Regulation of nausea and vomiting by cannabinoids

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Considerable evidence demonstrates that manipulation of the endocannabinoid system regulates nausea and vomiting in humans and other animals. The anti-emetic effect of cannabinoids has been shown across a wide variety of animals that are capable of vomiting in response to a toxic challenge. CB1 agonism suppresses vomiting, which is reversed by CB1 antagonism, and CB1 inverse agonism promotes vomiting. Recently, evidence from animal experiments suggests that cannabinoids may be especially useful in treating the more difficult to control symptoms of nausea and anticipatory nausea in chemotherapy patients, which are less well controlled by the currently available conventional pharmaceutical agents. Although rats and mice are incapable of vomiting, they display a distinctive conditioned gaping response when re-exposed to cues (flavours or contexts) paired with a nauseating treatment. Cannabinoid agonists (Δ9-THC, HU-210) and the fatty acid amide hydrolase (FAAH) inhibitor, URB-597, suppress conditioned gaping reactions (nausea) in rats as they suppress vomiting in emetic species. Inverse agonists, but not neutral antagonists, of the CB1 receptor promote nausea, and at subthreshold doses potentiate nausea produced by other toxins (LiCl). The primary non-psychoactive compound in cannabis, cannabidiol (CBD), also suppresses nausea and vomiting within a limited dose range. The anti-nausea/anti-emetic effects of CBD may be mediated by indirect activation of somatodendritic 5-HT1A receptors in the dorsal raphe nucleus; activation of these autoreceptors reduces the release of 5-HT in terminal forebrain regions. Preclinical research indicates that cannabinoids, including CBD, may be effective clinically for treating both nausea and vomiting produced by chemotherapy or other therapeutic treatments.

LINKED ARTICLES
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Abbreviations
2-AG, 2-arachidonoylglycerol; 5-HT, 5-hydroxytryptamine; 5-HT3, 5-hydroxytryptamine receptor 3; 5-HT4, 5-hydroxytryptamine receptor 4; 5-HT1B, 5-hydroxytryptamine receptor 1B; 5-HTP, 5-hydroxytryptophan; 5,7-DHT, 5,7-dihydroxytryptamine; 8-OH-DPAT, 8-hydroxy-N,N-dipropyl-2-aminotetralin; Δ9-THC, Δ9-tetrahydrocannabinol; AN, anticipatory nausea; AP, area postrema; CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; CBD, cannabidiol; DMNX, dorsal motor nucleus of the vagus; DRN, dorsal raphe nucleus; DVC, dorsal vagal complex; FAAH, fatty acid amide hydrolase; GABA, inhibitory G protein subunit; LiCl, lithium chloride; MAGL, monoacylglycerol-lipase; M6G, morphine-6-glucuronide; MRN, medial raphe nucleus; NADA, N-arachidonoyl-dopamine; NTS, nucleus of the solitary tract; S. murinus, Suncus murinus; TRPV1, transient receptor potential vanilloid 1

Introduction
A major advance in the control of acute emesis in chemotherapy treatment was the finding that blockade of one subtype of the 5-hydroxytryptamine (5-HT) receptor, the 5-HT3 receptor, could suppress the acute emetic response (retching and vomiting) induced by cisplatin in the ferret and the shrew (Costall et al., 1986; Miner and Sanger, 1986; Ueno et al., 1987; Matsuki et al., 1988; Torii et al., 1991). In clinical trials with humans, treatment with 5-HT3 antagonists often combined with the corticosteroid dexamethasone during the first chemotherapy treatment reduced the incidence of acute vomiting by approximately 70% (e.g. Bartlett and Koczwara, 2002; Aapro et al., 2003; Ballatori and Roila, 2003; Hickok et al., 2003; Andrews and Horn, 2006). However, the 5-HT3 antagonists are less effective at suppressing acute nausea than...
they are at suppressing acute vomiting (Morrow and Dobkin, 1988; Bartlett and Koczvara, 2002; Hickok et al., 2003) and they are ineffective at reducing instances of delayed (24 h later) nausea and vomiting (Morrow and Dobkin, 1988; Grelot et al., 1995; Rudd et al., 1996; Rudd and Naylor, 1996; Tsukada et al., 2001; Hesketh et al., 2003) and anticipatory (conditioned) nausea and vomiting (Nesse et al., 1980; Morrow and Dobkin, 1988; Hickok et al., 2003).

More recently, NK, receptor antagonists (e.g. aprepitant) have been developed that not only decrease acute vomiting, but also decrease delayed vomiting induced by cisplatin-based chemotherapy (Van Belle et al., 2002); however, these compounds alone and in combination with 5-HTI antagonist/dexamethasone treatment are also much less effective in reducing nausea (e.g. Hickok et al., 2003; Andrews and Horn, 2006; Slatkin, 2007), which is the symptom reported to be the most distressing to patients undergoing treatment with 5-HTI antagonists (de Boer-Dennert et al., 1997). Considerable evidence suggests that another system that may be an effective target for treatment of chemotherapy-induced nausea, delayed nausea/vomiting and anticipatory nausea (ANI)/vomiting is the endo-cannabinoid system (e.g. for review, Parker and Limebeer, 2008).

