Δ⁹-Tetrahydrocannabinol selectively acts on CB₁ receptors in specific regions of dorsal vagal complex to inhibit emesis in ferrets

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Van Sickle, Marja D., Lorraine D. Oland, Ken Mackie, Joseph S. Davison, and Keith A. Sharkey. Δ⁹-Tetrahydrocannabinol selectively acts on CB₁ receptors in specific regions of dorsal vagal complex to inhibit emesis in ferrets. Am J Physiol Gastrointest Liver Physiol 285: G566–G576, 2003. First published June 4, 2003; 10.1152/ajpgi.00113.2003.—The aim of this study was to investigate the efficacy, receptor specificity, and site of action of Δ⁹-tetrahydrocannabinol (THC) as an antiemetic in the ferret. THC (0.05–1 mg/kg ip) dose-dependently inhibited the emetic actions of cisplatin. The ED₅₀ for retching was ~0.1 mg/kg and for vomiting was 0.05 mg/kg. A specific cannabinoid (CB₁) receptor antagonist SR-141716A (5 mg/kg ip) reversed the effect of THC, whereas the CB₂ receptor antagonist SR-144528 (5 mg/kg ip) was ineffective. THC applied to the surface of the brain stem was sufficient to inhibit emesis induced by intragastric hypertonic saline. The site of action of THC in the brain stem was further assessed using Fos immunohistochemistry. Fos expression induced by cisplatin in the dorsal motor nucleus of the vagus (DMNX) and the mediobasal subnucleus of the nucleus of the solitary tract (NTS), but not other subnuclei of the NTS, was significantly reduced by THC rostral to obex. At the level of the obex, THC reduced Fos expression in the area postrema and the dorsal subnucleus of the NTS. The highest density of CB₁ receptor immunoreactivity was found in the DMNX and the mediobasal subnucleus of the NTS. Lower densities were observed in the area postrema and dorsal subnucleus of the NTS. Caudal to obex, there was moderate density of staining in the commissural subnucleus of the NTS. These results show that THC selectively acts at CB₁ receptors to reduce neuronal activation in response to emetic stimuli in specific regions of the dorsal vagal complex.

Area postrema; CB₂ receptor; dorsal motor nucleus of the vagus; nucleus of the solitary tract

EMESIS IS AN IMPORTANT PROTECTIVE GASTROINTESTINAL reflex (11). It is also an unpleasant and serious side effect of chemotherapy for the treatment of cancer and is a symptom of many diseases including diabetes and human immunodeficiency virus/autoimmune deficiency syndrome (3, 22, 29). Cannabinoids (CBs) are known to be effective antiemetics in humans, although they are not widely used because of their side effect profile (34). In a previous study (37), we showed in the ferret that CBs are effective antiemetics against the centrally acting opiate morphine 6-glucuronide through an action at CB₁ receptors. Other investigators (14, 33) have also found that CB₁ receptor agonists reduce emesis in the ferret and the shrew. An interesting extension of these observations was that in the ferret (37) and shrew (13) evidence was presented suggesting that an endogenous CB system may be present in the brain stem centers that modulate emesis, because specific CB₁ receptor antagonists potentiated the emetic response in the ferret and caused emesis when given alone to the shrew. These data are consistent with the hypothesis that the sites of CB₁ receptor distribution in the brain stem are the sites of neuronal activation in emesis and that CBs will inhibit emesis at these sites. We have tested this hypothesis by using Fos expression as a marker of neuronal activation (7, 9) in a system in which we first characterized the behavioral effects of the cancer chemotherapeutic and emetic agent cisplatin and Δ⁹-tetrahydrocannabinol (THC), a well-characterized CB derived from the cannabis plant.

Diverse emetic stimuli are known to activate the dorsal vagal complex (DVC) of the medulla in two ways. The DVC comprises the area postrema, nucleus of the solitary tract (NTS), and dorsal motor nucleus of the vagus (DMNX) (24). Activation of the DVC through the bloodstream activates neurons of the area postrema and the dorsal medial parts of the NTS by direct contact with blood-borne factors through their leaky blood-brain barrier (16, 25, 30). Neuronal activation in the NTS occurs through afferent inputs from the vagus and splanchnic nerves, carrying sensory information from the gastrointestinal tract (18, 26). The NTS is an important site of integration with higher centers, including the cortex. The DMNX carries signals to initiate the motor program of reverse peristalsis, which, when coordinated with appropriate respiratory movements, results in emesis (11).

