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Delta-9-tetrahydrocannabinol differentially suppresses cisplatin-induced emesis and indices of motor function via cannabinoid CB_1 receptors in the least shrew

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Abstract

We have recently shown that the cannabinoid CB_1 receptor antagonist, SR 141716A, produces emesis in the least shrew (*Cryptotis parva*) in a dose- and route-dependent manner. This effect was blocked by delta-9-tetrahydrocannabinol (Δ^9-THC). The present study investigates the cannabinoid receptor mechanisms by which Δ^9-THC produces its antiemetic effects against cisplatin (20 mg/kg, ip)-induced emesis as well as its cannabimimetic activity profile (motor reduction) in the least shrew. Intraperitoneal administration of Δ^9-THC (1, 2.5, 5 and 10 mg/kg) dose-dependently reduced both the percentage of animals vomiting ($ID_{50} = 1.8 \pm 1.6$ mg/kg) and the frequency of vomits ($ID_{50} = 0.36 \pm 1.18$ mg/kg) in a potent manner. The lowest significantly effective antiemetic dose of Δ^9-THC for the latter emesis parameters was 2.5 mg/kg. Although Δ^9-THC reduced the frequency of vomits up to 98%, it failed to completely protect all tested shrews from vomiting (80% protection). The cannabinoid CB_1 antagonist (SR 141716A) and not the CB_2 antagonist (SR 144528), reversed the antiemetic effects of Δ^9-THC in a dose-dependent fashion. Δ^9-THC (1, 5, 10 and 20 mg/kg, ip) suppressed locomotor parameters (spontaneous locomotor activity, duration of movement and rearing frequency) in a biphasic manner and only the 20-mg/kg dose simultaneously suppressed the triad of locomotor parameters to a significant degree. Subcutaneous (1–10 mg/kg) and intraperitoneal (0.05–40 mg/kg) injection of some doses of SR 141716A caused significant reductions in one or more components of the triad of locomotor parameters but these reductions were not dose dependent. Subcutaneous injection of SR 141716A (0.2, 1, 5 and 10 mg/kg) reversed the motor suppressant effects of a 20-mg/kg dose of Δ^9-THC (ip) in a dose-dependent manner. Relative to its motor suppressant effects, Δ^9-THC is a more potent antiemetic agent. Both effects are probably mediated via CB_1 receptors in distinct loci.

Keywords: Emesis; Antiemetic; Δ^9-THC; SR 141716A; SR 144528; Cisplatin; CB_1 receptor; CB_2 receptor; Rearing; Locomotor activity

1. Introduction

The health aspects of marijuana (*Cannabis sativa*) and related compounds are controversial. Nonetheless, it is clear that the main psychoactive component of marijuana, delta-9-tetrahydrocannabinol (Δ^9-THC), has significant antiemetic efficacy in cancer patients receiving chemotherapy (see, for reviews, Gralla, 1999; Gralla et al., 1984; Mitchelson, 1992; Voth and Schwartz, 1997). Not only Δ^9-THC, but also its tested synthetic analogs (nabilone and levonantradol) seem to be superior or equivalent to dopamine D_2 antagonists in their antiemetic efficacy in patients treated with chemotherapy. However, the efficacy of tested cannabinoids is not as good as the more potent antiemetics such as the 5-HT_3 receptor antagonists. Unlike the relatively larger body of clinical findings, only scant animal studies on the antiemetic effects of cannabinoids are available. Nonetheless, several cannabinoids (Δ^9-THC, Δ^8-THC, 7-hydroxy-Δ^9-THC, nabilone and HU 210) appear to be effective in preventing cisplatin- or apomorphine-induced emesis in the cat and pigeon models of emesis (Ferrari et al., 1999; London et al., 1979; McCarthy and Borison, 1981; McCarthy et al., 1984; Stark, 1982). There are at least two types of cannabinoid receptors, which are designated as cannabinoid CB_1 and CB_2 sites (Matsuda, 1997; Pertwee, 1997), for which potent and selective antagonists have been developed (Rinaldi-Carmona et al., 1994, 1998). Until recently, the cannabinoid

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receptor mediating the antiemetic effects of cannabinoids was not known. It appears highly likely that the CB_1 receptor mediates Δ^9-THC's antiemetic effect since the cannabinoid CB_1 antagonist (SR 141716A) produces emesis in the least shrew (*Cryptotis parva*) in a dose- and route-dependent manner (Darmani, 2001). Moreover, Δ^9-THC and its synthetic analogs (CP 55, 940 and WIN 55, 212-2) were shown to prevent this induced behavior. However, it is not yet known whether cannabinoid receptor antagonists can reverse the ability of Δ^9-THC in preventing the vomiting produced by the chemotherapeutic agent cisplatin.

Cannabinoids also display appetite stimulant, anticonvulsant, antinociceptive, hypothermic and psychotropic properties (Formukong et al., 1989; Martin et al., 1995; Mattes et al., 1994; Onaivi et al., 1996; Rinaldi, 1994). A number of clinical studies have shown that many patients who found Δ^9-THC an effective antiemetic also experienced a "psychological high" (Chang et al., 1979; Consroe and Sandyk, 1992; Lucas and Laszlo, 1980; Orr and McKernan, 1980; Sallan et al., 1975, 1980). Thus, it becomes imperative to investigate whether psychoactivity contributes to the antiemetic action of Δ^9-THC. Numerous animal models for evaluating the psychoactivity profile of cannabinoids have been developed (Martin et al., 1995). Reductions in motor activity parameters appear to be an index for the initial evaluation and for establishing a general cannabimimetic pharmacological profile in animals.

