Published in final edited form as:

Eur J Pharmacol. 2010 February 25; 628(1-3): 195. doi:10.1016/j.ejphar.2009.11.030.

Pranlukast prevents cysteinyl leukotriene-induced emesis in the least shrew (*Cryptotis parva*)

Seetha Chebolu^{a,1}, Yaozhi Wang^{a,1}, Andrew P. Ray^a, and Nissar A. Darmani^a

Seetha Chebolu: schebolu@westernu.edu; Yaozhi Wang: yaw007@ucsd.edu; Andrew P. Ray: aray@westernu.edu; Nissar A. Darmani:

^a Department of Basic Medical Science, College of Osteopathic Medicine of the Pacific, Western University of Health Sciences, 309 East Second Street, Pomona, CA 91766, USA

Abstract

Many chemotherapeutic agents activate multiple signaling systems, including potentially emetogenic arachidonic acid metabolites. Of these messengers, the emetic role of the leukotriene family has been neglected. The aims of this study were to test the emetic potential of key leukotrienes (LTA₄, LTB₄, LTF₄, and the cysteinyl leukotrienes LTC₄, LTD₄ and LTE₄), and to investigate whether the leukotriene CysLT₁ receptor antagonist pranlukast or mixed leukotriene CysLT_{1/2} receptor antagonist Bay u9773 can prevent the LTC₄ induced emesis. Least shrews were injected with varying doses of one of the six tested leukotrienes and vomiting parameters measured for 30 minutes. LTC₄ and LTD₄ were most efficacious, and significantly increased both the frequency and percentage of animals vomiting at doses from 0.1 and 0.05 mg/kg, respectively. The other tested leukotrienes were either weakly emetic or ineffective at doses up to 4 mg/kg. The relative emetogenic activities of the cysteinyl leukotrienes (LTC₄=LTD₄>LTE₄) suggest leukotriene CysLT₂ receptors have a key role in emesis. However, pranlukast dose-dependently, and at 10 mg/kg completely, blocked LTC₄-induced vomiting, implicating a leukotriene CysLT₁ receptor-mediated emetic effect. Bay u9773 dose-dependently reduced the percentage of animals vomiting, but did not significantly reduce vomiting frequency. Fos immunoreactivity, measured subsequent to LTC₄-induced vomiting to define its putative anatomical substrates, was significantly increased in the enteric nervous system and medullary dorsal vagal complex following LTC₄ (P < 0.05) versus vehicle injections. This study is the first to show that some leukotrienes induce emesis, possibly involving both central and peripheral leukotriene CysLT₁ and/or leukotriene CysLT₂ receptors.

Keywords

Leukotriene A₄; Leukotriene B₄; Fos; Dorsal vagal complex; Enteric nervous system

Corresponding Author: Nissar A. Darmani, Address: Department of Basic Medical Sciences, College of Osteopathic Medicine of the Pacific, Western University of Health Sciences, 309 East Second Street, Pomona, CA 91766, USA., Tel.: 1 909 469 5654; Fax: 1 909 469 5577. ndarmani@westernu.edu (Nissar A. Darmani).

¹Present Address: Department of Neuroscience, University of California at San Diego, 9500 Gilman Drive # 0662, La Jolla, CA 92093, USA

