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Cannabinoids of diverse structure inhibit two DOI-induced 5-HT_{2A} receptor-mediated behaviors in mice

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Abstract

We have recently shown that the selective cannabinoid CB_1 receptor antagonist SR 141716A produces robust frequencies of head-twitch response (HTR) and ear-scratch response (ESR) in drug-naive mice. Both behaviors were potently blocked by the selective 5-$HT_{2A/C}$ receptor antagonist SR 46349B. Selective 5-$HT_{2A/C}$ agonists such as DOI also produce these behaviors in mice. The purpose of the present study was to: (1) investigate whether Δ^9-tetrahydrocannabinol (Δ^9-THC) and its analogs [Δ^8-tetrahydrocannabinol (Δ^8-THC), HU-210, CP 55,940, and WIN 55,212-2] can prevent the DOI-induced behaviors and (2) to see whether any correlation exists in the ID_{50} potency order of these cannabinoids in inhibiting the DOI-induced HTR and ESR relative to their published ED_{50} potency profiles in producing the tetrad of behaviors in mice. Thus, at 0 min, different groups of mice were injected intraperitoneally with either vehicle or varying doses of the following cannabinoids: Δ^9-THC (0.25–20 mg/kg), Δ^8-THC (2.5–20 mg/kg), HU-210 (0.02–0.5 mg/kg), CP 55,940 (0.004–0.5 mg/kg), and WIN 55,212-2 (0.5–10 mg/kg). Twenty minutes later, each mouse received an intraperitoneal injection of DOI (1 mg/kg) and the frequencies of DOI-induced behaviors (mean ± S.E.M.) were recorded for the next 20 min. The tested cannabinoids reduced the frequencies of both DOI-induced HTR and ESR in a dose-dependent fashion. HU-210 was the most potent inhibitor of HTR, whereas CP 55,940 was most effective against ESR. The ID_{50} potency order of cannabinoids in blocking the HTR is: HU-210 > CP 55,940 > WIN 55,212-2 > Δ^9-THC > Δ^8-THC, which is identical to their published order of potency in producing the tetrad of behaviors in mice. On the other hand, they had the following ID_{50} potency order against the ESR: CP 55,940 > HU-210 > WIN 55,212-2 > Δ^9-THC > Δ^8-THC. The tested cannabinoids were 3–30 times more potent in preventing the ESR than the HTR. The data show that cannabinoids inhibit 5-HT_{2A} receptor-mediated functions in a potent but differential manner.

Keywords: Head-twitch response; Ear-scratch response; 5-HT_{2A} receptor; DOI; Δ^9-THC; Δ^8-THC; HU-210; CP 55,940; WIN 55,212-2

1. Introduction

A number of recent studies suggest that acute administration of Δ^9-tetrahydrocannabinol (Δ^9-THC) or its synthetic analogs can alter serotonergic function. Indeed, such cannabinoid agonists have been shown to inhibit both the electrically and Ca^{2+}-induced serotonin (5-hydroxytryptamine; 5-HT) release in the mouse brain cortical slices (Nakazi et al., 2000) as well as reducing 5-HT turnover in some discrete rat brain loci (Molina-Holgado et al., 1993). Also, cannabinoids can block serotonin release as well as the function of serotonin transporter in the peripheral tissues (Kenny et al., 1999; Volfe et al., 1985). At the receptor level, cannabinoids are reported to block the 5-HT_3 receptor-mediated inward currents induced by 5-HT_3 receptor agonists in a dose-dependent but noncompetitive manner in the rat nodose ganglion neurons (Fan, 1995). Such agents also alter the binding parameters of ^{3}H-5-HT and ^{3}H-ketanserin (a 5-HT_2 antagonist) to 5-HT receptors in the rat and bovine brain homogenates (Cheer et al., 1999; Kimura et al., 1996, 1998). Furthermore, behavioral studies indicate that the selective cannabinoid CB_1 receptor antagonist SR 141716A produces a number of behaviors in both cannabinoid-tolerant and drug-naive rodents (Aceto et al., 1995, 1996; Cook et al., 1998; Darmani and Pandya, 2000; Rubino et al., 1998). The receptor mechanism(s) by which SR 141716A produces the head-twitch response (HTR) and ear-scratch response (ESR) in mice were recently investigated in this laboratory. Acute injection of SR 141716A in drug-naive mice caused a dose-dependent increase in the

