal Care (CCAC) and the National Institutes of Health guidelines. The protocols were approved

by the Institutional Animal Care Committee, which is accredited by the CCAC. Male Sprague-Dawley rats, weighing between 262-330 g on the day of conditioning, obtained from Charles River Laboratories (St Constant, Quebec) were used in Experiment 1. They were single-housed in Plexiglas cages in the colony room at an ambient temperature of 21°C with a 12/12 light/dark schedule (lights off at 8 AM) and maintained on *ad-libitum* food and water. Male *S. murinus* (house musk shrews) bred and raised at the University of Guelph colony were used in Experiment 2. They were single-housed in cages in a colony room at an ambient temperature of 21°C on a 14/10 light dark schedule (lights off at 9 PM) as described in Parker et al. (2009).

Drugs

Both CBG and CBD were prepared in a vehicle (VEH) solution of 45% 2-hydroxypropyl-β-cyclodextrin (Sigma) with sterile water. CBD (provided by Dr. Raphael Mechoulam, Hebrew University) was prepared as a 5 mg/2 ml solution of the VEH and administered at a volume of 2 ml/kg, ip (5 mg/kg), the optimal dose previously demonstrated to interfere with conditioned gaping in rats (Parker *et al.*, 2002) and vomiting in shrews (Parker *et al.*, 2004). CBG (also provided by Dr. Raphael Mechoulam, Hebrew University) was prepared as 1 mg/2 ml, 5 mg/2 ml, and 10 mg/2 ml in Experiment 1a and as a 5 mg/2ml solution in Experiments 1b and 2 and was always administered at a volume of 2 ml/kg, ip. The 8-OH-DPAT was prepared in saline at a concentration of 0.01 mg/ml and administered at a volume of 1 ml/kg, sc. Lithium chloride (LiCl, Sigma) was prepared in a 0.15 M solution with sterile water and

administered ip at a volume of 20 ml/kg (127.2 mg/kg) in Experiments 1a and 1b and 60 ml/kg (390 mg/kg) in Experiment 2.

Apparatus

A clear Plexiglas chamber (22.5 x 26 x 20 cm) with an opaque Plexiglas lid was placed on a table with a clear Plexiglas top for Taste Reactivity (TR) procedures with rats and to monitor vomiting in the shrews in different rooms. A mirror beneath the chamber on a 45˚ angle facilitated viewing of the rat's ventral surface. The room was dark with two 60 W white lights on either side of the chamber. A video camera (Sony DCR-HC48) with fire-wire feed to a computer was used to record the behaviour from the mirror beneath the chamber.

Procedures

Experiment 1: Interaction of CBG and CBD or 8-OH-DPAT on LiCl-induced nausea in rats

<u>Experiment 1a: CBG and CBD</u>. All rats were surgically implanted with intra-oral cannulae as described by Limebeer *et al.* (2010). Three days later, the rats received an adaptation trial to the TR procedure. The rats were placed individually into the chamber with their cannulae attached to an infusion pump (Model KDS100; KD Scientific, Holliston, MA) via an infusion tube inserted through the ceiling of the chamber. They

were infused with reverse osmosis water for 2 min at a rate of 1 ml/min, following which they were returned to their home cages.

Twenty-four hr following adaptation, the rats received a conditioning trial in which they were administered a pretreatment and a treatment injection. The pretreatment injection was either VEH or CBG (1 mg/kg, 5 mg/kg, or 10 mg/kg) followed 15 minutes later by a treatment injection of either VEH or CBD (5 mg/kg). This design resulted in the following groups: VEH-VEH (n = 8), and VEH-CBD (n = 9), CBG 1 mg/kg-VEH (n = 8), CBG 1 mg/kg-CBD (n = 8), CBG 5 mg/kg-VEH (n = 9), CBG 5 mg/kg-CBD (n = 8), CBG 10 mg/kg-VEH (n = 8) and CBG 10 mg/kg-CBD (n = 9). Thirty min after the treatment injection, the rats were individually placed in the chamber and intra-orally infused with a 0.1% saccharin solution for 2 min at a rate of 1 ml/min while their orofacial and somatic responses were video-recorded. Immediately following the saccharin infusion, the rats were injected with 20 ml/kg of 0.15 M LiCl and returned to their home cages. Ninety-six hr following the conditioning trial, the rats individually received a single drug-free test trial in which they were returned to the chamber and intra-orally infused with the 0.1% saccharin solution for 2 min (1 ml/min) while their orofacial and somatic responses were video-recorded. The rats were then returned to their home cages.

For two days following the test trial the rats received consumption tests to assess conditioned taste avoidance. At 9 a.m. on the first day, having been water deprived for 17 hr, the rats received two graduated drinking tubes, one with the 0.1% saccharin solution and one with water. The tubes were placed on the lids of the home cages, in the usual location of their water bottles, and the amount of solution consumed was recorded

at 30, 60, 120, 240, and 360 min to obtain a measure of taste avoidance. On the second day, the rats were given an identical test, except that they received only one drinking tube with the saccharin solution and consumption was measured at the same time intervals and additionally at 24 hr.

The videotapes from the conditioning and test trials were scored by observers blind to the experimental condition using “The Observer” (Noldus, NL) event-recording program. The behavioural measure of interest was gaping, the most reliable measure of conditioned nausea (Breslin *et al.*, 1992). Gaping is defined as large openings of the mouth and jaw, with lower incisors exposed.

Conditioned taste avoidance was assessed in both a two bottle test and a single bottle test. For the two bottle test, the amount consumed of 0.1% saccharin solution and water was transformed into a saccharin preference ratio which was defined as the amount of saccharin solution consumed divided by the total amount of saccharin and water consumed (Saccharin solution/[Saccharin solution + Water]).

<u>Experiment 1b: CBG and 8-OHDPAT:</u> Experiment 1b was conducted identically to Experiment 1a, except that during the conditioning trial, the rats were pretreated with either VEH or CBG (5 mg/kg) followed 15 min later by a treatment injection of Saline or 8-OH-DPAT (0.01 mg/kg) with the following groups: VEH-Saline (n=8), CBG-Saline (n=8), VEH-8-OH-DPAT (n=7) or CBG -8-OH-DPAT (n=8). Thirty min later, rats were intraorally infused with 0.1% saccharin solution and were immediately injected with LiCl as in Experiment 1a.