Most animal emesis studies are confined to large animals such as cats, dogs, or ferrets. Different species can exhibit differential sensitivity to a given emetic (King, 1990). Utilization and maintenance of such large animals are cost prohibitive and, therefore, alternative emesis models have been characterized. Indeed, in the 1980s, Japanese researchers introduced a smaller animal (adult weight between 50 and 100 g), the house musk shrew (*Suncus murinus*), as an alternative experimental vomiting model (Matsuki et al., 1988). *S. murinus* is endogenous to Asia and Africa. Shrews are short-legged, mouse-like mammals with long, pointed snouts and short, dense fur, usually dark brown in color. Shrews are placed in the order of Insectivora and are amongst the most ancient of mammals (Churchfield, 1990). Shrews are considered closer to man than rodents, lagomorphs and carnivores in the phylogenetic system. Currently, some 266 shrew species are recognized. Relative to *S. murinus*, the least shrew (*C. parva*) is much smaller (adult weighing 4–6 g) and lives in Central and North America. It was recently introduced as a new animal model of vomiting for various emetic stimuli (Darmani, 1998, 2001; Darmani et al., 1999; Dukat et al., 2000). It is a relatively easy and inexpensive animal model for testing emetic and the antiemetic effects of various drugs. The purpose of the present study was to: (1) determine whether Δ^9-THC can prevent emesis produced by the chemotherapeutic agent cisplatin in the least shrew (Darmani, 1998), (2) show whether selective cannabinoid CB_1 (SR 141716A) or CB_2 (SR 144528) antagonists (Rinaldi-Carmona et al., 1994, 1998) can block the antiemetic action of Δ^9-THC against cisplatin-induced emesis and (3) demonstrate whether the cannabimimetic psychoactivity profile (i.e., motor inhibition) of Δ^9-THC is related to its antiemetic effects.

2. Materials and methods

2.1. Animals and drugs

Shrews (*C. parva*) were bred and maintained in the animal facilities of the Kirksville College of Osteopathic Medicine. Both male and female shrews (4–6 g, 45–70 days old) were used throughout the study. The animals were kept on a 14:10-h light–dark cycle at a humidity controlled room temperature of $21 \pm 1°C$ with ad libitum supply of food and water. The feeding and maintenance of shrews are fully described elsewhere (Darmani, 1998; Darmani et al., 1999). Δ^9-THC and cisplatinum (II) diamine dichloride ($Pt(NH_3)_2Cl_2$) were purchased from Research Biochemicals, Natick, MA. SR 141716A and SR 144528 were generously donated by Sanofi Recherche (Montpellier, France). All drugs were dissolved in a 1:1:18 solution of ethanol:emulphor:0.9% saline to twice the stated drug concentrations. These drug concentrations were further diluted by the addition of an equal volume of saline. This procedure was necessary because the 1:1:18 vehicle mixture can cause emesis in up to 20% of animals by itself. The final vehicle mixture rarely induced emesis. All drugs were administered at a volume of 0.1 ml/10g of body weight. All animals received care according to the "Guide for the Care and Use of Laboratory Animals," DHSS Publication, revised, 1985.

2.2. Experimental protocols

2.2.1. Emesis studies

The present protocols were based upon our previous emesis studies in the least shrew (Darmani, 1998, 2001; Darmani et al., 1999). All experiments were performed between 0800 and 1700 h. Since published studies in dogs have shown that Δ^9-THC by itself can induce nondose-dependent vomiting (Lowe, 1946; Shannon et al., 1978), the emetic effects of varying doses of Δ^9-THC in the shrew was investigated first. On the test day, the shrews were transferred to the experimental room and were allowed to acclimate for at least 1 h prior to experimentation. To habituate the shrews to the test environment, each animal was randomly selected and transferred to a $20 \times 18 \times 21$ cm clean clear plastic cage and offered four meal worms (*Tenebrio* sp.) 30 min prior to experimentation. Different groups of shrews were then injected intraperitoneally with vehicle ($n = 11$) or varying doses of Δ^9-THC (1, 5 and 10 mg/kg, 9–10 shrews per group). Immediately following injection, each shrew was placed in the observation cage

and the frequency of vomiting (mean ± S.E.M. of frequency of oral expulsion of food or liquid material) was recorded for each individual shrew for the next 60 min. These data showed that intraperitoneal administration of the cited doses of Δ^9-THC caused nondose-dependent emesis in the least shrew, which was not significantly different from vehicle injected control group.

Previous studies in this laboratory have shown that intraperitoneal administration of the chemotherapeutic agent cisplatin can induce vomiting in the least shrew in a dose-dependent manner (Darmani, 1998). Indeed, a 20-mg/kg ip dose of cisplatin can induce vomiting in 90–100% of the least shrew. The latter dose was chosen to demonstrate the possible antiemetic effects of Δ^9-THC on cisplatin-induced vomiting. The antiemetic doses of Δ^9-THC were based upon our preliminary experiments as well as other published studies (Feigenbaum et al., 1989; McCarthy et al., 1984). Shrews were offered four meal worms prior to drug administration. Different groups of shrews were first injected intraperitoneally with varying doses of Δ^9-THC (0, 1, 2.5, 5 or 10 mg/kg, $n = 8–10$ per group) and a 20-mg/kg dose of cisplatin (ip). Each shrew was then observed individually for the next 60 min immediately following the two simultaneous injections. The frequency of emesis was recorded as described above. Another group of shrews ($n = 11$) received two corresponding vehicle injections and were observed in an identical fashion. This experiment revealed that Δ^9-THC prevented cisplatin-induced emesis in a dose-dependent fashion and its 5- and 10-mg/kg doses produce similar maximal antiemetic effects.

This laboratory has previously shown that intraperitoneal administration of the selective cannabinoid CB_1 antagonist SR 141716A produces emesis in the least shrew at doses of 10 and 20 mg/kg (Darmani, 2001). When administered subcutaneously, SR 141716A was a less efficient emetogenic agent because only the 40-mg/kg dose caused a significant degree of vomiting. Thus, in the next experiment substantially lower subcutaneous doses of SR 141716A (1–10 mg/kg) were used to reverse the antiemetic effect of a 5-mg/kg dose of Δ^9-THC against cisplatin (20 mg/kg, ip)-induced vomiting. Thus, at 0 time different groups of shrews were injected subcutaneously with either vehicle ($n = 8$) or varying doses of SR 141716A (1, 5 and 10 mg/kg) and were then offered four meal worms. Ten minutes later, each shrew received intraperitoneally Δ^9-THC (5 mg/kg) and cisplatin (20 mg/kg) and the emesis frequency was recorded for the next 60 min as described above. SR 141716A reversed the antiemetic effect of Δ^9-THC on cisplatin-induced vomiting. To show whether subcutaneous administration of SR 141716A can affect the ability of cisplatin to produce emesis, we investigated the effect of a 10-mg/kg sc dose of SR 141716A on cisplatin (20 mg/kg)-induced vomiting in the above manner except Δ^9-THC was excluded. The selective cannabinoid CB_2 antagonist SR 144528 does not produce emesis by itself (Darmani, 2001). A 10-mg/kg sc dose of SR 144528 was used to determine the reversibility of the antiemetic effect of a 5 mg/kg dose of Δ^9-THC on cisplatin-induced emesis in the same manner as described for the CB_1 antagonist. Furthermore, the effect of a 10-mg/kg sc dose of SR 144528 on cisplatin (20 mg/kg, ip)-induced emesis was investigated in a manner similar to that described for the CB_1 antagonist.