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frequency of both HTR (a homolog of head-shakes in rats) and ESR (Darmani and Pandya, 2000). Although Δ^9-THC blocked SR 141716A-induced HTR at relatively high doses, the most potent blocker of the cited behaviors was the selective 5-$HT_{2A/C}$ antagonist SR 46349B ($ID_{50} = 0.08$ and 0.6 mg/kg, respectively). Both the HTR and the ESR have a serotonergic origin since selective serotonin 5-$HT_{2A/C}$ agonists, such as DOI, potently induce, whereas 5-$HT_{2A/C}$ antagonists block the induced behaviors (Darmani et al., 1990a,b). It seems certain that these behaviors are mediated via the activation of 5-HT_{2A} sites since mice lacking 5-HT_{2A} receptors (i.e., 5-HT_{2A} knockout mice) fail to produce either the HTR or the ESR in response to DOI administration (Gingrich et al., 1999). The discussed studies suggest functional interactions occur between the cannabinoid and the serotonergic neurotransmitter systems.

Δ^9-THC and its synthetic analogs (HU-210, CP 55,940, and WIN 55,212-2) induce a number of symptoms in mice that are collectively known as the tetrad of behaviors, which include hypoactivity, hypothermia, antinociception, and catalepsy (Abood and Martin, 1992). Δ^9-THC is almost equipotent in producing these symptoms, whereas its synthetic analogs show differing potencies in generating these effects. The overall ED_{50} potency order of these cannabinoids in producing the tetrad of behaviors in mice is HU-210 > CP 55,940 > WIN 55,212-2 > Δ^9-THC (Abood and Martin, 1992; Pertwee, 1997). Structurally, these cannabinoids belong to three distinct classes of cannabinoid agonists (Mechoulam et al., 1999; Pertwee, 1997). Both Δ^9- and Δ^8-THC (a double bond isomer of Δ^9-THC) are the naturally occurring psychoactive constituents of the marijuana plant and structurally belong to the ''classical cannabinoid'' group, which is made of dibenzopyran derivatives. HU-210 also belongs to this group and is a dimethylheptyl derivative of Δ^8-THC. Currently, HU-210 is one of the most potent cannabimimetic agents available. CP 55,940 is another potent cannabinoid agonist that lacks a pyran ring and is structurally related to the ''nonclassical cannabinoid'' group. WIN 55,212-2 is a pravadoline derivative and belongs to the aminoalkylindole group of cannabinoids. The purpose of the present study was: (1) to determine whether Δ^9-THC and its analogs can modify the frequency of HTR and ESR produced by the 5-$HT_{2A/C}$ agonist DOI and (2) to investigate whether the cannabimimetic structure activity potency profile of the cited cannabinoids in modifying DOI-induced behaviors is similar to their: (a) reported order of potency in producing the tetrad of behaviors in mice and (b) published affinity rank order for cannabinoid receptors in mice brain.

2. Method

2.1. Animals

Male albino ICR mice (18–24 g) were used throughout the study. Animals were housed in groups of five on a 12/12 light/dark cycle at a room temperature of 22 ± 1°C with 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 facilities are certified by the American Association of Accreditation of Laboratory Care. These studies were approved by the Institutional Animal Care and Use Committee of KCOM.

2.2. Drugs

The following drugs were purchased from Research Biochemicals (Natick, MA): Δ^9-THC, Δ^8-tetrahydrocannabinol (Δ^8-THC), DOI [(±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane], and *R*(+)-WIN 55,212-2 [*R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[[1,2,3-*de*]-1,4-benzoxazin-yl]-(1-naphthalenyl)methanone mesylate. HU-210 [(6*aR*)-*trans*-3-(1,1-dimehylheptyl)-6*a*, 7,10*a*-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[*b*,*d*]pyran-9-methanol] was obtained from Tocris Cookson (Ballwin, MO). CP 55,940 was generously donated by Pfizer (Groton, CT). DOI was dissolved in distilled water. Other 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. All drugs were administered intraperitoneally 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 and usually cannot be mistaken for other head movements, such as lateral head-shakes (lateral movement of head from side to side) or head-jerks (up and down jerking). The head-twitch frequency was scored by a multiple tally counter by a trained observer. The ESR is a rapid scratching movement of the head, neck, or lateral area by either hindlimb. The frequency of ESR episodes was also scored by a tally counter. An ESR episode produced by a particular hindlimb consisted of one or more repetitive scratches with less than 2 s in between. If the interval between consecutive scratches by a particular hindlimb was greater than 2 s, the scratches were considered as separate episodes. If the scratches were produced by alternative hind legs, then each scratch was considered as a separate episode. The experimenter was semiblind in that he knew what agent was being tested but the cannabinoid doses were not known to him.