Experiment 2: Interaction of CBG and CBD on LiCl-induced vomiting in shrews

The shrews were moved into the experimental room from the adjacent colony room and given four meal worms in an empty cage 15 min prior to receiving the pretreatment injection of either VEH or CBG (5 mg/kg). Fifteen min later, they were treated with an injection of either VEH or CBD (5 mg/kg), and 30 min later injected with LiCl (390 mg/kg). The shrews were then individually placed into the chamber and the frequency of vomiting episodes (expulsion of fluids from the stomach) displayed over the next 45 min was measured by an observer blind to experimental conditions. The groups were: CBG-CBD (n=8), CBG-VEH (n=8), VEH-CBD (n=11), VEH-VEH (n=11).

Data Analysis.

In Experiments 1a and 1b, the number of gapes displayed by each rat was entered into a two factor ANOVA the factors of pretreatment drug (Experiment 1a: VEH, 1 mg/kg CBG, 5 mg/kg CBG, 10 mg/kg CBG; Experiment 1b: VEH or CBG) and treatment drug (Experiment 1a: VEH or CBD; Experiment 1b: Saline or 8-OH-DPAT). For conditioned taste avoidance, since the intake measures were cumulative and therefore not independent, preference ratios for each time point was entered into a 4 x 2 between groups ANOVA. For Experiment 2, the number of vomiting episodes was entered into a 2 by 2 between groups ANOVA with the factor of pretreatment drug (CBG or VEH) and treatment drug (CBD or VEH). Significance was defined as $p < 0.05$.

Results

Experiment 1: Interaction of CBG and CBD or 8-OH-DPAT on LiCl-induced nausea in rats

Experiment 1a: CBG and CBD. CBD attenuated LiCl-induced conditioned gaping reactions; this attenuated nausea-like behaviour was prevented by pretreatment with 5 or 10 mg/kg of CBG (and marginally by 1 mg/kg of CBG). The lowest dose of CBG also suppressed LiCl-induced conditioned gaping on its own.

Figure 1 presents the mean number of gaping reactions elicited by intra-oral infusion of 0.1% saccharin solution on the conditioning and test trial for each pretreatment (VEH, 1 mg/kg, 5 mg/kg, and 10 mg/kg CBG) and treatment (VEH and CBD) drug. On the test trial, the 4 x 2 between groups ANOVA revealed significant effects of pretreatment, $F(3, 59) = 4.2$; $p = 0.01$, and a significant pretreatment by treatment interaction, $F(3, 59) = 3.4$; $p = 0.024$. As assessed by LSD, for each pretreatment group, CBD significantly attenuated gaping in the VEH pretreatment group ($p < 0.001$), but not in any other pretreatment group. In addition, separate one-way ANOVAs for each treatment drug revealed a significant effect for both the CBD treatment drug, $F(3, 30) = 3.5$; $p = 0.025$, and the VEH treatment drug, $F(3, 29) = 4.1$; $p = 0.015$. Among the CBD treated rats, those pretreated with VEH displayed significantly fewer gapes than those pretreated with either 5 or 10 mg/kg of CBG (p's < 0.025), but they only marginally differed from those pretreated with 1 mg/kg of CBG ($p = 0.063$).

Among the VEH treated rats, those pretreated with 1 mg/kg CBG displayed significantly fewer gapes than any other pretreatment group (p's < 0.025).

The groups did not differ in mean saccharin preference in the two-bottle test or in mean saccharin consumption in the one bottle test. Figure 2 presents the mean cumulative saccharin preference ratios during the two-bottle test for the various groups across the 360 minutes of testing. Separate 4 x 2 ANOVAs for each time period revealed no significant effects. As well, the mean amount of saccharin solution consumed in the subsequent one-bottle test across 24 hr of testing revealed no significant group differences at any time point (data not depicted).

Experiment 1b: CBG and 8-OH-DPAT. The classic 5-HT_{1A} receptor agonist, 8-OH-DPAT also suppressed LiCl-induced conditioned gaping reactions in rats as has been previously reported (Limebeer & Parker, 2003) and CBG reversed this effect. Figure 3 presents the mean number of gaping reactions elicited by LiCl-paired saccharin solution in Experiment 1b. As is apparent, the 2 x 2 ANOVA revealed a significant pretreatment by treatment interaction, $F(1, 26) = 4.2$; $p < 0.05$. Rats pretreated with VEH displayed fewer gapes during the test when they were treated with 8-OH-DPAT prior to conditioning ($p < 0.01$), but this effect was reversed by pretreatment with CBG. Although not depicted, as in Experiment 1a, the groups did not differ in mean saccharin preference in the two bottle test or in mean saccharin consumption in the one-bottle test.

Experiment 2: Vomiting in shrews

In Experiment 2, CBD suppressed LiCl-induced vomiting in *S. murinus,* an effect which was reversed by pretreatment with CBG. Figure 4 presents the mean number of vomiting episodes elicited by LiCl for each pretreatment/treatment group. The 2 x 2 between groups ANOVA revealed a significant interaction, $F (1, 34) = 5.7$; $p < 0.025$. Subsequent planned comparisons among all groups revealed that Group VEH-CBD displayed significantly less vomiting than any other group (p's < 0.01), which did not differ among themselves.

Discussion

The present results showed that both CBD and 8-OH-DPAT attenuated conditioned gaping produced by LiCl in rats, as has previously been reported (eg. Parker *et al.*, 2002; Limebeer and Parker, 2003). Both effects were prevented by pretreatment with CBG. CBD may produce this anti-nausea-like effect by activating the 5-HT_{1A} receptor, as the 5-HT_{1A} receptor antagonist WAY100135 also prevents CBD's suppression of gaping (Rock *et al.*, 2011). Cascio et al. (2010) reported that at high concentrations CBG antagonizes the 5-HT_{1A} receptor agonist 8-OH-DPAT in [^{35}S]GTPγS binding assays in mouse brain membranes, suggesting that CBG acts as a 5-HT_{1A} receptor antagonist. In support of this interpretation, CBG prevented the suppression of gaping by 8-OH-DPAT. As well, our finding that CBG prevented CBD's suppression of gaping, similar to WAY100135, provides further evidence that CBG may act as a 5-HT_{1A}

receptor antagonist and is also consistent with Cascio et al.'s (2010) finding that CBG is a neutral 5-HT_{1A} receptor antagonist and not an inverse receptor agonist.

In Experiment 2, CBG also prevented the CBD-induced suppression of LiCl-induced vomiting in shrews. Like nausea in rats, the suppression of vomiting produced by CBD is reversed by the 5-HT_{1A} receptor antagonist, WAY100135 (Rock *et al.*, 2011). The effect of CBD on toxin-induced vomiting in shrews is biphasic with low doses (1-10 mg/kg) suppressing vomiting, but higher doses (20-40 mg/kg) either producing no effect (Darmani, 2001b) or actually potentiating vomiting (Kwiatkowska *et al.*, 2004; Parker *et al.*, 2004). The effect of CBD on LiCl-induced conditioned nausea-like behaviour in rats may also be biphasic (eg. Rock *et al.*, 2011).

