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Δ^9-THC and related cannabinoids suppress substance P-induced neurokinin NK_1-receptor-mediated vomiting via activation of cannabinoid CB_1 receptor

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Abstract

Δ^9-THC suppresses cisplatin-induced vomiting through activation of cannabinoid CB_1 receptors. Cisplatin-evoked emesis is predominantly due to release of serotonin and substance P (SP) in the gut and the brainstem which subsequently stimulate their corresponding 5-HT_3- and neurokinin NK_1-receptors to induce vomiting. Δ^9-THC can inhibit vomiting caused either by the serotonin precursor 5-HTP, or the 5-HT_3 receptor selective agonist, 2-methyserotonin. In the current study, we explored whether Δ^9-THC and related CB_1/CB_2 receptor agonists (WIN55,212-2 and CP55,940) inhibit vomiting evoked by SP (50 mg/kg, i.p.) or the NK_1 receptor selective agonist GR73632 (5 mg/kg, i.p.). Behavioral methods were employed to determine the antiemetic efficacy of cannabinoids in least shrews. Our results showed that administration of varying doses of Δ^9-THC (i.p. or s.c.), WIN55,212-2 (i.p.), or CP55,940 (i.p.) caused significant suppression of SP-evoked vomiting in a dose-dependent manner. When tested against GR73632, Δ^9-THC also dose-dependently reduced the evoked emesis. The antiemetic effect of Δ^9-THC against SP-induced vomiting was prevented by low non-emetic doses of the CB_1 receptor inverse-agonist/antagonist SR141716A (< 10 mg/kg). We also found that the NK_1 receptor antagonist netupitant can significantly suppress vomiting caused by a large emetic dose of SR141716A (20 mg/kg). In sum, Δ^9-THC and related cannabinoids suppress vomiting evoked by the nonselective (SP) and selective (GR73632) neurokinin NK_1 receptor agonists via stimulation of cannabinoid CB_1 receptors.

Keywords

Vomiting; Δ^9-THC; SR141716A; cannabinoid CB_1 receptor; Substance P; GR73632

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1. Introduction

Vomiting is a protective reflex (Carpenter, 1990) which helps to remove ingested toxins from the gastrointestinal tract (GIT) (Horn, 2008). It is also a side-effect of drugs including cancer chemotherapeutics such as cisplatin (Andrews et al., 1998). Cisplatin-evoked acute and delayed emesis are mainly due to co-release of serotonin (5-hydroxytryptamine, 5-HT), dopamine, and substance P (SP) from enterochromaffin cells of the upper GIT, the vagus and/or other GIT nerves, as well as the brainstem (Andrews et al., 1998). These neurotransmitters (5-HT, dopamine, and SP), or corresponding receptor emetic drugs induce vomiting either by: i) activating their respective local serotonin 5-HT3 (5-HT_3)- and SP neurokinin 1- (NK_1) receptors present primarily on vagal afferents in the GIT, which eventually stimulate brainstem emetic loci, or ii) getting into the brainstem via the bloodstream to directly activate the brainstem emetic loci present in the area postrema (AP), the nucleus of the solitary tract (NTS), and the dorsal motor nucleus of the vagus (DMNX), collectively called the dorsal vagal complex (DVC) (Darmani and Ray, 2009).

The observation that NK_1 receptor antagonists could suppress both the acute- and the delayed-phases of cisplatin-induced nausea and vomiting (CINV), encouraged clinical trials of several drugs including: ezlopitant, vofopitant, aprepitant and netupitant in patients undergoing chemotherapy (Andrews and Rudd 2004; Karthaus et al., 2019). Interestingly, various reports have demonstrated that NK1 receptor antagonists have a distinctive antiemetic profile from 5-HT_3 receptor antagonists in their ability to inhibit both acute- and delayed phases of cisplatin-induced vomiting, as well as vomiting evoked by peripheral (e.g., abdominal vagal afferent electrical stimulation) and centrally-acting emetogens (e.g., apomorphine). Among 5-HT_3 receptor antagonists, palonosetron in combination with NK_1 receptor antagonists is recommended for the prevention of severe CINV caused by high-dose cisplatin therapy (Herrstedt et al., 2017). The mixture of palonosetron with an NK_1 receptor antagonist such as netupitant seemingly has synergistic antiemetic efficacy against both acute and delayed emesis (Rojas et al., 2014; Darmani et al., 2011). In fact, when these two drugs were given together with dexamethasone, over 90% control of cisplatin-induced vomiting has been described (Aapro et al., 2014; Keating, 2015). Unfortunately, although the combined use of NK_1 receptor- as well as 5-HT_3 receptor-antagonists, has substantially lowered rates of cisplatin-mediated acute and delayed emesis, a marked number of patients continue to suffer from CINV (Karthaus et al., 2019). Furthermore, there is no available treatment exclusively for nausea or a collective anti-nausea/ antiemetic drug which would suppress both nausea and vomiting irrespective of the source (Andrews and Sanger, 2014). Subsequently, nausea is still negatively impacting patients' quality of life.