2.4. Experimental protocols

A 1-mg/kg ip injection of DOI produces robust frequencies of both HTR and ESR in mice (Darmani and Gerdes, 1995; Darmani et al., 1996). Thus, in the present

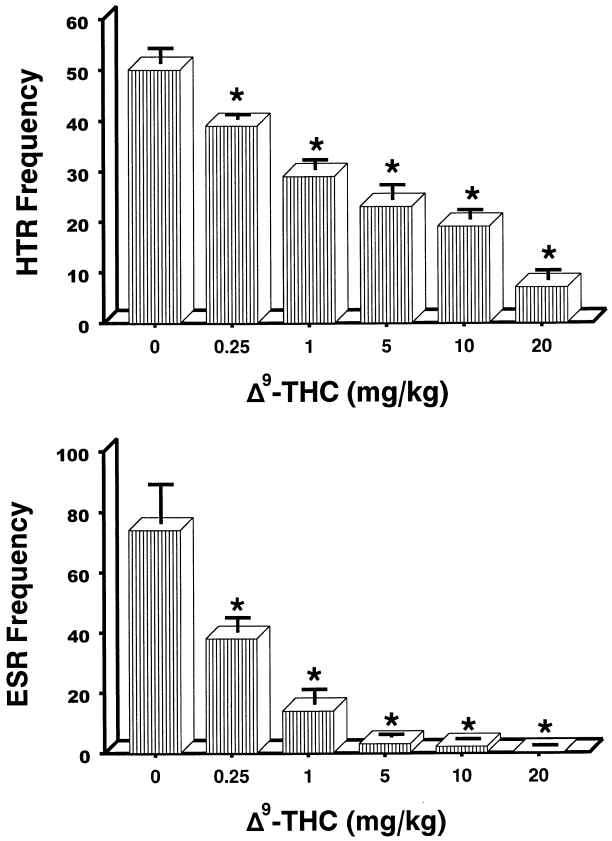

Fig. 1. Dose-dependent inhibitory effects of Δ^9-THC on the frequencies of HTR and ESR in mice induced by the selective 5-$HT_{2A/C}$ agonist DOI. The cited doses of Δ^9-THC ($n=7-9$ per group) were injected intraperitoneally 20 min prior to DOI administration (1 mg/kg ip). Data are presented as mean (±S.E.M.) for the 20-min observation period following DOI administration. * Significantly different from vehicle control at $P<.05$.

study this dose of DOI was chosen to induce the cited behaviors in mice. To investigate whether cannabinoid agonists may modify the ability of DOI to produce HTR and ESR, mice were allowed to habituate to the test environment in plastic cages (40 × 25 × 26 cm) lined with wood chippings for 20 min prior to treatment. Initially, pilot studies were carried out to establish the tested doses of cannabinoids to be used in the present investigation. Either vehicle ($n=8$) or varying doses of the following cannabinoids were injected intraperitoneally into different groups of mice: (1) Δ^9-THC (0.25, 1, 5, 10, and 20 mg/kg, $n=7-9$ per group); (2) Δ^8-THC (2.5, 5, 10, and 20 mg/kg, $n=5-8$ per group); (3) HU-210 (0.02, 0.1, 0.3, 0.5 mg/kg, $n=6-8$ per group); (4) CP 55,940 (0.004, 0.02, 0.1, 0.25, and 0.5 mg/kg, $n=6-8$ per group); and (5) WIN 55,212-2 (0.5, 1, 2.5, 5, and 10 mg/kg, $n=6-8$ per group). Twenty minutes later, each mouse was injected with a 1-mg/kg ip dose of DOI and the frequencies of both HTR and ESR (mean ± S.E.M.) were recorded for the next 20 min as described above. The controls were staggered so that one to two vehicle-injected animals were observed per cannabinoid dose–response.

2.5. Statistical analysis

The HTR and ESR data were analyzed by a one-way analysis of variance (ANOVA) followed by Dunnett's *t* test as post hoc analysis. A *P* value of $<.05$ was necessary to achieve statistical significance. The ID_{50} (the effective dose that attenuated DOI-induced responses by 50%) was calculated by the use of a computerized program (Graph Pad Inplot, San Diego, CA).