Interestingly, at the lowest dose evaluated here (but not higher doses), CBG appears to have produced an anti-nausea-like effect among the VEH pretreated rats; the mechanism for this effect is unknown. This finding may, however, be related to the concentration specific *in vitro* effects of CBG; that is at low concentrations, CBG stimulates GTPγS binding to mouse brain membranes and this effect disappears at higher concentrations to be replaced by its action as a 5-HT_{1A} receptor antagonist (Cascio *et al.*, 2010).

Treatment with CBD or 8-OH-DPAT did not modify the strength of conditioned taste avoidance in the saccharin preference test or in the one-bottle test of saccharin consumption in Experiment 1. Instead, as has been previously reported (Parker *et al.*, 2002; Limebeer and Parker, 2003), the effect of CBD or 8-OH-DPAT was selective to conditioned gaping (the behaviour selectively produced by nausea) and not conditioned taste avoidance (the behaviour that is non-selective to nausea). Since the rats treated with

CBD or 8-OH-DPAT did not show attenuated conditioned taste avoidance, these attenuated conditioned gaping reactions cannot simply be explained as interference with learning. Instead, it is more likely that CBD and 8-OH-DPAT suppressed the nausea-like behaviour produced by LiCl, resulting in attenuated conditioned gaping reactions; that is, the nauseating aspect of the unconditioned stimulus (US) was attenuated. CBG reversed this effect; however, even with the nausea suppressed, LiCl produced a sufficiently strong change in state (as do rewarding drugs for instance) that even in the absence of nausea, the rats displayed a conditioned avoidance of the taste. These findings replicate previous reports that taste avoidance is not modified by anti-nausea treatments that dramatically attenuate conditioned gaping reactions (eg. Parker *et al.*, 2008). Thus, conditioned gaping is a more selective measure of nausea in rats.

Chemotherapy-induced nausea remains a significant clinical problem. Marihuana may be used to treat nausea; however, people who smoke marihuana are exposed to over 60 cannabinoids, some of which counteract one another. Although the Δ^9-THC content in the more potent strains of marihuana has increased over the past 10 years, the concentration of both CBD and CBG has remained constant (Mehmedic *et al.*, 2010). Our findings suggest that it may be more effective to treat nausea with specific cannabinoids that have proven anti-nausea effects, such as CBD, rather than with marihuana, which is psychoactive and contains cannabinoids, such as CBG, that prevent the anti-nausea effects of other cannabinoids.

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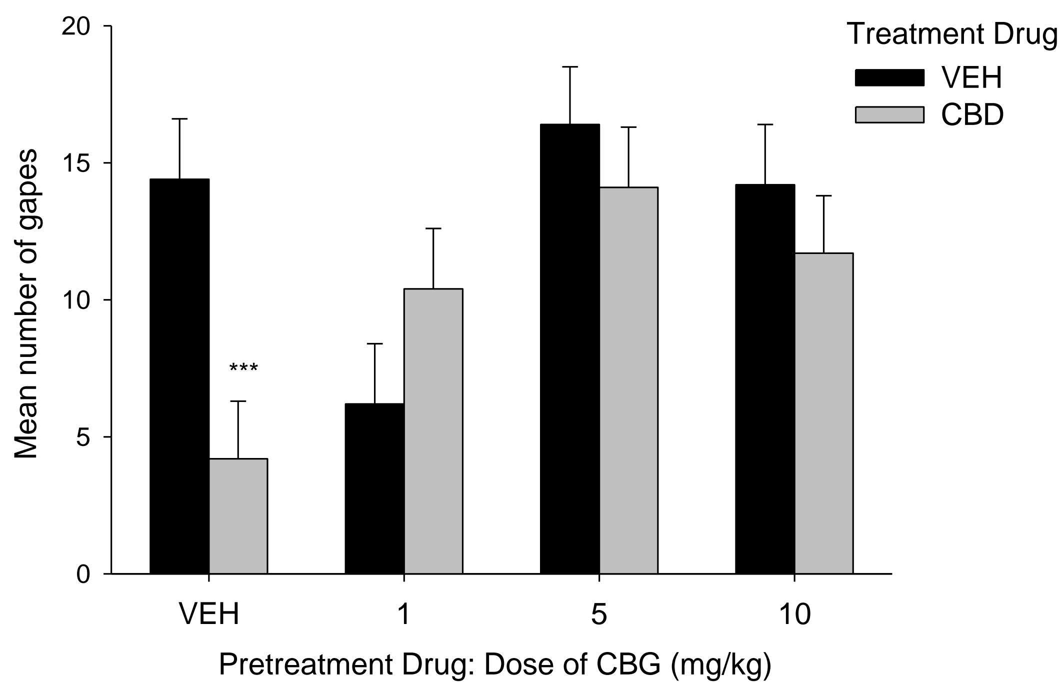

Figure 1. Mean (±SEM) number of gaping responses elicited by a 2 minute intra-oral infusion of 0.1% saccharin solution on the test trial for each pretreatment (VEH, 1 mg/kg, 5 mg/kg and 10 mg/kg CBG) and treatment (VEH and CBD) drug. All rats were conditioned with LiCl. *Asterisks* indicate significant results (***$p < 0.001$).