**Anti-emetic effects of cannabinoids in human clinical trials**

The cannabis plant has been used for several centuries for a number of therapeutic applications (Mechoulam, 2005), including the attenuation of nausea and vomiting. Ineffective treatment of chemotherapy-induced nausea and vomiting prompted oncologists to investigate the anti-emetic properties of cannabinoids in the late 1970s and early 1980s, before the discovery of the 5-HTI antagonists. The first cannabinoid agonist, nabilone (Cesamet), which is a synthetic analogue of Δ⁹-THC was specifically licensed for the suppression of nausea and vomiting produced by chemotherapy. Furthermore, synthetic Δ⁹-THC, dronabinol, entered the clinic as Marinol in 1985 as an anti-emetic and in 1992 as an appetite stimulant (Pertwee, 2009). In these early studies, several clinical trials compared the effectiveness of Δ⁹-THC with placebo or other anti-emetic drugs. Comparisons of oral Δ⁹-THC with existing anti-emetic agents generally indicated that Δ⁹-THC was at least as effective as the dopamine antagonists, such as prochlorperazine (Carey et al., 1983; Ungerleider et al., 1984; Crawford and Buckman, 1986; Cunningham et al., 1988; Tramer et al., 2001; Layeeque et al., 2006).

There is some evidence that cannabis-based medicines may be effective in treating the more difficult to control symptoms of nausea and delayed nausea and vomiting in children. Abramavov et al. (1995) evaluated the anti-emetic effectiveness of Δ⁹-THC, a close but less psychoactive relative of Δ⁹-THC, in children receiving chemotherapy treatment. Two hours before the start of each cancer treatment and every six hours thereafter for 24 h, the children were given Δ⁹-THC as oil drops on the tongue or in a bite of food. After a total of 480 treatments, the only side effects reported were slight irritability in two of the youngest children (3.5 and 4 years old); both acute and delayed nausea and vomiting were controlled.

Surprisingly, only one reported clinical trial (Meiri et al., 2007) has compared the anti-emetic/anti-nausea effects of cannabinoids with those of the more recently developed 5-HTI antagonists and none has compared cannabinoids with the NKI antagonist, aprepitant. Meiri et al. (2007) compared the efficacy and tolerability of dronabinol, ondansetron or the combination for delayed chemotherapy-induced nausea and vomiting in a 5 day, double-blind, placebo-controlled study. Patients that were receiving moderately to highly emetogenic chemotherapy were all given both dexamethasone and ondansetron, with half also receiving placebo and half receiving dronabinol prechemotherapy on Day 1. On Days 2–5, they received placebo, dronabinol, ondansetron or both dronabinol and ondansetron. The results of the study indicated that the efficacy of dronabinol alone was comparable with ondansetron in the treatment of delayed nausea and vomiting, for the total response of no vomiting/retching and nausea less than 5 mm on a visual analogue scale. Rates of absence of nausea were 71% with dronabinol, 64% with ondansetron and 15% with placebo; also the dronabinol group reported the lowest nausea intensity on a visual analogue scale (10.1 mm vs. 24 mm with ondansetron and 48.4 mm with placebo). However, the combined treatment (ondansetron and dronabinol) was no more effective than either agent alone. The dose of dronabinol used in the present study was at least 50% less than in previous studies resulting in a low incidence of CNS-related adverse effects, which did not differ from the incidence in the ondansetron-treated group. Although the study was not explicitly designed to evaluate the effects of combined therapy on acute nausea and vomiting, the combined active treatment group reported less nausea and vomiting on the chemotherapy treatment day than the placebo group.

All reported clinical trials for the effectiveness of cannabinoid compounds on chemotherapy-induced nausea and vomiting have involved oral use of cannabinoids, which may be less effective than sublingual or inhaled cannabinoids, given the need to titrate the dose (Hall et al., 2005). Recently, in 2005, Sativex (GW Pharmaceuticals), a combination of Δ⁹-THC and the non-psychoactive plant cannabinoid, cannabidiol (CBD), was made available as a sublingual spray for the relief of neuropathic pain in patients with multiple sclerosis and in cancer patients with advanced pain (Johnson et al., 2010). However, to the best of our knowledge, the effectiveness of this compound in reducing nausea and vomiting has not been evaluated. Many patients have a strong preference for smoked marijuana over the synthetic cannabinoids delivered orally (Tramer et al., 2001). Several reasons for this have been suggested: (i) advantages of self-titration with the smoked marijuana; (ii) difficulty in swallowing the pills while experiencing emesis; (iii) faster speed of onset for the inhaled or injected Δ⁹-THC than oral delivery; (iv) a combination of the action of other cannabinoids with THC that are found in marijuana. Although many marijuana users have claimed that smoked marijuana is a more effective anti-emetic than oral THC, no controlled studies have yet been published that evaluate this possibility.
Effects of cannabinoids on vomiting in animal models

To evaluate the anti-emetic potential of drug therapies, animal models have been developed. Since rats and mice do not vomit in response to a toxin challenge, it is necessary to use other animal models of vomiting. There is considerable evidence that cannabinoids attenuate vomiting in emetic species (reviewed in Parker et al., 2005; Parker and Limebeer, 2008). Cannabinoid agonists have been shown to reduce vomiting in cats (McCarthy and Borison, 1981), pigeons (Feigenbaum et al., 1989; Ferrari et al., 1999), ferrets (Simoneau et al., 2001; Van Sickle et al., 2001; 2003; 2005), least shrews, Cryptotis parva (Darmani, 2001a,b,c; 2002; Darmani and Johnson, 2004; Darmani et al., 2005; Ray et al., 2009; Wang et al., 2009) and the house musk shrew, Suncus murinus (Kwiatkowska et al., 2004; Parker et al., 2004). As well as attenuating acute vomiting produced by cisplatin, Δ⁹-THC also attenuates delayed vomiting in the least shrew (Ray et al., 2009).