2.2.2. Locomotor studies

On test day, shrews were transported to the test area in their home cages from the animal quarters and were allowed to acclimate for at least 1 h to a semidark environment. The reduced light condition was necessary for the computerized video tracking, motion analysis and behavior recognition system [Ethovision (version 2.0), Noldus Information Technology, Costerweg, Netherlands] to work efficiently. The parameters of Ethovision were set to record the following triad of locomotion activities: (1) spontaneous locomotor activity in terms of the total distance moved in meters (moving was recorded when a shrew traveled a distance greater than 2 cm in the plane of the observation cage), (2) total duration of movement in seconds (the summed time recorded for any type of movement) and (3) rearing frequency (a rearing event was recorded as a 20% reduction in shrew surface area when a shrew stood upright as seen by the overhead video camera). Our preliminary experiments indicated a 20% change in surface area for shrews is equivalent to 90% to 110% of manual recording of rearing frequency. Different versions of this system have been previously used for determination and validation of these locomotor parameters in different animal species (Spruijt et al., 1994; Winberg et al., 1993; Young et al., 1997).

After acclimation to the dark laboratory environment, shrews were further acclimated in white plastic dummy observation cages ($28 \times 28 \times 14$ cm) for 1 h prior to testing. In the first experiment, different groups of shrews were injected intraperitoneally with either vehicle ($n = 12$) or varying doses of Δ^9-THC (1, 5, 10 and 20 mg/kg, $n = 7–8$ per group). Each shrew was then individually placed in an observation cage of the same dimension and the locomotor parameters were recorded for 50 min starting 10 min after injection. Δ^9-THC significantly reduced all three locomotor parameters in shrews at its 20-mg/kg dose. In the second experiment, the effect of varying doses of subcutaneously administered SR 141716A (0, 1, 5 and 10 mg/kg; $n = 8–10$ per group) was determined for 30 min immediately following injection. At certain doses, SR 141716A administration reduced one or more components of the triad of activity parameters in the least shrew. Because intravenously administered SR 141716A is reported to increase spontaneous locomotor activity in mice (Compton et al., 1996), we further investigated the effect of intraperitoneal injection of SR 141716A. The intravenous route was not attempted since it is extremely difficult to administer drugs via this route in a such small animal. Thus, in the third experiment, the effects of the following intraperitoneally administered doses of SR 141716A was investigated: 0, 0.05, 0.2, 1, 5,

10, 20 and 40 mg/kg ($n = 7–12$ per group) under the above experimental conditions. In the final experiment, the effect of different subcutaneous doses of SR 141716A was investigated on the locomotor reducing properties of the 20-mg/kg dose of Δ^9-THC. Thus, at 0 time, different shrews were injected with varying subcutaneous doses of SR 141716A (0, 1, 5 and 10 mg/kg, $n = 7–9$ per group). At 30 min, each shrew received intraperitoneally a 20-mg/kg dose of Δ^9-THC. Ten minutes later (i.e., at 40 min), the locomotor activity parameters were recorded for the next 50 min as described earlier.

2.3. Statistical analysis

The emesis data were analyzed by the Kruskal–Wallis nonparametric one-way analysis of variance (ANOVA) and post hoc analysis by Dunn's multiple comparison test. A P value of $< .05$ was considered significantly different. For some emesis data, the two-tailed Mann–Whitney test was used. The ID_{50} values (the inhibitory dose that prevented emesis in 50% of shrews, or the dose that reduced emesis frequency by 50%) were calculated by the use of a computerized program (GraphPad InPlot, San Diego, CA). A one-way analysis of variance (ANOVA) followed by either Dunnett's or Bonferroni's multiple comparison tests were used to analyze the locomotor data.

3. Results

3.1. Emesis

As with our previous study (Darmani, 2001), intraperitoneal administration of the final concentration of the vehicle solvent (ethanol:emulphor:saline) occasionally produced emesis (1 out of 11 shrews tested). Intraperitoneal administration of 1-, 5- or 10-mg/kg doses of Δ^9-THC in the above solvent caused emesis in 20%, 30% and 20% of tested animals, respectively. However, the cited doses of Δ^9-THC did not produce emesis that was significantly different from that produced by the vehicle.

Administration of the 20-mg/kg ip dose of cisplatin induced vomiting in all of the tested shrews with a mean vomiting frequency of 9 ± 1.6 (Fig. 1). This figure also shows the ability of the cited doses of Δ^9-THC (ip) in preventing emesis induced by cisplatin (20 mg/kg, ip). Indeed, Δ^9-THC reduced the percentage of shrews vomiting in response to cisplatin in a dose-dependent manner with an ID_{50} of 1.8 ± 1.6 mg/kg ($K_{W_{4,43}} = 27.5$, $P < .0001$). Furthermore, the post hoc test showed that relative to the vehicle-injected, cisplatin-treated (V + C) control group, significant reductions (70%, 80% and 80%) in the number of animals vomiting occurred with the 2.5- ($P < .05$), 5- ($P < .01$) and 10-mg/kg ($P < .01$) doses of Δ^9-THC. Thus, even at the highest dose tested, Δ^9-THC failed to completely prevent emesis in cisplatin-exposed animals. Δ^9-THC pretreatment

