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Reversal of SR 141716A-induced head-twitch and ear-scratch responses in mice by Δ^9-THC and other cannabinoids

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Abstract

Recently, we have shown that cannabinoids of diverse structure block the ability of the selective 5-$HT_{2A/C}$ agonist DOI to produce the head-twitch response (HTR) and the ear-scratch response (ESR) in mice. The cannabinoid CB_1 antagonist/inverse agonist SR 141716A also induces these behaviors in mice. The purposes of the present study were: (1) to investigate whether Δ^9-tetrahydrocannabinol (Δ^9-THC) and other cannabinoids HU-210 and WIN 55, 212-2 can prevent SR 141716A-induced HTR and ESR and (2) to evaluate any correlation between the ID_{50} potency order of the cited cannabinoids in blocking SR 141716A-induced HTR and ESR and their ED_{50} order of potency in reducing spontaneous locomotor activity and rearing behavior. For the SR 141716A reversal study, different groups of mice were injected intraperitoneally with either vehicle or varying doses of the following cannabinoids: Δ^9-THC (2.5–20 mg/kg), Δ^8-THC (5-20 mg/kg), HU-210 (0.05–0.5 mg/kg), CP 55, 940 (0.5–2.5 mg/kg) and WIN 55, 212–2 (2.5–10 mg/kg). Thirty minutes later, each mouse received SR 141716A (2.5 mg/kg ip) and the frequencies of the induced behaviors (mean ± S.E.M.) were recorded for the next 30 min. The effects of the cited doses of cannabinoids were also examined on spontaneous locomotor activity and rearing frequency for a 20-min duration 10 min after cannabinoid injection. The tested cannabinoids reduced the frequencies of HTR and ESR in SR 141716A-injected mice. These agents also attenuated the cited naturally occurring repertoire of motor parameters in mice. Although large potency differences were observed among the cited cannabinoids, each tested cannabinoid was relatively equipotent in preventing locomotor parameters and SR 141716A-induced behaviors. The ID_{50} potency order of cannabinoids in blocking SR 141716A-induced HTR and ESR were similar (HU-210 > CP 55, 940 > WIN 55, 212-2 $\geq$ Δ^9-THC = Δ^8-THC), and are comparable with: (1) their ED_{50} potency order in attenuating both spontaneous locomotor activity and rearing behavior (HU-210 > CP 55, 940 > WIN 55, 212-2 > Δ^9-THC = Δ^8-THC) and (2) their published ED_{50} potency order for producing the tetrad of behaviors in mice as well as their rank order of binding affinities for cannabinoid CB_1 receptors. The present data show that cannabinoids of diverse structure prevent SR 141716A-induced HTR and ESR, and inhibition of these behaviors by cannabinoids could be used as a new index of cannabimimetic activity.

Keywords: Head-twitch response; Ear-scratch response; Locomotor activity; Rearing; Δ^9-THC; Δ^8-THC; HU-210; CP 55, 940; WIN 55, 212-2

1. Introduction

Naturally occurring [Δ^9-tetrahydrocannabinol (Δ^9-THC) and Δ^8-THC] and synthetic cannabinoids (HU-210, CP 55, 940 and WIN 55, 212-2) produce a number of effects in mice (hypoactivity, catalepsy, hypothermia and antinociception) that are collectively known as the tetrad of behaviors (Abood and Martin, 1992; Compton et al., 1992a,b, 1993). These behaviors have a central origin and are thought to be mediated via the cannabinoid CB_1 receptor (Compton et al., 1996; Lichtman and Martin, 1997; Rinaldi-Carmona et al., 1994). In addition, cannabinoids also produce a number of other effects such as changes in learning behavior, aggression and blood pressure, ataxia, etc. The second cannabinoid receptor (CB_2) appears to be predominantly located in the periphery (Pertwee, 1997, 1999). Development of potent and selective cannabinoid CB_1 (SR 141716A) and CB_2 (SR 144528) receptor antagonists have helped to decipher the diverse actions of cannabinoids (Rinaldi-Carmona et al., 1994, 1998).

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SR 141716A by itself produces a number of behaviors [e.g., head-twitch response (HTR) and ear-scratch response (ESR)] in drug naive mice (Cook et al., 1998; Darmani and Pandya, 2000). While the neurotransmitter mechanisms by which SR 141716A mediates the cited behaviors seems to be multiple, it does appear that modulation of serotonergic activity plays a paramount role (Darmani and Pandya, 2000). Indeed, in the latter study, the selective 5-$HT_{2A/C}$ antagonist SR 46349B was shown to potently block SR 141716A-induced HTR and ESR with respective ID_{50} values of 0.08 and 0.6 mg/kg. Several other lines of evidence also suggest possible interactions between the cannabinoid and the serotonin neurotransmitter systems. For example, cannabinoid agonists inhibit both electrically and Ca^{2+}-induced serotonin release in mouse brain cortical slices (Nakazi et al., 2000), as well as reducing serotonin turnover in discrete rat brain loci (Molina-Holgado et al., 1993). The function of serotonin transporter and serotonin release in peripheral tissues are also attenuated by these psychotropoic agents (Kenny et al., 1999; Volfe et al., 1985). At the serotonin receptor level, structurally diverse cannabinoids block the 5-HT_3 receptor-mediated inward currents induced by 5-HT_3 receptor agonists (Fan, 1995). In addition, cannabinoids appear to modulate binding parameters of ^{3}H-5-HT and ^{3}H-ketanserin (a 5-HT_2 receptor antagonist) to 5-HT receptors in the rat and bovine brains (Cheer et al., 1999; Kimura et al., 1996, 1998).

