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The cannabinoid CB_1 receptor antagonist SR 141716A reverses the antiemetic and motor depressant actions of WIN 55, 212-2

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Abstract

The dibenzopyran cannabinoids (delta-9 (Δ^9)-tetrahydrocannabinol and nabilone) are clinically used to suppress nausea and vomiting produced by chemotherapeutic agents such as cisplatin. The purpose of this investigation was to investigate the antiemetic potential of the aminoalkylindole cannabinoid receptor agonist WIN 55, 212-2 [*R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl) methyl] pyrolol [1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl) methanone mesylate] against cisplatin-induced vomiting. Different doses of WIN 55, 212-2 (0, 1, 2.5 and 5 mg/kg, i.p.) reduced both the frequency of cisplatin (20 mg/kg, i.p.)-induced emesis (ID_{50} = 0.5 mg/kg) as well as the percentage of shrews vomiting (ID_{50} = 1.2 mg/kg) in a dose-dependent manner. Significant reductions in emesis frequency occurred from 2.5 mg/kg dose of WIN 55, 212-2, whereas significant total protection from vomiting was afforded at its 5 mg/kg dose. The antiemetic actions of a 5-mg/kg dose of WIN 55, 212-2 against cisplatin (20 mg/kg, i.p.)-induced vomiting were reversed by nonemetic subcutaneous doses (0, 0.25, 0.5 and 1 mg/kg) of the cannabinoid CB_1 receptor antagonist/inverse agonist SR 141716A [*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide] (ID_{50} = 0.27 and 0.47 mg/kg, respectively) but not by a 5-mg/kg dose of the cannabinoid CB_2 receptor antagonist SR 144528 [*N*-[(1*S*)-endo-1,3,3-trimethylbicyclo [2.2.1] heptan-2-yl]5-(4-chloro-3-methylphenyl)-1-(4-methybenzyl) pyrazole-3-carboxamide]. The effects of the cited doses of WIN 55, 212-2 were also investigated on several motor parameters (spontaneous locomotor activity, duration of movement and rearing frequency). Significant reductions in motor parameters were only observed at its highest tested dose (ID_{50} = 1.97, 2.75 and 2.8 mg/kg; respectively). SR 141716A (0, 0.5, 1, 5 and 10 mg/kg) also reversed the motor suppressant effects of a 5-mg/kg dose of WIN 55, 212-2 (ID_{50} = 0.39, 0.1 and 0.3 mg/kg, respectively) and significant reversals were seen from its 0.5 and 1 mg/kg doses. These results suggest that WIN 55, 212-2 reduces both emesis and indeces of locomotion via the stimulation of cannabinoid CB_1 receptors. However, cannabinoid CB_1 receptors in different loci are most likely responsible for the antiemetic and motor suppressive effects of WIN 55, 212-2 since reduction in the frequency of vomiting occurred at lower doses relative to its sedative actions.

Keywords: Vomiting; Locomotor activity; Rearing; Reversal; WIN 55, 212-2; SR 141716A; SR 144528; Cannabinoid CB_1 receptor antagonist

1. Introduction

Both the cloning of cannabinoid (CB_1 and CB_2) receptors (Matsuda et al., 1990; Munro et al., 1993) and synthesis of their potent and selective antagonists (Rinaldi-Carmona et al., 1994, 1998) have helped to open new avenues for cannabinoid research. The current progress may ultimately lead to potential cannabinoid use in the clinic for the numerous therapeutic applications for which cannabinoids are being evaluated (Pertwee, 1999). Besides their established antiemetic action in the clinic (reviews: Gralla, 1999; Voth and Schwartz, 1997), cannabinoid receptor agonists also possess appetite stimulant, anticonvulsant, antinociceptive and hypothermic properties (Formukong et al., 1989; Mattes et al., 1994; Pertwee, 1999). Although the antiemetic activity of the main psychoactive component of marijuana plant, delta-9-tetrahydrocannabinol (Δ^9-tetrahydrocannabinol) and its synthetic analogs (nabilone and levonantradol) seem to be superior or equivalent to dopamine D_2 receptor antagonists in cancer patients receiving chemotherapy, their efficacy is not as good as the more potent antiemetics such as the 5-HT_3 receptor antagonists (Gralla, 1999). Until recently, the receptor mechanism by which cannabinoids produce their antiemetic action was not known. It seems that cannabinoids prevent chemotherapy-induced emesis via activation of the cannabinoid CB_1 receptor since its selective antagonist SR 141716A [*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-di-

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chlorophenyl)-4-methylpyrazole-3-carboxamide] induces emesis in the least shrew (*Cryptotis parva*) in a dose- and route-dependent manner (Darmani, 2001a). This effect was prevented by Δ^9-tetrahydrocannabinol and its analogs CP 55, 940 [(−)-3-[2-hydroxy-4-(1,1-dimethylheptyl]-4-[3-hydroxy propyl] cyclohexan-1-ol] and WIN 55, 212-2 [*R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl) methyl] pyrolol [1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl) methanone mesylate]. Moreover, nonemetic doses of SR 141716A was recently shown to prevent the antiemetic action of Δ^9-tetrahydrocannabinol against cisplatin-induced emesis (Darmani, 2001b).