The key psychoactive component of the marijuana plant, delta 9-tetrahydrocannabinol (Δ^9-THC) (Janoyan et al., 2002) and a number of its analogues/ formulations (Δ^8-THC, nabilone, and dronabinol) have been used against acute- and delayed-phases of chemotherapy-evoked vomiting in patients (Voth and Schwartz, 1997). Overwhelming clinical evidence indicate that Δ^9-THC pretreatment reduces emesis in some patients receiving cancer chemotherapy (Voth and Schwartz, 1997). Two oral synthetic formulations, dronabinol and nabilone, have been approved by the US Food and Drug Administration for antiemetic use against CINV. Evidence also supports cannabinoids effectiveness against nausea during the delayed phase

of chemotherapy-evoked emesis which is poorly controlled by 5-HT_3 receptor- and NK receptor-antagonists (Slatkin, 2007). Unlike the relatively large body of findings regarding the antiemetic potential of 5-HT_3 receptor- and NK_1 receptor- antagonists, only limited studies on the antiemetic effects of cannabinoids against diverse emetogens are available in vomit-competent animals (Darmani, 2002).

The mechanisms by which Δ^9-THC and its structural analogs produce their antiemetic effects were revealed following the cloning of cannabinoid CB_1 and CB_2 G-protein coupled receptors (CB_1 receptor and CB_2 receptor) (Di Marzo, 2008). CB_1 receptors are distributed throughout the central and the peripheral nervous system (Pertwee et al., 2010). CB_2 receptors are often localized in immune tissues in the periphery (Darmani, 2010). Δ^9-THC and associated cannabinoids behave as broad-spectrum agonist antiemetics in a CB_1 receptor antagonist-sensitive manner (Darmani, 2010). We and others have previously tested the antiemetic efficacy of Δ^9THC against diverse emetogens such as: i) cisplatin (Ray et al., 2009b), ii) the 5-HT precursor 5-hydroxytryptophan (5-HTP), iii) 5-HT_3 receptor agonists, iv) Δ_2 receptor agonists (Darmani and Crim, 2005), and v) the CB_1 receptor antagonist/inverse-agonist SR141716A. Since cisplatin evokes vomiting via the release of 5-HT, dopamine and substance P (SP) (etc), in the current study we investigated the antiemetic potential of Δ^9-THC against vomiting evoked by the neurokinin NK_1 receptor agonists SP and GR73632. Thus, we used the least shrew to evaluate: 1) the potential of Δ^9-THC and its analogs (WIN55,212-2 and CP55,940) to suppress vomiting produced by SP or the NK_1 receptor selective agonist GR73632, 2) the ability of low doses of the cannabinoid CB_1 receptor antagonist/inverse-agonist SR141716A, to reverse the antiemetic potential of Δ^9THC against SP-induced vomiting, and 3) the ability of NK_1 receptor selective antagonist netupitant to suppress emesis evoked by a large dose of SR141716A since the latter agent potentiates the neuronal release of SP (Lever and Malcangio, 2002).

2. Materials and methods

2.1 Animals

Male and female (4 – 6 g, 35 – 60 days old) adult least shrews from our animal facility were used. Shrews were housed in groups of 5 – 10 on a 14:10 light: dark cycle, at a humidity-controlled room temperature of 21 ± 1°C, with an ad libitum supply of food and water (Darmani et al., 2003b) (Darmani, 2001d). All animals received care according to the Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services Publication, revised, 2011). All experimental procedures were conducted between 8:00 am and 17:00 pm. All of the procedures used in this study were approved by the Western University Institutional Animal Care and Use Committee of Western University of health Sciences (Application number R17IACUC036).

2.2 Drugs

Δ^9-YHC, R(+)-WIN55,212-2, and substance P (SP) were purchased from Sigma/RBI (St. Louis, MO). CP55,940 was provided by Pfizer (Groton, CT) and SR141716A by Safoni-Synthelabo Recherche (Montpellier, France). Netupitant was a gift from the Helsinn Health Care (Lugano, Switzerland). GR73632 was purchased from Tocris Cookson Inc. (Ellisville,

MO). All other reagents were obtained from Sigma (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA).

Δ^9-THC, WIN55,212-2, CP55,940, SR141716A, and netupitant were dissolved to twice the stated drug dose in a 1:1:18 solution of ethanol:Emulphor™:0.9% saline. The drug doses were then diluted further with an equal volume of saline. This step was mandatory because the 1:1:18 vehicle mixture can stimulate vomiting by itself in up to 20% of animals (Darmani, 2001c). SP and GR73632 were dissolved in sterile distilled deionized water. All drugs were administered at a volume of 0.1 ml/10 g of body weight.

2.3 Emesis studies

The present protocols were based upon our previous emesis studies (Darmani, 2001b, c; Darmani et al., 1999). All experiments were performed between 8:00 am and 17:00 pm. On the day of the experiment, shrews were brought in experimental room from the animal facility, weighed, transferred to a 20 × 18 × 21 cm clean clear plastic individual cages, and allowed to acclimate for 1 h during which daily food was withdrawn. Drug-naive male and female shrews were randomly allocated to the control and the experimental groups regardless of their cage of origin. The shrews were given four meal worms (*Tenebrio sp.*) each 30 min before the administration of emetogens, to help identify wet vomits as described previously (Darmani et al., 1999). No animal was dropped from the experiment. For behavioral experiments, we present both the mean vomit frequency and the percentage of shrews vomiting. We have utilized the % vomit data to calculate "ID_{50} values."