NTS is subdivided anatomically into subnuclei with differing functions (8, 19, 27). Primary visceral afferent

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fibers coming into the brain through the solitary tract, project to different subnuclei with a viscerotopic arrangement. Gastrointestinal afferent fibers project to the subnucleus gelatinosus, whereas the cardiovascular, respiratory, and pulmonary afferent fibers project to the dorsal/dorsolateral, interstitial, and ventral/ventrolateral subnuclei, respectively. All visceral organ systems (except pelvic) also project to the commissural subnucleus in the caudal NTS, which becomes the medial subnucleus in the intermediate NTS (20). Ascending projections to forebrain autonomic nuclei arise mostly from the commissural and medial subnuclei of the NTS (26).

The ferret is a commonly used animal model for studies of emesis because of its sensitivity to emetics and the anatomic similarities to humans in the neuroanatomy of the brain stem and stomach (2, 21). We used cispatin in this species to explore the antiemetic actions of THC, to test whether this was mediated by CB1 receptors. We also examined the patterns of neuronal activation before and after CB1 receptor antagonism to correlate them with the distribution of CB1 receptors determined by immunohistochemistry.

MATERIALS AND METHODS

Behavioral experiments. Adult male ferrets (900–1500 g; Mustela putorius furo, Marshall Research Labs) were used for all studies (n = 3–5/group). These experiments were conducted in accordance with the guidelines established by the Canadian Council on Animal Care and were approved by the University of Calgary Health Sciences Animal Care Committee. Animals were fasted overnight before behavioral experiments, but had free access to water.

Ferrets were anesthetized with halothane and maintained at 1.5–2.0%. In the first treatment group, the vehicle used to dissolve THC (2% DMSO, 1% Tween 80 in physiological saline) was administered alone 15 min before the emetic agent cispatin. A small incision was made to expose the left jugular vein for the administration of cispatin (10 mg/kg iv). The incision was closed and then the unanesthetized ferret was observed for episodes of retching (de

1 h after the last emetic episode) to count the number of instances of retching (def

by jaw tone and conjunctival re

The incision was closed and then the unanesthetized ferret was observed for episodes of retching (defined by rhythmic abdominal contractions with an open mouth) and vomiting (defined by retching with the expulsion of saliva and gastric juices). In the second treatment group, THC (0.05 to 1.0 mg/kg ip; Lipomed, Arlesheim, Switzerland) was administered 15 min before cispatin. The third treatment group, the selective CB1 receptor antagonist, SR-141716A (5 mg/kg ip, Sanofi Research), or the selective CB2 receptor antagonist, SR-144528 (5 mg/kg ip; Sanofi Research), was administered 15 min before THC (0.5 mg/kg ip for SR-144528 and 1.0 mg/kg for SR-141716A), which was again administered 15 min before cispatin. One hour after the emetic episodes, ferrets from each treatment group were deeply anesthetized with pentobarbital sodium (65 mg/kg ip) and perfusion fixed as above, and the brains were removed to be fixed overnight in 4% paraformaldehyde at 4°C. Brains were then transferred to 30% sucrose in 0.1 M phosphate buffer at 4°C overnight. Sections of brain stem were cut at 30 μm and floating sections were incubated in one of three CB1 receptor antibodies at 4°C for 48 h. Antibodies used in this study were raised to the NH2 terminus of the human CB1 receptor (1:500; Affinity BioReagents) and the NH2 (Affinity BioReagents) and COOH terminus of the rat CB1 receptor (1:500; requests for this antibody may be directed to: kmackie@uwashington.edu) (17, 36). Specificity was confirmed by preincubation of the antibodies with the peptides used to raise the antibodies (10 nM in diluted antibody). Sections were washed with 0.1% Triton X-100 (in PBS) and incubated for 2 h in donkey anti-rabbit Cy3 as the secondary antisera. Tissues were examined by using a Zeiss Axiosplan fluorescence microscope and photographed with a digital camera (Sensys Photometrics, Tucson, AZ).