Currently, very little is known regarding the antiemetic structure activity relationship of different cannabinoids in any species. Cannabinoid receptor agonists can be classified according to their chemical structure into four groups (Pertwee, 1999). The first of these is the "classical cannabinoid" group, which is made of dibenzopyran derivatives and includes Δ^9-tetrahydrocannabinol. The second, "nonclassical cannabinoid" group, consists of bicyclic and tricyclic analogs of Δ^9-tetrahydrocannabinol that lack a pyran ring, e.g. CP 55, 940. The third group are aminoalkylindoles and their prototype is the pravadolline derivative WIN 55, 212-2. The fourth is the "eicosanoid" group, which contains arachidonic acid derivatives such as anandamide.

Some clinical studies have suggested that the antiemetic action of cannabinoids may be related to their sedative effect and/or production of a "psychological high" (Chang et al., 1979; Lucas and Laszlo, 1980; Orr and McKernan, 1980; Sallan et al., 1975, 1980). This work was undertaken to investigate the antiemetic potential and the cannabimimetic profile of WIN 55, 212-2 in the least shrew. This insectivore species has recently been characterized as a new animal model for various emetic stimuli (Darmani, 1998, 2001a,b; Darmani et al., 1999; Dukat et al., 2000). The least shrew are small (adult 4–6 g in weight), short-legged, mouse-like mammals with long, pointed snouts and short dense dark brown fur, which reside in Central and North America (Churchfield, 1990). This species is very active, and unlike most laboratory animals, do not come to rest after acclimation to their environment. Reduction in motor activity is one component of the tetrad of behaviours used for the initial evaluation, and for establishing a general cannabimimetic pharmacological profile in rodents (Martin et al., 1995). As shown for other species (Ferrari et al., 1999), the least shrew provides a good opportunity for comparing the antiemetic and motor depressant profile of WIN 55, 212-2 and other cannabinoids. Thus, the purpose of the present study was: (1) to determine whether WIN 55, 212-2 can prevent emesis produced by the chemotherapeutic agent cisplatin in the least shrew (Darmani, 1998); (2) to show whether selective cannabinoid CB_1 and CB_2 receptor antagonists (SR 141716A and SR 144528, respectively) can reverse the antiemetic action of WIN 55, 212-2 against cisplatin-induced emesis, and (3) to demonstrate whether motor suppressive action of WIN 55, 212-2 is related to its antiemetic activity.

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 ± 1 °C with ad lib supply of food and water. The feeding and maintenance of shrews are fully described elsewhere (Darmani, 1998; Darmani et al., 1999). *R*(+)-WIN 55, 212-2 [*R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl) methyl] pyrolol [1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl) methanone mesylate] and *cis*-platinum (II) diamine dichloride ($Pt(NH_3)_2Cl_2$) were purchased from Research Biochemicals Natick, MA. SR 141716A [*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide] and SR 144528 [*N*-[(1*S*)-endo-1,3,3-trimethylbicyclo [2.2.1] heptan-2-yl]5-(4-chloro-3-methylphenyl)-1-(4-methybenzyl) pyrazole-3-carboxamide] were given to us by Professor R. Theobald which were generously donated by Sanofi Recherche (Montpellier, France). All drugs were dissolved in a 1:1:18 solution of ethanol: mulphor: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 induced emesis in up to 10% of shrews. In our preliminary studies, we also used ethanol as the dissolving agent for cannabinoid agonists and antagonists. However, concentrations of ethanol required to maintain intermediate to larger doses of the cited agents dissolved in saline would produce unwanted behaviours such as cataplexy in the shrew and thus the cited special solvent containing a small amount of ethanol was used as the dissolving medium. All drugs were administered at a volume of 0.1 ml/10 g 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. Emesis studies

The present protocols were based upon our previous emesis studies in the least shrew (Darmani, 1998, 2001a,b; Darmani et al., 1999). All experiments were performed between 0800 and 1700 h. Since published studies in dogs and shrews have shown that Δ^9-tetrahydrocannabinol by itself can induce nondose-dependent vomiting (Lowe, 1946; Shannon et al., 1978; Darmani, 2001b), the emetic effects

of varying doses of WIN 55, 212-2 in the shrew were 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 4 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 WIN, 55, 212-2 (1, 2.5 and 15 mg/kg; five shrews per group). Immediately following injection, each shrew was placed in the observation cage and the frequency of vomiting (oral ejections of food or liquid; mean ± S.E.M.) was recorded for each individual shrew for the next 60 min. These data showed that intraperitoneal administration of the cited doses of WIN 55, 212-2 did not produce emesis in the least shrew.

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, 2001b). Indeed, a 20-mg/kg intraperitoneal dose of cisplatin can produce vomiting in 90–100% of least shrews. The latter dose was chosen to demonstrate the possible antiemetic effects of WIN 55, 212-2 on the cisplatin-induced vomiting. The antiemetic doses of WIN 55, 212-2 were based upon our preliminary experiments as well as published studies (Darmani, 2001a,b). Shrews were offered 4 meal worms prior to drug administration. Different groups of shrews were first injected intraperitoneally with varying doses of WIN 55, 212-2 (0, 1, 2.5, or 5; $n = 8$–9 per group) and a 20-mg/kg dose of cisplatin (i.p.). 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 WIN 55, 212-2 prevented cisplatin-induced emesis in a dose-dependent manner and its 5 mg/kg dose produces maximal antiemetic effects.