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1. Introduction

One of the most distressing side effects of antitumor treatment is chemotherapy-induced vomiting (Slatkin, 2007). Although its full mechanism has continued to elude discovery, chemotherapy-induced vomiting appears to be a multifactorial process with dopamine, serotonin, substance P, and eicosanoids all contributing to its genesis (Andrews and Rudd, 2004; Minami et al., 2003; Darmani et al., 2009). One key eicosanoid, arachidonic acid, is released from cell membrane phospholipids by phospholipase A2. Depending on the cell and tissue type, arachidonic acid is then metabolized into different compounds by two key enzymes: 1) cyclooxygenases, which lead to formation of prostaglandins; and 2) lipoxygenases, which catalyze its conversion to hydroperoxyeicosatetraenoic acid (5-HPETE) and then to the rapidly metabolized intermediate leukotriene A₄ (LTA₄). LTA₄ is then converted to leukotriene B₄ via LTA₄ hydrolase or conjugated with glutathione to form the cysteinyl (or peptidyl) leukotriene, leukotriene C₄ (LTC₄). Enzymatic cleavage of specific portions of the glutathione moiety produces the other cysteinyl leukotrienes, leukotrienes D₄ (LTD₄), and E₄ (LTE₄) (Barnes, 1997; Capra et al., 2006), and also leukotriene F₄ (LTF₄), which is related to LTC₄ but like LTB₄ does not bind the cysteinyl leukotriene receptors. Two G-protein coupled receptors (GPCR) specific for cysteinyl leukotrienes (leukotriene CysLT₁ and leukotriene CysLT₂) have been cloned and characterized (Kanaoka and Boyce, 2004; Figueroa et al., 2001; Heise et al., 2000). Leukotriene CysLT₁ is expressed mostly in respiratory and gastrointestinal tissues and leukotriene CysLT₂ in cardiovascular and brain tissues (Capra et al., 2006).

Despite the knowledge that arachidonic acid and its related metabolites (prostaglandins E₂ and F_{2α} and their analogs) are potent emetogens (Darmani, 2002; Kan et al., 2003; Kan et al., 2006), little attention has been paid to the emetic potential of its other metabolites such as the leukotrienes. The only study that has indirectly attempted to determine the emetogenicity of the leukotrienes is the unsuccessful use of the leukotriene biosynthesis inhibitor MK-886 to block chemotherapy-induced vomiting in ferrets (Sam et al., 2007). Rather, extensive research on cysteinyl leukotrienes has examined their key role in asthma. Therefore, the aims of this study were 1) to test key leukotrienes as putative emetogens; and 2) to investigate if the currently available leukotriene CysLT₁ receptor antagonists could prevent leukotriene-induced vomiting. For this, we used the previously characterized (Barnes, 1997; Evans, 2003) human leukotriene CysLT₁ receptor selective antagonist pranlukast, and the mouse leukotriene CysLT_{1/2} dual antagonist Bay u9773 (4-[[[(1R,2E,4E,6Z,9Z)-1-[(1S)-4-carboxy-1-hydroxybutyl]-2,4,6,9-pentadecatetraen-1-yl]thio]benzoic acid). Additionally, immunohistochemistry for Fos protein was used to indicate neuronal activation related to leukotriene-induced vomiting and provide information on its anatomical substrates. In this study we used the least shrew (*Cryptotis parva*), an emesis-competent mammal whose responses to common emetogens are well defined and correlate well with human responses (Darmani, 1998; Darmani et al., 2008).

2. Materials and Methods

2.1 Animals

Adult least shrews (*C. parva*) were bred in the animal facility at Western university. Previous studies had demonstrated no gender differences, so both males and females were used. They were housed in groups on a 14:10 light:dark cycle, fed with food and water ad libitum. All the shrews used were 45–60 days old and weighed between 4–5 g. All experiments were conducted between 9:00 and 16:00 h and in accordance with Western University IACUC standards.

2.2 Drugs

Pranlukast, Bay u9773, LTA₄, LTB₄, LTC₄, LTD₄, LTE₄ and LTF₄ were purchased from Cayman Chemicals. All the leukotrienes were supplied in ethanol. The ethanol was evaporated under nitrogen and the drug was dissolved to twice the stated concentrations in a 1:1:18 solution of ethanol: emulphor™: 0.9% saline. This solution was then diluted with an equal volume of saline to a final ethanol concentration of 2.5%. Pranlukast and Bay u9773 were dissolved to twice the stated concentrations in DMSO and this was then diluted with an equal volume of distilled water to a final DMSO concentration of 50%.