Both SP and its selective NK_1 receptor agonist GR73632, can induce robust vomiting in least shrews at 5 and 50 mg/kg (i.p.) doses, respectively (Darmani et al., 2008). At time zero, different groups of shrews were injected with either: 1) Δ^9-THC (0, 0.5, 2.5, or 5 mg/kg; n = 8 shrews/group, respectively via i.p. except for 1.25 mg/kg where n = 10) or (0, 5, 10, or 20 mg/kg; n = 8 shrews/group, respectively via s.c. except for 2.5 mg/kg dose where n = 6); 2) WIN55,212-2 (0, 1, or 2.5 mg/kg; n = 8 shrews/group, respectively via i.p. except for 5 mg/kg dose where n = 10), or 3) CP55,940 (0.025, 0.05, or 0.1 mg/kg; n = 8 shrews/group, respectively via i.p. except for 0 mg/kg dose where n = 9). Following treatment, each shrew was offered four mealworms and was placed in an observation cage for 30 min prior to the administration of SP (50 mg/kg, i.p.). Additional shrews were injected with Δ^9-THC (0, 10, or 20 mg/kg; n = 8 shrews/group, respectively via i.p. except for 5 mg/kg dose where n = 6) for 30 min prior to the administration of GR73632 (5 mg/kg, i.p.) and were observed for the next 30 min. In another set of experiments, various doses of the selective NK_1 receptor-antagonist netupitant (0, 1, 2.5, 5, or 10 mg/kg; n = 6 shrews/group, i.p.), were injected in different groups of shrews 30 min prior to a single emetic dose of the CB_1 receptor antagonist/inverse-agonist SR141716A (20 mg/kg, i.p.). Throughout the study respective vehicles were included for each experimental condition. After the administration of each emetogen, the vomit frequency (oral ejections of food or liquid rejected for 30 min; mean ± S.E.M.) was recorded for each shrew.

To demonstrate whether the antiemetic effects of Δ^9-THC is a CB_1 receptor-mediated event, non-emetic (s.c.) doses of SR141716A (0, 5, or 10 mg/kg; n = 8 shrews/ group, s.c.) were used to prevent the antiemetic effect of a fully effective antiemetic dose of Δ^9-THC (20

mg/kg, s.c.) against SP (50 mg/kg, i.p.)-induced vomiting. Thus, at time 0, different groups of shrews were injected with Δ^9-THC and one of the discussed non-emetic doses of SR141716A, then were offered four mealworms. After 30 min, each shrew received SP (50 mg/kg, i.p.), and the frequency of evoked vomiting was recorded for the next 30 min as described earlier.

2.4 Statistical analyses

Assuming that type 1 error rate was set at 0.05, sample size estimates for behavioral studies was based on a power of 80% to detect 30% change between control and treated (assuming an expected standard deviation of 20% of mean values). This analysis results in a requirement for 8 animals in each group. The frequency of emesis data was analyzed by Kruskal–Wallis H (KW) nonparametric one-way analysis of variance (ANOVA) and post-hoc analysis by Dunn's multiple comparisons test. The incidence of emesis (percentage of animals vomiting) was analyzed by the Chi-square test to determine whether there were differences between groups. When appropriate, pairwise comparisons were also made by this method. The ID_{50} values (the inhibitory dose that prevented emesis in 50% of shrews) were calculated using a nonlinear regression test using GraphPad (InPlot, San Diego, CA). A *P*-value of < 0.05 was necessary to achieve statistical significance.

3. Results

Δ^9-THC reduced substance P-evoked emesis in a dose- and route-dependent manner (Figs. 1 and 2). Kruskal–Wallis nonparametric ANOVA test showed that relative to vehicle-pretreated control group, intraperitoneally (i.p.)-administered Δ^9-THC (0 – 5 mg/kg) significantly reduced the mean frequency of SP-induced vomiting during the 30-min observation period (KW (4, 37) = 16.31; $P < 0.01$) (Fig. 1A). Significant reductions in the frequency of SP-induced vomits occurred at its 1.25 ($P < 0.01$) and 5 mg/kg ($P < 0.001$) doses (Fig. 1A). In addition, the Chi-square test indicated that Δ^9-THC (i.p.) significantly protected shrews from SP-evoked vomiting (χ^2 (4, 37) = 14.5; P = 0.0001) (Fig. 1B). Significant reductions in the percentage of shrews vomiting occurred at its 1.25 (60%; $P < 0.01$), 2.5 (62.5%; $P < 0.01$, and 5 (87.5%; $P < 0.001$) mg/kg doses (Fig. 1B). Δ^9-THC potently protected shrews from SP-induced vomiting with a percentage ID_{50} inhibition value of 1.10 (0.55 - 2.10) mg/kg. Likewise, relative to control animals, subcutaneous (s.c.) administration of Δ^9-THC (0–20 mg/kg) significantly reduced the mean frequency of SP-induced vomiting (KW (4, 33) = 19.1; $P < 0.001$) (Fig. 2A). Moreover, compared to the vehicle-treated animals where all the animals vomited, the frequency of SP-induced vomits was totally abrogated at a relatively much higher dose of Δ^9-THC (20 mg/kg, s.c.; $P < 0.0001$) (Fig. 2A). The s.c.-administered Δ^9-THC protected shrews from SP-evoked vomiting (χ^2 (4, 33) = 19.25; $P < 0.0001$) (Fig. 2B) with significant reductions in the percentage of shrews vomiting at its 10 (50%; $P < 0.05$) and 20 mg/kg doses (100%; $P < 0.0001$) (Fig. 2B). Δ^9-THC (s.c.) protected shrews from SP-induced emesis with a percentage ID_{50} inhibition value of 7.2 (4.10 – 12.50) mg/kg.