Behavioral studies have indicated that both the HTR and ESR behaviors are 5-HT_{2A} receptor-mediated phenomena since selective 5-$HT_{2A/C}$ agonists (e.g., DOI) potently induce, whereas 5-$HT_{2A/C}$ antagonists block the cited behaviors in mice (Darmani et al., 1990a,b). The introduction of 5-HT_{2A} receptor knockout mice has confirmed the latter conclusion since these animals fail to produce either the HTR or the ESR in response to DOI injection (Gingrich et al., 1999). We have recently shown that cannabinoids of diverse structure (HU-210; CP 55, 940; WIN 55, 212-2; Δ^9-THC and Δ^8-THC) were able to block the ability of DOI to produce HTR and ESR in mice in a dose-dependent but differential manner (Darmani, 2001b). The tested cannabinoids were 3–30 times more potent in attenuating the ear-scratchings than reducing HTRs. Furthermore, HU-210 was the most potent inhibitor of HTR, whereas CP 55, 940 was most effective against scratchings. Structurally, these cannabinoids belong to three distinct classes of cannabinoid agonists (Mechoulam et al., 1999; Pertwee, 1997, 1999). Both Δ^9-THC and Δ^8-THC are members of the ''classical cannabinoid'' group and are made of dibenzopyran derivatives. HU-210 is one of the most potent cannabimimetic agents and is a dimethylheptyl derivative of Δ^8-THC. Lacking a pyran ring, CP 55, 940 is another potent cannabinoid agonist, which belongs to the ''nonclassical cannabinoid'' group. The pravadoline derivative, WIN 55, 212-2, is a less potent cannabinoid agonist, which belongs to the aminoalkylindole class of cannabinoids. Currently, very little is known regarding the structure activity relationship of different cannabinoid agonists in blocking the ability of the CB_1 antagonist SR 141716A to produce the discussed behaviors. The purposes of the present study were two-fold: (1) to evaluate whether structurally diverse cannabinoid agonists can alter the mean frequencies of HTR and ESR behaviors produced by SR 141716A in a similar or differential manner and (2) to determine whether the cannabimimetic structure activity potency profile of cannabinoids in modifying SR 141716A-induced behaviors is related to their potency profile in reducing locomotor activity parameters (spontaneous locomotor activity and rearing frequency) in drug naive mice.

2. Materials and methods

2.1. Animals

Male albino ICR mice (22–35 days old) were used throughout the study. Animals were kept in groups of no more than six on a 12L/12D cycle at a temperature of 22 ± 1 °C with an ad-lib supply of food and water. All animals received care according to the ''Guide for the Care and Use of Laboratory Animals,'' DHSS Publication (revised, 1985). The American Association of Accreditation of Laboratory Care has certified the KCOM animal facilities and the Institutional Animal Care and Use Committee of KCOM has approved these studies.

2.2. Drugs

The following drugs were purchased from Sigma/Research Biochemical (Natick, MA): Δ^9-THC, Δ^8-THC and *R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]-pyrrolo[[1,2,3-de]-1, 4-benzoxazin-yl]-(1-naphthalenyl)methanone mesylate] (*R*(+)-WIN 55, 212-2). HU-210 was obtained from Tocris Cookson (Ballwin, MO). Pfizer (Groton, CT) generously donated CP 55, 940. SR 141716A was donated by Sanofi Recherche (Monpellier, France). All drugs were dissolved in a 1:1:18 solution of ethanol, emulphor and 0.9% saline. Emulphor (EL-620, a polyoxyethylated vegetable oil, GAF (Linden, NJ)) is currently available as ALKmulphor. Drugs were administered via an intraperitoneal route at a volume of 10 ml/kg of body weight.

