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Delta-9-tetrahydrocannabinol and cannabidiol, but not ondansetron, interfere with conditioned retching reactions elicited by a lithium-paired context in *Suncus murinus*: An animal model of anticipatory nausea and vomiting

Linda A. Parker [a,*], Magdalena Kwiatkowska [a], Raphael Mechoulam [b]

[a] *Department of Psychology, Wilfrid Laurier University, Waterloo, Ontario, Canada N2L 3C5*
[b] *Department of Medicinal Chemistry and Natural Products, Hebrew University of Jerusalem, Jerusalem, Israel*

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Abstract

Chemotherapy patients report not only acute nausea and vomiting during the treatment itself, but also report anticipatory nausea and vomiting upon re-exposure to the cues associated with the treatment. We present a model of anticipatory nausea based on the emetic reactions of the *Suncus murinus* (musk shrew). Following three pairings of a novel distinctive contextual cue with the emetic effects of an injection of lithium chloride, the context acquired the potential to elicit conditioned retching in the absence of the toxin. The expression of this conditioned retching reaction was completely suppressed by pretreatment with each of the principal cannabinoids found in marijuana, Δ^9-tetrahydrocannabinol or cannabidiol, at a dose that did not suppress general activity. On the other hand, pretreatment with a dose of ondansetron (a 5-HT_3 antagonist) that interferes with acute vomiting in this species, did not suppress the expression of conditioned retching during re-exposure to the lithium-paired context. These results support anecdotal claims that marijuana, but not ondansetron, may suppress the expression of anticipatory nausea.

Keywords: Chemotherapy; Nausea; Emesis; Cannabinoids; Cannabidiol; THC; Marijuana; Ondansetron; Serotonin; Classical conditioning; Shrew; Learning

1. Introduction

Cancer patients undergoing chemotherapy treatment suffer the side effects of nausea and vomiting. When not alleviated by anti-emetic treatment during the initial sessions, anticipatory nausea and vomiting are also reported by chemotherapy patients upon re-exposure to the cues associated with the treatment [1,2]. Anticipatory nausea and vomiting (ANV) is best understood as a classically conditioned response [1]; that is, cues present at the time of exposure to the toxin acquire aversive properties as the result of the pairings. Interestingly, the human literature suggests that anti-emetic treatments, such as ondansetron, which are very effective in attenuating unconditioned nausea and vomiting produced by the chemotherapy treatment, are ineffective in attenuating ANV [3–5]. Little is known about pharmacological treatment of ANV.

Animal models of acute emesis have been critical to the development of pharmacological treatments to reduce nausea and vomiting in human patients. Although rats and mice are not capable of vomiting, considerable research with dogs, cats and ferrets has resulted in the development of highly effective anti-emetics [6]. Research with emetic species is limited by the cost of housing relatively large animals, which reduces the number of animals that may be included in a study. However, animal models of emesis have been recently developed [7,8] with the mouse-sized *Suncus murinus* (musk shrew) and the even smaller least shrew [9]. The insectivore shrew retches and vomits when administered most emetic treatments.

Although there has been considerable experimental investigation of unconditioned retching and vomiting elicited by toxins, there have been relatively few reports of conditioned retching and vomiting; that is, emetic reactions elicited by re-exposure to a toxin paired cue. Conditioned retching has been reported to occur in coyotes, wolves, hawks and ferrets when they are presented with cues previously paired with lithium-induced toxicosis [10–12].

* Corresponding author.
E-mail address: lparker@wlu.ca (L.A. Parker).

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Animal models of emesis provide tools for the evaluation of the anti-emetic potential of drug compounds. The 5-hydroxytryptamine-3 (5-HT_3) receptor antagonist drugs, such as ondansetron, have been shown to be highly effective in suppressing emetic reactions in animal models of vomiting and in human patients [6]. In fact, 5-HT_3 antagonists have reduced the incidence of vomiting in chemotherapy patients by 70%–80% [6]. Although highly effective in suppressing acute vomiting following a chemotherapy session, 5-HT_3 antagonists are ineffective in reducing ANV if it develops [3–5].