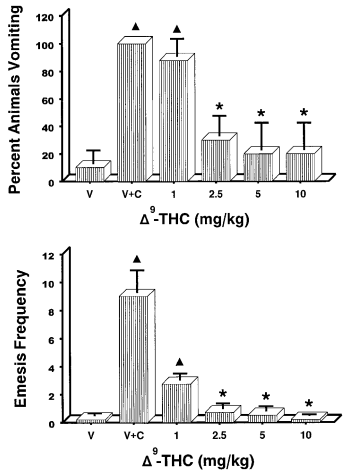

Fig. 1. Represents the antiemetic dose–response effects of Δ^9-THC on cisplatin (20 mg/kg, ip)-induced emesis in the least shrew. The top graph depicts reduction in percentage of shrews vomiting whereas the bottom graph shows attenuation in the frequency of vomiting. (V) = a control group that received twice vehicle (ip) injections. (V + C) = a group receiving an intraperitoneal injection of cisplatin plus an intraperitoneal injection of vehicle. The remaining groups received cisplatin plus an intraperitoneal injection of the cited doses of Δ^9-THC. Emesis parameters were recorded for 60 min postinjection. * Significantly different from (V + C) group at $P < .05$ by Dunn's multiple comparisons test. ▲Significantly different from vehicle-injected control group (V).

also significantly reduced the frequency of cisplatin-induced vomiting with an ID_{50} value of 0.36 ± 1.18 mg/kg ($K_{W_{4,43}} = 37.8$, $P < .0001$). The pattern of reduction was also dose dependent (69.4%, 92.2%, 94.4% and 97.8%, respectively). Moreover, significant effects were seen at the 2.5- ($P < .001$), 5- ($P < .001$) and 10-mg/kg ($P < .001$) doses of Δ^9-THC. Only 1 of the 10 shrews injected twice with vehicle (i.e., control V) exhibited vomiting (Fig. 1).

Fig. 2 describes the ability of subcutaneously administered SR 141716A to reverse the antiemetic action of Δ^9-THC (5 mg/kg, ip) against cisplatin (20 mg/kg, ip)-induced vomiting. In the absence of SR 141716A, the 5-mg/kg dose of Δ^9-THC prevented emesis in 9 of 11 tested shrews (i.e., 18% vomited; Fig. 2). The percentage of shrews vomiting increased (63.6%, 50% and 80%, respectively) in response to administration of 1-, 5- and 10-mg/kg doses of SR 141716A with an ID_{50} of 0.42 ± 21.3 mg/kg ($K_{W_{3,38}} = 12.9$, $P < .01$). However, due to large intergroup variability, Dunn's multiple comparison test showed a significant enhancement in the number of shrews vomiting only at the 10-mg/kg dose ($P < .05$). The frequency of vomiting also increased (1111%, 1122% and 1622% relative to control, respectively) with increasing doses of SR 141716A with an ID_{50} of 0.39 ± 4.98 mg/kg ($K_{W_{3,38}} = 15.2$, $P < .004$). However, again due to the large intergroup variation, a significant increase in vomiting frequency ($P < .05$) was only seen at the 10-mg/kg dose. The two-tailed Mann–Whitney test showed that a 10-mg/kg dose of the CB_2 antagonist SR 144528 failed to alter the ability of the 5-mg/kg dose of Δ^9-THC to prevent cisplatin (20 mg/

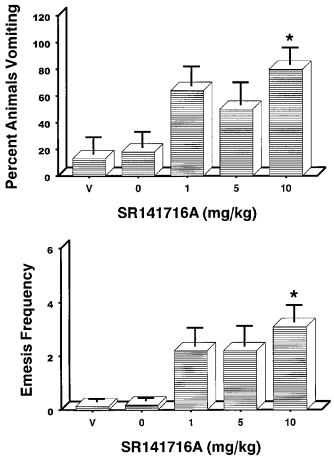

Fig. 2. Represents the ability of the cited subcutaneous doses of SR 141716A to reverse the antiemetic effects of a 5-mg/kg (ip) dose of Δ^9-THC against cisplatin (20 mg/kg, ip)-induced emesis. SR 141716A blocked the ability of Δ^9-THC to protect shrews from vomiting (top graph) as well as reversing the Δ^9-THC-induced reduction in emesis frequency (bottom graph). At 0 time shrews received SR 141716A and 10 min later Δ^9-THC plus cisplatin. Emesis parameter were recorded for the next 60 min. (V) = a control group that received the corresponding vehicle injections by subcutaneous and intraperitoneal routes. * Significantly different from the 5 mg/kg Δ^9-THC plus 20 mg/kg cisplatin-treated control group that had received no SR 141716A (i.e., column 0) at $P < .05$ by Dunn's multiple comparisons test.

kg)-induced vomiting ($P < .05$). Indeed, in both control ($n = 11$) and SR 144528-injected shrews ($n = 8$), only one shrew vomited per treatment group.

The Mann–Whitney test also showed that relative to controls, a 10-mg/kg dose of either SR 141716A or SR 144528 could not alter vomiting produced by cisplatin (Fig. 3). Thus, both the vomiting frequency and the percentage of animals exhibiting vomiting were not significantly altered by the cannabinoid antagonists.

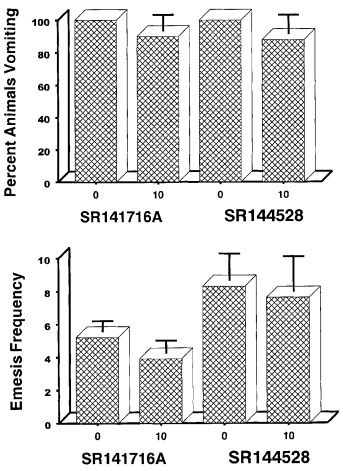

Fig. 3. Shows lack of effect of a 10-mg/kg dose (sc) of either the CB_1 antagonist (SR 141716A) or the CB_2 antagonist (SR 144528) on cisplatin (20 mg/kg, ip)-induced vomiting.