2.3. Measurement of HTR and ESR

The HTR in mice is analogous to wet-dog shakes in rats. It is a distinctive behavior that cannot be mistaken for other head movements such as lateral head shakes (lateral movement of the head from side to side) or head-jerks (up and down jerking of the head). The ESR is a rapid scratching movement of the head, neck or loin area by either hind limb. An ESR episode produced by a particular hind limb consisted of one or more repetitive scratches with less than a 2-s

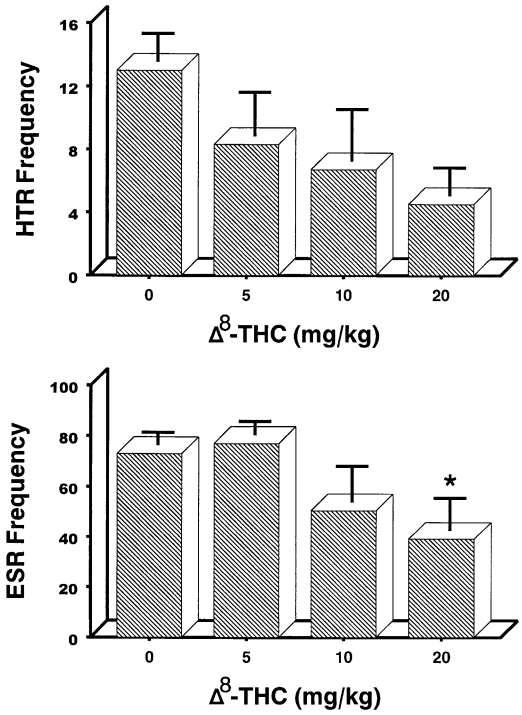

Fig. 1. Dose-dependent inhibitory effects of Δ^8-THC on the frequencies of HTR and ESR in mice induced by the selective cannabinoid CB_1 antagonist SR 141716A. The cited doses of Δ^8-THC were injected intraperitoneally 30 min prior to SR 141716A administration (2.5 mg/kg ip). Data are presented as mean (±S.E.M.) for the 30-min observation period following SR 141716A administration. * Significantly different from vehicle control at $P < .05$.

interval between scratches. When the interval between scratches was greater than 2 s, the scratches were considered separate episodes. Also, when alternative hind legs produced scratches, they were considered separate episodes. The HTR and ESR frequencies were recorded using a multiple tally counter by a trained observer.

2.4. Measurement of locomotor activity and rearing behavior

Both locomotor activity and rearing behavior were measured using a computerized video tracking, motion analysis and behavior recognition system, Ethovision (Version 2.0, Noldus Information Technology, Costerweg, Netherlands) (Darmani, 2001a). On the test day, mice were transported to the test room 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 Ethovision to work efficiently. The two parameters examined were total distance moved in meters (spontaneous locomotor activity) and rearing frequency. Rearing was recorded when a 5% reduction in mouse body surface area occurred while a mouse stood upright as seen by the overhead video camera. Both parameters were recorded for a 20-min duration 10 min after an intraperitoneal injection of a cannabinoid.

2.5. Experimental protocols

To investigate whether cannabinoid agonists modified the ability of SR 141716A to produce HTR and ESR, mice were acclimated to the test environment [plastic cage ($40 \times 25 \times 26$ cm) lined with cedar wood chips] for 30 min prior to first injection. Either vehicle ($n = 9$) or varying doses of the following cannabinoids were injected intraperitoneally in different groups of mice: (1) Δ^9-THC (2.5, 5, 10 and 20 mg/kg; $n = 6–8$ per dose), (2) Δ^8-THC (5, 10 and 20 mg/kg; $n = 6–8$ per dose), (3) HU 210 (0.05, 0.1 and 0.5 mg/kg; $n = 6$ per dose), (4) CP 55, 940 (0.5, 1.0 and 2.5 mg/kg; $n = 5–6$ per dose), (5) WIN 55, 212-2 (2.5, 5.0 and 10 mg/kg; $n = 6$ per dose). Thirty minutes later, each mouse was injected with a 2.5 mg/kg dose of SR 141716A. Frequencies of HTR and ESR (mean ± S.E.M.) were recorded for the next 30 min as described previously.

To determine the effects of the various cannabinoid agonists on locomotor parameters, each mouse was individually placed in plastic cages ($28 \times 28 \times 28$ cm) and acclimated to the test environment for a period of 30 min prior to injection. At 30 min, different groups of mice were injected intraperitoneally with either vehicle ($n = 14$) or varying doses of one of the following cannabinoids [(1) Δ^9-THC (2.5, 5, 10 and 20 mg/kg; $n = 8$ per dose); (2) Δ^8-THC (5, 10, 20 and 40 mg/kg; $n = 8$ per dose); (3) HU-210 (0.05, 0.1 and 0.5 mg/kg; $n = 8$ per dose); (4) CP

