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Authors

Parker, LA
Limebeer, CL
Rock, EM
[et al.](#)

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The FAAH inhibitor URB-597 interferes with cisplatin- and nicotine-induced vomiting in the *Suncus murinus* (house musk shrew)

LA Parker¹, CL Limebeer¹, EM Rock¹, DL Litt¹, M. Kwiatkowska¹, and D. Piomelli²

¹Department of Psychology, University of Guelph, Guelph, Ontario

²Department of Pharmacology, University of California at Irvine, Irvine, CA

Abstract

Considerable evidence implicates the endocannabinoid system as a neuromodulator of nausea and vomiting. The action of anandamide (AEA) can be prolonged by inhibiting its degradation, through the use of URB597 (URB), a Fatty Acid Amide Hydrolase (FAAH) enzyme inhibitor. Here we present evidence that the FAAH inhibitor, URB, interferes with cisplatin- and nicotine - induced vomiting in the *Suncus murinus*. In Experiment 1, shrews were injected with URB (0.9 mg/kg) or vehicle 120 min prior to the behavioral testing. They received a second injection of AEA (5 mg/kg) or vehicle 15 min prior to being injected with cisplatin (20 mg/kg) or saline and the number of vomiting episodes were counted for 60 min. In Experiment 2, shrews were injected with vehicle or URB (0.9 mg/kg) 120 min prior to receiving an injection of nicotine (5 mg/kg) or saline and the number of vomiting episodes were counted for 15 min. Experiment 3 evaluated the potential of the CB1 antagonist, SR141716, to reverse the effect of URB on nicotine-induced vomiting. URB attenuated vomiting produced by cisplatin and nicotine and the combination of URB+AEA suppressed vomiting produced by cisplatin. The effect of URB on nicotine-induced vomiting was reversed by SR141716. These data suggest that the EC system plays a tonic role in the regulation of toxin-induced vomiting.

Keywords

Emesis; Endocannabinoid; shrew; FAAH; Nicotine; Cisplatin

1. Introduction

Considerable evidence implicates the endocannabinoid system as a neuromodulator of nausea and vomiting [see 1, 2]. The anti-emetic properties of cannabinoid drugs extend beyond humans [3] to include other emetic species such as pigeons [4], ferrets [5, 6], cats [7], least shrews, *Cryptotis parva* [e.g. 8] and the house musk shrew *Suncus murinus* [9, 10]. The anti-emetic properties of ⁹-THC are reversed by pretreatment with the cannabinoid 1 (CB₁) antagonist/inverse agonist, SR141716 [8, 6, 10]. SR141716 also produces vomiting on its own at higher doses [8]. The natural ligands for CB receptors,

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Corresponding author: Linda A Parker, Dept of Psychology, University of Guelph, Guelph Ontario N1G 2W1, (Phone) 519-824-4120, 56330, (Fax) 519-837-8629, parkerl@uoguelph.ca.

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arachidonylethanolamide or anandamide (AEA) and 2-arachidonyl glycerol (2-AG), have been shown to be involved in emesis in a variety of studies. AEA has weak anti-emetic effects in both shrews [11] and ferrets [6] but 2-AG induces vomiting via its downstream metabolites such as arachidonic acid and prostaglandins in shrews [11].

CB₁ receptors are found throughout the brain, including the areas involved in emetic reactions in the brainstem [6]. Emetic stimuli activate the dorsal vagal complex (DVC) of the medulla, including the area postrema (AP), nucleus of the solitary tract (NTS), and the dorsal motor nucleus of the vagus (DMNX) by 2 pathways [12]: 1) Activation of the DVC through the bloodstream activates neurons of the AP and dorsal medial parts of the NTS through a leaky blood-brain barrier. 2) Activation of the DVC through the NTS occurs through afferent inputs from the vagus and splanchnic nerves, carrying sensory information from the gastrointestinal tract. The NTS integrates the information that arrives to the DVC with higher centers. The DMNX carries signals to initiate the motor program of reverse peristalsis resulting in emesis [12].