Anti-emetic effect of cannabinoids: mechanisms of action

The mechanism of action of the suppression of nausea and vomiting produced by cannabinoids has recently been explored with the discovery of the endocannabinoid system and the development of animal models of nausea and vomiting. Recent reviews on the gastrointestinal effects of cannabinoids have concluded that cannabinoid agonists act mainly via peripheral CB₁ receptors to decrease intestinal motility (Pertwee, 2001), but may act centrally to attenuate emesis (Van Sickle et al., 2001). The dorsal vagal complex (DVC) is involved in the vomiting reactions induced by either vagal gastrointestinal activation or several humoral cytotoxic agents. The DVC is considered to be the starting point of a final common pathway for the induction of emesis in vomiting species. The DVC consists of the area postrema (AP), nucleus of the solitary tract (NTS) and the dorsal motor nucleus of the vagus (DMNX) in the brainstem of rats, ferrets and the least shrew. CB₁ receptors, as well as the catabolic enzyme of anandamide, fatty acid amide hydrolase (FAAH), have been found in areas of the brain involved in emesis, including the DMNX (Van Sickle et al., 2001).

CB₁ receptors in the NTS are activated by Δ⁹-THC and this activation is blocked by the selective CB₁ antagonist/inverse agonist, SR-141716, known as rimonabant (Darmani et al., 2005) and AM 251 (Van Sickle et al., 2003). In fact, at higher doses than those required to reverse the anti-emetic effects of Δ⁹-THC, rimonabant produces emesis on its own in the least shrew (Darmani, 2001c) and AM-251 potentiates cisplatin-induced emesis in the ferret (Van Sickle et al., 2001). Molecular markers of activation also implicate the role of central CB₁ receptors in the anti-emetic effects of Δ⁹-THC. Cisplatin pretreatment results in c-fos expression in the DMNX, specific subnuclei of the NTS and AP, which is significantly reduced by pretreatment with Δ⁹-THC (Van Sickle et al., 2001; 2003). Endogenous cannabinoid ligands, such as anandamide and 2-arachidonyl glycerol (2-AG), as well as synthetic cannabinoids, such as WIN 55,212–2, also act on these receptors (Simoneau et al., 2001). However, Darmani and Johnson (2004) provide evidence that both central and peripheral mechanisms contribute to the actions of Δ⁹-THC against emesis produced by 5-hydroxytryptophan (5-HTP), the precursor to 5-HT in the least shrew. At lower doses, Δ⁹-THC acts centrally as an anti-emetic, but at higher doses (10 mg·kg⁻¹) it acts peripherally.

Although anandamide has been reported to have anti-emetic properties in the ferret (Van Sickle et al., 2001) and the least shrew (Darmani, 2002), the role of 2-AG in the regulation of nausea and vomiting is less clear. Darmani (2002) found that 2-AG (2.5–10 mg·kg⁻¹, i.p.) produces emesis in the least shrew, most likely via its downstream metabolites, because its emetic activity can be blocked by both rimonabant and the the COX inhibitor, indomethacin. An evaluation of changes in endocannabinoid levels elicited by cisplatin revealed that cisplatin increased levels of 2-AG in the brainstem, but decreased intestinal levels of both 2-AG and anandamide (Darmani et al., 2005). Darmani et al. (2005) suggested that the central elevation of 2-AG may contribute to the emetic potential of cisplatin (in addition to mobilizing the release of known emetic stimuli such as 5-HT, dopamine and substance P). On the other hand, Van Sickle et al. (2005) reported that 2-AG is anti-emetic in ferrets treated with the emetogenic agent morphine-6-glucuronide (M6G). CB₂ receptors in the brainstem may play a role in the regulation of emesis by 2-AG, at least when CB₁ receptors are co-stimulated. The anti-emetic effects of 2-AG (0.5–2.0 mg·kg⁻¹) in ferrets were reversed by both CB₁ (AM251) and CB₂ (AM630) antagonists, but the anti-emetic effects of anandamide were only reversed by AM251. Therefore, 2-AG, unlike anandamide, may selectively activate these brainstem CB₂ receptors (Van Sickle et al., 2005). Finally, consistent with the anti-emetic effects of 2-AG in the ferret, the monoacylglycerol-lipase ( MAGL) inhibitor, JZL-184 (Long et al., 2009a,b), which elevates endogenous 2-AG, dose-dependently suppresses vomiting in the S. murinus (Sticht et al., 2010). Furthermore, in vitro data revealed that JZL 184 inhibited MAGL expression in shrew tissue.

The FAAH inhibitor, URB597, alone and in combination with exogenously administered anandamide has been shown to interfere with vomiting produced by M6G in the ferret (Van Sickle et al., 2005) and in the least shrew (Sharkey et al., 2007) and with nicotine and cisplatin in S. murinus (Parker et al., 2009a). Although inhibition of FAAH elevates multiple endocannabinoid-like molecules that show activity at multiple target receptors, the anti-emetic effects of URB 597 were reversed by pretreatment with rimonabant, indicating a CB₁ mechanism of action. There may be a species difference in this effect, because URB597 (5 or 10 mg·kg⁻¹) administered to the least shrew did not modify toxin-induced vomiting (Darmani et al., 2005); yet in this latter study URB597 was administered only 10 min prior to cisplatin at a time that may not have produced sufficient inhibition of FAAH prior to the onset of the toxin effect (Fegley et al., 2005). In experiments with the S. murinus, a much lower dose (0.9 mg·kg⁻¹) administered 2 h prior to the toxin challenge suppressed vomiting.