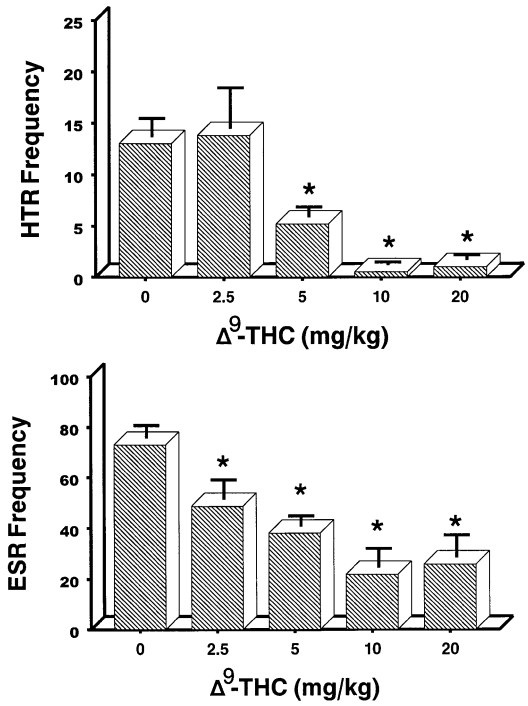

Fig. 2. Represents the dose-dependent inhibitory action of Δ^9-THC on the ability of the selective cannabinoid CB_1 antagonist SR 141716A to produce HTR and ESR in mice. The cited doses of Δ^9-THC (ip) were administered 30 min prior to injection of SR 141716A (2.5 mg/kg ip). The frequencies of HTR and ESR (mean ± S.E.M.) were recorded for 30 min following SR 141716A injection. * Significantly different from vehicle control at $P < .05$.

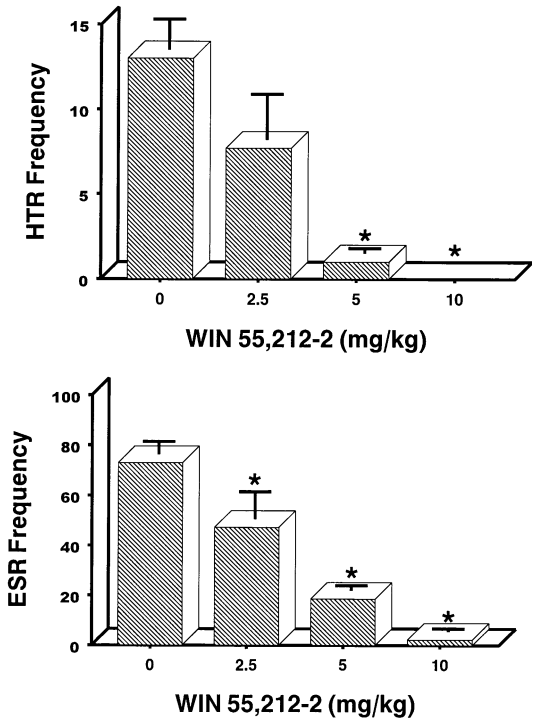

Fig. 3. Inhibition of SR 141716A-induced HTR and ESR by WIN 55, 212-2. The cited doses of WIN 55, 212-2 (ip) were administered 30 min prior to SR 141716A (2.5 mg/kg ip) injection. The behaviors (mean ± S.E.M.) were recorded for 30 min following SR 141716A injection. * Significantly different from vehicle control at $P < .05$.

55, 940 (0.5, 1.0 and 2.5 mg/kg; $n = 8$ per dose); (5) WIN 55, 212-2 (2.5, 5 and 10 mg/kg; $n = 8$ per dose)] and placed in observational chambers ($28 \times 28 \times 28$ cm) where the selected locomotor parameters were recorded as described previously.

2.6. Statistical analysis

Both the frequencies of HTR and ESR, and locomotor activity data were evaluated using a one-way analysis of variance (ANOVA) followed by Dunnett's t test as a post-hoc analysis. A P value of $< .05$ was necessary to achieve significance. Both the ID_{50} (the effective dose that attenuated SR 141716A-induced responses by 50%) and ED_{50} (the cannabinoid dose that reduced locomotor activity or rearing frequency by 50%) values were calculated using the statistical program Graphpad Prism (San Diego, CA).

3. Results

3.1. HTR and ESR

In accordance with our previous findings (Darmani and Pandya, 2000), intraperitoneal administration of a 2.5 mg/kg dose of SR 141716A produced robust frequencies of HTR (13 ± 2) and ESR (73 ± 5) in mice. The induced behaviors were attenuated by varying doses of different cannabinoids (Figs. 1–5). Δ^8-THC was the least potent of the tested cannabinoids (Fig. 1). Although different doses (5, 10 and 20 mg/kg) of Δ^8-THC tended to reduce the SR 141716A-induced HTR frequency (36%, 48% and 65% reductions, respectively), one-way ANOVA failed to show an overall significant effect [$F(3,26) = 2.6$, $P = .07$] (Fig. 1, top graph). However, Dunnett's post-hoc t test indicated a significant reduction was observed at the 20 mg/kg dose of Δ^8-THC ($ID_{50} = 9.98$ mg/kg, 95% CL = 3.4–26.2). On the other hand, both statistical tests revealed that Δ^8-THC significantly reduced the ESR frequency with an ID_{50} of 18.6 mg/kg (95% CL 6.9–50) [$F(3,26) = 3.1$, $P < .045$] and a significant effect (46%) was seen at the 20 mg/kg dose (Fig. 1, bottom graph). Relative to Δ^8-THC, Δ^9-THC was more potent in blocking the ability of SR 141716A to induce the cited behaviors (Fig. 2). Indeed, the 5, 10 and 20 mg/kg doses of Δ^9-THC significantly reduced (60%, 96% and 92%, respectively) SR 141716A-induced HTRs with an ID_{50} of 4.4 mg/kg (95% CL = 3.4–5.7) [$F(4,30) = 10.7$, $P < .0001$] (Fig. 2, top graph). Likewise, Δ^9-THC more potently ($ID_{50} = 2.6$ mg/kg, 95% CL 1.1–6.1) blocked the induced ESR frequency and significant reductions (33%, 48%, 70% and 65%, respectively) were seen from the lowest (2.5 mg/kg) tested dose [$F(4,30) = 11.2$, $P < .0001$] (Fig. 2, bottom graph).