Central CB₁ receptors have been shown to be involved in the control of emesis in the ferret [6, 13]. Cannabinoid agonists were effective antiemetics against the centrally acting opiate morphine 6-glucuronide through activation at CB₁ receptors [6]. Furthermore, when ⁹-THC was applied to the surface of the brainstem, emesis induced by intragastric hypertonic saline was inhibited (van Sickle et al 2003). Finally, cisplatin-induced Fos expression was induced in the AP, DMNX and the medial subnucleus of the NTS, but not regions of the NTS that were not involved in emesis [13]. ⁹-THC (administered ip) reduced Fos expression in each of these areas. Additionally, van Sickle et al [14] recently reported that CB₂ receptors are also expressed in the DVC, which may play the role of facilitating the action of endocannabinoids on CB₁ receptors to suppress toxin-induced emesis.

AEA is an endogenous agonist for cannabinoid receptors [15] which is rapidly degraded by the fatty acid amide hydrolase (FAAH) [16] that is distributed throughout the brain and periphery [17]. The action of AEA can be prolonged by inhibiting its degradation, through the use of URB597 (URB), a FAAH enzyme inhibitor, that can increase basal levels of AEA in the rat brain [18]. It has been shown that systemic administration of URB in rats potentiates the hypothermic actions of AEA [18]. URB has been reported to suppress vomiting produced by morphine 6 glucuronide in the ferret [19], but has been reported to be ineffective in suppressing cisplatin induced vomiting in the least shrew [20]. Here we present evidence that the FAAH inhibitor, URB597, interferes with cisplatin- and nicotine - induced vomiting in the *Suncus murinus*.

2. Methods

2.1 Subjects

The shrews were bred and raised in a colony at Wilfrid Laurier University, and the University of Guelph. Both males (30–50 gm) and females (19–35 gm) were used as subjects and were equally distributed among the groups. The sexes did not significantly differ in vomiting frequency in any analysis; therefore, males and females were pooled in all reported analyses.

2.2 Drugs

All drugs were administered intraperitoneally (ip), except nicotine which was administered subcutaneously (sc). URB-597 (URB; .9 mg/kg, synthesized in the Piomelli laboratory), anandamide (AEA; 5 mg/kg) and SR141716 (SR; 2.5 mg/kg) were prepared in a solution of 1 ml ethanol/1 ml Cremaphor (Sigma)/18 ml physiological saline. Previous work with the *Suncus* suggests that equivalent behavioural effects with rats requires increasing the shrew

dose by a factor of 3 [1, 9, 10, 26]; therefore the dose was 3 times the most effective dose (0.3 mg/kg) across a variety of tests with rats [18, 21, 22, 23]. Cisplatin (20 mg/kg) was prepared in physiological saline. Nicotine (5 mg/kg) was prepared in a physiological saline solution. The URB and AEA vehicle comparison groups were injected with the 1 ml alcohol/1 ml Cremaphor/18 ml saline solutions and the cisplatin and nicotine vehicle comparison groups were injected with physiological saline.

2.3 Procedures

Experiment 1: Effect of URB and/or AEA on cisplatin-induced vomiting—Each animal was offered four meal worms (*Tenebrio* sp.) in its home cage 15 min prior to pretreatment injections. The shrews received the first pretreatment injection (URB or VEH) 120 min prior to the behavioral testing (this is the optimal pretreatment interval to elevate AEA according to Fegley et al [18]) and a second pretreatment injection (AEA or VEH) 15 min prior to the behavioral testing. Five min after the second pretreatment injection, the shrews were injected with either cisplatin or saline. The groups which varied by pretreatment drugs and treatment drugs were as such: VEH-VEH-saline (n=6), VEH-VEH-cisplatin (n=8), VEH-AEA-saline (n=5), VEH-AEA-cisplatin (n=7), URB-VEH-saline (n=6), URB-VEH-cisplatin (n=7), URB-AEA-saline (n=5), URB-AEA-cisplatin (n=8). Male (N=28) and female (N=24) shrews were assigned to groups in approximately equal number (as feasible).