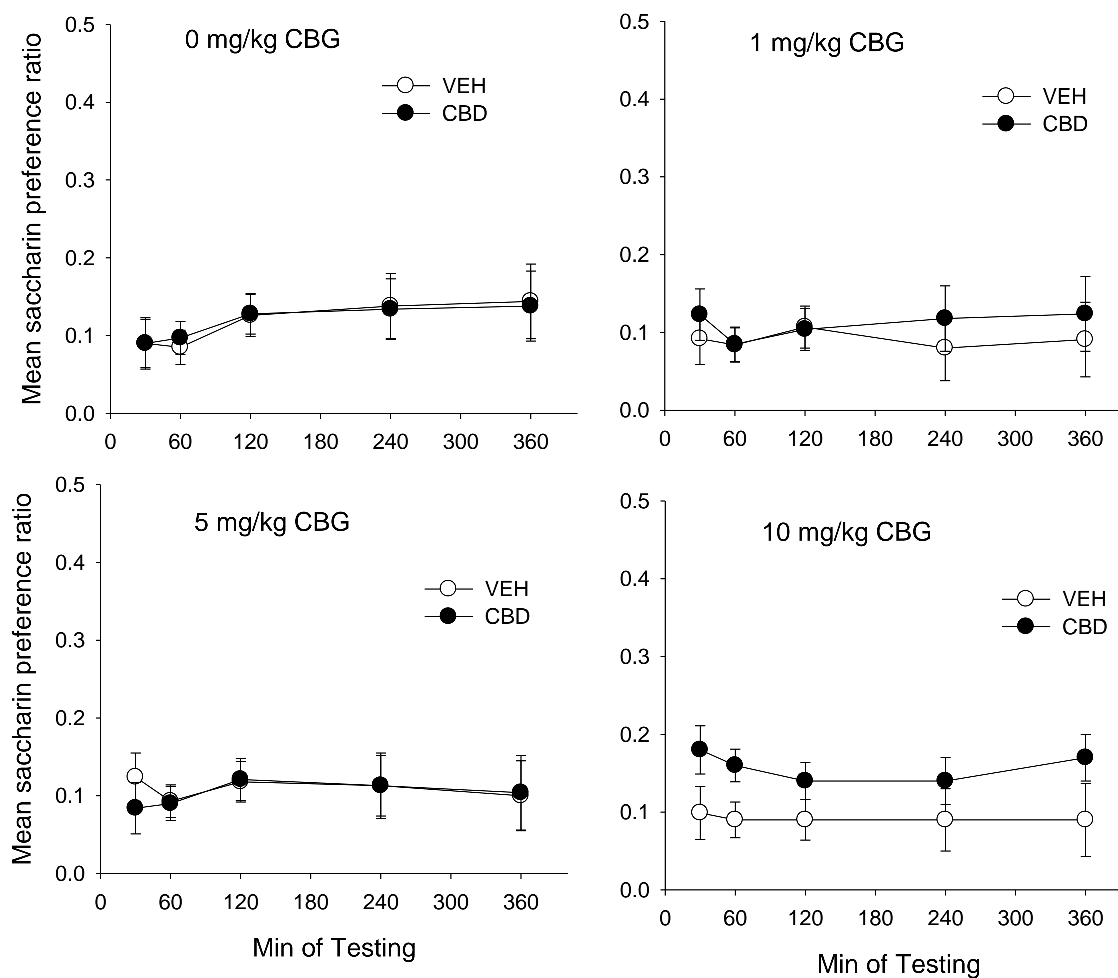

Figure 2. Mean (±SEM) saccharin preference ratio (Saccharin solution/[Saccharin solution + Water]) for the various conditioning groups during the preference test at 30, 60, 120, 240, and 360 minutes. All rats were conditioned with LiCl.

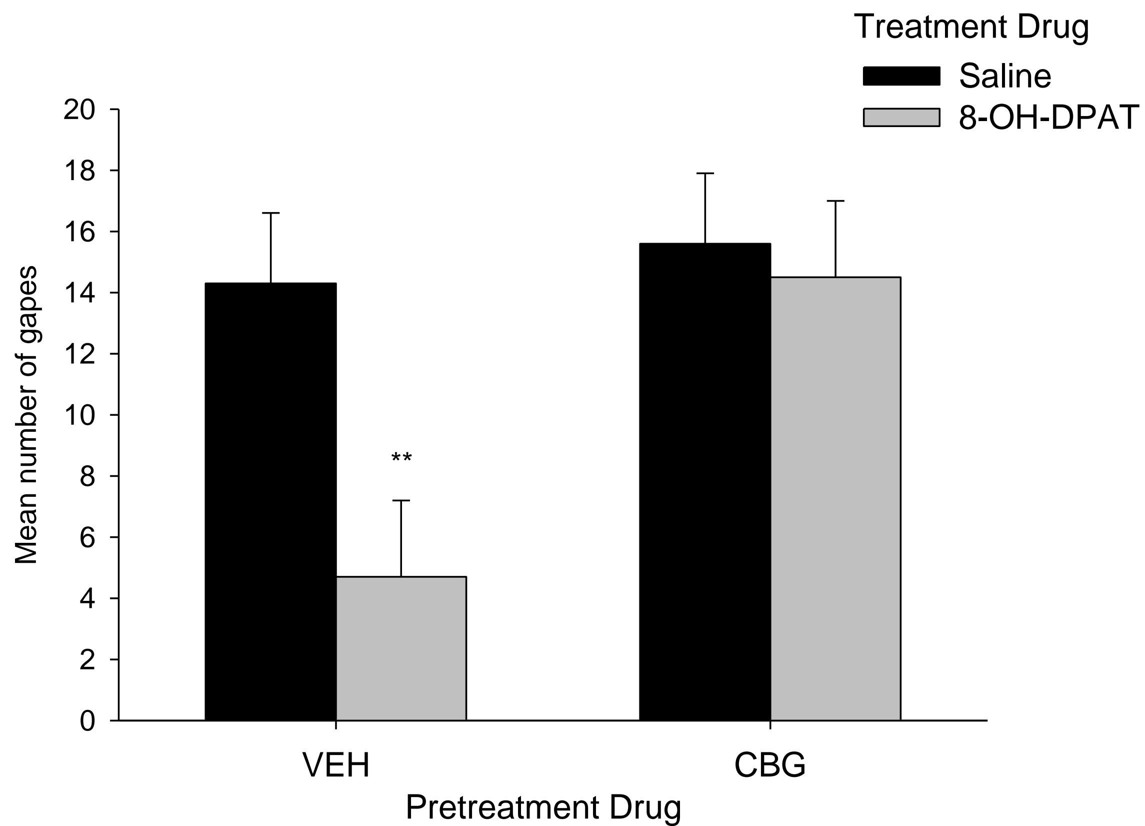

Figure 3. Mean (±SEM) number of gaping responses elicited by a LiCl-paired saccharin solution in Experiment 1b when rats were pretreated with VEH or CBG and treated with Saline or 8-OH-DPAT prior to the conditioning trial.