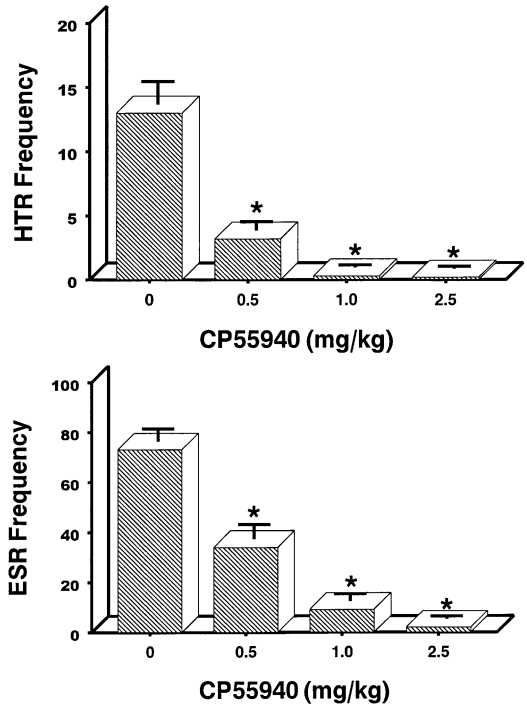

Fig. 4. The inhibitory actions of CP 55, 940 on the ability of the selective cannabinoid CB_1 antagonist SR 141716A to induce HTR and ESR. The cited doses of CP 55, 940 were administered (ip) 30 min prior to SR 141716A (2.5 mg/kg ip) injection. The frequencies of HTR and ESR (mean ± S.E.M.) were recorded for 30 min following SR 141716A injection. * Significantly different from vehicle control at $P < .05$.

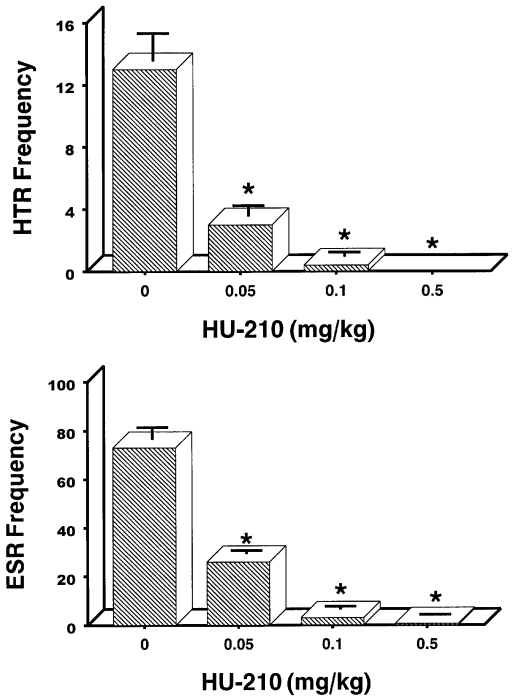

Fig. 5. Attenuation of the ability of the cannabinoid CB_1 antagonist SR 141716A to produce HTR and ESR by the cannabinoid agonist HU-210. The cited doses of HU-210 were administered (ip) 30 min prior to SR 141716A (2.5 mg/kg ip) injection. The frequencies of HTR and ESR (mean ± S.E.M.) were recorded for 30 min following SR 141716A administration. * Significantly different from vehicle control at $P < .05$.

The aminoalkylindole cannabinoid WIN 55, 212-2 reduced SR 141716A-induced HTR ($ID_{50} = 2.7$ mg/kg, CL = 2.6–2.9) and ESR ($ID_{50} = 3.2$ mg/kg, CL = 2.9–3.5) with a similar potency to Δ^9-THC (Fig. 3). Indeed, the HTR was significantly attenuated by 92% and 100% at its 5 and 10 mg/kg doses [$F(3,23) = 14.4$, $P < .0001$], whereas the ESR exhibited significant reductions (35%, 75% and 97%) from the lowest tested dose (2.5 mg/kg) of WIN 55, 212-2 [$F(3,23) = 29.1$, $P < .0001$] (Fig. 3). Prior administration of CP 55, 940 blocked both the HTR ($ID_{50} = 0.37$ mg/kg, 95% CL = 0.12–1) and ESR ($ID_{50} = 0.47$ mg/kg, 95% CL 0.3–0.7) in a more potent manner (Fig. 4). Significant reductions (75%, 98% and 98%, respectively) in the HTR frequency were observed from the lowest (0.5 mg/kg) tested dose (Fig. 4, top graph) [$F(3,22) = 25.7$, $P < .001$]. Significant reductions in the ESR frequency followed a similar pattern, 53%, 87% and 97%, respectively, for the cited doses of CP 55, 940 (Fig. 4, bottom graph) [$F(3,22) = 52.5$, $P < .001$]. The most potent cannabinoid in reducing both the frequencies of HTR ($ID_{50} = 0.034$ mg/kg, 95% CL 0.03–0.035) and ESR (0.043 mg/kg, 95% CL 0.03–0.05) was HU-210 (Fig. 5). Indeed, the HTR frequency was reduced by 77%, 97% and 100% from the lowest tested dose (Fig. 4, top graph) [$F(3,23) = 27.6$, $P < .0001$]. A similar pattern of reduction in the ESR (64%, 96% and 99%, respectively) was also observed at 0.05, 0.1 and 0.5 mg/kg doses of HU-210 (Fig. 5, bottom graph) [$F(3,23) = 100.5$, $P < .0001$].