This laboratory has previously shown that intraperitoneal administration of the selective cannabinoid CB_1 receptor antagonist SR 141716A produces emesis in the least shrew at its 10 and 20 mg/kg doses (Darmani, 2001a). When administered subcutaneously, SR 141716A was a less efficient emetogenic agent since only its 40 mg/kg dose caused a significant degree of vomiting. Thus, in the next experiment substantially lower subcutaneous doses of SR 141716A (0.25–1 mg/kg) were used to reverse the antiemetic effect of a 5-mg/kg dose of WIN 55, 212-2 against cisplatin (20 mg/kg, i.p.)-induced vomiting. Thus, at 0 time different groups of shrews were injected subcutaneously with either vehicle ($n = 8$) or varying doses of SR 141716A (0.25, 0.5 and 1 mg/kg) and were then offered four meal worms. Ten minutes later, each shrew received intraperitoneally WIN 55, 212-2 (5 mg/kg) and cisplatin (20 mg/kg) and the emesis frequency was recorded for the next 60 min as described above. A 5-mg/kg subcutaneous dose of SR 144528 was used to reverse the antiemetic effect of a 5-mg/kg dose of WIN 55, 212-2 on cisplatin-induced emesis in the same manner as described for the CB_1 receptor antagonist.

2.3. Locomotor studies

On the test day, shrews were brought in their home cages from animal quarters and were allowed to acclimate for at least 1 h to a semi-dark environment. The reduced light condition was necessary for the computerized video tracking, motion analysis, and behaviour 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 plain 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 is recorded as a 20% decrease in surface area when shrews stand upright as seen by the overhead video camera]. Our preliminary experiments indicated a 20% change in the surface area for shrews is equivalent to 90% to 110% of our 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; Darmani, 2001b).

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 WIN 55, 212-2 (1, 2.5 and 5 mg/kg, $n = 7$ per group). Then each shrew was individually placed in an observation cage of the same dimension and the discussed locomotor parameters were recorded for 50 min starting at the 10-min post-injection period. WIN 55, 212-2 significantly reduced all three parameters in shrews at its 5 mg/kg dose. In the next experiment, the effect of different subcutaneous doses of SR 141716A was investigated on the locomotor reducing properties of the 5 mg/kg dose of WIN 55, 212-2. Thus, at 0 time, different shrews were injected with varying subcutaneous doses of SR 141716A (0, 0.5, 1, 5 and 10 mg/kg; $n = 8$–11 per group). At 30 min, each shrew received intraperitoneally a 5-mg/kg dose of WIN 55, 212-2. Ten minutes later (i.e. at 40 min), the locomotor activity parameters were recorded for the next 50 min as described earlier.

2.4. Statistical analysis

The frequency of emesis data were analyzed by the Kruskal–Wallis (KW) nonparametric one-way analysis of variance (ANOVA) and posthoc analysis by Dunn's multiple comparisons test. A P value of < 0.05 was necessary to achieve statistical significance. The incidence of emesis (number of animals vomiting) were analyzed by the Fisher's exact test to determine whether there were differences between groups. When appropriate, pairwise comparisons were also made by this method. 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 which 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 Dunnett's multiple comparisons test were used to analyze the locomotor data.

3. Results

3.1. Emesis

As with our previous studies (Darmani, 2001a,b), intraperitoneal administration of the final concentration of the special solvent (ethanol:emulphor:saline) produced emesis in up to 10% of shrews. Intraperitoneal administration of 1, 2.5 or 5 mg/kg doses of WIN 55, 212-2 in the above solvent did not cause significant emesis.

The 20-mg/kg intraperitoneal dose of cisplatin induced vomiting in all of the tested shrews with a mean vomiting frequency of 9 ± 1.6 (Fig. 1). The Fisher's exact test showed that WIN 55, 212-2 reduced the percentage of shrews vomiting in response to cisplatin in a dose-dependent manner with an ID_{50} of 1.2 ± 7.6 mg/kg (Fig. 1A) ($\chi^2(4,38) = 23.94$, $P < 0.000015$). Furthermore, relative to the vehicle-injected-cisplatin-treated control group, a significant reduction (87.5%, $P < 0.004$) in the number of animals vomiting occurred in the 5 mg/kg dose of WIN 55, 212-2. WIN 55, 212-2 pretreatment also significantly reduced the frequency of cisplatin-induced vomiting with an ID_{50} value of 0.5 ± 1.72 mg/kg ($KW(4,38) = 29.29$, $P < 0.0001$) (Fig. 1B). The pattern of reduction in the number of vomitings was also dose-dependent (69.1, 82.7 and 94.5%, respectively). Although reduction caused by the 1-mg/kg dose of WIN 55, 212-2 just failed to attain significance, larger doses of this cannabinoid significantly [2.5 ($P < 0.05$), and 5 mg/kg ($P < 0.001$)] attenuated the vomiting frequency. Only 1 of the 10 shrews injected twice with vehicle exhibited vomiting (Fig. 1).