Ten min after the treatment injection, the shrews were placed in the clear Plexiglas observation chamber (22.5 × 26 × 20 cm), which was illuminated by four 60W lights suspended from the chamber's floor. A mirror was mounted at a 45° angle beneath the chamber floor, which allowed for the observation of the ventral surface of the shrew for better viewing of vomiting reactions. An observer counted the number of vomiting episodes (expulsion of fluids from stomach).

Experiment 2: Effects of URB on nicotine-induced vomiting—As in Experiment 1, the shrews were initially offered four meal worms in their home cage 15 min prior to pretreatment injections. The shrews received the pretreatment injection (URB or VEH) 120 min before receiving the treatment injection (nicotine or saline). The AEA pretreatments were not included in Experiment 2, because they did not facilitate the suppressive effects of URB on vomiting in Experiment 1. The shrews were then immediately placed in the observation chamber for 15 min and the frequency of vomiting episodes was measured. The groups were: VEH-saline (n=7), VEH-nicotine (n=8), URB-saline (n=8), URB-nicotine (n=8) with males (N=18) and females (N=14) distributed across the groups matched as closely as possible.

Experiment 3: Effect of SR141716 on URB suppression of nicotine-induced vomiting—Fifteen min following presentation of 4 meal worms, the shrews were injected with URB or VEH, 2 hr prior to the test. The groups treated with SR141716 were injected 30 min prior to the test. All shrews were injected with nicotine and placed in the observation chamber for 15 min and the frequency of vomiting was measured. The groups were: VEH-nicotine (n=8), URB-nicotine (n=8), VEH-SR-nicotine (n=6), URB-SR-nicotine (n=6).

3. Results

Experiment 1: Effect of URB and/or AEA on cisplatin-induced vomiting

As can be seen in Figure 1, URB and URB + AEA attenuated cisplatin-induced vomiting. The mean number of vomits displayed by the shrews was analyzed as a 4 (pretreatment groups) by 2 (treatment groups) analysis of variance (ANOVA). The analysis revealed

significant effects of pretreatment, $F(3, 44) = 3.3$; $p = .029$, treatment, $F(1, 44) = 27.4$; $p < .001$, and a pretreatment by treatment interaction, $F(3, 44) = 3.3$; $p = .029$. Among the shrews treated with cisplatin, subsequent Bonferroni T tests revealed that those pretreated with URB-VEH ($p = .04$) and URB-AEA ($p = .013$) displayed significantly fewer episodes of vomiting than did those pretreated with VEH-VEH; however, VEH-AEA did not differ from any group.

Experiment 2: Effect of URB on nicotine-induced vomiting

As can be seen in Figure 2, URB pretreatment also attenuated nicotine-induced vomiting in the shrews. The 2 by 2 ANOVA revealed significant effects of pretreatment, $F(1, 27) = 20.0$; $p < .001$; treatment, $F(1, 27) = 173.5$; $p < .001$; and a pretreatment by treatment interaction, $F(1, 27) = 20.0$; $p < .001$. Among the nicotine treated shrews, those pretreated with VEH vomited more than those pretreated with URB ($p < .01$). However, Group URB-nicotine displayed more vomiting episodes than either group not treated with nicotine ($ps < .001$), suggesting that the dose of 0.9 mg/kg of URB did not completely prevent NIC-induced vomiting.

Experiment 3: Effect of SR141716 on URB suppression of nicotine-induced vomiting

Figure 3 reveals that the CB₁ antagonist, SR141716, reversed the suppressant effect of URB on nicotine-induced vomiting. A one-way ANOVA revealed a significant pretreatment effect, $F(3, 24) = 6.3$; $p < .01$. Bonferroni post-hoc comparison tests revealed that Group URB displayed significantly ($p < .05$) fewer vomiting episodes than any other group.