A relative of the cannabinoid system, vanilloid TRPV1 receptors have recently been shown to regulate emesis in the ferret (Sharkey et al., 2007). The TRPV1 receptor is targeted by capsaicin (the burning component of chili peppers) as well as resiniferatoxin, which can produce pro-emetic and anti-
emetic effects at similar doses in *S. murinus* (Andrews et al., 2000), but produces anti-emetic effects in ferrets (Andrews and Bhandari, 1993; Andrews et al., 2000; Yamakuni et al., 2002). Recent evidence indicates that anandamide and the endovanilloid, N-arachidonoyl-dopamine (NADA), are endogenous agonists for both CB₁ and TRPV1 receptors (Di Marzo and Fontana, 1995; van der Stelt and DiMarzo, 2004). Extensive colocalization of CB₁ and TRPV1 receptors have been demonstrated (Cristino et al., 2006). Both endogenous (anandamide, NADA) and synthetic (arvanil or O-1861) ‘hybrid’ agonists of CB₁ and TRPV1 receptors have been shown to exert more potent pharmacological effects in *vivo* (Di Marzo et al., 2001) than ‘pure’ agonists of each receptor type, particularly when acting on cells co-expressing the two receptor types (Hermann et al., 2003). Sharkey et al. (2007) found that anandamide, NADA and arvanil were all anti-emetic in the ferret; these effects were attenuated by the CB₁ receptor inverse agonist AM251 and the TRPV1 antagonists iodoseresiniferatoxin and AMG9810. TRPV1 receptors were localized in the ferret NT S and were co-localized with CB₁ in the mouse brainstem.

**CB₁/5-HT interactions**

Recent findings indicate that the cannabinoid system interacts with the 5-hydroxytryptaminergic system in the control of emesis (e.g. Kimura et al., 1998). The DVC not only contains CB₁ receptors, but is also densely populated with 5-HT₁ receptors (Himni et al., 1996; 1998), potentially a site of anti-emetic effects of 5-HT₁ agonists. Cannabinoid receptors are co-expressed with 5-HT₁ receptors in some neurones in the CNS (Hermann et al., 2002). The first evidence of an interaction between cannabinoids and 5-HT₁ receptors was revealed by the finding that anandamide, WIN55 212 and CP55940 inhibit 5-HT₁ receptor-mediated inward currents with IC₅₀ values in the nanomolar concentration range in rat nodose ganglion cells (Fan, 1995). Subsequently, Δ⁹-THC, anandamide and several synthetic cannabinoids were shown to directly inhibit currents through human 5-HT₁ receptors (Barrand et al., 2002). Since WIN 55,212-2 did not displace a 5-HT₁ antagonist ([³H]-GR65630) from the ligand binding site, the results suggest that cannabinoids inhibit 5-HT₁ receptors noncompetitively by binding to an allosteric modulatory site of the receptor (Barrand et al., 2002). Indeed, anandamide produced analgesia in CB₁/ CB₂ knockout mice that was prevented by pretreatment with the 5-HT₁ antagonist, ondansetron (Racz et al., 2008). In the regulation of vomiting, low doses of Δ⁹-THC and ondansetron that were ineffective alone completely suppressed cisplatin-induced vomiting in the *S. murinus* (Kwiatkowska et al., 2004) and the combination of low doses of tropisetron and Δ⁹-THC were more efficacious in reducing emesis frequency in the least shrew than when given individually (Wang et al., 2009). Additionally, cannabinoids have been shown to reduce the ability of 5-HT₁ agonists to produce emesis (Darmani and Johnson, 2004) and this effect was prevented by pretreatment with rimonabant. Cannabinoids may act at CB₁ presynaptic receptors to inhibit the release of newly synthesized 5-HT (Schlicker and Kathmann, 2001; Howlett et al., 2002; Darmani and Johnson, 2004). Indeed, Darmani et al. (2003) reported that rimonabant (which produces vomiting in the least shrew) increases brain 5-HT levels and turnover at doses that induce vomiting in the shrew.

**CBD: a special case**

Another major cannabinoid found in marijuana is CBD. Unlike Δ⁹-THC, CBD does not produce intoxicating effects and has a low affinity for the CB₁ and CB₂ receptors (Mechoulam et al., 2002). At a low dose, CBD (5 mg·kg⁻¹, i.p.) inhibits cisplatin-induced (Kwiatkowska et al., 2004) and LiCl-induced (Parker et al., 2004) vomiting and anticipatory retching (Parker et al., 2006) in *S. murinus*. As has been reported by others (e.g. Pertwee, 2004), the effects of CBD are biphasic with high doses (20–40 mg·kg⁻¹, i.p.) potentiating toxin-induced vomiting in the *S. murinus* (Parker et al., 2004; Kwiatkowska et al., 2004), but a dose as high as 20 mg·kg⁻¹ of CBD had no effect on 2-AG-induced emesis in the least shrew (Darmani, 2002). A wide range of doses was not effective in reducing motion-induced emesis in the *S. murinus* (Cluny et al., 2008), which may reflect a different mechanism of action of motion and toxin-induced vomiting (Cluny et al., 2008).