Fig. 2 describes the ability of subcutaneously administered SR 141716A to reverse the antiemetic action of WIN 55, 212-2 (5 mg/kg, i.p.) against cisplatin (20 mg/kg, i.p.)-induced vomiting. In the absence of SR 141716A, the 5 mg/kg dose of WIN 55, 212-2 prevented emesis in seven out of nine tested shrews (i.e. 22% vomited) (Fig. 2A). The percentage of shrews vomiting increased (56%, 75% and 100%, respectively) in response to pre-administration of different (0.25, 0.5 and 1 mg/kg) doses of SR 141716A with an ID_{50} of 0.27 ± 1.56 mg/kg ($\chi^2(3,31) = 12.21$, $P < 0.004$). However, due to large intergroup variability, a significant enhancement in the number of shrews vomiting was observed at the 1-mg/kg dose of SR 141716A ($P < 0.014$). It's 0.5 mg/kg dose just failed to attain significance ($P = 0.057$). The frequency of vomiting also increased (70%, 545% and 1415% relative to control, respectively) with increasing doses of SR 141716A with an ID_{50} of 0.47 ± 1.8 mg/kg ($KW(3,31) = 19.23$, $P < 0.0002$) (Fig. 2B). However, once again due to the large intergroup variation, a significant increase in vomiting frequency ($P < 0.01$) was only seen at its 1 mg/kg dose. At such doses, SR 141716A by itself does not induce

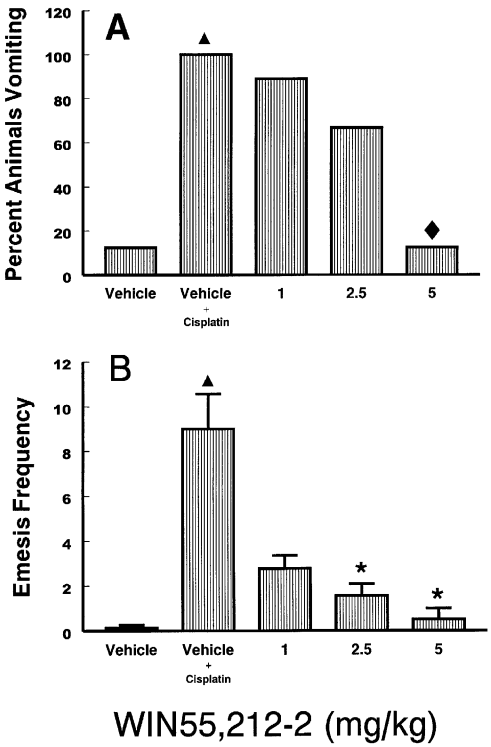

Fig. 1. This figure represents the antiemetic dose–response effects of WIN 55, 212-2 on cisplatin (20 mg/kg, i.p.)-induced emesis in the least shrew. Graph A depicts reduction in percentage of shrews vomiting, whereas graph B shows attenuation in the frequency of vomiting. Vehicle = a control group which received two vehicle (i.p.) injections. Vehicle + cisplatin = a group receiving an i.p. injection of cisplatin plus an i.p. injection of vehicle. The remaining groups received cisplatin plus an i.p. injection of the cited doses of WIN 55, 212-2. Emesis parameters were recorded for 60 min post-injection. ♦ Significantly different from vehicle + cisplatin group at $P < 0.05$ by Fisher's exact test; * Significantly different from vehicle + cisplatin group at $P < 0.05$ by Dunn's multiple comparisons test; ▲ Significantly different from vehicle-injected control group by their corresponding statistical tests.

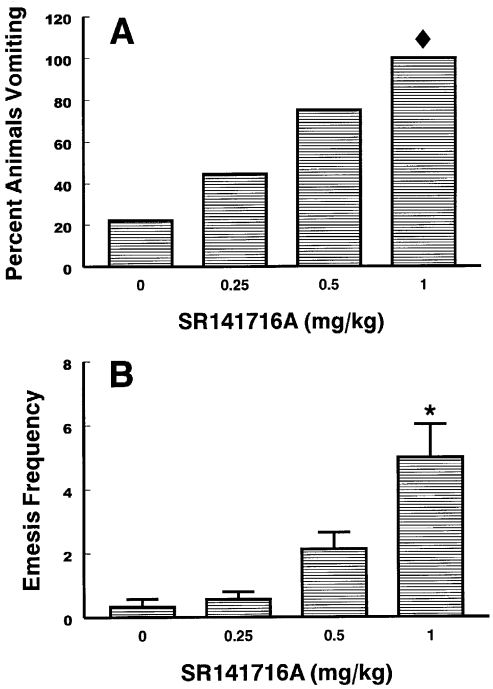

Fig. 2. This figure represents the ability of the cited subcutaneous doses of SR 141716A to reverse the antiemetic effects of a 5-mg/kg (i.p.) dose of WIN 55, 212-2 against cisplatin (20 mg/kg, i.p.)-induced emesis. SR 141716A blocked the ability of WIN 55, 212-2 to protect shrews from vomiting (graph A) as well as reversing the WIN 55, 212-2-induced reduction in emesis frequency (graph B). At 0 time shrews received either vehicle or the cited doses of SR 141716A and 10 min later, WIN 55, 212-2 plus cisplatin. Emesis parameters were recorded for the next 60 min. ♦ Significantly different from the 5 mg/kg WIN, 212-2 plus 20 mg/kg cisplatin-treated control group which had received no SR 141716A (i.e. column 0) at $P < 0.05$ by the Fisher's exact test; * Significantly different from the 5 mg/kg WIN 55, 212-2 plus 20 mg/kg cisplatin-treated control group which had received no SR 141716A (i.e. column 0) at $P < 0.05$ by Dunn's multiple comparisons test.