3.2. Locomotor activity and rearing

Intraperitoneal administration of Δ^8-THC caused dose-dependent decreases in both spontaneous locomotor activity (i.e., total distance moved) ($ED_{50} = 10.51$ mg/kg, 95% CL 6.3–17.4) and the rearing frequency in mice ($ED_{50} = 9.2$ mg/kg, 95% CL 6.1–13.7) (Fig. 6). However, only the 20 and 40 mg/kg doses of Δ^8-THC caused significant reductions (86% and 77%, respectively) in the total distance moved by mice [$F(4,41) = 10.5$, $P < .0001$] (Fig. 6, top graph). The rearing frequency was significantly reduced (54%, 89% and 81%, respectively) by the 10, 20 and 40 mg/kg doses of Δ^8-THC [$F(4,41) = 16.2$, $P < .0001$] (Fig. 6, bottom graph). Δ^9-THC had an equivalent inhibitory action on both locomotion ($ED_{50} = 9.3$ mg/kg, 95% CL 5.2–16.8) and rearing ($ED_{50} = 8.4$ mg/kg, 95% CL 5.4–12.8) in mice (Fig. 6). Indeed, both locomotor activity (65% and 68% reduction) and rearing frequency (70% and 81% reduction) were significantly affected at the 10 and

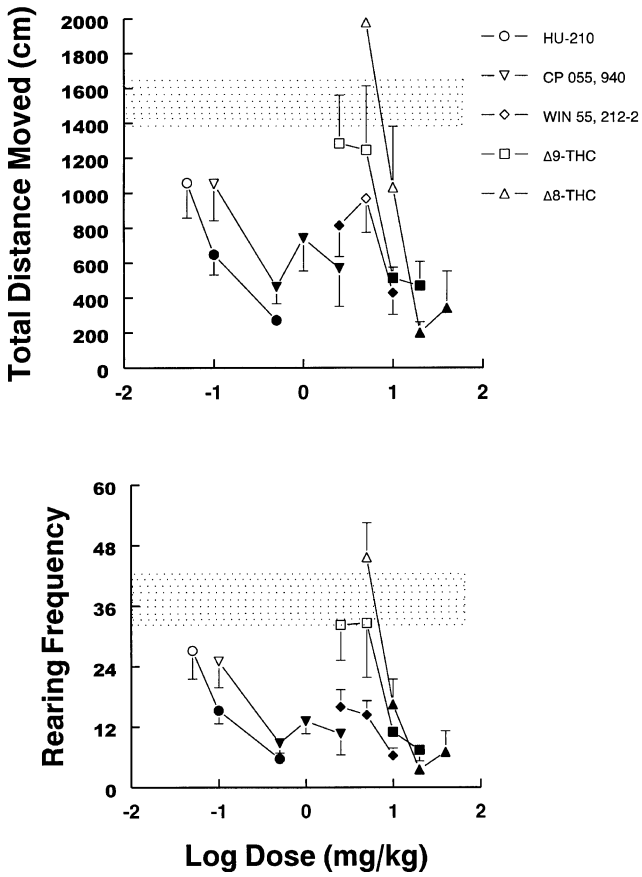

Fig. 6. Log–dose response inhibitory effects of diverse cannabinoids on spontaneous locomotor activity (total distance moved) and rearing frequency in mice. The cited doses of cannabinoids (0 = HU-210; 0.05, 0.1 and 0.5 mg/kg; ▽ = CP 55, 940; 0.5, 1 and 2.5 mg/kg; ◇ = WIN 55, 212-2; 2.5, 5 and 10 mg/kg; □ = Δ^9-THC; 2.5, 5, 10 and 20 mg/kg; and △ = Δ^8-THC; 5, 10, 20 and 40 mg/kg) or vehicle were administered (ip) to different groups of mice and the motor parameters were recorded for 20 min by Ethovision 10 min after injection. Filled symbols indicate significant difference from vehicle control (dotted lines, mean ± S.E.M.).

20 mg/kg doses [$F(4,41) = 4.85$, $P < .003$ and $F(4,41) = 5.81$, $P < .0008$, respectively].