The anti-emetic effect of CBD does not appear to be mediated by its action at CB₁ receptors, because it is not reversed by the CB₁ antagonist, rimonabant (Kwiatkowska et al., 2004; Parker et al., 2004). Recent evidence indicates that CBD may act as an indirect agonist on the 5-HT₁₃ autoreceptors, to reduce the availability of 5-HT (Russo et al., 2005; E.M. Rock et al., unpubl. obs.). Known 5-HT₁₃ autoreceptor agonists such as 8-OH-DPAT, buspiron, and LY228729, have been found to suppress vomiting in emetic species such as pigeons (Wolff and Leander, 1994; 1995; 1997), shrews (Okada et al., 1994; Andrews et al., 1996; Javid and Naylor, 2006), cats (Lucot and Crampton, 1989; Lucot, 1990) and dogs (Gupta and Sharma, 2002). Indeed, Russo et al. (2005) reported that CBD displaces the agonist [³H]-8-OH-DPAT from a cloned human 5HT₁₃ receptor in a concentration-dependent manner. Furthermore, CBD was shown to act as an agonist at the SH₃ receptor, because, like 5HT, it increased GTP binding to the receptor coupled G protein, Gi, characteristic of a receptor agonist. Finally, the agonist CBD was shown to reduce CAMP production, characteristic of Gi activation.

Recently, our laboratory has investigated the mechanism of action for the anti-emetic effects of CBD. Consistent with previous results, CBD (5 mg·kg⁻¹, s.c.) was shown to be effective in suppressing vomiting in the *S. murinus* induced by either nicotine, LiCl or cisplatin (20 mg·kg⁻¹, but not 40 mg·kg⁻¹). Interestingly, this CBD-induced suppression of vomiting was reversed by systemic pretreatment with the 5-HT₁₃ antagonist WAY100135 (E.M. Rock et al., unpubl. obs.), suggesting that the anti-emetic effect of CBD may be mediated by activation of somatodendritic autoreceptors. This activation of the 5-HT₁₃ receptors results in a reduction of the rate of firing of 5-HT neurones, ultimately reducing the release of forebrain 5-HT (Blier and de Montigny, 1987). It is this reduction in 5-HT release that is probably mediating CBD’s anti-emetic effects. In addition, a recent finding suggests that CBD may also act as an allosteric modulator of the 5-HT₁ receptor (Yang et al., 2010); CBD reversibly inhibited 5-HT-evoked currents in 5-HT₁₃ receptors expressed in *Xenopus laevis* oocytes in a concentration-dependent manner (1 μM), but did not alter the specific binding of a 5-HT₁₃ antagonist. These findings

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suggest that allosteric inhibition of 5-HT₃ receptors by CBD may also contribute to its role in the modulation of emesis.

**Effects of cannabinoids on nausea in animal models**

Nausea is more resistant to effective treatment with new anti-emetic agents than is vomiting (e.g. Andrews and Horn, 2006) and therefore remains a significant problem in chemotherapy treatment and as a side effect from other pharmacological therapies, such as anti-depressants. Even when the cisplatin-induced emetic response is blocked in the ferret by administration of a 5-HT₃ receptor antagonist, c-fos activation still occurs in the AP, suggesting that an action here may be responsible for some of the other effects of cytotoxic drugs, such as nausea or reduced food intake (Reynolds et al., 1991). In rats, the gastric afferents respond in the same manner to physical and chemical (intragastric copper sulphate and cisplatin) stimulation that precedes vomiting in ferrets, presumably resulting in nausea that precedes vomiting (Hillsley and Grundy, 1998; Billig et al., 2001). Furthermore, 5-HT₃ antagonists that block vomiting in ferrets also disrupt this preceding neural afferent reaction in rats. That is, in the rat the detection mechanism of nausea is present, but the vomiting response is absent. Nauseogenic doses of cholecystokinin and LiCl induce specific patterns of brainstem and forebrain c-fos expression in ferrets that are similar to c-fos expression patterns in rats (Reynolds et al., 1991; Billig et al., 2001). In a classic review paper, Borrisson and Wang (1953) suggest that the rats' inability to vomit can be explained as a species-adaptive neurological deficit and that, in response to emetic stimuli, the rat displays autonomic and behavioural prodromata (salivation, papillary dilation, tachypnoea and tachycardia).

**Conditioned taste avoidance: a nonslective measure of nausea in rats**

The typical measure used in the literature to evaluate the nauseating potential of a drug is conditioned taste avoidance. However, taste avoidance is not only produced by nauseating doses of drugs, it is also produced by drugs that animals choose to self-administer or that establish a preference for a distinctive location (e.g. Berger, 1972; Wise et al., 1976; Reicher and Holman, 1977). In fact, when a taste is presented prior to a drug self-administration session, the strength of subsequent avoidance of the taste is a direct function of intake of the drug during the self-administration session (Wise et al., 1976; Grigson and Twinning, 2002). This paradoxical phenomenon was initially interpreted as another instance of taste aversion learning. Because Garcia et al. (1974) had developed a model to account for taste aversion produced by emetic agents, it was reasonable for early investigators to assume that rewarding doses of drugs also produce taste avoidance because they produce a side effect of nausea that becomes selectively associated with a flavour (Reicher and Holman, 1977). However, in an animal capable of vomiting, the *S. murinus*, rewarding drugs do not produce a conditioned taste avoidance, in fact they produce a conditioned taste preference and a conditioned place preference (Parker et al., 2002a). Since rats are incapable of vomiting, it is likely that conditioned taste avoidance produced by rewarding drugs in this species is based upon a learned fear of anything that changes their hedonic state (e.g. Gamzu, 1977) when that change is paired with food previously eaten.