vomiting (Darmani, 2001a). A 5-mg/kg dose of the CB_2 antagonist SR 144528 failed to alter the ability of a 5-mg/kg dose of WIN 55, 212-2 in preventing cisplatin (20 mg/kg)-induced vomiting. Indeed, in the control group ($n = 9$) two shrews vomited, whereas none of the SR 144528-injected animals ($n = 8$) vomited.

3.2. Locomotion

One-way analysis of variance (ANOVA) indicated that intraperitoneal administration of 1 to 5 mg/kg doses of WIN 55, 212-2 significantly attenuated spontaneous locomotor activity (i.e. total distance moved) with an ID_{50} of 1.97 ± 2.4 mg/kg in the least shrew in the 50 min observation period (Fig. 3A) ($F(3,29) = 6.53$, $P < 0.002$). However, Dunnett's posthoc *t*-test revealed that injection of low doses of WIN, 55, 212-2 (1–2.5 mg/kg) failed to significantly modify the latter parameter relative to the vehicle control group. Only its highest tested-dose (5 mg/kg), sharply and significantly blocked the behaviour by 90% ($P < 0.01$). WIN 55, 212-2 also significantly reduced the total duration of movement in shrews ($ID_{50} = 2.75 \pm 2$ mg/kg) (Fig. 3B) ($F(3,29) = 18.04$, $P < 0.0001$). However, a significant effect (71% reduction, $P < 0.01$) was only seen at its 5 mg/kg dose. In a similar manner, WIN 55, 212-2 administration reduced the frequency of rearings with an ID_{50} of 2.8 ± 2.1 mg/kg in the least shrew (Fig. 3C) ($F(3,29) = 4.32$, $P < 0.01$). Dunnett's *t*-test revealed that only the 5 mg/kg dose of WIN 55, 212-2 caused significant inhibition (94%) ($P < 0.05$).

Fig. 4 represents the ability of the cited subcutaneous doses of SR 141716A to reverse the motor inhibitory

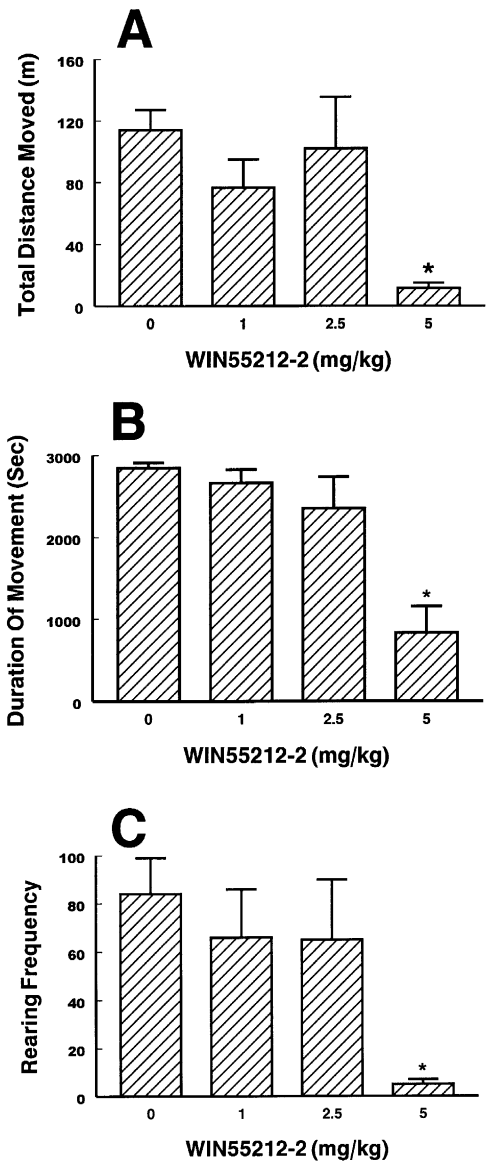

Fig. 3. The dose–response effects of WIN 55, 212-2 on the triad of motor behaviours in the least shrew. The cited behaviours were recorded for 50 min by a computerized video tracking, motion analysis, and behaviour recognition system (Ethovision) 10 min after WIN 55, 212-2 administration. * Significantly different from vehicle-injected control group at $P < 0.05$ by Dunnett's *t*-test.