Fos immunohistochemistry. Sections of brain stem were cut at 30 μm and floating sections of the brain stem were incubated in 10% normal donkey serum (NDS) diluted in 0.1% Triton X-100 (in PBS) for 1 h followed by incubation in sheep anti-Fos (1:1,000, OA-11-824, Genosys Biotechnologies, Cambridge, UK) in PBS with 0.1% Triton X-100 and 10% NDS for 48 h at 4°C. Sections were washed in PBS and then incubated in biotinylated anti-sheep IgG secondary antibody solution for 60 min at room temperature. Staining was revealed by using the avidin biotin complex technique [Vectastain Elite avidin-biotin complex (ABC) Kit, Vector]. Briefly, sections were incubated in ABC reagent for 60 min at room temperature and then in a solution containing 3,3’-diaminobenzidine (0.5 mg/ml) for 30–60 s. Brain stem sections were then mounted on slides to dry for 24 h, dehydrated for 4 min each in 50, 70, 95, and 100% ethanol, and then cleared in xylene, after which they were mounted and viewed. The boundaries of the nuclei and subnuclei of the NTS were assessed in adjacent sections stained with cresyl violet based on our previous work (7–9).

The number of Fos immunoreactive neuronal nuclei was determined bilaterally for the area postrema, DMNX, and all subnuclei of the NTS at three levels along the rostrocaudal extent of the DVC, 0.5–1.0 mm rostral and 0.5 mm caudal to obex, and at obex. Three sections per level per animal were counted and averaged (n = 4 animals). Data were compared by using ANOVA with Tukey’s post test (Pricam; GraphPad Software, San Diego, CA).

Central THC administration. Adult male ferrets were anesthetized with halothane and a tracheotomy was performed so that retching episodes did not interfere with anesthetic administration. A blood pressure transducer was inserted and secured in the right carotid artery. The neck incision was then stapled closed around the tubes, and the ferret was turned onto its stomach. A midline incision was made over the base of the skull, and blunt dissection through the neck muscle exposed the dura, which was cut open over the cisterna magna. Once the brain stem was exposed, the halothane was lowered to 0.8%, and the ferret was left for 10 min. During this time the level of anesthetic was monitored by jaw tone and conjunctival reflex. Microdroplets of 0.01 mg/kg THC (~2.5 μl total) were applied to the surface of the brain stem, followed by a 15-min wait (n = 3 ferrets). In a separate control group (n = 3), 2.5 μl of the vehicle used to dissolve THC were applied to the brain stem surface. Hypertonic saline (2 M) was administered directly into the stomach through a feeding tube at 1 ml/min over 10 min. Hypertonic saline was used because it is a reliable emetic in the lightly...
anaesthetized ferret, whereas injectable emetics such as cisplatin are unreliable in this model. The ferret was then observed visually and by recordings of blood pressure for 60 min.

RESULTS

Antiemetic actions of THC in the ferret are mediated by CB1 receptors. We first investigated whether the CB1 receptor agonist THC was an antiemetic using cisplatin to evoke emesis. Cisplatin administered to the ferrets, after the injection of the vehicle for THC (n = 5), evoked 17.4 ± 5.5 episodes of retching and vomiting and 5.6 ± 2.1 episodes of vomiting alone in a 3-h period (Fig. 1A). With the administration of increasing doses of THC, the numbers of episodes of retching and vomiting were reduced with the complete elimination of emetic episodes at a dose of 1 mg/kg THC (n = 4, Fig. 1, A and B). To examine whether this was due to activation of CB1 receptors, the specific CB1 receptor antagonist SR-141716A was used. SR-141716A reversed the effects of THC. Thus SR-141716A increased the number of episodes of retching and vomiting evoked by cisplatin in the presence of THC to 10.8 ± 1.4 episodes of retching and 4.0 ± 0.8 episodes of vomiting (n = 4, Fig. 1C). When THC (n = 3) or SR-141716A (n = 3) was administered alone without cisplatin, no episodes of retching or signs of emesis were observed. The administration of SR-141716A with cisplatin evoked 16 ± 3.2 episodes of retching and vomiting and 4.3 ± 1.5 episodes of vomiting (n = 3), which is not significantly different from cisplatin alone.