2.3 Experimental protocols

On the day of the experiment, shrews were brought from the animal facility, separated into individual cages and allowed to adapt for at least two hours. One hour before the experiment food was withheld. The experiment was initiated when the shrews were injected intraperitoneally (i.p.) with either the vehicle for control animals, or one of the different leukotrienes at varying doses (0.025, 0.0375, 0.05, 0.1, 0.25, 0.5, 1, 2 or 4 mg/kg, n = 5–18 per dose). Each individual shrew received one dose of one leukotriene only. Shrews were given 4 mealworms each prior to the injections, to aid in identifying wet vomits as described previously (Darmani et al., 1998). The shrews were observed for 30 min for vomiting behavior (number of animals vomiting within groups and frequency of vomits). Animals were used once and then euthanized with an overdose of pentobarbital (100 mg/kg i.p.) following the behavioral studies. Some were transcardially perfused with paraformaldehyde solution following anesthesia for use in subsequent immunohistochemical (Fos immunoreactivity) studies (see below).

For the agonist-antagonist studies, pranlukast (0, 2.5, 5 or 10 mg/kg, n = 7–11 per dose) or Bay u9773 (1 or 5 mg/kg, n=6–8 per dose) was injected at 0 min. Immediately after this injection, shrews were given 4 mealworms each, and 30 min later the leukotriene agonist (1 mg/kg LTC₄) was injected. The agonist was chosen on the basis at which it caused maximum emesis in all tested animals at the lowest dosage. Each treated shrew was observed for 30 min post agonist injection and the number of animals vomiting within groups and frequency of vomits were recorded. Animals were euthanized with an overdose of pentobarbital (100 mg/kg i.p.) following the behavioral studies.

Immunohistochemical procedures were used to support the behavioral data and identify potential anatomical sites of action of leukotriene-mediated vomiting, and were performed as described elsewhere (Ray et al., 2009b). The number of Fos-immunoreactive (Fos-positive) nuclei resulting from LTC₄-induced vomiting versus non-vomiting, vehicle-injected shrews was compared in brain and gut regions previously found to be associated with emesis (i.e., the dorsal vagal complex and enteric nervous system of the small intestine). The brains, as well as intestinal sections 2 cm long, beginning 1 cm from the stomach, were taken from transcardially perfused shrews used in either control or LTC₄-treated behavioral groups. All tissue was processed immunohistochemically using the same methods (Ray et al., 2009b) regardless of its source.

2.4 Statistical analysis

The vomiting frequency data and dose-dependence were analyzed using the Kruskal–Wallis non-parametric analysis of variance (ANOVA). Animals within a group which did not vomit were still counted (as a zero frequency) for the purpose of statistical analysis. The percentage of animals vomiting across groups at different doses was compared using the Mann-Whitney *U* test. The mean numbers of Fos-positive nuclei across vomiting and non-vomiting conditions were counted by an observer blind to the drug condition and analyzed via Student's *t*-test. Nuclei were counted on a per section basis in all tissues. In the ENS, sections were also subdivided into 20µm lengths for counting because the oblique sectioning made identifying

individual enteric ganglia difficult in some sections. In all cases, a P -value < 0.05 was necessary for statistical significance.

3. Results

3.1 Leukotrienes dose-response emesis studies

The different leukotrienes tested were LTA₄, LTB₄, LTC₄, LTD₄, LTE₄ and LTF₄. As mentioned, LTC₄, LTD₄, and LTE₄ are the cysteinyl leukotrienes.

3.1.1 Cysteinyl leukotrienes—Dose-response vomiting behavior data for the cysteinyl leukotrienes are presented in Fig. 1. Both LTC₄ (Fig. 1A, B) and LTD₄ (Fig. 1C, D) showed dose-dependent increases in vomiting behaviors. Compared to vehicle injection, LTC₄ demonstrated significantly higher frequencies of vomiting ($P < 0.05$) at doses greater than 0.05 mg/kg, and significant percentage of animals vomited ($P < 0.05$) at doses greater than 0.1 mg/kg. LTD₄ had a significantly greater frequency of vomiting ($P < 0.05$) and percentage of animals vomiting ($P < 0.05$) at all tested doses from 0.05 – 2 mg/kg. Only LTC₄ produced 100% vomiting, at the 1 mg/kg dosage ($U = 84, 0$; $P < 0.0005$), while LTD₄ produced 91% vomiting at the same dosage ($U = 126, 6$; $P < 0.0005$). LTE₄ demonstrated significant increases in the vomiting frequency ($P < 0.05$) and the percentage of animals vomiting ($P < 0.05$) at doses greater than 1 and 2 mg/kg, respectively (Fig. 1E, F).