3.2. Locomotion

Intraperitoneal administration of 1–20-mg/kg doses of Δ^9-THC significantly attenuated spontaneous locomotor activity (i.e., total distance moved) in the least shrew during the 50-min observation period (Fig. 4, top graph), $F(4,38) = 6.83$, $P < .003$. However, Dunnett's post hoc t test revealed that injection of low to moderate doses of Δ^9-THC (1–10 mg/kg) failed to significantly modify the latter parameter relative to the vehicle control group. Only the highest dose tested (20 mg/kg) sharply and significantly blocked the behavior by 100%. Although the 5-mg/kg dose of Δ^9-THC tended to attenuate locomotor activity by 46% ($P > .05$), its 10-mg/kg dose caused a highly variable degree of locomotor activity with a mean value identical to the control value. The action of the latter dose of Δ^9-THC was reevaluated and again a similar effect was observed (data not shown). Inclusion of the 10-mg/kg data interfered with the ID_{50} computation of the reduction in spontaneous locomotor activity. Δ^9-THC also significantly reduced the total duration of movement in shrews (Fig. 4, middle graph), $F(4,38) = 3.2$, $P < .02$. Indeed, the cited doses of Δ^9-THC dose-dependently attenuated movement duration by 2.7%, 21%, 25% and 39.7%, respectively. However, a significant effect was only seen at the 20-mg/kg dose. Thus, unlike the

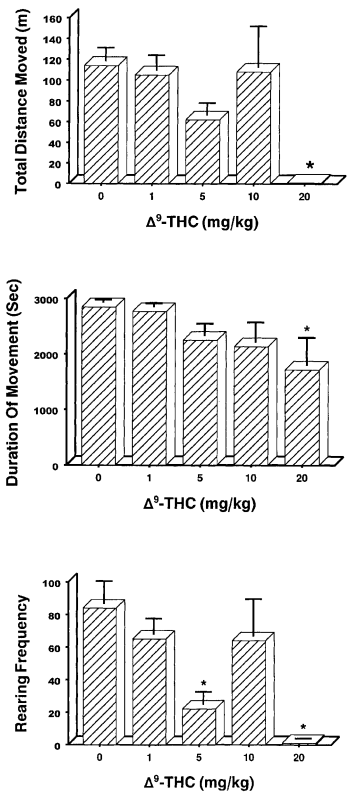

Fig. 4. The biphasic dose–response effects of Δ^9-THC on the triad of motor behaviors in the least shrew. The cited behaviors were recorded for 50 min by a computerized video tracking, motion analysis and behavior recognition system (Ethovision) 10 min after Δ^9-THC administration. * Significantly different from vehicle-injected control group at $P < .05$ by Dunnett's t test.

discussed total blockade of spontaneous locomotor activity, the total duration of all types of movement was only reduced by about 40% in the 20-mg/kg Δ^9-THC-treated shrews. Δ^9-THC administration also totally blocked the frequency of rearings in the least shrew (Fig. 4, bottom graph), $F(4,38)=5.93$, $P<.0008$. Once again, inhibition of rearing behavior appears to be biphasic since the cited doses of Δ^9-THC caused the following respective reductions: 22.6%, 73.8%, 23.8% and 99.1%. Indeed, Dunnett's t test revealed that only the 5- and 20-mg/kg doses of Δ^9-THC caused significant ($P<.05$) effects.

Subcutaneous administration of the CB_1 antagonist SR 141716A also altered some components of the triad of locomotor parameters in the least shrew in the 30 min observation period (Fig. 5). Indeed, its 5-mg/kg dose significantly reduced both spontaneous locomotor activity (61% decrease, $P<.05$) and the rearing frequency (47% decrease, $P<.05$), $F(3,30)=3.5$, $P<.03$; and $F(3,30)=3.39$, $P<.03$; respectively). However, SR 141716A had no effect on the duration of movement. Intravenous administration of SR 141716A has been shown to enhance locomotor activity in mice (Compton et al., 1996). Since intravenous injection in the least shrew is impractical, the effects of a wide dose range (0.05, 0.2, 1, 5, 10, 20 and 40 mg/kg) of intraperitoneal administration of SR 141716A was investigated (Fig. 6). Overall, the intraperitoneal injection of SR 141716A produced suppressive effects similar to its subcutaneous route. The 5-, 10- and 40-mg/kg doses of SR 141716A caused significant ($P<.05$) reductions, 47%, 50% and 55%, respectively) in spontaneous locomotor activity, $F(7,55)=2.8$, $P<.01$. Its 5-mg/kg dose significantly reduced (13%, $P<.05$) duration of movement, $F(7,55)=2.07$, $P<.05$, whereas its 0.2-mg/kg dose reduced (69%, $P<.05$) the rearing frequency ($F(7,55)=2.69$, $P<.02$). Other doses of SR 141716A failed to induce significant effects on the triad of motor behaviors.

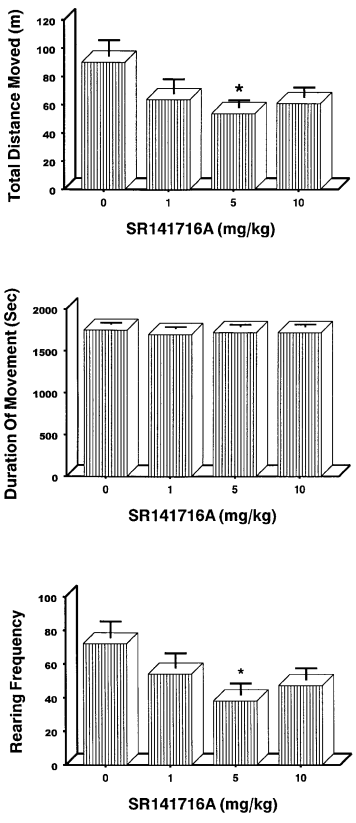

Fig. 5. The effect of subcutaneous administration of the cited doses of SR 141716A on the triad of motor parameters in the least shrew. The cited behaviors were recorded by Ethovision for 30 min immediately following SR 141716A injection. * Significantly different from vehicle control group at $P<.05$ by Dunnett's t test.