To further address the specificity of the response, we investigated the effects of the selective CB2 receptor antagonist SR-144528. SR-144528 had no effect on the number of emetic episodes evoked by cisplatin in the presence of a submaximal dose of THC (n = 3, 0.5 mg/kg). This dose, which reduced the episodes of retching to 4.8 ± 1.0, was used because it allowed us to evaluate both a further reduction and also an increase in emetic episodes in the presence of SR-144528.

CB1 receptor activation selectively reduced Fos expression in discrete subnuclei of NTS, DMNX, and area postrema. Cisplatin treatment induced Fos expression in specific regions of the DVC. Fos expression was highest in the area postrema and the medial subnucleus of the NTS at all levels of the DVC that were examined (Fig. 2). Consistent Fos expression was also observed in the dorsal/dorsolateral, ventral/ventrolateral, and intermediate subnuclei of the NTS and the DMNX. Only a few Fos immunoreactive nuclei were observed in the interstitial or gelatinosus subnuclei of the NTS. As shown in Fig. 3, THC reduced Fos expression in the DVC evoked by cisplatin treatment in specific subnuclei of the NTS and the area postrema.

In the rostral DVC (0.5 mm rostral to obex), THC treatment significantly reduced the number of Fos immunoreactive nuclei per section in the medial subnucleus of the NTS from 65.6 ± 10.6 with cisplatin to 26.0 ± 4.4 (Fig. 4, P < 0.05, n = 4). This was reversed by pretreatment with the CB1 receptor antagonist SR-141716A to 80.0 ± 9.3 Fos immunoreactive nuclei per section, which was not significantly different from cisplatin alone (P > 0.05, n = 5). Within the medial subnucleus of the NTS, the density of Fos expression was highest in the area immediately dorsal to the DMNX after cisplatin treatment. In the same sections, THC reduced Fos immunoreactivity in the DMNX from 19.0 ± 2.9 to 7.3 ± 2.1 nuclei and the reduction was reversed by SR-141716A to 12.5 ± 0.3 nuclei (Fig. 4, P < 0.05). The majority of nuclei with Fos immunore-
activity in the DMNX were those of smaller cells rather than the large vagal motor neurons. There were no significant differences among the Fos immunoreactivity in the subnucleus gelatinosus or the medial, dorsal/dorsolateral, ventral/ventrolateral, intermediate, or interstitial subnuclei at this level for the three treatments (data not shown).

At the obex (Fig. 3), Fos expression in the area postrema in animals treated with cisplatin was reduced from 100.6 ± 19.6 to 42.0 ± 11.6 nuclei by THC treatment and was then significantly increased to 135.8 ± 33.7 nuclei by SR-141716A (Fig. 3, P < 0.05). In the same sections, Fos immunoreactivity was reduced in the medial subnucleus of the NTS from 55.4 ± 7.1 to 42.8 ± 3.0 nuclei by THC and, after SR-141716A pretreatment, was significantly increased to 66.5 ± 4.1 nuclei (P < 0.05). Also, around the obex, the number of Fos immunoreactive nuclei in the dorsal/dorsolateral subnucleus of the NTS was reduced from 7.5 ± 1.1 to 3.8 ± 0.3 nuclei by THC, and the reduction was significantly reversed by SR-141716A to 10.5 ± 1.8 nuclei. Other subnuclei of the NTS at this level showed no significant changes in Fos immunoreactivity among treatments and, in contrast to more rostral regions, the DMNX showed no significant change in Fos immunoreactive nuclei (Fig. 3).

In the caudal DVC, the only subnucleus that showed any significant changes among treatments was the ventral/ventrolateral subnucleus of the NTS in which Fos expression was reduced from 4.0 ± 0.8 to 1.3 ± 0.6 nuclei by THC (Fig. 4, P < 0.05). Pretreatment with SR-141716A increased Fos to 3.3 ± 0.7 nuclei, but this did not reach significance (P > 0.05).