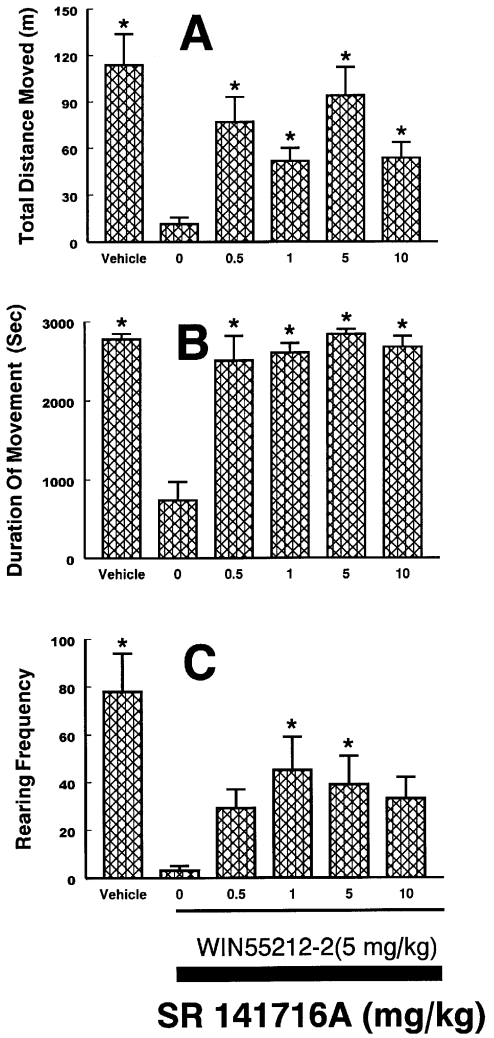

Fig. 4. This figure represents the ability of the cited subcutaneous doses of SR 141716A to reverse the motor depressant effects of a 5-mg/kg (i.p.) dose of WIN 55, 212-2 on the cited triad of motor parameters. Vehicle = a control group which had received both an i.p. and s.c. injections of vehicle; (0) = a control group which received a 5-mg/kg, i.p, dose of WIN 55, 212-2 plus a subcutaneous injection of vehicle. * Significantly different from control (0) by Dunnett's *t*-test at $P < 0.05$.

effects of a 5-mg/kg intraperitoneal dose of WIN 55, 212-2. Also shown in this figure is an additional control representing the possible effects of the vehicle injections (i.e. one i.p. and one s.c.). The cited doses of SR 141716A reversed the inhibitory action of WIN 55, 212-2 on the cited motor parameters in a dose-dependent fashion. Indeed, relative to the control group (i.e. 0 mg/kg SR 141716A + 5 mg/kg WIN 55, 212-2), SR 141716A significantly reversed the WIN 55, 212-2-induced suppression of spontaneous locomotor activity with an ID_{50} of 0.39 ± 2.44 mg/kg (Fig. 4A) ($F(5,53) = 6.76$, $P < 0.0001$). Furthermore, Dunnett's multiple comparisons test showed that significant reversals [576% ($P < 0.01$), 354% ($P < 0.05$), 723% ($P < 0.01$) and 371% ($P < 0.05$), respectively] occurred from the lowest tested dose of SR 141716A. The cited doses of SR 141716A also reversed [238% ($P < 0.01$), 252% ($P < 0.01$), 283% ($P < 0.01$) and 262% ($P < 0.01$), respectively] the ability of WIN 55, 212-2 in reducing the duration of movement with an ID_{50} of 0.1 ± 1.25 mg/kg (Fig. 4B) ($F(5,53) = 24.08$, $P < 0.0001$). Likewise, SR 141716A pretreatment reversed the inhibition of the rearing behaviour produced by WIN 55, 212-2 with an ID_{50} of 0.23 ± 1.91 mg/kg (Fig. 4C) ($F(5,53) = 5.32$, $P < 0.0005$). However, significant reversals were only seen at its 1 (1400%, $P < 0.01$) and 5 mg/kg (1200%, $P < 0.05$) doses.

4. Discussion

This study shows that along with the other classes of cannabinoid agonists (see Introduction), the aminoalkylindole cannabinoid WIN 55, 212-2, also possesses antiemetic efficacy against cisplatin-induced emesis. WIN 55, 212-2 appears to be a more efficacious antiemetic in reducing the frequency of cisplatin-induced emesis ($ID_{50} = 0.5$ mg/kg) than totally protecting shrews from vomiting ($ID_{50} = 1.2$). Indeed, a 5-mg/kg dose of WIN 55, 212-2 was required to completely protect 7 of the 8 tested shrews from vomiting ($P < 0.05$), whereas its 2.5 mg/kg dose only afforded protection in three of the nine tested shrews ($P > 0.05$). On the other hand, the 2.5 mg/kg dose of WIN 55, 212-2 significantly attenuated ($P < 0.05$) the frequency of vomits by 82%. Furthermore, its lowest tested dose (1 mg/kg) also reduced the vomit frequency (69%) but just failed to attain significance. Other cannabinoids possess a similar pattern of antiemetic action since they are more potent in reducing the amount or frequency of vomits rather than affording total emesis protection in both patients (Chang et al., 1979; Gralla et al., 1984; Lucas and Laszlo, 1980; Sweet et al., 1981; Sallan et al., 1980) and animals (Darmani, 2001b; Feigenbaum et al., 1989; Ferrari et al., 1999) treated with chemotherapeutic agents. Several basic and clinical reports indicate that Δ^9-tetrahydrocannabinol by itself can induce a nondose-dependent degree of emesis in 20–30% of test subjects in several but not all vomiting species (Darmani, 2001b; Lowe, 1946; Shannon et al., 1978; London et al., 1979; Feigenbaum et al., 1989; McCarthy et al., 1984; Noyes et al., 1975; Frytak et al., 1979; Orr and McKernan, 1980). Unlike Δ^9-tetrahydrocannabinol (Darmani, 2001b), WIN 55, 212-2 did not produce emesis in any of the tested shrews. The antiemetic potency of WIN 55, 212-2 is similar to Δ^9-tetrahydrocannabinol (ID_{50} 1.86) against cisplatin (20 mg/kg)-induced vomiting in the least shrew (Darmani, 2001b). Biochemical studies support the present results since Δ^9-tetrahydrocannabinol and WIN 55, 212-2 have a similar affinity for the cannabinoid CB_1 receptor (Pertwee, 1999). However, we have previously shown that relative to Δ^9-tetrahydrocannabinol ($ID_{20} = 15.2$ mg/kg), WIN 55, 212-2