3. Results

In the present study, administration of a 1-mg/kg dose of DOI in mice pretreated with the cannabinoid vehicle produced robust frequencies of both HTR (50 ± 3) and ESR (74 ± 13), which are similar to our published studies (Darmani and Gerdes, 1995; Darmani et al., 1996). The naturally occurring cannabinoid Δ^9-THC attenuated the frequency of both DOI-induced HTR ($ID_{50}=4.67$ mg/kg,

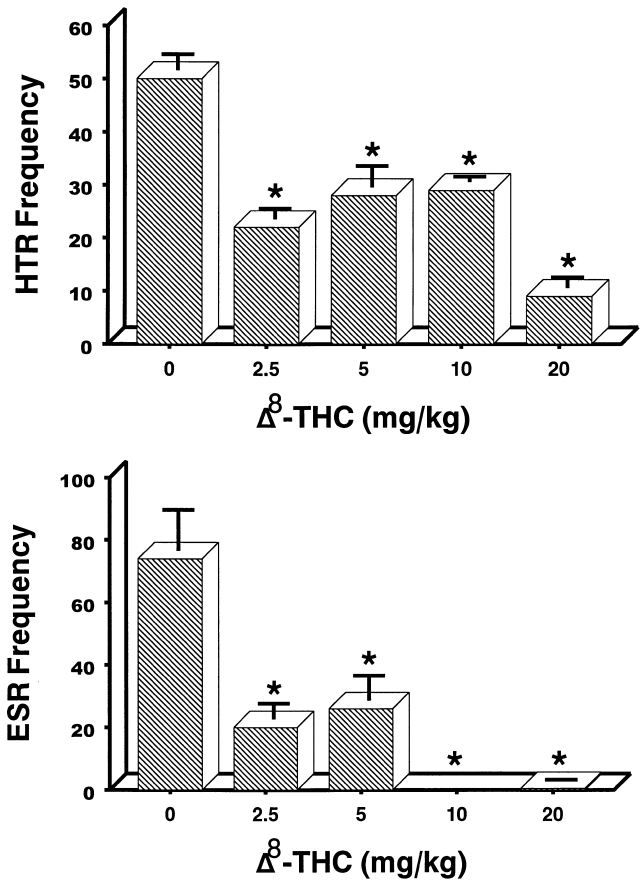

Fig. 2. Represents the dose-dependent inhibitory action of Δ^8-THC on the ability of the selective 5-$HT_{2A/C}$ agonist DOI to produce HTR and ESR in mice. The cited doses of Δ^8-THC were administered 20 min prior to injection of DOI (1 mg/kg ip). The frequencies of HTR and ESR (mean ± S.E.M., $n=5-8$ per group) were recorded for 20 min following DOI injection. * Significantly different from vehicle control at $P<.05$.

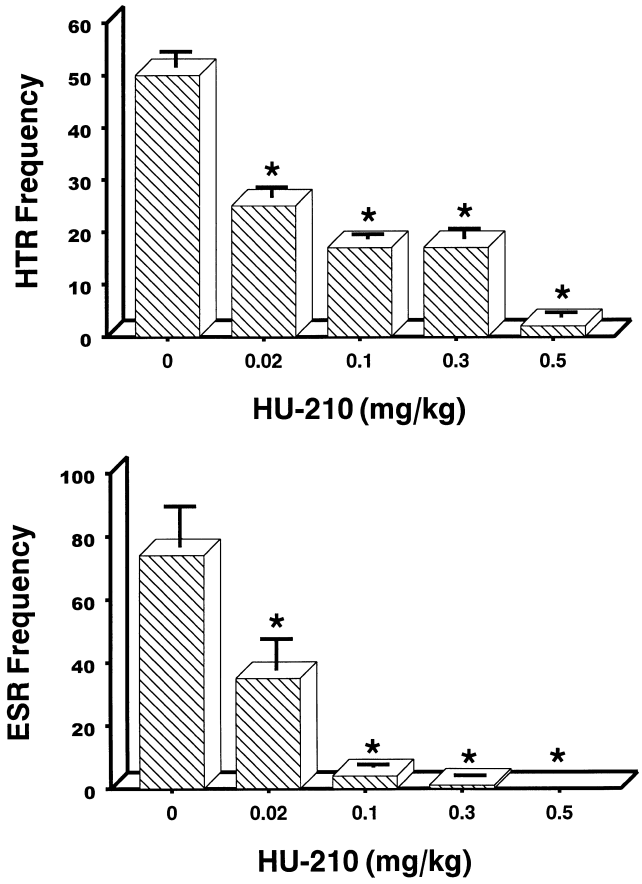

Fig. 3. Inhibition of DOI-induced HTR and ESR by HU-210. The cited doses of HU-210 ($n=6-8$ per group, intraperitoneal) were administered 20 min prior to DOI (1 mg/kg ip) injection. The behaviors (mean ± S.E.M.) were recorded for 20 min following DOI injection. * Significantly different from vehicle control at $P<.05$.