Regions of Fos expression induced by cisplatin that are sensitive to CB1 receptor activation are also sites of CB1 receptor expression. At ~0.5–1.0 mm rostral to the obex, CB1 receptor immunoreactive terminals were concentrated in the DMNX and the medial subnucleus of the NTS with all three CB1 receptor antisera (Fig. 5A). Only with the antisera to the NH2 terminus of the human CB1 receptor were fibers localized within the solitary tract (TS) itself (Fig. 5B). The majority of the subnucleus gelatinosus of the NTS contains little CB1 receptor immunoreactivity, as does the intermediate subnucleus of the NTS, the interstitial subnucleus of the NTS, and tissue immediately surrounding the TS. Moderate terminal immunoreactivity was seen with all CB1 receptor antisera in the dorsal/dorsolateral subnucleus of the NTS and the
most dorsomedial aspect of the subnucleus gelatinosus.
Single immunoreactive neuronal perikarya were present
in the dorsal/dorsolateral, interstitial, intermediate, and
the ventral/ventrolateral subnucleus of the NTS with the
antisera to the NH₂ terminus of the human CB₁ receptor.

The area of the medial subnucleus dorsolateral to the
DMNX and medial to the TS shows less dense immuno-
reactivity. This is the position of the subnucleus centralis
(6, 12), which has not been completely described in the
ferret.

Fig. 4. Number of Fos positive nuclei/section in specific subnuclei of the DVC in ferrets treated with vehicle/
cisplatin (10 mg/kg iv, solid bar, n = 5), THC (1 mg/kg ip)cisplatin (open bar, n = 4) or SR-141716A (5 mg/kg
ip)/THC/cisplatin (gray bar, n = 4). In the rostral DVC (0.5 mm rostral to the obex), THC treatment significantly
reduced the number of Fos immunoreactive nuclei per section in only the mn of the NTS (A) and the DMNX (B).
This effect was reversed by the CB₁ receptor antagonist SR-141716A in both subnuclei. In the caudal DVC, THC
treatment significantly reduced the number of Fos immunoreactive nuclei per section in the v/vln only (C).
SR-141716A pretreatment did not reverse this effect. *Significant difference (P < 0.05, ANOVA followed by
Tukey's post test comparison) of THC/cisplatin from vehicle/cisplatin alone and from SR-14176A/THC/cis-
platin. **Significant difference (P < 0.05) of THC/cisplatin from THC/cisplatin/SR-141716A alone.
At the obex, CB1 receptor immunoreactivity in the medial subnucleus is most dense close to the dorsal aspect of the DMNX and becomes less dense as it extends laterally toward the TS (Fig. 5). Antiser to the human CB1 receptor revealed neuronal perikarya in the interstitial, intermediate, and ventral/ventrolateral subnuclei, although there was not much terminal staining with any of the antiser at this level. The DMNX, dorsal/dorsolateral, and medial subnuclei of the NTS and the subnucleus gelatinosus of NTS (which is much smaller at this level) show an even distribution of intense staining. The area postrema shows less dense immunoreactivity, but at higher magnification, immunoreactive terminals are clearly present in this tissue.

Immediately caudal to the obex, distribution of immunoreactivity is much the same as in the obex (Fig. 5). Sections show the same pattern of staining in the DMNX. Moderate staining appears in the commissural subnucleus of the NTS. There is less immunoreactivity in the area of the TS and the ventral/ventrolateral subnuclei, but they are not very well defined at this level.

**CB1 receptor activation by THC occurs centrally in the brain stem to inhibit emesis.** A low dose of THC, which was ineffective against cisplatin in the unanesthetized ferret (0.01 mg/kg), applied to the surface of the brain stem completely inhibited the emesis induced by intragastric hypertonic saline. Without THC application in control experiments, hypertonic saline induced 5.0 ± 1.2 episodes of retching and vomiting (Fig. 6C, n = 3, P < 0.05). The mean rise in arterial blood pressure in the THC-treated group was 8.7 ± 1.8 mmHg and the mean rise in arterial blood pressure was 62.0 ± 11.1 mmHg in the control group (Fig. 6D, P < 0.05). Episodes of retching and vomiting began to occur within an average of 4 min from the end of the hypertonic saline infusion.