To determine whether the antiemetic effect of Δ^9-THC was mediated via CB_1 receptor, reversal of the antiemetic capacity of an effective dose of Δ^9-THC (20 mg/kg, s.c.) against

SP-induced (50 mg/kg, i.p.) emesis was investigated via co-treatment with various doses of the CB_1 receptor antagonist/inverse-agonist, SR141716A (0, 5, or 10 mg/kg, s.c.). Our results demonstrate that a 10 mg/kg (s.c.) dose of SR14171A significantly prevented the antiemetic action of Δ^9-THC. Thus, relative to the control group (i.e. 0 mg/kg SR14171A + 20 mg/kg Δ^9-THC) where there was no vomit, SR14171A significantly prevented the ability of Δ^9-THC-to block SP-evoked emesis (FW (2, 21) = 8.65; P = 0.01) (Fig. 3A) with a significant reduction at its 10 mg/kg dose (P < 0.01) (Fig. 3A). SR14171A also significantly reversed the protective efficacy of Δ^9-THC against SP-evoked vomiting (χ^2 (2, 21) = 9.25; P < 0.01) with a significant reversal at 10 mg/kg (87.5%; P < 0.01) (Fig. 3B). SR14171A prevented the antiemetic efficacy of Δ^9-THC against SP-induced with a percentage ID_{50} inhibition value of 7.04 (4.03 – 11.87) mg/kg.

As with SP, intraperitoneal injection of various doses of Δ^9-THC (i.p.) significantly suppressed the mean frequency of GR73632 (5 mg/kg, i.p.)-induced vomiting (KW (3, 26) = 16.71; P < 0.001) with significant reductions at its 10 (P < 0.01) and 20 mg/kg (P < 0.001) doses (Fig. 4A). Δ^9-THC (i.p.) also significantly protected shrews from the evoked vomiting (χ^2 (3, 26) = 12.3; P < 0.001) (Fig. 4B) with a significant effect at 20 mg/kg (75%; P < 0.01) (Fig. 4B). In addition, Δ^9-THC protected shrews from GR73632-induced vomiting with a percentage ID_{50} inhibition value of 11.10 (6.50 – 18.60) mg/kg.

Large doses (20 - 40 mg/kg) of SR141716A can evoke vomiting in the least shrew (Darmani et al., 2003a). Thus, we used a single fully effective emetic dose of SR141716A (20 mg/kg, i.p.) to evaluate the antiemetic potential of the neurokinin NK_1 receptor selective antagonist, netupitant. Netupitant (0, 1, 2.5, 5, or 10 mg/kg, i.p.) caused a dose-dependent decrease in the frequency of SR141716A-induced vomiting (KW (4, 25) = 22.5; P < 0.001) with significant reductions at its 5 (P < 0.01) and 10 mg/kg (P < 0.01) doses (Fig. 5A). Netupitant also significantly protected shrews from SR141716A-evoked vomiting (χ^2 (4, 25) = 14.70; P = 0.0001) (Fig. 5B) with significant protection at its 5 (66.70%; P = 0.01) (Fig. 5B) and 10 mg/kg (83.33%; P < 0.01) doses. Moreover, netupitant potently reduced GR73632-induced vomiting with a percent ID_{50} inhibition value of 4.60 (2.50 - 8.80) mg/kg.

Finally, we investigated the antiemetic potential of two synthetic cannabinoids WIN55,212-2 and CP55,940 against SP (50 mg/kg, i.p.)-evoked vomiting. WIN55,212-2 (0, 1, 2.5, or 5 mg/kg, i.p.) caused dose-dependent decreases in the frequency of SP-induced emesis (KW (3, 30) = 25.20; P < 0.0001) with significant effects at 2.5 (P = 0.01) and 5 mg/kg (P = 0.001) doses (Fig. 6A). Moreover, WIN55,212-2 significantly protected shrews from SP-evoked vomiting (χ^2 (3, 30) = 21.44; P < 0.0001) at its 2.5 (62.50%; P < 0.01) and 5 mg/kg (90.91%; P < 0.0001) doses (Fig. 6B). WIN55,212-2 protected shrews from SP-evoked vomiting with a percent ID_{50} inhibition value of 1.70 (0.73 - 3.52) mg/kg. Likewise, pretreatment with varying doses of CP55,940 (0, 0.025, 0.05, or 0.1 mg/kg, i.p.) produced substantial decreases in the mean frequency of SP-evoked vomits (KW (3, 29) = 17.10; P < 0.001) with significant reduction at 0.1 mg/kg (P < 0.001) (Fig. 7A). CP55,940 also significantly protected shrews from SP-evoked vomiting (χ^2 (3, 29) = 17.02; P < 0.0001) at its 0.1 mg/kg dose (100%; P < 0.0001) (Fig. 7B). Moreover, CP55,940 potently protected shrews from SP-induced vomiting with a percent ID_{50} inhibition value of 0.06 (0.03 - 0.17) mg/kg.