The aminoalkylindole cannabinoid WIN 55, 212-2 also dose-dependently but more potently attenuated both locomotor ($ED_{50} = 5.3$ mg/kg, 95% CL 0.9–32) and rearing ($ED_{50} = 2.1$ mg/kg, 95% CL 0.6–7.1) behaviors in mice (Fig. 6). Total distance moved was significantly affected (44% and 71%, respectively) at doses 2.5 and 10 mg/kg [$F(3,34) = 7.7$, $P < .0005$] (Fig. 6, top graph). Moreover, rearing frequency was significantly reduced (57%, 62% and 84%, respectively) at all tested doses of WIN, 212-2 [$F(3,34) = 17$, $P < .0001$] (Fig. 6, bottom graph). Fig. 6 also represents the potent inhibitory effects of CP 55, 940 (0, 0.1, 0.5, 1 and 2.5 mg/kg) on both locomotor activity ($ED_{50} = 0.6$ mg/kg, 95% CL 0.05–4.2) and rearing frequency ($ED_{50} = 0.2$ mg/kg, 95% CL 0.04–1.4) in mice. The total distance moved was significantly reduced at its 0.5 (68%), 1 (49%) and 2.5 mg/kg (61%) doses [$F(4,41) = 6.3$, $P < .0005$] (Fig. 6, top graph). The rearing frequency was also significantly reduced by 57%, 62% and 84% from its 0.5 mg/kg dose, respectively [$F(4,41) = 11.3$, $P < .0001$] (Fig. 6, bottom graph). HU-210 dose-dependently and most potently blocked both locomotor activity ($ED_{50} = 0.09$ mg/kg, 95% CL 0.05–0.18) and rearing frequency ($ED_{50} = 0.09$ mg/kg, 95% CL 0.05–0.19) with identical ID_{50} values (Fig. 6). Although at 0.05 mg/kg HU-210 had no significant effect, it did significantly reduce both locomotor (56% and 81%) and rearing (60% and 84%) at higher tested doses [$F(3,34) = 13.4$, $P < .0001$ and $F(3,34) = 13.5$, $P < .001$, respectively].

4. Discussion

Numerous studies have reported on the ability of the CB_1 antagonist SR 141716A to reverse a plethora of effects produced by endogenous, plant derived and synthetic cannabinoid agonists (reviews: Mechoulam et al., 1999; Pertwee, 1997, 1999). A number of these studies also suggest that in addition to its antagonist nature, SR 141716A also possesses inverse agonist action both in vitro and in vivo. Only scant published studies are available, however, in delineating whether cannabinoid agonists can reverse the effects of SR 141716A. Recent studies in this laboratory have shown that cannabinoids of diverse structure prevent emesis produced by SR 141716A in the least shrew in an ID_{50} rank order similar to their order of binding affinities for cannabinoid CB_1 receptors (Darmani, 2001c).

Since SR 141716A also induces the HTR and ESR in mice (see Section 1), the initial aim of the present study was to investigate whether different structural analogs of cannabinoids could prevent the latter induced effects. In a study concerning the mechanisms by which SR 141716A produces the cited behaviors, it was shown that although large doses of Δ^9-THC (10–20 mg/kg) were ineffective in preventing the ESR produced by a 10 mg/kg dose of SR 141716A, a 20 mg/kg dose of this cannabinoid significantly reduced the induced HTR by 69% (Darmani and Pandya, 2000). In the present study, we show that 5–20 mg/kg doses of Δ^9-THC can significantly reduce the frequencies of both HTR (60–92%) and ESR (48–65%) when a smaller behavioral inducing dose of SR 141716A (2.5 mg/kg) is utilized. Relative to Δ^9-THC, Δ^8-THC is a two-fold less active cannabinoid both in man and in a wide variety of animal tests (Razdan, 1986). Likewise, in the present study, Δ^8-THC was two (HTR) and seven (ESR) times less active than Δ^9-THC in preventing SR 141716A-induced behaviors. The aminoalkylindole cannabinoid WIN 55, 212-2, showed approximately similar inhibitory action to that of Δ^9-THC with regards to induced HTR (1.6 times) and ESR (0.8 times) behaviors. Previously, it has been shown that intravenously administered WIN 55, 212-2 is also equipotent to Δ^9-THC in producing catalepsy, but was three times less potent in reducing body temperature and 11 times more potent in decreasing spontaneous locomotor activity in mice (Compton et al., 1992a). The most potent synthetic cannabinoid available HU-210 was 60 and 130 times more effective than Δ^9-THC in blocking the HTR and ESR behaviors, respectively. The other tested potent cannabinoid CP 55, 940 showed intermediate inhibitory potency (12- and 5.5-fold, respectively) relative to Δ^9-THC against the SR 141716A-induced effects. Thus, the inhibitory ID_{50} potency profile of the tested cannabinoids in reversing both SR 141716A-induced HTR and ESR (HU-210 > CP 55, 940 > WIN 55, 212-2 $\geq \Delta^9$-THC > Δ^8-THC) is generally similar with: (1) their published ED_{50} potency order for producing the tetrad of behaviors in mice (Compton et al., 1992a,b; Little et al., 1988, 1989) and (2) their rank order of binding affinities for cannabinoid receptors (Abood and Martin, 1992; Pertwee, 1997).