3.1.2. Non-cysteinyl leukotrienes—As shown in Fig. 2A/B, LTA₄ administration caused significant emesis only at its highest tested (4 mg/kg) dose (frequency, $P < 0.01$; percentage vomiting, $U = 114, 42$; $P < 0.02$) when compared to the vehicle treated group. LTB₄ failed to induce significant vomiting at doses up to 4 mg/kg (Fig. 2C, D). On the other hand, LTF₄ injection caused emesis in 36–40 % of shrews ($P < 0.05$, $U = 84, 36$; $P < 0.05$, $U = 42, 18$, respectively) at 1 and 4 mg/kg doses, statistically significant when compared against vehicle treated controls. However, LTF₄ showed a significant increase in emesis frequency at 1 mg/kg ($P < 0.05$) but not at the 4 mg/kg dose ($P > 0.05$) (Fig. 2E, F).

As noted in Fig. 1A and B, the most potent emetic responses occurred with LTC₄ at the 1 mg/kg dose. Hence, LTC₄ was chosen as the agonist for the subsequent agonist-antagonist studies.

3.2 The effects of pranlukast and Bay u9773 pretreatment on LTC₄-induced vomiting

Pranlukast pretreatment (i.p.) blocked LTC₄-induced emesis in a dose-dependent manner (Fig. 3). As shown in Fig. 3A/B, when compared with the vehicle injection, pranlukast pretreatment significantly reduced the frequency of vomiting at 5 ($P = 0.005$) and 10 mg/kg ($p < 0.0005$) by 80% and 100% respectively. The percentage of animals vomiting was also significantly reduced at the 5 ($U = 52.5, 10.5$; $P < 0.01$) and 10 mg/kg ($U = 56, 0$; $P < 0.0005$) doses (Fig. 3B). To control for possible effects on emesis generated by the antagonists themselves, each was tested alone for vomiting behavior induction. No dose of pranlukast (1–10 mg/kg) induced significant vomiting by itself (no shrews vomited at all). After Bay u9773 injection, 1 in 5 shrews (1 mg/kg) and 1 in 6 shrews (5 mg/kg) vomited one time each, but the effect was not significant ($P > 0.05$).

Pretreatment (i.p.) with Bay u9773 produced a dose-dependent decrease in vomiting induced by LTC₄ ($P < 0.05$). A significant decrease in the percentage of animals vomiting was noted at its 5 mg/kg dose (100% vs. 33%, $P < 0.05$) (Fig. 3D). However, only a trend towards reduction in the frequency of induced emesis was noted and the difference failed to achieve significance at either doses of Bay u9773 (Fig. 3C).

3.3 Fos immunoreactivity following LTC₄ injections

Representative photomicrographs demonstrating Fos-IR in the brain and intestine are presented in Fig. 4. The number of Fos-immunopositive nuclei following LTC₄-induced vomiting seen in the enteric nervous system and the dorsal vagal complex is shown in Fig. 5 and compared to non-vomiting, vehicle-injected controls. The dorsal vagal complex was subdivided for counting purposes into its traditionally-described component nuclei, the dorsal motor nucleus of the vagus, nucleus of the solitary tract, and area postrema (Ray et al., 2009b). Fos-IR nuclei were significantly more numerous in the LTC₄-injected shrews relative to vehicle-injected, non-vomiting controls, in the dorsal motor nucleus of the vagus ($P < 0.05$, Fig. 5A) and nucleus of the solitary tract ($P < 0.05$, Fig. 5B), but not in the area postrema (Fig. 5C). The enteric nervous system also demonstrated significantly more Fos-IR nuclei ($P < 0.05$, Fig. 5D) following LTC₄ injection.