4. Discussion

This study addressed the antiemetic effects of Δ^9-THC and related cannabinoids against SP-induced emesis. Our results demonstrate that Δ^9-THC causes significant inhibition of SP-evoked vomiting in a dose- and route-dependent manner. Indeed, intraperitoneal administration of Δ^9-THC was 6.5 times more efficacious in protecting 50% of shrews from vomiting than its s.c. injection. More importantly, the antiemetic effect of Δ^9-THC against SP-induced vomiting was blocked by the CB_1R inverse-agonist/antagonist, SR141716A. Furthermore, Δ^9-THC (i.p.) significantly prevented vomiting caused by the NK_1 receptor selective agonist, GR73632, in a dose-dependent manner. Finally, netupitant, a potent and selective NK_1 receptor antagonist (Rizzi et al., 2012) caused significant and dose-dependent decreases in both the mean vomit frequency and the percentage of animals vomiting in response to administration of an emetic dose of SR141716A. Collectively, our findings strongly support the concept that Δ^9-THC prevents vomiting evoked by nonselective (SP) as well as the selective (GR73632) neurokinin NK_1 receptor agonists via activation of cannabinoid CB_1 receptors.

Clear differences in Δ^9-THC ' antiemetic efficacy as a function of route of administration are to be expected. Relative to the s.c. route where no noticeable antiemetic effect occurred at a 5 mg/kg dose of Δ^9-THC, the latter dose via the i.p. route nearly completely suppressed the mean frequency of SP-induced vomiting. Likewise, s.c. administration of Δ^9-THC (0.1 - 30 mg/kg) does not seem to affect mice locomotor activity (Compton et al., 1992), whereas its i.p. injection can significantly lower both motion and rearing behaviors (Janoyan et al., 2002). One explanation for this difference relates to the highly lipophilic nature of Δ^9-THC, which is expected to be absorbed from the s.c. route into the general circulation more gradually (Sharma et al., 2012). Indeed, intraperitoneally administrated drugs are exposed to a large surface of peritoneal membrane, which often allows their rapid absorption, and distribution (Lukas et al., 1971).

Another finding of this investigation is administration of relatively smaller non-emetic doses of SR141716A can prevent the antiemetic action of a totally effective dose of Δ^9-THC against SP-evoked emesis. Prior studies have demonstrated that SR141716A pretreatment is able to prevent the antiemetic efficacy of Δ^9-THC against LiCl- and cisplatin-induced vomiting in the house musk shrew (Kwiatkowska et al., 2004; Parker et al., 2004). Likewise, in ferrets, the ability of Δ^9-THC to attenuate cisplatin- or morphine-6-glucuronide-induced emesis has been reported to be blocked by SR141716, or its analog AM251 (Van Sickle et al., 2001). Published studies from our laboratory also demonstrate that SR14171A can prevent the antiemetic effects of Δ^9-THC against cisplatin- or apomorphine-evoked emesis in least shrews (Darmani, 2001c; Ray et al., 2009b; Wang et al., 2009). Furthermore, Δ^9-THC suppresses cisplatin-evoked emesis in least shrews through the stimulation of CB_1R both in the brainstem and the GIT (Darmani and Johnson, 2004; Ray et al., 2009b). Equally, vomiting caused by the selective NK_1 receptor agonist GR73632 involves both central and peripheral mechanisms (Darmani et al., 2008; Ray et al., 2009a). Based on these facts and current findings, we postulate that stimulation of CB_1 receptors by Δ^9-THC leads to suppression of SP-induced vomiting in both the brainstem and the GIT, however, this entails further investigation.

As a selective NK_1 receptor competitive antagonist, netupitant binds to and prevents the activity of SP on NK_1 receptor (Rizzi et al., 2012; Spinelli et al., 2014), thereby inhibiting NK_1 receptor binding by the endogenous SP. As with other NK_1 receptor antagonists (Rojas et al., 2014), netupitant has a broad-spectrum antiemetic efficacy and can attenuate vomiting caused by diverse emetogens including the L-type calcium channel agonist FPL64176 (Zhong et al., 2018), the SERCA inhibitor thapsigargin (Zhong et al., 2016), as well as cancer chemotherapeutics such as cisplatin (Darmani et al., 2015; Rudd et al., 2016). Currently, we tested the antiemetic potential of netupitant against a large single emetic dose of the selective CB_1 receptor antagonist SR141716A (20 mg/kg, i.p.) and found increasing doses of netupitant reduced the number of shrews vomiting in response to SR14171A with an ID_{50} inhibition value of 4.60 (2.50 - 8.80) mg/kg. Moreover, a 10 mg/kg dose of netupitant protected 83% of the shrews from the induced emesis. The latter dose of netupitant can completely protect least shrews from vomiting evoked by a 5 mg/kg (i.p.) dose of the NK_1 receptor selective agonist GR73632 (Zhong et al., 2019). In line with our behavioral findings, cannabinoid agonists (e.g. HU210) seem to attenuate SP release in cultured rat dorsal ganglion cells (Oshita et al., 2005), while corresponding CB_1 receptor antagonists (e.g. SR141716A) potentiate capsaicin-evoked SP release in the mouse spinal cord (Lever and Malcangio, 2002). However, exogenous administration of either SP (50 mg/kg, i.p.) or GR73632 (5 mg/kg, i.p.) can cause the release of endogenous SP in the least shrew brainstem emetic nuclei (Darmani et al., 2008; Zhong et al., 2019). In this setting, an NK_1 receptor antagonist such as netupitant would probably prevent the evoked endogenous SP release in the brainstem and thus would prevent the vomiting caused by either GR73632, SP or SR141716A. We plan to investigate the interaction between CB_1- and NK_1-receptors in detain in future studies. Overall, these findings support the involvement of endogenous SP in SR14171A-mediated emesis.