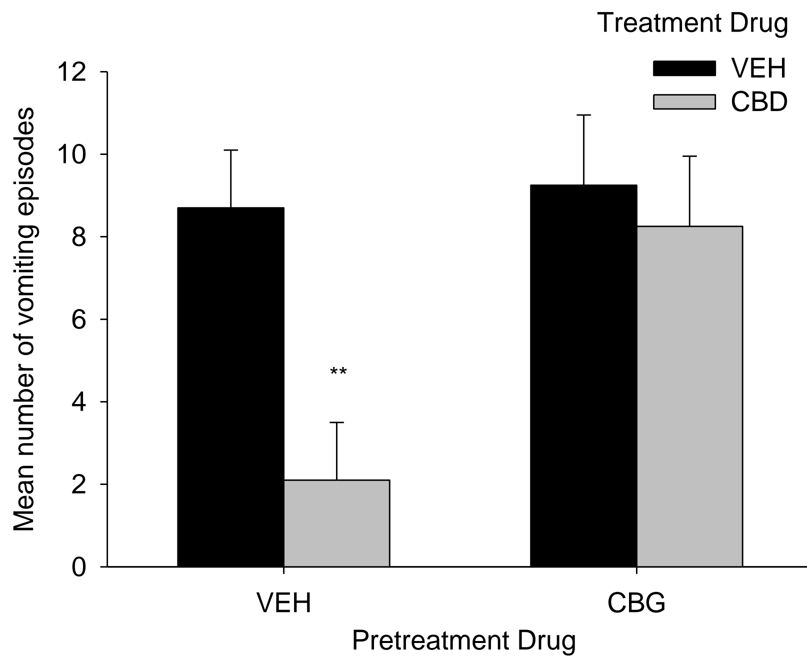

Figure 4. Mean (+SEM) number of LiCl-induced vomiting episodes 30 min following treatment with VEH or 5 mg/kg CBD among shrews pretreated 15 min earlier with VEH or 5 mg/kg CBG. Asterisks indicate significant results (**$p < 0.01$)

CHAPTER 4

General Discussion

The series of experiments conducted for this dissertation provide evidence that CBD, a non-psychoactive cannabinoid found in cannabis, exerts its anti-emetic effect in *S. murinus* (Kwiatkowska *et al.*, 2004; Parker *et al.*, 2004) and anti-nausea-like effect in rats (conditioned gaping, Parker *et al.*, 2002) by indirect agonism of somatodendritic 5-HT_{1A} autoreceptors located in the DRN. Other therapeutic effects of CBD, including ischemic injury (Hayakawa *et al.*, 2004; Mishima *et al.*, 2005; Hayakawa *et al.*, 2007a), hepatic encephalopathy (Magen *et al.*, 2010), anxiety (Campos and Guimaraes, 2008a; Gomes *et al.*, 2011) and depression (Zanelati *et al.*, 2010), are each attenuated by pretreatment with 5-HT_{1A} receptor antagonists. Evidence presented in this thesis indicates that CBD's anti-emetic and anti-nausea-like effects are also 5-HT_{1A}-dependent.

Systemically administered WAY100135 (in *S. murinus*), and WAY100135 or WAY100635 (in rats) blocked systemic CBD's anti-emetic and anti-nausea-like effects, respectively. Furthermore, when injected into the DRN, CBD prevented conditioned gaping and this effect was reversed by systemic WAY100635, indicating that it is likely these somatodendritic 5-HT_{1A} autoreceptors located within the DRN upon which CBD is exerting its anti-nausea-like action. In addition intra-DRN administration of WAY100635 blocked the systemic CBD-induced suppression of conditioned gaping and this effect was selective to the DRN, as those rats receiving WAY100635 outside of the DRN did not show the reversal of the CBD-induced suppression of gaping. In fact, rats in this group reacted in a similar manner to those in SAL-CBD, indicating that WAY100635

was not effective when administered outside of the DRN. As the DRN is a localized site of somatodendritic 5-HT_{1A} autoreceptors, and stimulation of these receptors decreases the firing rate of 5-HT afferents to terminal brain areas (Verge *et al.*, 1985; Sotelo *et al.*, 1990), these results suggest that CBD may in fact be reducing the firing rate of 5-HT afferents to terminal forebrain regions, thereby creating its anti-nausea-like effects. Future studies will explicitly evaluate the effect of CBD on the firing rate of DRN 5-HT neurons both *in vitro* and *in vivo*.

CBD, albeit at a rather high concentration of 16 μM, binds to and activates human 5-HT_{1A} receptors that have been transfected into Chinese hamster ovary cells (Russo *et al.*, 2005), but lower, more physiologically relevant doses had not yet been investigated. Our *in vitro* experiments showed that at concentrations of up to 10 μM, CBD does not displace [^{3}H]8-OH-DPAT from specific binding sites on rat brainstem membranes, nor does CBD stimulate [^{35}S]GTPγS binding to rat brainstem membranes. However, CBD (100 nM) does enhance the stimulatory effect of 8-OH-DPAT on [^{35}S]GTPγS binding to rat brainstem membranes. In line with these findings, our *in vivo* studies demonstrated the enhanced suppression of conditioned gaping with subthreshold combined doses of CBD and 8-OH-DPAT. CBD does not however, alter the rate at which [^{3}H]8-OH-DPAT dissociates from specific binding sites in these membranes *in vitro*, indicating that CBD does not activate 5-HT_{1A} receptors in an allosteric manner. Therefore, CBD is likely producing its effects by augmenting the action of endogenous 5-HT on the 5-HT_{1A} receptor, because CBD enhanced the action of 8-OH-DPAT both *in vitro* (% stimulation of [^{35}S]GTPγS binding above basal) and *in vivo* (combined subthreshold doses having an

enhanced suppression effect on conditioned gaping), without having a direct agonist action on the 5-HT_{1A} receptor in brainstem preparations.

The hypothesis that CBD is likely augmenting the action of endogenous 5-HT at the 5-HT_{1A} receptor is further strengthened by our finding that the concentration-response curve of CBD for its *in vitro* potentiation of 8-OH-DPAT $[^{35}S]GTP\gamma S$ binding is bell-shaped. Similarly, the *in vivo* 5-HT_{1A} mediated effects of CBD on emesis (Kwiatkowska *et al.*, 2004), nausea-like-behavior (Rock *et al.*, 2008), ischemic injury (Mishima *et al.*, 2005), anxiety (Campos and Guimaraes, 2008a) and depression (Zanelati *et al.*, 2010) also exhibit bell-shaped dose response curves. Specifically for vomiting in *S. murinus*, low doses suppress toxin-induced vomiting, but high doses can actually potentiate it (Kwiatkowska *et al.*, 2004; Parker *et al.*, 2004). This bell-shaped dose response curve highlights the importance of selecting a dose of CBD to optimally manage nausea and vomiting.