95% CL = 1.53–14.2) and ESR ($ID_{50}=0.25$ mg/kg, 95% CL = 0.22–0.3) behaviors (Fig. 1). Indeed, relative to the control group, significant reductions (22%, 42%, 54%, 62%, and 86%, respectively) in the HTR frequency occurred at 0.25, 1, 5, 10, and 20 mg/kg doses of Δ^9-THC [$F(5,39)=44.8$, $P<.0001$]. Thus, the highest tested dose of Δ^9-THC failed to completely prevent the induced HTR. On the other hand, Δ^9-THC completely blocked the production of ESR, and relatively, a greater reduction in this behavior was observed for each of the cited doses of Δ^9-THC [$F(5,39)=21.8$, $P<.0001$]. The naturally occurring double bond isomer of Δ^9-THC, Δ^8-THC, also reduced the capacity of DOI to produce HTR ($ID_{50}=6.9$ mg/kg, 95% CL = 1.13–54.3) and ESR ($ID_{50}=1.21$ mg/kg, 95% CL = 0.3–4.8) (Fig. 2). Relative to the control group, significant reductions (56%, 44%, 42%, and 82%, respectively) in HTR frequency were observed at 2.5, 5, 10, and 20 mg/kg doses of Δ^8-THC [$F(4,27)=33.7$, $P<.0001$]. In a manner similar to Δ^9-THC, Δ^8-THC more potently blocked the production of DOI-induced ESR (73%, 65%, 100%, 99.5%, respectively) [$F(4,27)=16$, $P<.0001$].

The most potent cannabinoid known thus far, HU-210, in a dose-dependent and potent manner attenuated both the frequency of DOI-induced HTR ($ID_{50}=0.046$ mg/kg, 95% CL = 0.0062–0.34) and ESR ($ID_{50}=0.015$ mg/kg, 95% CL = 0.0071–0.03) (Fig. 3). Relative to the control group, significant attenuations in DOI-induced HTR (50%, 66%, 66%, and 96%, respectively) occurred at 0.02, 0.1, 0.3, and 0.5 mg/kg doses of HU-210 [$F(4,27)=69.2$, $P<.0001$]. This cannabinoid also potently prevented the ESR behavior with significant reductions (53%, 95%, 99%, and 100%, respectively) apparent from its lowest tested dose [$F(4,27)=17.3$, $P<.0001$]. The nonclassical cannabinoid CP 55,940 also blocked the DOI-induced HTR ($ID_{50}=0.11$ mg/kg, 95% CL = 0.042–0.26) and ESR ($ID_{50}=0.0045$ mg/kg, 95% CL = 0.002–0.009) in a potent and dose-dependent fashion (Fig. 4). For this cannabinoid, significant reductions (24%, 38%, 76%, and 96% relative to control) in HTR frequency occurred at its 0.02, 0.1, 0.25, and 0.5 mg/kg doses [$F(5,32)=65.6$, $P<.0001$]. The induced ESR behavior was much more sensitive to the inhibitory action of CP