**DISCUSSION**

In the present study, we have shown that THC dose-dependently reduced emesis induced by the chemotherapeutic agent cisplatin through an action at CB1 receptors. The specific CB2 receptor antagonist SR-144528 was found to have no effect on the antiemetic action of THC. Investigation of neuronal activation in the DVC demonstrated not only that THC treatment reduced Fos expression but that this could be reversed by using pharmacological blockade of the CB1 receptor. In the DMNX, medial subnucleus of the NTS, dorsal/dorsolateral subnucleus of the NTS, and the area postrema, neuronal activation in response to emesis was significantly reduced by CB1 receptor antagonism and returned to control levels by CB1 receptor antagonism. These same areas of the DVC showed dense CB1 receptor immunoreactivity. Taken together, these data provide strong support for the idea that CB modulation of the emetic response to cisplatin occurs in the DVC of the brain stem at CB1 receptors. These data are consistent with the hypothesis that endogenous CBs are physiological regulators of vagal reflexes that include emesis.

Our results show that THC was effective at reducing cisplatin-induced emesis. Cisplatin induces an early phase of emesis by causing serotonin (5-HT) release from enterochromaffin cells in the gut. This response can be inhibited by 5-HT3 receptor antagonists such as ondansetron (3, 18). THC has previously been shown (14) to reduce emesis induced by cisplatin in the shrew through CB1 receptors. Interestingly, a CB1 receptor antagonist, SR-141716A, was able to induce emesis when administered alone in the shrew (13), possibly suggesting a high level of emetic tone controlled by endogenous CBs in this species. The presence of endogenous CBs modulating emesis was also suggested by the potentiation of the emetic response to morphine 6-glucuronide by a CB1 receptor antagonist AM 251 in the ferret (37). Why potentiation was not seen in the present study when the CB1 receptor antagonist was given alone is not clear. Perhaps the response itself is at a maximum. Alternatively, it may be that the centrally acting emetic morphine 6-glucuronide can be potentiated as its site of action is in the brain stem, whereas the peripheral emetic cisplatin activates pathways with a lesser degree of endocannabinoid tone. It may be that tonic activation of CB1 receptors occurs in discrete subnuclei in the DVC, because SR-141716A not only antagonized the reduction in Fos expression but raised it to levels higher than control in the AP, medial, and dorsal/dorsolateral subnucleus of the NTS. This selectivity is also shown where THC had no effect on Fos expression such as the intermediate and interstitial subnuclei of the NTS, in which SR-141716A did not change the baseline (emesis induced) Fos counts.

CB1 receptor agonists are effective at reducing emesis induced by morphine 6-glucuronide, which acts centrally (37). Recent work by Simoneau et al. (33) showed similar behavioral findings by using centrally acting opioids to induce emesis. The synthetic CB WIN 55,212-2 reduced the number of emetic episodes in the ferret. This effect was reversed by using AM 251, whereas a CB2 receptor antagonist (AM 630) had no effect. Behavioral evidence showing effectiveness against both central and peripheral emetic agents suggests a common central site of action for CBs as antiemetics. Consistent with this hypothesis is the functional result that a dose of THC 100-fold lower than the effective dose to reduce emesis in response to cisplatin applied directly to the brain stem surface abolished emesis.

The DVC has been implicated as the site in the brain stem of neuronal activation in emesis (11, 18). With the use of Fos as a marker, various emetic stimuli have been found to activate neurons in specific subnuclei of the NTS, the DMNX, and the area postrema (4, 7, 9, 31, 39). The NTS and DMNX have been interpreted as sites of integration of gastrointestinal primary afferent activity with vestibular, cortical, or other regions of the brain that lead to coordinated activation of the motor nuclei that initiate emesis (11, 18). The area postrema is thought to be the chemoreceptor trigger zone in emesis because neurons in this region are activated by circulating agents in the cerebrospinal fluid (5, 10, 30).
There is also a primary afferent input to neurons in this region.