Another approach to understanding the role that nausea plays in the establishment of taste avoidance in rats is to evaluate the potential of anti-nausea treatments to interfere with avoidance of a flavour paired with an emetic treatment. Early work suggested that anti-nausea agents interfered with the expression of previously established taste avoidance produced by LiCl (Coil et al., 1978); however, more recent find- ings suggest that similar anti-nausea treatments (Goudie et al., 1982; Rabin and Hunt, 1983; Parker and McLeod, 1991) and different anti-nausea treatments (Gadusek and Kalat, 1975; Limebeer and Parker, 2000; 2003; Parker et al., 2002b; 2003) failed to interfere with the expression of LiCl-induced taste avoidance. Furthermore, there is considerable evidence that anti-nausea treatments either do not interfere with the establishment of conditioned taste avoidance learning (Rabin and Hunt, 1983; Rudd et al., 1998; Limebeer and Parker, 2000; Parker et al., 2002b) or at least only interfere with the establishment of very weak LiCl-induced taste avoidance (Wegener et al., 1997; Gorzalka et al., 2003). Two prominent anti-nausea treatments include drugs that reduce 5-HT availability and drugs that elevate the activity of the endocannabinoid system in rats (see Parker et al., 2005; 2009b; Parker and Limebeer, 2008). These treatments interfere with the establishment and/or the expression of conditioned disgust reactions, but not conditioned taste avoidance (for review, see Parker, 2003; Parker et al., 2009b).

**Conditioned gaping: a selective measure of nausea in rats**

Over the past number of years, our laboratory has provided considerable evidence that conditioned nausea in rats may be displayed as conditioned disgust reactions (Parker, 1982; 1995; 1998; 2003; Limebeer and Parker, 2000; 2003; Limebeer et al., 2004; Parker et al., 2008; 2009b) using the taste reactivity (TR) test (Grill and Norgren, 1978). Rats display a distinctive pattern of disgust reactions (including gaping, chin rubbing and paw treatment) when they are intragastrically infused with a bitter tasting quinine solution. Rats also display this disgust pattern when infused with a sweet tasting solution (that normally elicits hedonic reactions of tongue protrusions) that has previously been paired with a drug that produces vomiting (such as LiCl or cyclophosphamide) in species capable of vomiting. Only drugs with emetic properties produce this conditioned disgust reaction when paired with a taste.

The most reliable conditioned disgust reaction in the rat is that of gaping (Breslin et al., 1992; Parker, 2003). If conditioned gaping reflects nausea in rats, then anti-nausea drugs should interfere with this reaction. Limebeer and Parker (2000) demonstrated that when administered prior to a saccharin-LiCl pairing, the 5-HT₃ antagonist, ondansetron, prevented the establishment of conditioned gaping in rats, presumably by interfering with LiCl-induced nausea. Since ondansetron did not modify unconditioned gaping elicited by bitter quinine solution, the effect was specific to nausea-
induced gaping. Subsequently, Limebeer and Parker (2003) demonstrated a very similar pattern following pretreatment with the 5-HT₁₅ autoreceptor antagonist, S-OH-DPAT, that also reduces 5-HT availability and serves as an anti-emetic agent in animal models. Most recently, Limebeer et al. (2004) reported that lesions of the dorsal raphe nucleus (DRN) and median raphe nucleus (MRN) that reduced forebrain 5-HT availability interfered with the establishment of LiCl-induced conditioned gaping consistent with reports that reduced 5-HT availability interferes with nausea. Since rats are incapable of vomiting, we have argued that the gape represents an ‘incipient vomiting response’. The orofacial characteristics of the rat gape are very similar to those of the shrew retch just before it vomits (Parker, 2003). Indeed, Travers and Norgren (1986) suggest that the muscular movements involved in the gaping response mimic those seen in species capable of vomiting.

Effects of cannabinoids on nausea in rats

Using the conditioned gaping response as a measure of nausea in rats, we have demonstrated that a low dose (0.5 mg·kg⁻¹, i.p.) of Δ⁹-THC interferes with the establishment and the expression of cyclophosphamide-induced conditioned gaping (Limebeer and Parker, 1999). The potent agonist, HU-210 (0.001–0.01 mg·kg⁻¹), also suppressed LiCl-induced conditioned gaping (Parker and Mechoulam, 2003; Parker et al., 2003) and this suppression was reversed by the CB₁ antagonist/inverse agonist, rimonabant, suggesting that the effect of HU-210 was mediated by its action at CB₁ receptors. When administered 30 min prior to the conditioning trial, rimonabant did not produce conditioned gaping on its own, but it did potentiate the ability of LiCl to produce conditioned gaping. This same pattern has been reported in the emesis literature (Van Sickle et al., 2001; Chambers et al., 2007). Van Sickle et al. (2001) reported that although the CB₁ antagonist/inverse agonist AM251 did not produce vomiting on its own, it potentiated the ability of an emetic stimulus to produce vomiting in the ferret.

More compelling evidence that the endocannabinoid system may serve as a regulator of nausea is the recent finding that prolonging the duration of action of anandamide by combination of URB597 (0.3 mg·kg⁻¹, i.p.) and the expression of cyclophosphamide-induced conditioned gaping reactions in rats (Cross-Mellor et al., 2007). Rats pretreated with URB597 (0.3 mg·kg⁻¹, i.p.) 2 h prior to a saccharin-LiCl pairing displayed suppressed conditioned gaping reactions in a subsequent drug free test. Rats given the combination of URB597 (0.3 mg·kg⁻¹, i.p.) and anandamide (5 mg·kg⁻¹, i.p.) displayed even greater suppression of conditioned gaping reactions. Although inhibition of FAAH produces an elevation of a variety of fatty acids that act at different receptors, the effect of URB597 on conditioned nausea was reversed by AM251, indicating that it was mediated by CB₁ receptors.