Although following intravenous administration Δ^9-THC has been shown to be twice as potent at Δ^8-THC in reducing spontaneous locomotor activity in mice (Martin et al., 1984), the present study shows that both agents are equipotent in reducing both locomotor activity and rearing behavior after their intraperitoneal injection. It appears that route of administration greatly affects the action of cannabinoids since subcutaneous injection of Δ^9-THC does not seem to affect mice locomotor activity during the 15-min observation following its administration (Compton et al., 1992a). Furthermore, though the current study shows that WIN 55, 212-2, CP 55, 940 and HU-210 are 1.8, 17 and 103 times more potent than Δ^9-THC in reducing locomotor activity, intravenous administration of these cannabinoids has revealed even greater potency differences among these agents (i.e., 10, 25 and 250 times greater effect than Δ^9-THC, respectively) (Abood and Martin, 1992). Reduction in rearing behavior also exhibited a similar inhibitory pattern in that both Δ^9-THC and Δ^8-THC had nearly identical ED_{50} values, whereas WIN 55, 212-2, CP 55, 940 and HU-210 were respectively 4, 36 and 93 times more potent than Δ^9-THC. Thus, the ED_{50}

potency rank order for inhibition of naturally occurring locomotor activity and rearing frequency follow a pattern (HU-210 > CP 55, 940 > WIN 55, 212-2 > Δ^9-THC = Δ^8-THC) which is comparable to overall rank order for the prevention of SR 141716A-induced behaviors. Even though these cannabinoids exhibit differential cannabimimetic inhibitory potency profile against each discussed behavior, each tested cannabinoid seems to inhibit locomotor activity, rearing behavior and SR 141716A-induced HTR and ESR with a similar 50% inhibitory effective dose (i.e., ID_{50} or ED_{50}). Thus, inhibition of SR 141716A-induced behaviors by cannabinoids may provide another index for the measurement of cannabimimetic activity. We have already proposed that inhibition of DOI-induced HTR and ESR by cannabinoids is a more sensitive index of cannabimimetic activity in animals (Darmani, 2001b). Indeed, although in the latter study intraperitoneal administration of the cited cannabinoids was shown to inhibit DOI-induced HTR one to five times more effectively than motor inhibition observed in the present study, reduction in DOI-induced ESR appeared to be the most sensitive evaluated behavior (6–122 times sensitive relative to motor inhibition) to cannabinoid inhibition (Darmani, 2001b). There appears to be one major difference in the ability of the tested cannabinoids in blocking the HTRs and ESRs produced by the cannabinoid CB_1 antagonist/inverse agonist SR 141716A and the serotonin 5-$HT_{2A/C}$ agonist DOI. Indeed, although each tested cannabinoid blocked SR 141716A-induced HTR and ESR equipotently, these agents were shown to be 3- to 30-fold more effective in preventing DOI-induced ESR than the HTR. The difference is probably due to the selective nature of DOI in directly stimulating postsynaptic 5-HT_{2A} receptors to produce these behaviors, whereas SR 141716A indirectly activates multiple neurotransmitter systems upstream of 5-HT_{2A} sites which subsequently activate the serotonergic system to produce HTR and ESR (see Section 1).

The tetrad of behaviors in mice were shown to be highly correlated with cortical brain cannabinoid CB_1 receptor affinity (Compton et al., 1993). Prefrontal cortex is an important locus for the production of HTR (Willins and Meltzer, 1997). However, other behavioral effects of cannabinoids are most likely to be dependent upon neural paths lying outside the cortex. The present and other discussed studies suggest that any agonist with affinity for the CB_1 receptor will produce the spectrum of cannabinoid pharmacological effects. The rat drug-discrimination paradigm has widely been considered indicative of cannabimimetic actions in man (Balster and Prescott, 1992). In the latter study, a strong positive correlation was obtained between CB_1 binding affinity and psychoactivity in humans. This suggests that in vitro cannabinoid receptor affinity is predictive of psychoactivity in humans. Since ED_{50} or ID_{50} rank order of the effects of diverse cannabinoids in the discussed animal tests follow the affinity potency of cannabinoids for the CB_1 receptors, these new animal behavioral tests (HTR and ESR) could be also considered as index of cannabinoid psychoactivity in humans.

In summary, the present results show that cannabinoids of diverse structure reverse the ability of the CB_1 antagonist/inverse agonist SR 141716A to produce HTR and ESR behaviors in mice. The ID_{50} potency profile of these cannabinoids in countering the induced behaviors is similar to their ED_{50} potency order in reducing locomotor activity and rearing behavior as well as their published affinity rank order for the cannabinoid CB_1 receptor. Therefore, antagonism of SR 141716A-induced HTR and ESR may be used as a new measure of cannabimimetic activity in mice.

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