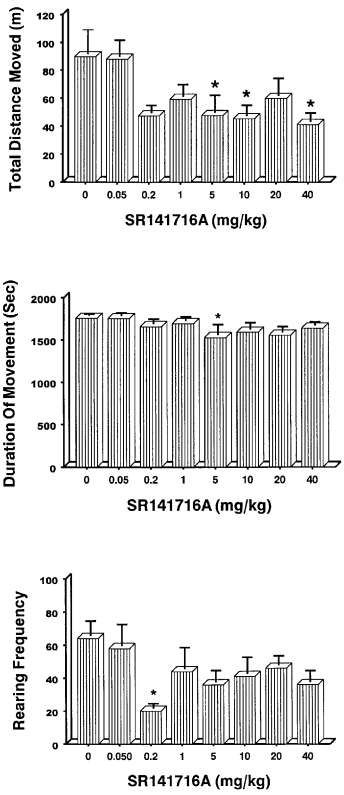

Fig. 6. The effects of intraperitoneal administration of the cited doses of SR 141716A on the triad of motor behaviors in the least shrew. The cited behaviors were recorded for 30 min by Ethovision immediately after SR 141716A administration. * Significantly different from vehicle control group at $P<.05$ by Dunnett's t test.

Fig. 7 represents the ability of the cited subcutaneous doses of SR 141716A to reverse the motor inhibitory effects of a 20-mg/kg ip dose of Δ^9-THC. Also shown in this figure are two additional controls, one representing the possible effects of the vehicle injections (i.e., V + V; one intraperitoneal and one subcutaneous) and the other showing the effect of a 1-mg/kg sc dose of SR 141716A plus an intraperitoneal injection of vehicle (i.e., 1 + V). The cited doses of SR 141716A reversed the inhibitory action of Δ^9-THC on the cited motor parameters in a dose-dependent fashion. Indeed, relative to the control group (i.e., 0 mg/kg SR 141716A + 20 mg/kg Δ^9-THC), SR 141716A significantly reversed the Δ^9-THC-induced blockade of spontaneous

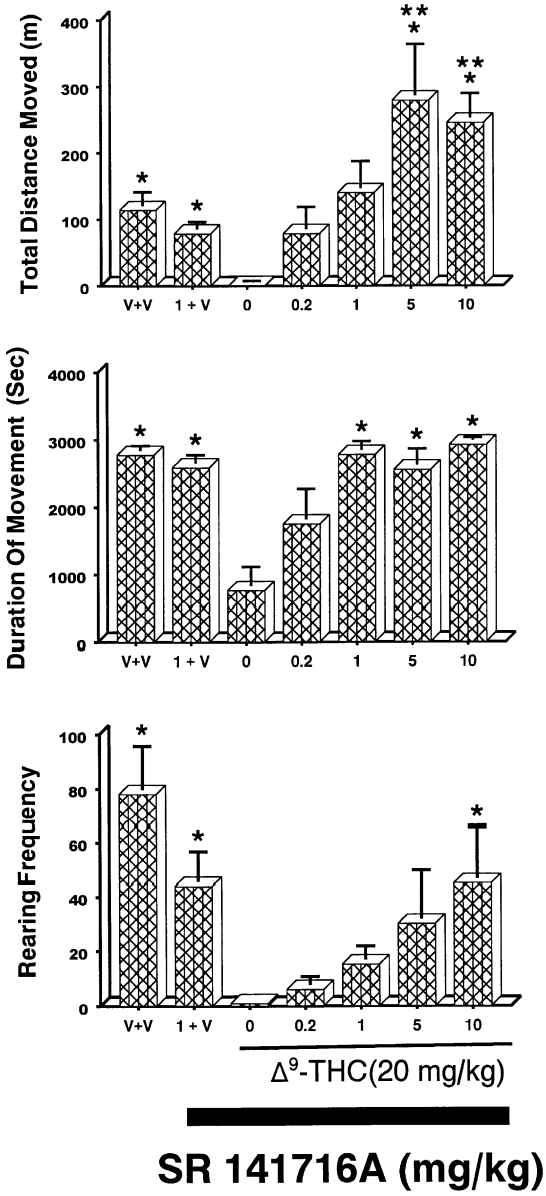

Fig. 7. Represents the ability of the cited subcutaneous doses of SR 141716A to reverse the motor depressant effects of a 20-mg/kg ip dose of Δ^9-THC on the cited triad of motor parameters. (V + V) = a control group that received both an intraperitoneal and subcutaneous injection of vehicle; (1 + V) = a control group receiving a 1-mg/kg dose of SR 141716A (sc) and an intraperitoneal injection of vehicle; (0) = a control group that received a 20 mg/kg ip dose of Δ^9-THC plus a subcutaneous injection of vehicle. * Significantly different from control (0) by Dunnett's t test at $P < .05$. ** Significantly different from controls (V + V) and (1 + V) by Bonferroni's multiple comparisons test.

locomotor activity at its 5- ($P < .001$) and 10-mg/kg doses ($P < .01$) with an ID_{50} of 0.94 ± 4 mg/kg (Fig. 7, top graph), $F(4,39) = 7.5$, $P < .0001$. Furthermore, Bonferroni's multiple comparison test showed that both the 5- and 10-mg/kg doses of SR 141716A in Δ^9-THC-pretreated shrews potentiated the locomotor activity over basal levels in vehicle (V + V)-injected control group as well as relative to the vehicle plus 1 mg/kg SR 141716A-treated control shrews (i.e., 1 + V), $F(6,59) = 7.1$, $P < .0001$, (Fig. 7, top graph). The cited doses of SR 141716A also reversed (129%, 263%, 234% and 282%, respectively) the ability of Δ^9-THC in reducing the duration of movement with an ID_{50} of 0.18 ± 1.8 mg/kg (Fig. 7, middle graph), $F(4,39) = 10.57$, $P < .0001$. In this case, the 1-, 5- and 10-mg/kg doses of the CB_1 antagonist attained significance ($P < .05$). However, relative to the (V + V) or (1 + V) control groups, no significance was observed. SR 141716A pretreatment also reversed the inhibition of the rearing behavior produced by Δ^9-THC pretreatment with an ID_{50} of 6 ± 1.92 mg/kg (Fig. 7, bottom graph), $F(4,39) = 2.69$, $P < .045$. However, a significant effect was only seen at its 10-mg/kg dose ($P < .05$). Furthermore, relative to the (V + V) and (1 + V) control groups, no significant effect was observed.