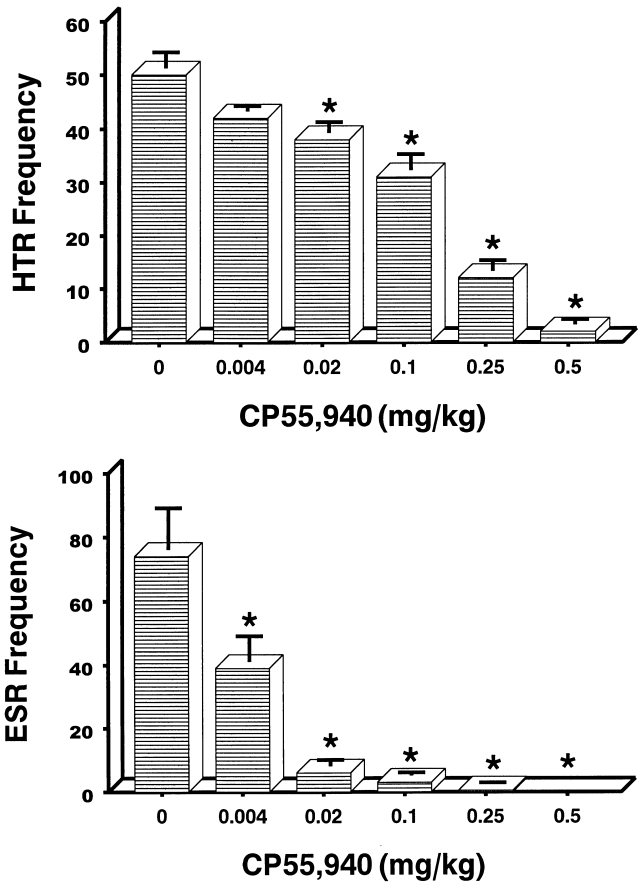

Fig. 4. The inhibitory actions of CP 55,940 on the ability of the selective 5-$HT_{2A/C}$ agonist DOI to induce HTR and ESR. The cited doses of CP 55,940 were administered (6–8 per group, intraperitoneal) 20 min prior to DOI (1 mg/kg ip) injection. The frequencies of HTR and ESR (mean ± S.E.M.) were recorded for 20 min following DOI injection. * Significantly different from vehicle control at $P<.05$.

55,940 since its lowest tested dose (0.004 mg/kg) significantly reduced the behavior by 47%. Furthermore, increasing doses of CP 55,940 further reduced (92%, 96%, 99%, and 100%, respectively) the ESR frequency [$F(5,32)=19.4$, $P<.0001$].

The aminoindole cannabinoid WIN 55,212-2 was relatively less potent than both HU-210 and CP 55,940 in reducing the frequency of both DOI-induced HTR ($ID_{50}=3.04$ mg/kg, 95% CL = 0.44–21.28) and ESR ($ID_{50}=0.1$ mg/kg, 95% CL = 0.06–0.18) (Fig. 5). The lowest tested dose of WIN 55,212-2 (0.5 mg/kg) significantly reduced the induced HTR by 49%. However, larger doses of this cannabinoid (e.g., 1, 2.5, and 5 mg/kg) failed to further reduce the induced behavior until its dosage was increased to 10 mg/kg, which caused a 94% reduction [$F(5,34)=34.6$, $P<.0001$]. Similar to other cannabinoids, WIN 55,212-2 was more potent in reducing the frequency of ESR, and significant reductions (82%, 96%, 91%, 99%, and 100%, respectively) were seen at its cited doses [$F(5,34)=18.7$, $P<.0001$].

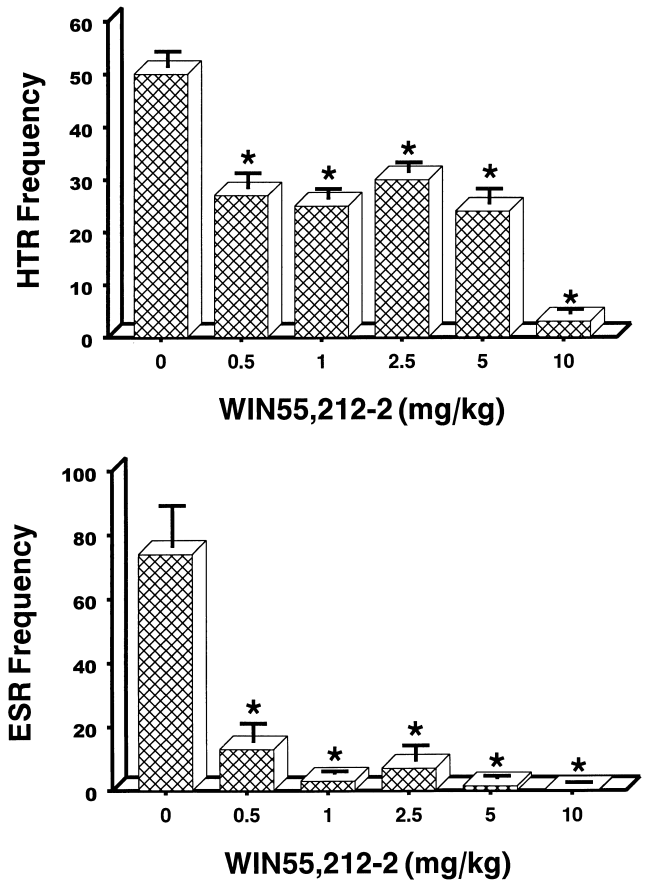

Fig. 5. Attenuation of the ability of the 5-$HT_{2A/C}$ agonist DOI to produce HTR and ESR by the cannabinoid agonist WIN 55,212-2. The cited doses of WIN 55,212-2 ($n=6-8$ per group) were administered intraperitoneally 20 min prior to DOI (1 mg/kg ip) injection. The frequencies of HTR and ESR (mean ± S.E.M.) were recorded for 20 min following DOI administration. * Significantly different from vehicle control at $P<.05$.