4. Discussion

This study is the first to show that the cysteinyl leukotrienes LTC₄ and LTD₄ are emetic in least shrews, and that pranlukast, an antagonist at the human cysteinyl leukotriene CysLT₁ receptor, can block the LTC₄-induced vomiting. Also of note, Bay u9773, the dual antagonist of both leukotriene CysLT_{1/2} receptor subtypes, demonstrated a dose-dependent decrease in LTC₄-induced emesis as well. The emetogenic activity of cysteinyl leukotrienes appears to be a specific effect, as the non-cysteinyl leukotrienes tested were at best weak, and more often ineffective, emetogens.

4.1 Behavioral effects of leukotrienes

As mentioned previously, the cysteinyl leukotrienes exert their effects by binding to two G-protein coupled receptors, CysLT₁ and CysLT₂. The rank order binding potency in mice and humans for leukotriene CysLT₁ is LTD₄ $\gg$ LTC₄ $>$ LTE₄, and for leukotriene CysLT₂ is LTC₄ = LTD₄ $\gg$ LTE₄ (for review see Liu et al., 2003; Evans, 2003). In this study, the rank order of emetic potency in least shrews for the cysteinyl leukotrienes was LTC₄ = LTD₄ $>$ LTE₄, and thus most closely resembles the binding affinity for the leukotriene CysLT₂ receptor. However, pranlukast efficiently blocked LTC₄-induced emesis, despite being considered a specific leukotriene CysLT₁ antagonist in humans. Thus, either pranlukast binds to leukotriene CysLT₂ receptors as well as leukotriene CysLT₁ receptors, or emesis expression is not strictly correlated with CysLT binding. The former explanation seems more likely based on data from other studies. For example, mouse leukotriene CysLT₁ and leukotriene CysLT₂ receptors were found to be similar to human receptors in binding and functional characteristics, but pranlukast still antagonized mouse leukotriene CysLT₂ receptors transfected into CHO cells (Evans, 2003; Ogasawara et al., 2002). Therefore, these data suggest that even though there is strong evidence of pranlukast being a specific antagonist at human leukotriene CysLT₁ receptors, species differences can affect its selectivity for non-human CysLT₁ receptors. Indeed, Bay u9773 has been shown to be a partial agonist at human leukotriene CysLT₂ receptors, but an antagonist at mouse leukotriene CysLT₂ receptors (Evans, 2003). Thus, species differences in shrew versus human cysteinyl leukotriene receptors might result in antagonism of least shrew leukotriene CysLT₂ receptors. Furthermore, the antiemetic activity of Bay u9773 was largely similar to that of pranlukast, and could not be used to differentiate between the activated receptor subtypes. Further work will be necessary to discern which receptor subtype is predominantly involved, and whether LTC₄ and LTD₄ stimulate the same or different receptors preferentially.

The similarities of the emetic potency in least shrew and the published receptor-binding profiles in other species suggest a structure-activity relationship may exist. Indeed, our data indicate a critical role for the glutathione moiety of the cysteinyl leukotrienes in LTC₄-mediated

vomiting. The intact glutathione molecule is a modified tripeptide with glutamate, cysteine, and glycine residues. LTC₄ has a complete glutathione conjugate bound to the lipid moiety via the cysteine's sulfur. LTD₄ is enzymatically cleaved to remove the glutamate residue, and demonstrates a slight reduction in emetic activity. LTE₄ is metabolized from LTD₄ via removal of the glycine residue, and is therefore left with only the cysteine residue. LTE₄ was only weakly emetogenic. The glycine residue appears to be important for receptor binding affinity since LTF₄, which lacks only the glycine residue, either does not bind, or at best very weakly binds the cysteinyl leukotriene receptors (Lord et al., 1985). Based on the data from this study in which LTC₄ and LTD₄ are significantly more emetogenic than LTE₄ and LTF₄, both of which demonstrate similarly weak emetogenicity, the minimum prerequisite for a significant emetic response appears to be the glycine residue, while retaining the entire tripeptide provides an optimal effect. It should be noted, though, that glutathione in itself is not emetogenic (Darmani, unpublished observations), and thus the correlation of vomiting behavior with glutathione is most likely related to its ability to enhance or otherwise stabilize leukotriene CysLT receptor binding by the leukotriene molecule.