Little is known regarding the antiemetic structure activity relationship of different cannabinoids in any species. We compared intraperitoneally injected Δ^9-THC potency against SP-evoked vomiting to that of related cannabinoid $CB_{1/2}$ receptor agonists WIN55,212-2 and CP55,940. We found that CP55,940 was the most potent antiemetic with the following ID_{50} order: CP55,940<<<Δ^9-THC = WIN55,212-2. Thus, while Δ^9-THC and WIN55,212-2 are essentially equipotent against SP-induced vomiting, they are 18 to 28 less efficacious than CP55,940. Moreover, while CP55,940 (0.1 mg/kg) completely protected shrews from the evoked vomiting, the other two cannabinoids could protect up to 90% of shrews from vomiting at a dose of 5 mg/kg. The present findings are consistent with our published studies where CP55,940 was 13–20 times more effective against cisplatin-induced emesis than WIN55,212-2 or Δ^9-THC (Darmani, 2001a, c). Other reports seem to substantiate our findings since Δ^9-THC and WIN55,212-2 have a similar affinity for the cannabinoid CB_1 receptor (Pertwee, 1999) and are both less potent than CP55,940 in suppression of locomotor activity in mice.

The role of CB_2 receptor in the antiemetic actions of cannabinoids is still elusive. Different CB_2 receptor antagonists were unsuccessfully utilized to reverse the antiemetic efficacy of various CB_1 /CB_2 receptor agonists against diverse emetic stimuli (Darmani, 2001a, d). The fact that CB_2 receptor is predominantly expressed in the immune tissues, and absent or very low in the emetic loci, imply that CB_2 receptor may not have a role in vomiting. In support

of this premise, our laboratory has shown that large doses of SR141716A and not SR144528 (a selective and potent cannabinoid CB2 receptor antagonist) can evoke vomiting in a dose-dependent manner and the induced emesis was blocked by Δ^9-THC and WIN55,212-2 and CP55,940 (Darmani, 2001d). Intriguingly, Van Sickle et al., 2005 have shown that in the ferret the antiemetic actions of the endocannabinoid 2-AG (but not anandamide, another endocannabinoid) could be prevented by a CB_2 receptor antagonist, which failed to prevent the antiemetic effects of Δ^9-THC against cisplatin-evoked vomiting. Neither the antiemetic effects of Δ^9-THC, WIN55,212-2, or CP55,940 could be reversed by SR144528 in the least shrew (Darmani, 2001a; Darmani et al., 2003b; Simoneau et al., 2001). Thus, the CB_2 receptor may have no or only a modest role in emesis.

In summary, the current results extend published findings that Δ^9-THC is a broad-spectrum antiemetic not only against conventional emetogens (such as cisplatin, dopamine or serotonin), but it also prevents vomiting caused by the neuropeptide SP and the corresponding neurokinin NK_1 receptor selective agonist GR73632. Δ^9-THC and related cannabinoids (WIN55,212-3 and CP55,940) appear to attenuate SP-evoked vomiting via activation of the cannabinoid CB_1 receptor. The exact molecular mechanisms by which CB_1 receptor antagonists block SP-evoked emesis remain unknown and warrants further investigation.

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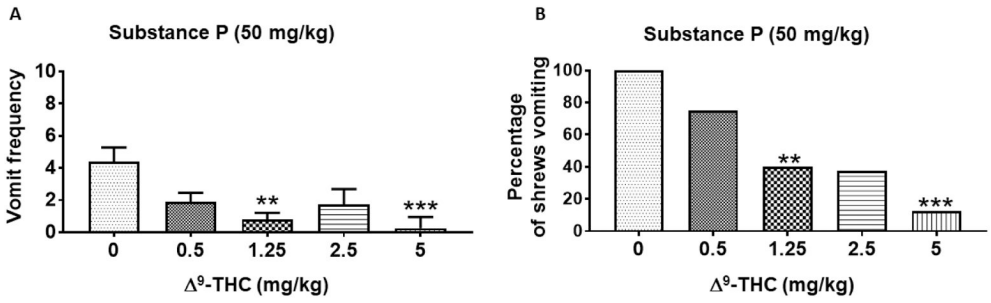

Figure 1. Antiemetic effects of different doses of intraperitoneally-administered Δ^9-THC against substance P (SP)-induced emesis in the least shrew.

Different groups of shrews received either vehicle (0 mg/kg) or varying doses of Δ^9-THC, 30 min prior to injection of a fully effective emetic dose of SP (50 mg/kg, i.p.). Emetic parameters were recorded for the next 30 min post emetic injection. The mean frequency of vomits (± S.E.M.) (graph A) and the percentage of animals vomiting (graph B) are presented. ** $P < 0.01$ and *** $P < 0.001$ versus 0 mg/kg control. N = 8- 10 shrews/ group.