4. Discussion

It is well established that selective serotonin 5-$HT_{2A/C}$ receptor agonists such as DOI produce both the HTR and the ESR in mice via the activation of serotonergic 5-HT_{2A} sites (see Introduction). The results presented in this manuscript clearly demonstrate that cannabinoids of diverse structure and potency (Δ^9-THC, Δ^8-THC, HU-210, CP 55,940, and WIN 55,212-2) block the ability of DOI to induce the cited behaviors in mice in a dose-dependent fashion. Cannabinoids mainly produce their effects via the activation of either CB_1 or CB_2 receptors (Pertwee, 1997). The cannabinoid receptor responsible for the prevention of DOI-induced behaviors is most likely to be CB_1 receptors because: (1) the selective cannabinoid CB_1 receptor antagonist SR 141716A can produce both the HTR (or its homolog wet-dog shakes in rats) and ESR behavior in drug-naive mice (Cook et al., 1998; Darmani and Pandya, 2000) and rats (Aceto et al., 1996; Rubino et al., 1998); (2) the selective 5-$HT_{2A/C}$ antagonist SR 46349B was shown to potently prevent SR 141716A-induced HTR and ESR (Darmani and Pandya, 2000); and (3) Δ^9-THC also prevents the ability of SR 141716A to induce the HTR and ESR (Darmani and Pandya, in press, and unpublished findings). In our earlier study (Darmani and Pandya, 2000), relatively larger doses of Δ^9-THC (e.g., 20 mg/kg) were required to suppress the HTR produced by a 10-mg/kg dose of SR 141716A. However, our recent findings with 1 mg/kg SR 141716A, smaller doses of Δ^9-THC (e.g., 5 mg/kg) were required to suppress the behavior. Thus, Δ^9-THC appears to be much more potent against DOI than SR 141716A. This is partly because SR 141716A can produce the cited behaviors via multiple pathways (Darmani and Pandya, 2000).

At this point, it should be stressed that mice and rats show species-specific differences in behaviors produced by DOI and SR 141716A. In response to DOI administration, mice mainly exhibit the HTR and ESR (Darmani et al., 1990a,b), whereas rats produce wet-dog shakes (a behavior similar to HTR in mice) and back muscle contractions (Darmani and Ahmad, 1999; Schreiber et al., 1995). On the other hand, injection of SR 141716A causes HTR (or wet-dog shakes) and the ESR in both species. At the present, it is not fully known whether SR 141716A can induce back muscle contractions in drug-naive rats; however, it has been shown that the CB_1 antagonist produces a similar behavior (myoclonic spasms) in Δ^9-THC-tolerant rats but not in their chronically vehicle-exposed control group (Aceto et al., 1996). As observed in mice, cannabinoids seem to alter DOI-induced behaviors in rats (Cheer et al., 1999). Indeed, in this one-dose study, Cheer et al. (1999) have reported that HU-210 (0.2 mg/kg ip) reduces the ability of DOI (1 mg/kg ip) to produce wet-dog shakes in rats by approximately 50%. On the contrary, the capacity of DOI to produce back-muscle contractions was potentiated by HU-210 pretreatment. The latter authors have

suggested that either different subtypes of 5-HT_2 receptors are responsible for the production of these behaviors or the same 5-HT_2 receptor subtype utilizes different signal transduction mechanisms to produce such differential actions. Previous studies have also utilized the latter notions to account for the observed differential adaptation mechanisms of HTR and ESR in mice following repeated DOI exposure (Darmani, 1992; Darmani and Gerdes, 1995; Darmani et al., 1992). However, unlike the discussed differential effects of HU-210 on DOI-induced wet-dog shakes and back-muscle contractions in the rat, all of the cannabinoids tested in this study prevented both behaviors induced by DOI in mice. Thus, it seems more probable that back-muscle contractions produced by DOI in the rat are mediated via the activation of 5-HT_{2C} sites, whereas the 5-HT_{2A} receptor is responsible for the production of other discussed behaviors in both species.