4.2 Role of central and peripheral systems in LTC₄-induced emesis

As mentioned, human leukotriene CysLT₁ is expressed mostly in respiratory and GI tissues and human leukotriene CysLT₂ in cardiovascular and brain tissues (Capra et al., 2006). This suggests that depending on the receptor subtype(s) stimulated, leukotriene-induced emesis in least shrews could be either due to peripheral (gut CysLT₂-containing), central (brain CysLT₁-containing), or combined loci. From an anatomical standpoint, it is likely that leukotrienes (via leukotriene CysLT₁ receptors) can stimulate vagal afferents terminating in the GIT (Hornby, 2001). Inflammatory mediators like leukotrienes are known to activate the intracellular Ca⁺² induced Ca⁺² release (CICR) pool, and bring about inhibition of a post-spike hyperpolarization (AHP_{slow}) current present in vagal afferent terminals, which results in an increase in firing frequency (Cordoba-Rodriguez et al., 1999). Furthermore, increased firing frequency in vagal afferents also correlates with vomiting due to other emetogens (Round and Wallis, 1986; Koga and Fukuda, 1992; Minami et al., 1995; Cordoba-Rodriguez et al., 1999), and could therefore be a mechanism through which leukotrienes induce emesis. Vagal afferent neurons reside in the nodose ganglion, and bifurcating fibers projecting from the same afferent neurons terminate in both the GIT and the nucleus of the solitary tract, thus inducing activity at both the enteric and central nervous system levels, and specifically in the dorsal vagal complex. Although this activity is one possible source of Fos-IR in the dorsal vagal complex, our Fos-IR data also demonstrated that both enteric and dorsal vagal complex nuclei are activated following leukotriene-induced vomiting. Taken together, the combined data suggest that cysteinyl leukotrienes can modulate emetic activity through both peripheral and central components. When combined with the studies described above, the data imply that facilitation of vagal activity via Ca⁺² signaling would be a likely mechanism for this activation.

Induction of Fos-IR nuclei in the dorsal motor nucleus of the vagus and nucleus of the solitary tract, but not in the area postrema, suggests that blood borne mediators do not have a significant role in leukotriene-induced emesis. This would be expected if injection of LTC₄ was acting directly on GIT tissue or GIT-localized afferent terminals, since leukotrienes undergo rapid breakdown and function primarily as paracrine signals (Jedlitschky et al., 1993). Alternatively, as indicated for some other emetogenic stimuli (Bhandari et al., 1992; Zaman et al., 2000; Ray et al., 2009a), cysteinyl leukotriene receptors in the area postrema could be located on dendrites extending into the area postrema from nucleus of the solitary tract neurons, and would therefore not increase Fos-IR in the area postrema. This effect is rarely observed, however, as the area postrema frequently demonstrates Fos-IR related to activation by blood-borne emetogens (Ray et al., 2009a). Based on the short half-life of leukotrienes after injection combined with the lack of Fos-IR in the area postrema, the most likely hypothesis is that leukotriene-induced

vomiting is initiated in the GIT via leukotriene CysLT₁ receptors, and central Fos-IR is induced upon activation of the nucleus of the solitary tract via vagal afferent fibers, rather than direct activation of dorsal vagal complex leukotriene CysLT₂ receptors via blood-borne leukotrienes. It should be noted, though, that Fos-IR is a correlative measure, and Fos-IR in the enteric nervous system could be due to the output (motor response) phase rather than the initiation phase of vomiting. In addition, evidence has accumulated that cysteinyl leukotrienes themselves can increase the permeability of the blood-brain barrier (Chio et al., 1992) or be transported by multidrug resistance proteins (Liu et al., 2009). Thus, a direct central mechanism cannot be completely ruled out.

In conclusion, our study is the first to show leukotrienes cause emetic behavior, which could be successfully blocked by the specific cysteinyl leukotriene receptor antagonists pranlukast and Bay u9773. Further work will be necessary to define the specific mechanism through which leukotrienes induce vomiting, as well as whether vomiting related to inflammation occurs separately but in parallel with vomiting caused by other emetogens (e.g. cisplatin), or whether it acts in concert with them to increase the severity of vomiting induced by those other emetogens.