The anti-emetic potential of CBD, is not only dose-dependent, but is also mediated by the nature of the emesis-inducing stimulus. In Chapter 2, Experiment 3, CBD suppressed vomiting produced by 20 mg/kg cisplatin, but was not effective in reducing vomiting at a dose of 40 mg/kg of cisplatin. This finding suggests that although CBD can prevent vomiting induced by some emetogenic therapies, CBD may not be effective in reducing acute vomiting produced by highly emetogenic therapies such as high dose cisplatin. CBD has also been shown to be ineffective in alleviating motion sickness (Cluny *et al.*, 2008), indicating that the type and dose of the illness-inducing agent is an important factor in the effectiveness of CBD. No known clinical trials have been performed to assess the potential efficacy of CBD alone to reduce acute vomiting in

either low or high emetogenic chemotherapy treatments, however the human literature does highlight the beneficial use of CBD in delayed nausea and vomiting, as opposed to the acute onset. Duran *et al.*'s (2010) human clinical trial with Sativex (combination of Δ^9-THC and CBD) indicates that significantly more patients receiving Sativex (in combination with a 5-HT_3 receptor antagonist) experienced no delayed nausea or vomiting in comparison to placebo controls. Further, Yang *et al.* (2010) have reported that CBD acts as an allosteric inhibitor of 5-HT_{3A} receptor mediated currents in *Xenopus laevis* oocytes, indicating that there may be a link between CBD and 5-HT_3 receptors as well. Since 5-HT_3 receptor antagonists such as OND are effective anti-emetic and anti-nausea agents (eg. Kwiatkowska *et al.*, 2004; Limebeer *et al.*, 2009) it is possible that allosteric inhibition of the 5-HT_3 receptor could be involved in CBD's regulation of nausea and vomiting. It would therefore, be of interest to determine the efficacy of Nabidiolex (a sublingual spray containing CBD alone) on its own and in combination with the 5-HT_3 receptor antagonist OND in human clinical trials. Indeed, Kwiatkowska *et al.* (2004) report that combined subthreshold doses of Δ^9-THC and OND completely suppressed vomiting in *S. murinus*. Future experiments will evaluate the potential of subthreshold doses of CBD and OND to interfere with toxin-induced vomiting in shrews and conditioned gaping in rats. This would certainly mirror a human clinical situation, as many patients are given 5-HT_3 receptor antagonists with their treatment.

The mechanism of action for CBD's anti-emetic and anti-nausea-like effects was further investigated in this dissertation by evaluating its interaction with cannabigerol (CBG), another cannabinoid, which acts *in vitro* as a 5-HT_{1A} receptor antagonist (Cascio *et al.*, 2010). CBG systemically blocked the systemic CBD-induced suppression of

vomiting and conditioned gaping, in a manner similar to that of the classic 5-HT_{1A} receptor antagonists, further supporting Cascio's results and adding to the evidence that CBD may be exerting it's anti-emetic and anti-nausea-like effect via 5-HT_{1A} receptor agonism.

Interestingly, at the lowest dose (1 mg/kg) evaluated, CBG also appeared to have an anti-nausea-like effect, but the mechanism for this effect is not known. Cascio *et al.* (2010) reported concentration-specific *in vitro* effects of CBG; at low concentrations CBG stimulated GTPγS binding to mouse brain membranes, but at higher concentrations it acted as a 5-HT_{1A} receptor antagonist. In addition, systemic CBG (5 mg/kg) also reversed systemic 8-OH-DPAT-induced (0.01 mg/kg) suppression of conditioned gaping, adding further support to the contention that CBG acts *in vivo* as a 5-HT_{1A} receptor antagonist. Therefore, it seems that CBG and CBD may be acting in opposition at the 5-HT_{1A} receptor, with CBD acting as an indirect receptor agonist and CBG exerting its action as a receptor antagonist.

Treatment with CBD or 8-OH-DPAT did not alter the strength of conditioned taste avoidance in Chapter 3, Experiment 1, nor did these treatments interfere with conditioned taste avoidance in the *in vivo* studies performed in Chapter 2 (data not reported). Instead, as has been previously reported (Parker *et al.*, 2002; Limebeer and Parker, 2003), the effects of CBD or 8-OH-DPAT were selective to conditioned gaping and not conditioned taste avoidance. Since the rats treated with CBD or 8-OH-DPAT did show conditioned taste avoidance (reduced saccharin consumption), the reduction in conditioned gaping reactions cannot simply be explained by interference with learning because these animals did learn about the taste and subsequently avoided the

consumption of it. Instead, it is more likely that CBD or 8-OH-DPAT reduced the experience of nausea caused by the emetic agent, resulting in attenuated conditioned gaping reactions. CBG, WAY100135 and WAY100635 reversed this effect on conditioned gaping, but had no effect on conditioned taste avoidance. Even in the absence of nausea, the emetic agents still produced a change in state, causing the rats to display conditioned taste avoidance. These findings replicate previous reports that taste avoidance is not modified by anti-nausea treatments that dramatically reduce conditioned gaping (eg. Parker *et al.*, 2008). Conditioned gaping is a selective measure of nausea in rats. LiCl-induced conditioned gaping responses are also eliminated by 5-7-DHT lesions of the DRN and MRN, which deplete forebrain 5-HT (Limebeer *et al.*, 2004) and also by decerebration (Grill and Norgren, 1978a), indicating that this selective measure of nausea may be mediated by the action of forebrain 5-HT.

In addition to their role in nausea and vomiting, 5-HT_{1A} receptors have anti-depressant action (see Lucki *et al.*, 1994 for review) and 5-HT_{1A} receptor KO mice display anti-depressant-like responses in a tail-suspension test (Heisler *et al.*, 1998). Most anti-depressants cause an increase in 5-HT concentration via re-uptake inhibition (see Celada *et al.*, 2004 for review). Initially, this increase in 5-HT is coupled with a 5-HT_{1A}-dependent inhibition of cell firing, ultimately resulting in an attenuation of this 5-HT increase by re-uptake inhibition. After chronic anti-depressant administration, these receptors become desensitized, resulting in recovery of firing in DRN 5-HT cells and an increase in 5-HT (see Blier and de Montigny, 1994). It is thought that these somatodendritic 5-HT_{1A} autoreceptors may be responsible for the initial delay in onset of action of anti-depressants, making these receptors a target for the acceleration of anti-

depressants' clinical effects. An acceleration of onset of anti-depressant action was achieved with the 5-HT_{1A} receptor antagonist pindolol, in human clinical trials (Artigas *et al.*, 1996; see Celada *et al.*, 2004 for review). It is therefore reasonable to suspect that CBD, perhaps acting as a 5-HT_{1A} receptor agonist, may induce depressive-like symptoms. (Zanelati *et al.*, 2010). However, a single dose of CBD actually exhibits anti-depressant-like effects in mice, as assessed by the forced swim test (FST, El-Alfy *et al.*, 2010; Zanelati *et al.*, 2010). Interestingly, the CBD-induced effect in the FST was 5-HT_{1A}-dependent, because it was blocked by a dose of WAY100635, which did not produce effects on its own. The important question though, is whether these compounds, specifically CBD, would show anti-depressant potential when administered chronically, potentially resulting in desensitization of the 5-HT_{1A} receptor.