As expected, the most potent tested cannabinoid available, HU-210, is also the most active blocker of HTR in this study. The attained ID_{50} potency profile of cannabinoids in blocking the HTR (HU-210 > CP 55,940 > WIN 55,212-2 > Δ^9-THC>Δ^8-THC) is identical with both 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 their rank order of binding affinities for cannabinoid receptors, (Abood and Martin, 1992; Pertwee, 1997). Thus, reduction in DOI-induced HTR provides another easily quantifiable but antiserotonergic measure of cannabimimetic activity. Although Δ^9-THC is generally equipotent in producing the different components of the tetrad of behaviors, other cannabinoids exhibit differential inhibitory potencies (Abood and Martin, 1992). Furthermore, cannabinoid-induced reduction in locomotor activity seems to be the most sensitive symptom of the tetrad of behaviors in mice for each of the cited cannabinoids. The ratio of intraperitoneal ID_{50} of each cannabinoid in preventing the HTR in this study vs. their corresponding published intravenous ED_{50} values in reducing locomotor activity seems to separate the tested cannabinoids into two groups. Indeed, intraperitoneally administered CP 55,940, Δ^8-THC, and Δ^9-THC are only 2.7, 3.6, and 4.7 times less potent in reducing the HTR than the reduction observed in locomotor activity following their intravenous administration. On the other hand, HU-210 and WIN 55,212-2 are 11.5- and 12.6-fold less active in blocking head-twitches. Differences in pharmacokinetic factors may account for the latter phenomenon since different routes of administration can differentially affect the potency of various cannabinoids (Compton et al., 1992b). Thus, relative to locomotor activity, the HTR seems to be a more sensitive measure of cannabimimetic activity for at least some cannabinoids since drugs are generally much more potent via the intravenous vs. the intraperitoneal route. The DOI-induced ESR appears to be even more sensitive to cannabinoids. Indeed, the calculated intraperitoneal ESR ID_{50} dosages for Δ^9-THC, Δ^8-THC, WIN 55,212-2, and CP 55,940 are 4, 1.6, 1.3, and 8.9 times less than their reported respective ED_{50} values for locomotor activity reduction obtained following their intravenous administration. Furthermore, these cannabinoids were 3–30 times more potent in preventing the DOI-induced ESR than the HTR. Thus, the present data clearly demonstrate that DOI-induced ESR is a much more sensitive parameter to measure cannabimimetic activity. Although HU-210 is the most potent blocker of HTR (i.e., 2.3 times more effective than CP 55,940), this agent is 3.3-fold less active than CP 55,940 in reducing the induced ESR by 50%. Thus, the ID_{50} potency order of the studied cannabinoids in blocking the latter behavior is CP 55,940 > HU-210 > WIN 55,212-2 > Δ^9-THC > Δ^8-THC. However, for some of these cannabinoids, their lowest tested dose was less than the calculated ESR ID_{50} values, which may cause some interpolation error.

Since cannabinoids reduce locomotor activity and induce catalepsy, it is expected that such agents would also reduce other motor behaviors such as the HTR and ESR. However, as discussed earlier, the observed behaviors in this study are much more sensitive to cannabinoids (particularly the ESR) relative to locomotor activity. Furthermore, cataleptic effects are observed following the administration of much larger doses of cannabinoids. Thus, other mechanisms may be responsible for the observed reductions in HTR and ESR. Although cannabinoids can reduce both 5-HT release and turnover (Molina-Holgado et al., 1993; Nakazi et al., 2000), these presynaptic effects should not directly alter the ability of DOI to induce the HTR and ESR in mice since these behaviors are mediated via postsynaptic 5-HT_{2A} sites (Gingrich et al., 1999; Heal et al., 1992). One possible mechanism by which cannabinoids may interfere with DOI-induced behaviors is a direct interaction at the postsynaptic 5-HT_{2A} receptors. Indeed, high concentrations of the endogenous cannabinoid anandamide were shown to significantly reduce the 5-HT_2 receptor density by 43% (Kimura et al., 1998). However, other cannabinoids are reported to either lack such an effect (Kimura et al., 1996) or enhance the affinity of 5-HT_2 sites for 5-HT (Cheer et al., 1999). A second possibility is that cannabinoids may interfere with the 5-HT_2 receptor signal transduction mechanisms. However, this mechanism appears unlikely since a 500-nM concentration of HU-210 had no significant effect on the 5-HT-stimulated phosphoinositide hydrolysis in the rat cerebral cortex (Cheer et al., 1999). It is most likely that cannabinoids potently block the discussed behaviors via cannabinoid CB_1 receptors by inhibiting some component of the polysynaptic pathways responsible for the production of HTR and ESR downstream of 5-HT_{2A} receptors.

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