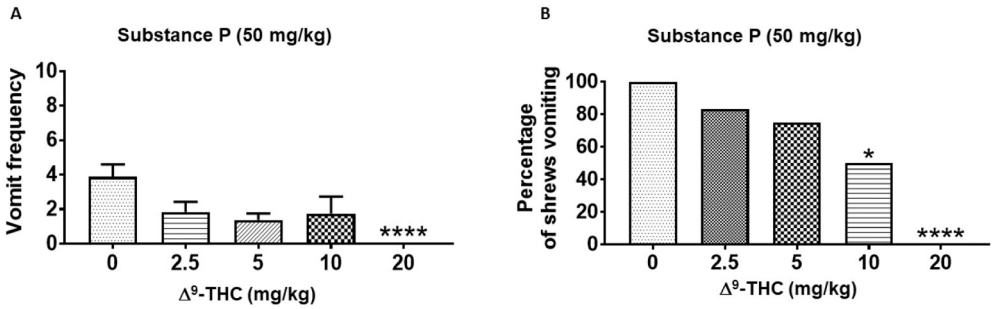

Figure 2. Antiemetic effects of different doses of subcutaneously-administered Δ^9-THC against substance P (SP)-induced emesis in the least shrew.

Different groups of shrews received either vehicle (0 mg/kg) or varying doses of Δ^9-THC, 30 min prior to a fully effective emetic dose of SP (50 mg/kg, i.p.). Emetic parameters were recorded for the next 30 min post emetic injection. The mean frequency of vomits (± S.E.M.) (graph A) and the percentage of animals vomiting (graph B) are presented. * $P < 0.05$ and *** $P < 0.001$ versus 0 mg/kg control. N = 6-8 shrews/ group.

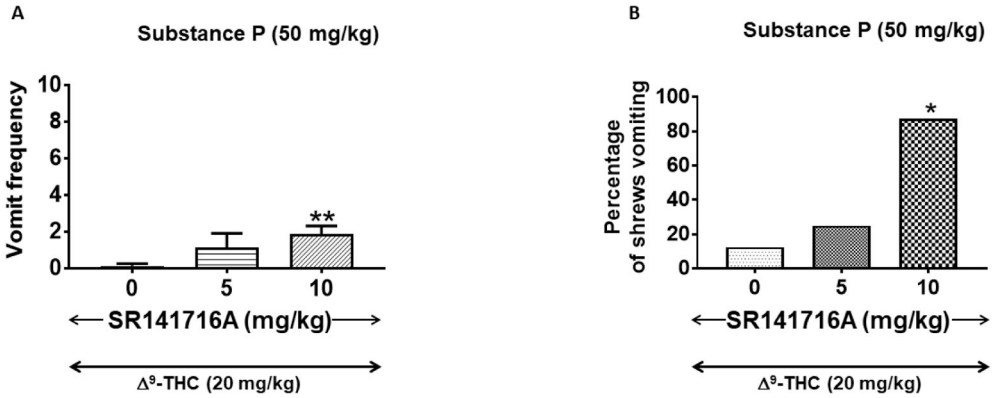

Figure 3. Ability of varying subcutaneously (s.c.)-administered doses of SR141716A to reverse the effects of a fully efficacious antiemetic dose Δ^9-THC (20 mg/kg, s.c.) against substance P (SP)-induced emesis in the least shrew.

Different groups of shrews received either 0 mg/kg SR14171A + 20 mg/kg Δ^9-THC, or varying doses of SR14171A + 20 mg/kg Δ^9-THC; 30 min prior to an emetic dose of SP (50 mg/kg, i.p.). SR14171A significantly reversed the Δ^9-THC-induced decrease in emesis frequency (graph A). SR141716A also reversed the ability of Δ^9-THC to protect shrews from vomiting (graph B). * $P < 0.05$ and ** $P < 0.01$ versus 0 mg/kg SR141716A + 20 mg/kg Δ^9-THC control group. N = 8 shrews per group.

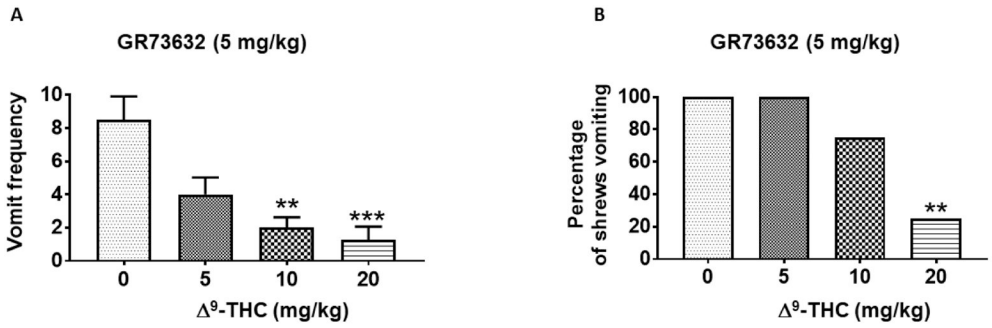

Figure 4. Antiemetic effects of intraperitoneally-administered Δ^9-THC against GR73632-induced emesis in the least shrew.

Different groups of shrews received either vehicle (0 mg/kg) or varying doses of Δ^9-THC, 30 min prior to an injection of an emetic dose of the neurokinin NK_1 receptor selective agonist, GR73632 (5 mg/kg, i.p.). Emetic parameters were recorded for the next 30 min post emetic injection. The mean frequency of vomits (± S.E.M.) (graph A) and the percentage of animals vomiting (graph B) are presented. ** $P < 0.01$ and *** $P < 0.001$ versus 0 mg/kg control. N = 6-8 shrews/ group.