Acknowledgments

This work was supported by NIH grant # R01CA115331 from the National Cancer Institute to Dr. Darmani.

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Fig. 1. The dose-dependent emetic effects of cysteinyl leukotrienes

Intraperitoneally administered cysteinyl leukotrienes (LTC₄, LTD₄ and LTE₄) at varying doses showed dose-dependent emetic effects in a 30 min post-injection observation period in the least shrew. Graphs A, C and E represent dose-response curves for the frequency of emesis (mean ± S.E.M.) for LTC₄, LTD₄ and LTE₄ respectively. Graphs B, D and F show the respective dose-response curves for the percentage of shrews in a given group that vomited in response to intraperitoneal administration of LTC₄, LTD₄ and LTE₄ respectively. * Represents significant (P < 0.05) differences from the vehicle control (0 mg/kg).

Fig. 2. The dose-dependent emetic effects of non-cysteinyl leukotrienes

Intraperitoneally administered non-cysteinyl leukotrienes (LTA₄, LTB₄ and LTF₄) at varying doses showed limited emetic effects in a 30 min post-injection observation period in the least shrew. Graphs A, C, and E represent dose-response curves for the frequency of emesis (mean ± S.E.M.) following intraperitoneal injection of LTA₄, LTB₄ and LTF₄, respectively. Graphs B, D and F show the respective dose-response curves for the percentage of shrews in a given group that vomited in response to LTA₄, LTB₄ and LTF₄ injection. * Represents significant ($P < 0.05$) differences from the vehicle control (0 mg/kg).

Fig. 3. Dose-dependent inhibitory effects of Pranlukast and Bay u9773 on vomiting parameters induced by LTC₄ injection

Prior intraperitoneal administration of the cited doses of either pranlukast (graphs A and B) or Bay u9773 (graphs C and D), attenuated both the frequency ($\pm$ S.E.M.) and the percentage of shrews vomiting in response to an intraperitoneal injection of 1 mg/kg dose of LTC₄ in the 30 min observation period following emetogen administration. * Represents a statistically significant ($P < 0.05$) reduction in vomiting versus vehicle-pretreated controls.

Fig. 4. Examples of Fos-immunoreactive nuclei in the dorsal vagal complex (DVC) and enteric nervous system (ENS)

Arrows point to examples of Fos-IR nuclei. Brain sections are cut sagittally, and intestinal sections are cut at an oblique angle to the longitudinal axis. A) Fos-IR nuclei in the dorsal vagal complex of vehicle-injected controls. Modest expression of Fos is normal in the brains of freely-behaving animals. B) Fos-IR in the NTS and AP of LTC₄-injected (1 mg/kg, i.p.) shrews. Note the increased expression in the NTS, but not the AP. The DMNX is not present at this level, but appeared similar to the NTS in terms of degree of labeling. C) Fos-IR in the intestine of vehicle-injected controls. Very little or no Fos was expressed in the ENS. D) Fos-IR was induced in the intestine of LTC₄-injected shrews. The sections shown in C and D were morphologically similar to jejunal tissue sections. In both sections, the ENS nerve plexi were in the darker band of tissue in the top right (C) or left (D) corner adjacent to the crypts. Fos-IR was primarily restricted to the nerve plexi. Abbreviations: AP- area postrema, C- intestinal crypts, ENS- enteric nervous system, NTS- nucleus of the solitary tract. Scale bar for all plates = 50 μ m.

Fig. 5. Counts of Fos-immunoreactive nuclei following vehicle and leukotriene C₄ injection-induced emesis

Means and standard errors of Fos-immunoreactive nuclei per section following vehicle and LTC₄ injections (1 mg/kg, i.p.) are graphed for each subdivision of the DVC (DMNX, NTS and AP) and for each 20 μ m length of the ENS. * Represents a significant ($P < 0.05$) increase in Fos-IR nuclei versus vehicle-pretreated controls. Abbreviations: AP- area postrema, DMNX- dorsal motor nucleus of the vagus nerve, DVC- dorsal vagal complex, ENS- enteric nervous system, NTS- nucleus of the solitary tract.