At doses (greater than 4 mg·kg⁻¹) that effectively suppress feeding in rats, the CB₁ antagonist/inverse agonist AM251 produces conditioned gaping reactions when explicitly paired with saccharin solution (McLaughlin et al., 2005) reflective of nausea. This finding suggests that the appetite suppressant effect of the newly marketed CB₁ antagonist/inverse agonist, rimonabant, may be partially mediated by the side effect of nausea, which is the most commonly reported side effect in human randomized control trials (Pil-Sunyer et al., 2006). On the other hand, the silent CB₁ antagonists, AM4113 and AM6527, which do not have inverse agonist properties, do not produce conditioned gaping (Sink et al., 2007; Limebeer et al., 2010). In addition, the peripherally restricted silent CB₁ antagonist, AM6545, which also suppresses feeding at equivalent doses of AM251 (Cluny et al., 2010; Randall et al., 2010; Tam et al., 2010), does not produce the side effect of nausea (Cluny et al., 2010). Finally, neither the silent antagonist, AM6527 (which crosses the blood–brain barrier) nor AM6545 (with limited CNS penetration), potentiate LiCl-induced nausea, an effect evident with low doses (2.5 mg·kg⁻¹) of systemic administration of AM-251 (Limebeer et al., 2010). AM251-induced conditioned nausea is thus mediated by inverse agonism of the CB₁ receptor. This effect may be mediated peripherally, because intracranial administration of AM251 at doses up to 1/10 the peripheral dose into the lateral ventricle or the 4th ventricle did not potentiate LiCl-induced nausea that is evident with systemic administration of this inverse agonist of the CB₁ receptor.

CBD reduces nausea by a non-cannabinoid mechanism of action

In addition, the non-intoxicating compound found in marihuana smoke, CBD (5 mg·kg⁻¹, i.p.) as well as its synthetic dimethylheptyl homologue (5 mg·kg⁻¹, i.p.), suppresses the establishment and the expression of LiCl-induced conditioned gaping (Parker et al., 2002b; Parker and Mechoulam, 2003). Recent research (Rock et al., 2010) demonstrates that the anti-nausea effects of CBD (5 mg·kg⁻¹, s.c.) are suppressed by systemic pretreatment with the 5-HT₁₅ receptor antagonists WAY100135 (10 mg·kg⁻¹, i.p.). In addition, the more selective 5-HT₁₅ receptor antagonist, WAY100635, administered systemically (0.1 mg·kg⁻¹, i.p.) or intracranially (21 ng in 0.5 μL) into the DRN, a site of somatodendritic 5-HT₁₅ autoreceptors, interferes with the CBD-induced suppression of LiCl-induced conditioned gaping in rats. This effect was selective to receptors located in the DRN, as those rats with misplaced cannulae that received CBD outside of the DRN did not show a similar effect. In addition, when administered directly into the DRN, CBD (10 μg·μL⁻¹) suppressed LiCl-induced gaping. These results suggest that CBD produces its anti-emetic/anti-nausea effects by activation of somatodendritic autoreceptors located in the DRN, reducing the release of forebrain 5-HT. Since depletion of forebrain 5-HT by 5,7-DHT lesions of the DRN and MRN also prevented the establishment of LiCl-induced conditioned gaping (Limebeer et al., 2004), nausea appears to be mediated by 5-HT action in forebrain regions. Research aimed at determining the forebrain regions (e.g. insular cortex) responsible for the sensation of nausea are currently being conducted in our laboratory (Tuerke et al., 2010).

Cannabinoids and AN in rats and shrews

AN often develops over the course of repeated chemotherapy sessions (Nesse et al., 1980; Morrow and Dobkin, 1988; Reynolds et al., 1991; Stockhorst et al., 1993; Aapro et al., 1994; Ballatori and Roila, 2003; Hickok et al., 2003). For instance, Nesse et al. (1980) described the case of a patient who had
severe nausea and vomiting during repeated chemotherapy treatments. After his third treatment, the patient became nauseated as soon as he walked into the clinic building and noticed a ‘chemical smell’, that of isopropyl alcohol. He experienced the same nausea when returning for routine follow-up visits, even though he knew he would not receive treatment. The nausea gradually disappeared over repeated follow-up visits. Nesse et al. (1980) reported that about 44% of the patients being treated for lymphoma demonstrated such AN. AN is best understood as a classically conditioned response (CR) (Pavlov, 1927).

Control over AN could be exerted at the time of conditioning or at the time of re-exposure to the conditioned stimulus (CS). If an anti-emetic drug is presented at the time of conditioning, then a reduction in AN would be the result of an attenuated unconditioned response (UCR); that is, reduced nausea produced by the toxin at the time of conditioning thereby attenuating the establishment of the CR. Indeed, when administered during the chemotherapy session, the 5-HT3 antagonist, granisetron, has been reported to reduce the incidence of AN in repeat cycle chemotherapy treatment (Aapro et al., 1994). On the other hand, if a drug is delivered prior to re-exposure to cues previously paired with the toxin-induced nausea, then suppressed AN would be the result of attenuation of the expression of the CR (conditioned nausea); the 5-HT3 antagonists are ineffective at this stage (Nesse et al., 1980; Morrow and Dobkin, 1988; Reynolds et al., 1991; Stockhorst et al., 1993; Aapro et al., 1994; Ballatori and Rola, 2003; Hickok et al., 2003).