more potently protected shrews from SR 141716A-induced emesis (ID_{50} = 3.95) (Darmani, 2001a). These differential findings suggest that either: (1) the emetic effects of SR 141716A and cisplatin are mediated via different mechanisms or sites, and/or (2) the potency rank order of cannabinoid agonists may not reflect their efficacy (Pertwee, 1999), and their relative maximal agonist effects can vary across different brain regions (Breivogel and Childers, 2000). Variability in some of the cited ED_{50} and ID_{50} values as revealed by their relatively larger S.E.M.'s may also account for these differences.

The second important finding of this study is that the antiemetic action of WIN 55, 212-2 is probably mediated via cannabinoid CB_1 receptors. Several findings support this notion: (1) low doses of the cannabinoid CB_1 receptor antagonist SR 141716A (0.25–1 mg/kg), counteracted the antiemetic action of an effective antiemetic dose of WIN 55, 212-2 in cisplatin-treated shrews by increasing both the frequency of emesis and the percent of shrews vomiting in a potent and dose-dependent manner; (2) unlike SR 141716A, a relatively larger dose of the selective cannabinoid CB_2 receptor antagonist SR 144528 (5 mg/kg) failed to counter this antiemetic effect; (3) Δ^9-tetrahydrocannabinol's antiemetic action against cisplatin-induced vomiting can be reversed by SR 141716A and not by SR 144528 (Darmani, 2001b); (4) large doses (10–40 mg/kg) of SR 141716A (and not SR 144528) can induce vomiting in the least shrew and the induced behaviour is cannabinoid agonist sensitive (Darmani et al., 2001a), and (5) WIN 55, 212-2 decreases rodent intestinal propulsive activity, defecation and gastrointestinal transit via a cannabinoid CB_1 receptor-mediated, SR 141716A-sensitive mechanism (Colombo et al., 1998; Izzo et al., 1999). Furthermore, SR 141716A was shown to increase the latter gastrointestinal effects when administered alone. Other cannabinoid receptor agonists produce similar depressive effects on such intestinal functions (Calignano et al., 1997; Krowicki et al., 1999; Shook and Burks, 1989). However, cannabinoid CB_2 receptor may also affect intestinal motility (Hanus et al., 1999). The subcutaneous doses of SR 141716A used for the reversal of antiemetic effects in the present study are 40–160 times lower than that required to produce significant emesis by itself via this route (Darmani, 2001a). Furthermore, up to 10 mg/kg, SR 141716A does not modify cisplatin-induced emesis (Darmani, 2001b). SR 141716A seems to be more potent in counteracting the antiemetic effect of WIN 55, 212-2 (5 mg/kg) than Δ^9-tetrahydrocannabinol (5 mg/kg) (Darmani, 2001b) against cisplatin-induced emesis. Indeed, a 10-mg/kg dose of SR 141716A was necessary to significantly reverse Δ^9-tetrahydrocannabinol's antiemetic action. Such differential effects of SR 141716A has also been noted in other test systems, and the dose of the cannabinoid CB_1 receptor antagonist required to block the pharmacological actions of cannabinoids may depend upon the specific effect of interest (Lichtman and Martin, 1997). Indeed, susceptibility of WIN 55, 212-2 and CP 55, 940 to antagonism by SR 141716A is respectively 5 and 33 times less in the myenteric plexus preparation than in the mouse vas deferens, whereas the susceptibility of Δ^9-Tetrahydrocannabinol to SR 141716A is the same in the two preparations (Pertwee et al., 1995, 1996).