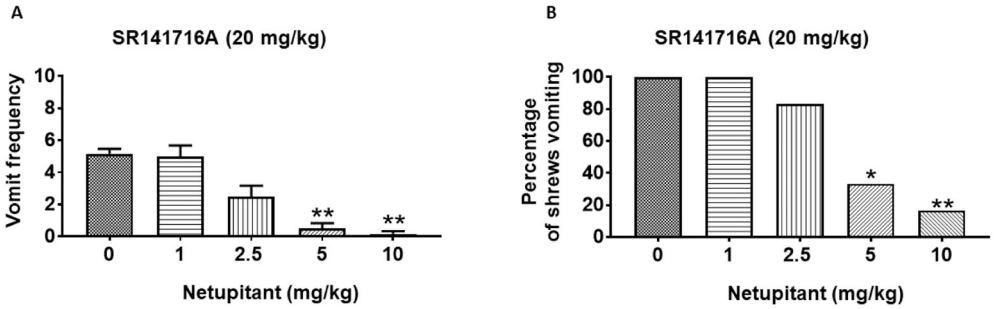

Figure 5. Antiemetic effects of intraperitoneally-administered NK_1R selective antagonist netupitant against SR141716A-induced emesis in the least shrew.

Different groups of shrews received either vehicle (0 mg/kg) or varying doses of netupitant, 30 min prior to an emetic dose of SR141716A (20 mg/kg, i.p.). Emetic parameters were recorded for the next 30 min post emetic injection. The mean frequency of vomits (± S.E.M.) (graph A) and the percentage of animals vomiting (graph B) are presented. * $P < 0.05$ and ** $P < 0.01$ versus 0 mg/kg control. N = 6 shrews/ group.

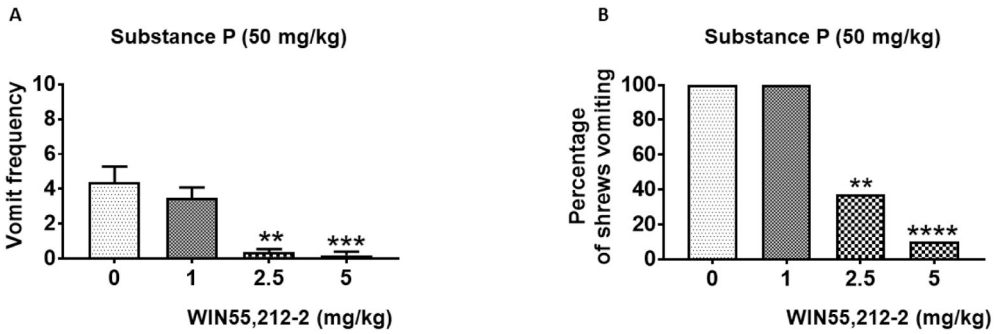

Figure 6. Antiemetic effects of intraperitoneally-administered WIN55,212-2 against substance P (SP)-induced emesis in the least shrew.

Different groups of shrews received either vehicle (0 mg/kg) or varying doses of WIN55,212-2, 30 min following an emetic dose of SP (50 mg/kg, i.p.). Emetic parameters were recorded for the next 30 min post emetic injection. The mean frequency of vomits (± S.E.M.) (graph A) and the percentage of animals vomiting (graph B) are presented. ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ versus 0 mg/kg control. N = 8-10 shrews/ group.

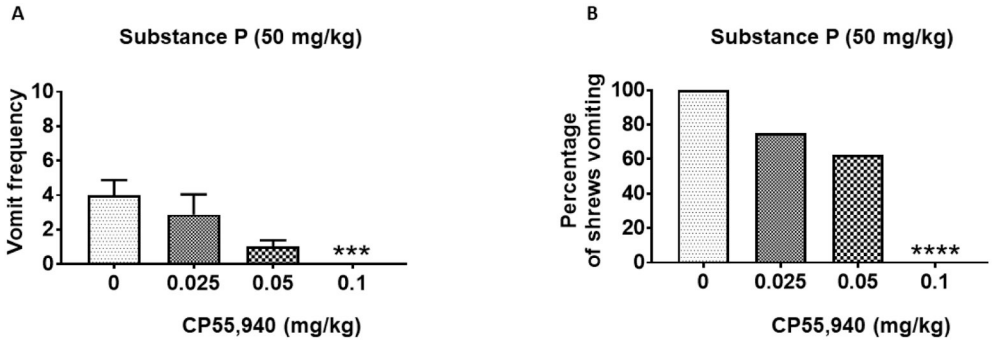

Figure 7. Antiemetic effects of intraperitoneally-administered CP55,940 against substance P (SP)-induced emesis in the least shrew.

Different groups of shrews received either vehicle (0 mg/kg) or varying doses of CP55,940, 30 min prior to an emetic dose of SP (50 mg/kg, i.p.). Emetic parameters were recorded for the next 30 min post emetic injection. The mean frequency of vomits (± S.E.M.) (graph A) and the percentage of animals vomiting (graph B) are presented. *** $P < 0.001$ and **** $P < 0.0001$ versus 0 mg/kg control. N = 8-9 shrews/ group.