Previous work (7, 9, 31) in the ferret and the cat shows a distribution of Fos immunoreactivity similar to that shown in the present study. The medial subnucleus of the NTS and the area postrema show the most extensive Fos expression after peripheral emetic stimuli in the ferret (9), and this expression was dependent on an intact vagus nerve (7). A model of fictive vomiting (where cats are decerebrated and paralyzed) showed only minor differences in Fos immunoreactivity when compared with conscious, treated cats (31). Similar to the ferret, Fos was detected in the area postrema, NTS (medial and subpostremal), intermediate reticular
zone of the lateral tegmental field, nucleus retroambiguus, dorsal vagal, and phrenic motor nuclei in this model.

Esophageal primary afferents synapse in the subnucleus centralis of the NTS in the rat. The area of the subnucleus centralis, ventral and medial to the TS, was found to have less dense CB1 receptor immunoreactivity. Fos immunoreactive nuclei were also less dense in this region after emesis. Gastric primary afferents project to the subnucleus gelatinosus of the NTS in which they synapse with NTS neurons or directly with dendrites of neurons from the DMNX (1). The possibility of the CB1 receptor functioning directly at the terminals of the primary afferent fibers from the stomach and duodenum is discussed further in Fig. 7 and below. Gastric projections also extend into the DMNX and NTS at the level of the obex and just caudal and rostral to it (26). THC was found to significantly reduce Fos immunoreactivity after cisplatin throughout the rostrocaudal extent of the medial subnucleus of the NTS to a greater degree than changes in other subnuclei. This may be due to the presence of neurons in the medial subnucleus of the NTS that project to the forebrain and other brain areas for functions such as sensation (26).

Interstitial and intermediate subnuclei of the NTS receive input from primary afferents of the palate and pharynx in the rat (1). The lack of change in the

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**Fig. 5.** A: distribution of CB1 receptor immunoreactivity in the DVC with human NH2-terminal (a–c), rat COOH-terminal (d–f), and rat NH2-terminal (g–i) CB1 receptor antibodies 0.5 mm caudal to the obex (a, d, and g), at the level of the obex (b, e, and h), and 0.5–1.0 mm rostral to the obex (c, f, and i). The human NH2-terminal antibody gave dense CB1 receptor immunoreactivity in the DMNX (X) and mn of the NTS. With this antibody, we also observed immunoreactive neurons in the hypoglossal nucleus (XII). At rostral levels, the sg of the NTS was only weakly immunoreactive. The AP contained punctate immunoreactive terminals illustrated at higher magnification in Fig. 6. Both rat CB1 receptor antibodies displayed patterns of immunoreactivity similar to that of the human; however, the rat COOH-terminal antibody gave more pronounced immunoreactivity in the AP, whereas the neuronal immunoreactivity of the rat NH2-terminal antibody in the hypoglossal nucleus was less intense than that seen with the human NH2-terminal receptor antibody. Scale bar = 100 μm. B: higher magnifications of human NH2-terminal CB1 receptor immunoreactivity in the AP (a) and regions adjacent to the solitary tract (TS) (b and c). a: Punctate immunoreactive terminals are evident in the AP. b: TS shows immunoreactive fibers (open arrowhead), and in the ventral/ventrolateral subnucleus of the NTS immunoreactive neuronal perikarya (filled arrowheads) were observed. c: Immunoreactive neurons were also evident in the intermediate (open arrowhead) and interstitial subnucleus (filled arrowhead). Scale bars = 50 μm.
expression of Fos in the interstitial and intermediate subnuclei in the ferret may be due to the low number of perikarya in these nuclei or their lack of involvement in the cisplatin-induced emetic stimulus. However, the presence of CB1 receptor immunoreactive neuronal perikarya indicates a function for endogenous CBs in response to sensory information from the pharynx and palate. These sites also represent potential postsynaptic sites of CB1 receptor distribution, similar to what has been described for some lamina II neurons in the spinal cord (32). Despite the low number of neurons in the dorsal/dorsolateral subnucleus at the level of obex and ventral/ventrolateral subnucleus caudal to the obex, the reduction of Fos immunoreactivity by THC and the reversal by SR-141716A were significant. These areas could be involved in receiving the sensory feedback from the cardiovascular and respiratory systems during emesis (26).