4. Discussion

This study shows that intraperitoneal administration of Δ^9-THC reduces the number of shrews vomiting in response to a 20-mg/kg emetic dose of cisplatin in a potent and dose-dependent manner ($ID_{50} = 1.86$ mg/kg). However, the employed doses of Δ^9-THC failed to completely prevent emesis in all of the cisplatin-treated shrews. Indeed, although its 2.5-mg/kg dose significantly protected 70% of shrews from vomiting, larger tested doses of Δ^9-THC (5 and 10 mg/kg) only afforded protection up to 80% of shrews. The observed incomplete blockade of emesis could be due to: (1) lack of full efficacy of Δ^9-THC against cisplatin-induced vomiting as reported both in clinical trials and animal studies (Gralla, 1999; McCarthy et al., 1984; Voth and Schwartz, 1997) and/or (2) Δ^9-THC may have emetic effects of its own in some test subjects. Indeed, in the present study, intraperitoneal injection of Δ^9-THC caused a nondose-dependent degree of emesis in 20–30% of shrews, which is not significantly different from vehicle-exposed control group. A similar degree of emesis seems to occur when Δ^9-THC is administered intravenously in dogs (Lowe, 1946; Shannon et al., 1978) or orally in man (Frytak et al., 1979; Noyes et al., 1975; Orr and McKernan, 1980). On the other hand, Δ^9-THC has not been reported to induce emesis in either the pigeon (Feigenbaum et al., 1989), or in the feline (McCarthy et al., 1984). Our preliminary studies also indicated that Δ^9-THC may occasionally induce vomiting when administered subcutaneously. Thus, the induced emesis is not dependent upon the route via which Δ^9-THC is administered. The vehicle used to dissolve Δ^9-THC may occasionally produce emesis in up to 10% of shrews. Δ^9-THC appears to be a more efficacious antiemetic in reducing the frequency of emesis ($ID_{50} = 0.36$ mg/kg). Indeed, although the lowest tested dose of Δ^9-THC (1 mg/kg), which had no effect on the percentage of shrews vomiting in response to cisplatin, did attenuate the frequency of emesis by nearly 70%. However, the latter reduction did not attain significance. On the other hand,

larger tested doses of Δ^9-THC (2.5–10 mg/kg) significantly reduced the emesis frequency by 92–98%. Clinical studies support these findings since Δ^9-THC appears to completely prevent emesis in only 10–60% of cancer patients receiving chemotherapy but reduces the frequency of nausea and emesis to a much greater extent (33–94% inhibition) (Chang et al., 1979; Gralla et al., 1984; Lucas and Laszlo, 1980; Neidhart et al., 1981; Sallan et al., 1980; Sweet et al., 1981). Cisplatin induces emesis in two phases (acute and delayed) and the purpose of the present study was to investigate the antiemetic action of Δ^9-THC against the acute phase of cisplatin-induced vomiting. The antiemetic effects of Δ^9-THC appears to be species dependent since it was shown to be ineffective in preventing apomorphine-induced emesis in the dog (Shannon et al., 1978) but can prevent the latter effect in the shrew (unpublished observations). Moreover, Δ^9-THC is a relatively less potent antiemetic against cisplatin-induced vomiting in the pigeon (Feigenbaum et al., 1989) but completely prevents it in cats (McCarthy et al., 1984). The present study shows that the least shrew appears to be an even more sensitive model for the antiemetic action of Δ^9-THC.

The most important finding of the present investigation is that subcutaneous administration of nonemetic doses of the selective cannabinoid CB_1 receptor antagonist SR 141716A, reversed the antiemetic action of an effective dose of Δ^9-THC (5 mg/kg) in cisplatin-exposed shrews in a dose-dependent manner. Indeed, both the percentage of animals vomiting as well as the frequency of vomits increased with larger doses of SR 141716A. The results suggest that Δ^9-THC prevents chemotherapy-induced emesis via the activation of cannabinoid CB_1 receptors since a 10-mg/kg dose of the selective CB_2 antagonist SR 144528 failed to reverse the antiemetic action of Δ^9-THC. This conclusion is further supported by our previous findings that at larger doses SR 141716A, and not SR 144528, can induce emesis in the least shrew in a dose- and route-dependent manner (Darmani, 2001). In addition, the previous study showed that Δ^9-THC and its synthetic analogs (CP 55, 940 and WIN 55, 212-2) reduced SR 141716A-induced emesis in a potency order consistent with an action on CB_1 receptors. Furthermore, indirect evidence from rodent studies suggests involvement of CB_1 receptors in the mediation of emesis. Indeed, different classes of cannabinoid agonists (anandamide, methanandamide, nabilone, Δ^9-THC, WIN 55, 212-2 and CP 55, 940) are reported to decrease the intestinal transit and gastric motility via SR 141716A-sensitive cannabinoid CB_1 receptors (Calignano et al., 1997; Colombo et al., 1998; Izzo et al., 1999; Krowicki et al., 1999; Shook and Burks, 1989). Moreover, these studies show that SR 141617A by itself increases intestinal ejection pressure as well as promoting defecation and gastrointestinal motility. Thus, taken together, the cannabinoid CB_1 receptor appears to be involved in the control of both emesis and intestinal motility function.