5-HT_{1A} receptor agonists also have anxiolytic properties (see De Vry, 1995 for review) which are blocked by 5-HT_{1A} receptor antagonists (Kataoka *et al.*, 1991; Shimizu *et al.*, 1992). 5-HT_{1A} receptor KO mice are more anxious than wild-types (Zhuang *et al.*, 1999), displaying responses indicative of elevated anxiety in open-field, elevated-zero maze and novel-objects test (Heisler *et al.*, 1998; Parks *et al.*, 1998) and conversely, mice overexpressing 5-HT_{1A} receptors show decreased anxiety in the elevated plus maze and the open field test (Kusserow *et al.*, 2004). Although the role of somatodendritic 5-HT_{1A} receptors cannot be ruled out, it seems that the postsynaptic 5-HT_{1A} receptors play a critical role in regulating anxiety (see Ohno, 2010 for review), as microinjections of the 5-HT_{1A} receptor agonist tandospirone into the dorsal hippocampus (site of postsynaptic 5-HT_{1A} receptors) produced an anxiolytic action (Kataoka *et al.*, 1991) and 5,7-DHT

lesions to the DRN did not alter the anxiolytic actions of 5-HT_{1A} receptor agonists (Shimizu *et al.*, 1992).

Studies in humans and animals suggest that CBD also has anxiolytic properties (see Zuardi *et al.*, 2006 for review). In humans, CBD (300 mg, in capsule form) decreased anxiety induced by a public speaking test (Zuardi *et al.*, 1993). In rats and mice, those treated with CBD spend more time in the open arms of the elevated plus maze, indicating CBD's anxiolytic effect in this model (Guimaraes *et al.*, 1990; Onaivi *et al.*, 1990). When injected into the dorsolateral periaqueductal gray (dPAG, a midbrain structure related to anxiety containing 5-HT_{1A} receptors) of rats, CBD produced anxiolytic-like effects and these effects were reversed by WAY100635 (Soares Vde *et al.*, 2010), but not by AM251(Campos and Guimaraes, 2008b), indicating that CBD's anti-anxiety effect is mediated by 5-HT_{1A} receptors, located postsynaptically in midbrain structures such as the dPAG.

In addition to CBD's other therapeutic properties, CBD also produces antitumor effects (see Guindon and Hohmann, 2011 for review). *In vitro*, CBD shows antiproliferative activity most likely by the induction of apoptosis in human glioma cell lines (Massi *et al.*, 2004). Vacanni *et al*. (2005) have demonstrated CBD's ability to inhibit migration of human glioma cells, and this effect was both CB_1 and CB_2 receptor-independent. CBD has also been shown to reduce human breast cancer cell proliferation, invasion and metastasis (Ligresti *et al.*, 2006; McAllister *et al.*, 2011), offering CBD as a novel compound that could potentially treat this very aggressive type of cancer. Furthermore, when administered systemically to mice, CBD (0.5 mg) inhibited the

growth of implanted human glioma cells (Massi *et al.*, 2004). Interestingly, mice pretreated with CBD prior to cisplatin exposure displayed significantly less signs of compromised renal function, which is an unfortunate side-effect of toxic cisplatin treatment (Pan *et al.*, 2009). As CBD exerts antitumor effects both *in vitro* and *in vivo*, this cannabinoid is an excellent antineoplastic agent candidate that could concomitantly reduce the nausea and vomiting associated with chemotherapy treatment.

In addition to nausea and vomiting, cancer patients also experience cancer-related anorexia and weight loss. Activation of the 5-HT system modulates feeding (see Vickers and Dourish, 2004 for review). Although drug discovery is currently interested in selective ligands to treat obesity, therefore decreasing body weight and appetite, the 5-HT system is an important target for altering appetite and body weight. In rats, low doses (0.015-0.06 mg/kg) of 8-OH-DPAT induce feeding (Dourish *et al.*, 1985a; Dourish *et al.*, 1985b; Bendotti and Samanin, 1986), and this effect is blocked by WAY100135 (Hartley and Fletcher, 1994). Additionally, it has been shown that the increased consumption in rats elicited by a 0.06 mg/kg dose of 8-OH-DPAT may be due to the drug-induced gnawing, as rats treated with 8-OH-DPAT gnawed on wood blocks, in addition to rat chow (Fletcher, 1987). On the other hand, high doses (0.125-4 mg/kg), decrease food intake, especially in tests that are of a short duration (Dourish *et al.*, 1985a). It is important to note however, that these high doses of 8-OH-DPAT produced serotonin-related stereotyped behaviour (forepaw treading, headweaving, wet dog shakes and flat body posture), inhibiting feeding behaviour. Once the stereotypy had dissipated, these rats did feed in long bouts, similar to those observed in the low dose group (Dourish *et al.*, 1985a). When microinjected directly into the DRN, 5-HT caused a dose-dependent

increase in food intake (Fletcher and Davies, 1990), making the 5-HT_{1A} receptor an important target for modulation of feeding.

Marihuana has also been shown to stimulate appetite in humans (Abel, 1971; Foltin *et al.*, 1986) and increase weight gain (Greenberg *et al.*, 1976). In animals, CB_1 receptor agonists such as Δ^9-THC and AEA have been shown to increase food intake (Williams *et al.*, 1998; Williams and Kirkham, 1999), while CB_1 receptor antagonists suppress food intake (Wiley *et al.*, 2005; Salamone *et al.*, 2007; Riedel *et al.*, 2009). CBD on the other hand, does not alter food intake in either fed or fasted rats (Wiley *et al.*, 2005; Scopinho *et al.*, 2011) but CBD, at the high dose of 20 mg/kg, does block the hyperphagic effects induced by 8-OH-DPAT and a CB_1 receptor agonist (Scopinho *et al.*, 2011). It is possible, as the authors indicate that CBD at this high dose may be acting at post-synaptic 5-HT_{1A} receptors to produce this effect on food intake. This may be another example of the biphasic effects of CBD, indicating that further research is needed to understand the role of CBD in appetite.