The CB1 receptor has been found to reduce neurotransmitter release at both excitatory and inhibitory synapses in the cerebellum and the hippocampus by several means. CB1 receptor is a G protein-coupled receptor that can inhibit adenylate cyclase, inactivate A-type K+ channels, and inhibit transmitter release by the direct binding of the β/γ-subunit of the G protein to the calcium channels in the postsynaptic membrane (23). Preliminary studies by Derbenev et al. (15) show that CB1 receptor activation reduces glutamatergic transmission to the DMNX in the brain stem of the rat. Whereas the behavioral data and immunohistochemistry support a site of CB1 action within the DVC, the site at which emetic inputs converge and emesis is initiated currently remains unknown.

The CB1 receptor may reduce emesis at three sites indicated in Fig. 7: 1) by an action on the terminals of the primary afferent fibers themselves, 2) by acting on the terminals of neurons of the NTS that project to the output neurons of the DMNX, and 3) by inhibiting transmitter release from interneurons of the NTS. The possibility that CB1 receptors are found on the dendrites of the DMNX that project up into the NTS could be inferred from the immunohistochemical data, and further ultrastructural studies are required to definitively identify the localization of CB1 receptors in the DVC. However, the majority of electrophysiological evidence and electron microscopy have revealed that CB1 receptors in the brain are located at presynaptic sites (38). The interpretation of the three possibilities is on the basis of the assumption that input to an unidentified group of neurons in the DMNX results in their activation and the initiation of the motor program in the stomach, via the vagus (18).

At the first putative site, THC-activating CB1 receptors would inhibit emesis by reducing afferent transmission. In this case, a reduction in Fos immunoreactivity would be shown in neurons downstream of synaptic connections. This is consistent with our data, which showed a reduction in the NTS and DMNX. However, the lack of CB1 receptor immunoreactivity in the subnucleus gelatinosus of the NTS would suggest that CB1 receptor is not present on some vagal afferent fibers from the stomach. Similarly, in our previous work (37), unilateral nodose ganglionectomy had no effect on the density of CB1 receptor immunoreactivity in the ferret brain stem, although this result may have been confused by the presence of contralateral projection fibers. CB1 receptor immunoreactive fibers are evident in the TS itself, but the origins and targets of these fibers are unknown.

At the second potential site of action, CB1 receptors are present on the terminals of neurons within the NTS that receive inputs from the primary afferents. These may be inhibitory interneurons, which then reduce the activity of inhibitory NTS neurons that project to the DMNX. However, to date, the presence of interneurons, which transmit within the NTS and do not project to other brain stem nuclei, has not been demonstrated (35).

At the third potential site, CB1 receptors are present on the terminals of NTS neurons, which project to the DMNX or area postrema. The medial subnucleus of the NTS has enhanced Fos immunoreactivity after the emetic stimulation that was reduced by THC through a CB1 receptor-mediated action. The downstream target of this activation, the DMNX, also showed a reduction in Fos immunoreactivity by THC. The area postrema was strongly excited in response to cisplatin. The dramatic reduction in Fos immunoreactivity that we have demonstrated after THC pretreatment may be due to a modulatory input to the area postrema from the NTS regulated by the CB1 receptor. The area postrema itself is required for the initiation of emesis, because animals with their area postrema ablated are incapable of vom-

![Image](image-url)
ing in response to cisplatin (28). However, assuming that the area postrema is the sole site of action, then CB1 receptors would have to be present directly on the area postrema neurons, and this localization has not been observed.

THC clearly appears to reduce emesis by reducing the neuronal activation in the DVC in response to cisplatin. Complex neuronal circuitry for this reflex within the NTS is consistent with a nucleus of integrative capacity as opposed to a simple relay center (26, 35). Thus the importance of demonstrating CB1 receptor modulation of the emetic reflex goes beyond the regulation of emesis to the modulation of other vagovagal and vagospinal reflexes that control other important gastrointestinal and possibly other functions.

In summary, we have shown that THC dose-dependently inhibits emesis induced by cisplatin through an action at CB1 receptors. The site of action of THC is in discrete nuclei of the DVC. These same areas are also discretely inhibited by THC. Therefore, the area postrema is not the sole site of action, then the modulation of other vagovagal and vagospinal reflexes that control other important gastrointestinal and possibly other functions.

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DISCLOSURES

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