Anecdotal evidence suggests that Δ9-THC alleviates AN in chemotherapy patients (Grinspoon and Bakalar, 1993; Iversen, 2000). Although there has been considerable experimental investigation of unconditioned retching and vomiting in response to toxins, there have been relatively few reports of conditioned retching; that is, emetic reactions elicited by re-exposure to a toxin paired cue. Conditioned retching has been observed to occur in coyotes, wolves and hawks upon re-exposure to cues previously paired with lithium-induced toxicosis (Garcia et al., 1977) and ferrets have been reported to display conditional emetic reactions during exposure to a chamber previously paired with lithium-induced toxicosis (Davey and Biederman, 1998).

The S. murinus displays conditioned retching when returned to a chamber previously paired with a dose of lithium that produced vomiting (Parker and Kemp, 2001). Furthermore, this conditioned retching reaction is suppressed by pretreatment with Δ9-THC. This effect was replicated more recently and extended to demonstrate that CBD also interferes with the expression of conditioned retching in the shrew, but the 5-HT3 antagonist ondansetron was completely ineffective (Parker et al., 2006). The doses employed were selected on the basis of their potential to interfere with toxin-induced vomiting in the S. murinus (Kwiatkowska et al., 2004, Parker et al., 2004).

Rats also display conditioned retching reactions when re-exposed to a context previously paired with LiCl-induced nausea (Limebeer et al., 2006; 2008; Rock et al., 2008). Following four pairings of a distinctive, vanilla odour-laced chamber with LiCl-induced illness, rats were returned to the context for 30 min and received a 1 min intraoral infusion of novel saccharin solution every 5 min. During the infusions, the rats displayed gaping reactions. Surprisingly, the rats also gaped during intervals when they were not being infused with saccharin while in the LiCl-paired context. It was further demonstrated that Δ9-THC, but not ondansetron, interfered with the conditioned gaping response during both infusion and inter-infusion intervals.

The finding that rats express conditioned gaping responses when re-exposed to an odour-laced context previously paired with LiCl during inter-infusion intervals (Limebeer et al., 2006) suggests that LiCl-paired cues in the absence of the flavour can elicit conditioned nausea. Meachum and Bernstein (1992) had previously shown the re-exposure to a lithium-paired odour cue elicited gaping reactions in rats. Recently, Limebeer et al. (2008) found that even in the absence of a flavoured solution or a distinctive odour, rats display conditioned gaping reactions during exposure to a distinctive context previously paired with a high dose of lithium, as well as a low dose of lithium and provocative motion. Most recently, Rock et al. (2008) reported that CBD (within a limited dose range 1–5 mg·kg⁻¹, but not 10 mg·kg⁻¹) and the FAAH inhibitor, URB597, prevented the expression of conditioned gaping elicited by the lithium-paired context. The effect of URB597 was reversed by rimonabant, indicating a CB₁ mechanism of action. Indeed, inhibition of FAAH by URB597 also prevented the establishment of LiCl-induced conditioned gaping elicited by the contextual cues when administered 2 h prior to each conditioning trial. These results suggest that cannabinoid compounds may be effective agents in the treatment of AN in chemotherapy patients.

Conclusions

Since the discovery of the mechanism of action of cannabinoids, our understanding of the role of the endocannabinoid system in the control of nausea and vomiting has greatly increased. In the ferret and shrew models, the site of action has been identified in the emetic area of the brainstem, the DVC. The shrew model, in particular, is cost effective for the evaluation of the anti-emetic properties of agents. The conditioned gaping response in the rat has provided a glimpse into the anti-nausea mechanisms of action of cannabinoids, in the absence of a vomiting response. Since nausea is a more difficult symptom to control than vomiting, the gaping model may serve as a useful tool for the development of new anti-nausea treatments, as well as for the evaluation of the potential side effects of nausea in newly developed pharmacological treatments. Recent work has also supported anecdotal reports that cannabis may attenuate AN. Using the S. murinus and the rat models of AN, both Δ9-THC and CBD effectively prevented conditioned retching and conditioned gaping (respectively) elicited by re-exposure to a lithium-paired chamber.

Although chemotherapy-induced vomiting is well controlled in most patients by conventionally available drugs, nausea (acute, delayed and anticipatory) continues to be a challenge. Nausea is often reported as more distressing than vomiting, because it is a continuous sensation (e.g. delboer-Dennert et al., 1997; Andrews and Horn, 2006). Indeed, this distressing symptom of chemotherapy treatment (even when vomiting is pharmacologically controlled) can become so

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severe that as many as 20% of patients discontinue the treatment (Jordan et al., 2005). Both preclinical and human clinical (e.g. Abrahamov et al. 1995; Meiri et al., 2007) research suggests that cannabinoid compounds may have promise in treating nausea in chemotherapy patients.

Animal models of vomiting have been valuable in elucidating the neural mechanisms of the emetic reflex (e.g. Hornby, 2001); however, the neural mechanisms of nausea are still not well understood (Andrews and Horn, 2006). One limitation in the preclinical screening of the nauseating side effects of compounds and the potential of compounds to treat nausea has been the lack of a reliable preclinical rodent model of nausea. For years researchers have been using conditioned taste avoidance in rats as a model of nausea, but it has been well documented that non-nauseating treatments also produce taste avoidance – it is not a selective measure of nausea (e.g. Parker et al., 2008). However, the considerable amount of evidence reviewed above indicates that conditioned disgust in rats elicited by an illness-paired flavour (e.g. Parker et al., 2008) or an illness-paired context (e.g. Rock et al., 2008) represents a selective and sensitive rodent model of nausea. This model may be a useful tool for elucidating the neurobiology of nausea and the role that the endocannabinoid system plays in the regulation of this distressing condition.

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References


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