4. Discussion

Although AEA alone did not significantly reduce vomiting, when its action was presumably prolonged by pretreatment with the FAAH inhibitor, cisplatin-induced vomiting was suppressed in the *Suncus murinus*. In fact, even when AEA was not exogenously administered, URB suppressed cisplatin-induced vomiting, suggesting that the action of the endocannabinoid was enhanced by pretreatment with the FAAH inhibitor. Furthermore, this anti-emetic effect was not selective to cisplatin-induced vomiting, because in Experiment 2, URB also attenuated nicotine-induced vomiting. Finally, the suppression of nicotine-induced vomiting by URB was reversed by SR141716, indicating a CB₁ receptor mechanism of action. These results are consistent with those reported by Sharkey et al (19) using Morphine 6 glucuronide as the emetic agent in ferrets.

Presumably, the anti-emetic effect of URB was mediated by its potential to prolong the activity of the endocannabinoid, AEA, which has previously been shown to have weak anti-emetic effects on its own in ferrets [6] and least shrews [11]. Although the effect of our treatments on tissue levels of AEA were not specifically assayed in the present experiment, URB has been shown to produce a slow accumulation of AEA in the brain with a maximal effect at 2 hr post-injection [18, 21]. Recently, Darmani et al [20] reported that very high doses of URB (5–10 mg/kg) administered 10 min prior to cisplatin did not prevent vomiting in the least shrew. However, in the present experiments, when a much lower dose of URB (0.9 mg/kg) was administered 2 hr before cisplatin or nicotine, vomiting was suppressed. It is therefore likely that the interval between URB injection and the induction of emesis was too short in the prior study to demonstrate the anti-emetic effects of FAAH inhibition. This explanation is further supported by other behavioral effects of URB produced by a 2 hr pretreatment interval. That is, URB can magnify the hypothermic response produced by AEA [18] and produce anxiolytic-like responses in both the zero-maze test and the isolation-induced ultrasonic vocalization test when administered 2 hr before behavioral testing [21].

These anxiolytic responses were significantly attenuated with an injection of SR141716, suggesting a CB₁ mechanism of action.

The results reported here suggest that FAAH inhibition produces an anti-emetic effect. Previous work from our laboratory suggests that FAAH inhibition can also produce an anti-nausea effect. Cross-Mellor et al [22] found that administration of URB alone and in combination with AEA reduced conditioned gaping reactions elicited by a Lithium Chloride (LiCl)-paired saccharin solution rats (a putative model of nausea [see 23 for review]). These effects were reversed by pretreatment with AM251, suggesting that they were CB₁ mediated. More recently, Rock et al [24] reported that URB also attenuated the expression of conditioned gaping reactions in rats elicited by re-exposure to a context previously paired with LiCl (a model of anticipatory nausea); this effect was reversed by CB₁ antagonism with SR141716. When administered prior to conditioning, URB also interfered with the establishment of conditioned gaping elicited by the LiCl-paired context. Interestingly, unlike URB or THC [25, 26], serotonin (5-HT₃) antagonists, the classic anti-emetic drugs, are not effective in suppression anticipatory nausea when it develops in humans [eg, 27] or in rats [25]. Therefore, FAAH inhibition by URB may be a potential alternative treatment for vomiting and for nausea in patients that do not respond to currently available treatments.

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Figure 1. Mean ($\pm$ sem) frequency of vomiting episodes among the various pretreatment groups in Experiment 1, treated with saline or cisplatin; * indicates significantly ($p < .05$) different from VEH-VEH-Cisplatin.

Figure 2. Mean ($\pm$ sem) frequency of vomiting episodes among the various pretreatment groups in Experiment 2, treated with saline or nicotine; ** indicates significantly ($p < .01$) different from VEH-Nicotine.

Figure 3.

Mean ($\pm$ sem) frequency of vomiting episodes among the various pretreatment groups in Experiment 3. All rats were treated with nicotine (5 mg/kg); * indicates significantly ($p < .05$) different from all other groups.