Another aspect of the present study was to compare the antiemetic effects of WIN 55, 212-2 with its motor depressive actions since some clinical findings suggest that sedation and/or production of "psychological high" may contribute to its antiemetic effects (see Introduction). Although direct comparison of emotional effects of cannabinoids in animals and humans is uncertain, numerous models for evaluating the psychoactivity profile of cannabinoid agonists have been developed (Martin et al., 1995). Reduction in motor activity parameters is one component of the tetrad of behaviours used for the evaluation of cannabimimetic pharmacological profile of cannabinoids in rodents. Although the main effects of cannabinoids on rodent locomotion is hypoactivity and catalepsy (Compton et al., 1992; 1996; Romero et al., 1996; Gifford et al., 1999; Ferrari et al., 1999), these agents may produce biphasic effects on movement which are time- and dose-dependent (Carlini et al., 1970; Davis et al., 1972; Sulcova et al., 1998; Sañudo-Peña et al., 1999, 2000; Darmani, 2001a). In the least shrew, intraperitoneal injection of WIN 55, 212-2 (1–5 mg/kg) reduced the triad of motor parameters [spontaneous locomotor activity (total distance moved), duration of movement; and the rearing frequency] in a dose-dependent manner with similar ID_{50} values (1.97–2.8 mg/kg). However, a significant reduction was only observed at its highest tested dose (5 mg/kg) which blocked the cited behaviours by 71–94%. Although significant motor inhibition and complete protection of emesis by WIN 55, 212-2 occurred at its highest dose tested, it did, however, attenuate the frequency of cisplatin-induced emesis at lower doses [56% at 1 mg/kg ($P > 0.05$) and 75% at 2.5 mg/kg ($P < 0.05$)]. Furthermore, a 2.5-mg/kg dose of Δ^9-tetrahydrocannabinol has been shown to significantly reduce the discussed cisplatin-induced emesis parameters in the least shrew while only its 20 mg/kg dose caused significant reductions in the triad of motor behaviours (Darmani, 2001b). In addition, the more potent cannabinoid receptor agonist HU 210 also exhibits antiemetic effects in pigeons at doses which do not significantly affect motor function (Ferrari et al., 1999). As in the case of Δ^9-tetrahydrocannabinol (Darmani, 2001b), the motor depressant effects of WIN 55, 212-2 was counteracted by SR 141716A in a potent and dose-dependent manner in the least shrew. However, much lower doses of SR 141716A were required to significantly reverse the motor suppressive effects of WIN 55, 212-2 than those required for the reversal of corresponding effects of Δ^9-tetrahydrocannabinol. The direct effect of SR 141716A on motor behaviours is not univocal and seems to be dependent upon the route of administration, species used and the

experimental conditions under which the motor behaviours are observed (Compton et al., 1996; Darmani, 2001b; Gallate and McGregor, 1999; Masserano et al., 1999; Poncelet et al., 1999; Rinaldi-Carmona et al., 1994). Most of these studies have found that SR 141716A by itself has no effect on locomotion while others indicate either enhancement or suppression of locomotor behaviours. In the least shrew, it has been shown that SR 141716A by itself does not induce a consistent effect on the discussed triad of locomotor parameters when administered either subcutaneously (1–10 mg/kg) or intraperitoneally (0.2–40 mg/kg) (Darmani, 2001b). However, some doses of SR 141716A did produce a nondose-dependent suppression of some components of the triad of motor behaviours.

Although antiemetic and motor depressive effects of WIN 55, 212-2 are cannabinoid CB_1 receptor-mediated effects, different brain loci are most likely to be responsible for these events. The highest density of cannabinoid CB_1 receptors is found in the basal ganglia and its subcortical structures that form the extrapyramidal system (Glass et al., 1997; Herkenham et al., 1991; Mailleux and Vanderhaegen, 1992; Tsou et al., 1998). Cannabinoids exert their motor effects through these extrapyrimidal structures (Sañudo-Peña et al., 1999, 2000). The area postrema, the medullary formation and the nucleus of solitary tractus are involved in the regulation of emesis (Mitchelson, 1992). While brain stem appears to be cannabinoid CB_1 receptor sparse, however, the solitary tract nucleus and the area postrema contain significant amounts of cannabinoid CB_1 receptors (Herkenham et al., 1991; Tsou et al., 1998). These brain loci in the medulla are probably involved in the antiemetic action of WIN 55, 212-2 and other cannabinoids. This conclusion is supported by a report that direct application of Δ^9-tetrahydrocannabinol to the dorsal surface of rat medulla attenuates gastric tone which in turn may reduce nausea and vomiting (Krowicki et al., 1999). However, cisplatin-induced vomiting is a complex phenomenon contributed to by the discussed central structures as well as the vagus, dorsal vagal complex and the myenteric complex (Veyrat-Follet et al., 1997; Naylor and Rudd, 1996). Moreover, cisplatin produces emesis in two phases (acute and delayed) and the present study was concerned with the prevention of the acute phase of the induced emesis. Interestingly, one aspect of clinical use of cannabinoids in chemotherapy is that patients who are protected from the acute phase of emesis also respond well during the delayed phase which serotonin 5-HT_3 receptor antagonists poorly control (Abrahamov et al., 1995; Chan et al., 1987; Dalzell et al., 1986; Naylor and Rudd, 1996).

In summary, similar to dibenzopyran derivatives (e.g. Δ^9-tetrahydrocannabinol), the aminoalkylindole cannabinoid WIN 55, 212-2 also possesses antiemetic properties. The antiemetic potency of WIN, 212-2 is similar to Δ^9-tetrahydrocannabinol against cisplatin-induced vomiting in the least shrew. Both cannabinoids reduce the frequency of vomiting at doses lower than those required to significantly affect locomotor parameters. The antiemetic and sedative actions of cannabinoids are probably mediated by different loci and both effects are cannabinoid CB_1 receptor-mediated since these events were reversed by SR 141716A.

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