Although the average Δ^9-THC content in cannabis has been increasing in recent years (Potter *et al.*, 2008), the concentration of both CBD and CBG has remained fairly constant (Mehmedic *et al.*, 2010). There is however, great variability in the Δ^9-THC and CBD content between cannabis samples. For example, in sinsemilla (flowering tops of the unfertilized female plant) there is almost a complete absence of CBD (Potter *et al.*, 2008), but in ditchweed (a wild cannabis found in the Midwestern United States) CBD is the major cannabinoid present (Mehmedic *et al.*, 2010). Further, the precursor for CBD, cannabidiolic acid (CBDA), is highly prevalent in hemp, in contrast to the precursor of Δ^9-THC, tetrahydrocannabinolic acid (THCA), which is highly prevalent in a medical

marihuana variety known as Purple Kush (Page *et al.,* 2011). It is currently unknown whether CBDA also has potential as an anti-emetic/anti-nausea drug. We are currently evaluating this possibility. In addition to variation within cannabis samples, there are also differences in use by marihuana smokers. For example, a clinical trial with 251 marihuana-dependent patients revealed that not only were there demographic differences in their preferred method of cannabis use (joints, blunts or pipes), but there was also wide variation of cannabis weight in grams per unit of use, translating to a variation in associated cost and dose (Mariani *et al.*, 2011). This variation in cannabis sample potency as well as individual use indicate that more systematic methods need to be integrated into human clinical trials with marihuana users.

This variability in human cannabis use, leads to great variation when assessing pharmacological effects of these drugs, causing researchers to rely on animal models. When injected into rats, [^{3}H]CBD (4 mg/kg, iv) is rapidly distributed (within 2 min) and slowly disappears from the blood and tissue, with a 10.9 h half-life, and complete elimination by 40 h (Siemens *et al.*, 1980) In contrast, when given orally (23.4 mg/kg) [^{3}H]CBD appeared in the blood within 2 h, with an 11.3 h half-life (Siemens *et al.*, 1980). When given ip, CBD and CBG (both at 120 mg/kg) lead to much higher mouse brain and plasma levels than when given orally (Deiana *et al.*, 2011). The plasma time course for [^{3}H]CBD and [^{3}H]THC in rats (1 mg/kg, iv) are similar, both rapidly penetrating the brain (Alozie *et al.*, 1980). When given orally to dogs in a capsule form, CBD (180 mg), cannot be detected in the plasma, indicating low bioavailability due to first pass metabolism (Samara *et al.*, 1988). Similar effects have been shown in human studies, such that more clinical effects are shown after IV administration rather than smoking, in

both light and heavy users (Lindgren *et al.*, 1981). Taken together, these results indicate higher detected cannabinoid levels when administered systemically rather than orally, pointing to the possible need for a systemically administered cannabinoid therapeutic.

Future Studies

The DRN is the site of the somatodendritic 5-HT$_{1A}$ autoreceptors, which reduce the rate of firing of 5-HT afferents to terminal regions when they are stimulated (Verge *et al.*, 1985; Sotelo *et al.*, 1990). The results presented here suggest that CBD may exert its anti-nausea-like effects by reducing the firing rate of 5-HT afferents to terminal forebrain regions, but which forebrain region is responsible for CBD's effects on conditioned gaping is unknown. A possible candidate is the insular cortex; the site of convergence for both gustatory and interoceptive information (Cechetto and Saper, 1987) and a site of DRN projections (Reep and Winans, 1982). Ablation of the rat insular cortex prevents the establishment of LiCl-induced gaping (Kiefer and Orr, 1992), unlike lesions of the basolateral or central amygdala (Rana and Parker, 2008). When electrically stimulated, the insular cortex produces vomiting in cats (Kaada, 1951) and humans (Catenoix *et al.*, 2008), along with a sensation of nausea in human patients (Penfield and Faulk, 1955). Reversible lidocaine lesions of the rat insular cortex interfere with lying on belly, an unconditioned illness behaviour (see Parker, 1984), which is produced by LiCl (Contreras *et al.*, 2007). The role of 5-HT availability in the insular cortex and its effect on conditioned gaping is currently being evaluated by our research group.

It seems likely that CBD is acting on the somatodendritic 5-HT$_{1A}$ autoreceptors located within the DRN, decreasing the firing rate of 5-HT afferents to terminal brain

areas (Verge *et al.*, 1985; Sotelo *et al.*, 1990), thereby creating its anti-nausea-like effects. This hypothesis could be confirmed using i*n vivo* microdialysis. In this experiment dialysate could be collected from the insular cortex (a proposed site for nausea) or perhaps the prefrontal cortex, during which a microinjection of either CBD or VEH into the DRN would occur, 30 min prior to an ip injection of either LiCl or SAL. If our hypothesis is correct, one would expect to see an increase in 5-HT levels in group VEH-LiCl, relative to group VEH-SAL after the injection. In addition, group CBD-LiCl would be expected to show a decrease in 5-HT levels relative to VEH-LiCl, if indeed CBD is acting as a 5-HT_{1A} receptor agonist to reduce the firing rate of 5-HT afferent to the areas being sampled.

We are also currently exploring whether Nabidiolex (an oromucosal spray containing CBD only, provided by GW Pharmaceuticals) may alleviate nausea and vomiting, and other illness-related symptoms, in dogs diagnosed with lymphoma who are undergoing chemotherapy in the veterinary college at the University of Guelph.

Conclusions

Smoked marihuana and oral Δ^9-THC have been shown to be effective in controlling nausea and vomiting in chemotherapy patients. In a pilot study investigating the use of inhalation marihuana in patients who were unresponsive to standard treatment, 34% of patients rated smoking marihuana as very effective and 44% as moderately effective (Vinciguerra *et al.*, 1988). Data indicates that inhalation of Δ^9-THC appears to be more effective than the oral route, (see Musty and Rossi, 2001 for review) but marihuana is much more than just Δ^9-THC. People who smoke marihuana are exposed to

over 60 cannabinoids, including Δ^9-THC, CBD and CBG. The results of this thesis suggest that marihuana containing high CBD content may also alleviate nausea and vomiting, however, high CBG content could reverse the beneficial effects of CBD. Therefore, the data presented in this thesis indicate that CBD alone may be effective in reducing nausea and vomiting. In addition, CBD may be a more favourable therapeutic option than Δ^9-THC, as it does not possess psychoactive effects.

The findings of this thesis shed light on the mechanism of action of the non-psychoactive cannabinoids CBD and CBG which are found in the cannabis plant. We have found evidence to indicate that CBD may be acting like a 5-HT_{1A} receptor agonist because its systemic and central effects were blocked by both systemic and central administration of a 5-HT_{1A} receptor antagonist. Therefore it seems that CBD may be acting at somatodendritic 5-HT_{1A} autoreceptors located in the DRN to reduce the firing rate of 5-HT neurons to ultimately suppress toxin-induced nausea and vomiting. CBG, on the other hand, may be acting as a 5-HT_{1A} receptor antagonist to block the effects of CBD and the classic 5-HT_{1A} receptor agonist 8-OH-DPAT on nausea and vomiting. These results also highlight the need for further investigation of the efficacy of CBD as a therapeutic for cancer patients, especially for the treatment of chemotherapy-induced nausea, for which there is no current effective treatment.

CHAPTER 5

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