Testimony of numerous patients indicates that marijuana reduces both acute and anticipatory nausea and vomiting associated with chemotherapy, thereby maintaining the resolve to continue with therapy [13,14]. Marijuana contains over 60 cannabinoids, but the most prominent of these are the psychoactive component, Δ^9-tetrahydrocannabinol (THC), and the non-psychoactive component, cannabidiol (CBD). Early clinical trials indicated that pure THC and the synthetic cannabinoid, nabilone (an analogue of THC) reduced unconditioned nausea and vomiting induced by chemotherapy treatments [15,16]. These findings have been experimentally validated in animal models [9,17,18]. THC suppresses unconditioned retching and vomiting elicited by the chemotherapeutic agent, cisplatin [9,17] and lithium [18] in the shrew. Furthermore, THC suppresses conditioned retching (ANV) elicited by a lithium-paired context [19]. CBD has also been shown to suppress lithium-induced vomiting in the *S. murinus*, within a limited dose range (5–10 mg/kg, intraperitoneally (ip)). Since CBD is not intoxicating these results suggest that it may serve as an effective cannabinoid treatment for nausea and vomiting.

The present investigation evaluated the potential of the cannabinoids, THC and CBD, and the 5-HT_3 antagonist, ondansetron (OND), to interfere with the expression of conditioned emetic reactions elicited by a contextual cue previously paired with lithium chloride in *S. murinus*. The doses employed were selected on the basis of their potential to interfere with toxin-induced vomiting in the *Suncus* [17,18]. In the human literature, if nausea and vomiting are not prevented during the initial chemotherapy treatments by pretreatment with a 5-HT_3 antagonist, such as OND, then ANV develops in over 40% of the patients [1,20]. Once ANV develops, then OND is not effective in treating these symptoms. If conditioned retching in the shrew is a model of ANV in humans, then ondansetron would not be expected to interfere with the expression of previously established lithium-induced conditioned retching in the shrew, unlike the cannabinoids. Additionally, the potential of the nonpsychoactive cannabinoid compound, CBD, to interfere with conditioned retching was newly evaluated.

2. Method

2.1. Subjects

A total of 46 shrews (23 male [30–50 g] and 23 female [20–35 g] served as subjects. All shrews were born and raised in the Wilfrid Laurier breeding colony (stock descents from animals raised at the University of Virginia.). The animals were housed individually in $28 \times 17 \times 12$ cm solid bottomed plastic cages with pine wood shavings and shredded paper towels for bedding. They were maintained on a 14:10 h light:dark cycle (lights on at 0700 h, EST) at 23+1 °C, and received food and water ad libitum, except as specified. The food was a 10:1 mixture of Purina Cat Chow (Ralston Purina) and Complete Mink Pellets. All animals were weaned at 20 days of age.

2.2. Drugs

All drugs were injected ip at a volume of 1 ml/kg. The pretreatment drugs were all prepared in a solution of 1 ml ethanol/1 ml Cremaphor (Sigma)/18 ml of physiological saline and delivered at a volume of 1 ml/kg. THC (provided by the National Institute on Drug Abuse [NIDA]) was prepared as a 3 mg/ml solution of the vehicle (VEH), CBD was prepared as a 5 mg/ml solution of the vehicle and Ondansetron (Sigma) was prepared as a 1.5 mg/ml solution. The dose of each agent selected (THC: 3 mg/kg; CBD: 5 mg/kg; OND: 1.5 mg/kg) has been previously shown to prevent toxin-induced vomiting in the shrew [17,18]. The treatment drug was 0.15 M lithium chloride (LiCl) or physiological saline (Sal) solution both at a volume of 60 ml/kg, because a dose of 390 mg/kg (60 ml/kg of 0.15 M) LiCl is required to reliably induce vomiting in the shrew [18].

2.3. Apparatus

The conditioning chamber ($22.5 \times 26 \times 20$ cm) was constructed of clear Plexiglas. A mirror hung at an angle beneath the chamber to facilitate viewing the ventral surface of the shrews. The room was illuminated by four 25-W light bulbs located 30 cm from either side of the test chamber. A digital videocamera was focused on the mirror beneath the chamber and the behavior of each shrew was recorded over each 45 min session.

2.4. Procedure

2.4.1. Experiment 1: conditioned retching elicited by a contextual cue

The shrews received three conditioning trials, separated by 72 h, during which the contextual chamber was paired with either lithium chloride ($n=5$) or with saline ($n=6$). On each trial, each shrew was injected with the appropriate solution and immediately placed in the test chamber for a period of 45 min. The frequency of vomiting and retching episodes were measured by an observer. The chambers were thoroughly washed with lemon scented soapy water between each animal's trials.

The test trials began 6 days after the final conditioning trial. The shrews received two test trials (separated by 72 h). On each trial, they were injected with 1 ml/kg of vehicle immediately prior to placement in the test chamber for 45 min and their behavior was videotaped for later scoring.