Several clinical trials have suggested that the antiemetic action of Δ^9-THC may be correlated with sedation and/or production of a psychological high (Chang et al., 1979; Lucas and Laszlo, 1980; Orr and McKernan, 1980; Sallan et al., 1980). The major effects of cannabinoids is hypoactivity and catalepsy in animals (Compton et al., 1996; Ferrari et al., 1999; Romero et al., 1996). However, detailed locomotor analysis studies show that cannabinoid agonists produce biphasic effects on movement, which are time- and dose-dependent (Carlini et al., 1970; Davis et al., 1972; Sañudo-Peña et al., 1999, 2000; Sulcova et al., 1998). Similar effects were obtained in the least shrew. Indeed, relative to its antiemetic efficacy, Δ^9-THC was less effective in reducing the triad of motor parameters (spontaneous locomotor activity, duration of movement and rearing), and only its highest tested dose significantly and simultaneously reduced these behaviors. Although its 5-mg/kg dose reduced both rearing and locomotor activity, only the former behavior was significantly reduced. Though the 20-mg/kg dose of Δ^9-THC decreased the movement duration by only 40% ($P<.05$), the other two motor parameters were totally blocked. This difference is due to the definition of computer measured movement analysis. Spontaneous motor activity was set to measure initiation of any horizontal movement greater than 2 cm whereas the duration of movement recorded all types of movement. Thus, although shrews exhibited a cataleptic posture (i.e., splayed fore- and hindlegs) at the 20-mg/kg dose of Δ^9-THC, they did exhibit continual fore- and hindleg treading (i.e., piano playing), which was computed in the duration of movement calculation by Ethovision. Indeed, catalepsy is a phenomenon that may or may not be accompanied by inhibition of movement (Klemm, 1989). Because of the biphasic inhibitory nature of Δ^9-THC on the discussed motor parameters, its ID_{50} values for each specific motor effect could not be accurately computed and therefore cannot be directly compared with its antiemetic ID_{50} values. However, unlike the discussed possible clinical association between the antiemetic, sedative and psychological effects of Δ^9-THC, the present findings in the least shrew clearly show a divergence between its antiemetic and sedative effects. The potent synthetic cannabinoid HU 210 also shows antiemetic effects at doses that do not affect other behavioral functions in the pigeon (Ferrari et al., 1999). Other clinical studies support this view since coadministration of the dopamine antagonist prochlorperazine promotes the antiemetic effects while reducing Δ^9-THC's "high" and its other side effects (Garb et al., 1980; Lane et al., 1990).

Several studies in different species have shown that the motor suppressant effects of Δ^9-THC and other cannabinoids are mediated via CB_1 receptors (Compton et al., 1996; Rinaldi-Carmona et al., 1994). Thus, a second aspect of the current study was to investigate whether SR 141716A can reverse the motor suppressing effects of Δ^9-THC. The direct effects of SR 141716A on motor

behaviors are not equivocal and appears to be dependent upon the route of administration, species used and the experimental conditions under which the motor behaviors are observed (Compton et al., 1996; Gallate and McGregor, 1999; Masserano et al., 1999; Poncelet et al., 1999; Rinaldi-Carmona et al., 1994). Most of these studies have found no effect on locomotion although enhancement or suppression of locomotor behaviors has also been noted. In this study, the effects of both subcutaneous and intraperitoneal administration of SR 141716A in the least shrew were investigated. Certain doses of SR 141716A partially (13–69% reduction) suppressed one or more components of the triad of motor behaviors via both routes of administration. As in our antiemetic reversal studies, different doses of SR 141716A were administered subcutaneously for the investigation of interactions of this CB_1 antagonist with Δ^9-THC. SR 141716A reversed the suppressive effects of Δ^9-THC (20 mg/kg) on the triad of motor parameters in a dose-dependent manner. SR 141716A was most effective in reversing Δ^9-THC-induced reduction on movement duration ($ID_{50} = 0.18$ mg/kg) as significant reversal was apparent at its 1-mg/kg dose. Significant reversal of spontaneous activity ($ID_{50} = 0.91$ mg/kg) required 5-mg/kg dose of SR 141716A, whereas the reversal of suppression of rearing behavior ($ID_{50} = 6$ mg/kg) achieved significance at its 10-mg/kg dose. This data clearly shows that the motor components that are least sensitive to Δ^9-THC are more potently reversed by SR 141716A. Although both agents suppressed behavior by themselves, their combination did not produce additive suppressant effects. Moreover, the 5- and 10-mg/kg doses of SR 141716A, not only reversed the suppressant action of Δ^9-THC on spontaneous locomotor activity but also significantly elevated the behavior over basal activity in vehicle (V + V)-injected control group. In agreement with the discussed rodent studies, the motor suppressant action of Δ^9-THC in the least shrew also seems to be mediated via CB_1 receptors.

The discussed results suggest that different brain loci are probably responsible for the mediation of antiemetic and motor suppressive actions of Δ^9-THC. The CB_1 receptor is one of the most widely distributed neurotransmitter receptors in the brain (Herkenham et al., 1991; Mailleux and Vanderhaeghen, 1992; Tsou et al., 1998). The densest binding in the entire rat brain is in the basal ganglia including some of its components such as the striatum and subthalamic nucleus. The motor effects of cannabinoids occur through these extrapyramidal structures (Sañudo-Peña et al., 1999, 2000). The vomiting reflex centers for various emetic stimuli include the area postrema, the medullary formation and the nucleus of the solitary tract, which abuts the area postrema (Mitchelson, 1992). The latter sites in the medulla probably mediate the antiemetic action of Δ^9-THC and other cannabinoids. Indeed, direct application of Δ^9-THC to the dorsal surface of rat medulla leads to a decrease in gastric tone that in turn may reduce nausea and vomiting (Krowicki et al., 1999). However, cisplatin-induced vomiting is a complex behavior that is mediated via central as well as peripheral mechanisms (Darmani, 1998) and Δ^9-THC may affect one or both of these mechanisms.

In summary, Δ^9-THC prevents cisplatin-induced emesis in the least shrew in a potent and dose-dependent manner. Relative to its antiemetic effects, the motor depressant actions of Δ^9-THC were only observed at comparatively large doses. Both antiemetic and motor depressant actions of Δ^9-THC were reversed in a dose-dependent manner by the selective cannabinoid CB_1 receptor antagonist SR 141716A. Reversal of antiemetic action of Δ^9-THC required larger doses of SR 141716A than its motor depressant actions. The CB_2 antagonist (SR 144528) failed to block the antiemetic effect of Δ^9-THC. It is concluded that CB_1 receptors located in distinct brain structures probably mediate the antiemetic and motor suppressant actions of Δ^9-THC and other cannabinoids.

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