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Cannabinoid pharmacological properties common to other centrally acting drugs

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Abstract

Cannabinoids produce a characteristic profile of in vivo effects in mice, including suppression of spontaneous activity, antinociception, hypothermia, and catalepsy. Measurement of these four properties, commonly referred to as the tetrad test, has played a key role in establishing the structure–activity relationship of cannabinoids acting at cannabinoid CB_1 receptors. The purpose of this study was to determine whether drugs acting at noncannabinoid CB_1 receptors produced a similar pharmacological profile. Mice were tested in this paradigm after being injected with Δ^9-tetrahydrocannabinol and selected drugs from other drug classes. Δ^9-Tetrahydrocannabinol dose-dependently produced all four effects with reversal by the cannabinoid CB_1 receptor antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride (SR 141716A). Amphetamine, scopolamine, morphine, desipramine, pimozide, pentobarbital, ethanol, and diazepam were not fully active in at least one of the tests. Antipsychotics showed the greatest similarity to those of cannabinoids in the tetrad tests, although there were also distinct differences. Clozapine, haloperidol, thioridazine, and chlorpromazine (but not pimozide) were fully active in all four tests; however, unlike with Δ^9-tetrahydrocannabinol, their effects were not blocked by SR 141716A. Further, whereas antipsychotics produced nearly 100% catalepsy, maximal catalepsy produced by Δ^9-tetrahydrocannabinol was 60%. The mechanism through which antipsychotics produce these effects in mice is uncertain, but it differs from cannabinoid CB_1 receptor activation that mediates the effects of cannabinoids. While results of previous research suggest that the tetrad tests are a useful tool in examination of structure–activity relationships of cannabinoid CB_1 receptor agonists, the present results suggest that they must be used cautiously in the search for novel cannabinoid receptors.

Keywords: Antipsychotic; Depressant; Cannabinoid; SR 141716A

1. Introduction

The marijuana plant (*Cannabis sativa*) contains a number of active and inactive cannabinoids, its primary psychoactive ingredient being Δ^9-tetrahydrocannabinol. In addition to plant-derived cannabinoids, other classes of cannabinoids have been identified, including bicyclic analogs such as (−)-*cis*-3-[2-hydroxy-4(1,1-dimethyl-heptyl)phenyl]-*trans*-4-(3-hydroxy-propyl)cyclohexanol (CP 55,940), aminoalkylindoles, and the endogenous cannabinoid anandamide and its analogs. Like many other psychoactive drugs, these various classes of cannabinoids produce a multitude of pharmacological effects. In rats and monkeys, they produce Δ^9-tetrahydrocannabinol-like discriminative stimulus effects and suppress operant behavior (Frankenheim et al., 1971; Wiley, 1999). In dogs, they produce static ataxia (Lichtman et al., 1998). In mice, cannabinoids produce a characteristic profile of in vivo effects that includes suppression of spontaneous activity, antinociception, hypothermia, and catalepsy (Martin et al., 1991). These pharmacological effects in mice are reversed by coadministration of *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride (SR 141716A) (Compton et al., 1996), an antagonist of brain cannabinoid CB_1 receptors (Rinaldi-Carmona et al., 1994), but not by *N*-(1*S*-endo-1,3,3-trimethylbicycloheptan-2-yl)-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide (SR 144528) (Wiley et al., 2002), an antagonist of peripheral cannabinoid CB_2 receptors (Rinaldi-Carmona et al., 1998). These results suggest that the effects are centrally mediated. Further evidence for central mediation via cannabinoid CB_1 receptors is the fact that potencies for pro-

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ducing these effects in mice are correlated (individually and collectively) with binding affinity for brain cannabinoid CB_1 receptors (Adams et al., 1995; Compton et al., 1993; Wiley et al., 1998). Indeed, these tests have been useful as a functional battery to delineate structure–activity relationships among different classes of cannabinoids that bind to and activate cannabinoid CB_1 receptors (e.g., Compton et al., 1993).

More recently, however, activity in these tests has been used to indicate cannabinoid activity in the absence of cannabinoid CB_1 receptor mediation, e.g., in cannabinoid CB_1 receptor knockout mice or by drugs that do not bind to cannabinoid CB_1 receptors (Di Marzo et al., 2000a,b, 2001). However, while this profile of in vivo effects in mice is characteristic of cannabinoids, it is crucial to note that drugs from other noncannabinoid classes are known to produce one or more of the same effects in mice as do cannabinoids. For example, central nervous system depressants suppress locomotor activity, and morphine is an efficacious antinociceptive agent. In order to delineate more clearly the degree to which cannabinoid action might be inferred from activity in these four tests in mice, we investigated the pharmacological specificity of the mouse tetrad tests as a whole in the present study. In addition to Δ^9-tetrahydrocannabinol, we tested selected noncannabinoid drugs that included a psychomotor stimulant (amphetamine), a tricyclic antidepressant (desipramine), an antimuscarinic agent (scopolamine), an opioid (morphine), several antipsychotics (pimozide, clozapine, chlorpromazine, thioridazine, and haloperidol), and several central nervous system depressants (diazepam, pentobarbital, and ethanol).

2. Materials and methods

2.1. Subjects

Male ICR mice (25–32 g), purchased from Harlan (Dublin, VA), were housed in groups of five. All animals were kept in a temperature-controlled (20–22 °C) environment with a 12-h light–dark cycle (lights on at 7:00 a.m.). Separate mice were used for testing each drug dose in the in vivo behavioral procedures. The mice were maintained on a 14:10-h light–dark cycle and received food and water ad libitum. The studies reported in this manuscript were carried out in accordance with guidelines published in ''Guide for the care and use of laboratory animals'' (National Research Council, 1996) and were approved by our Institutional Animal Care and Use Committee.

2.2. Apparatus

Measurement of spontaneous activity in mice occurred in standard activity chambers interfaced with a Digiscan Animal Activity Monitor (Omnitech Electronics, Columbus, OH). A standard tail-flick apparatus (described by Dewey et al., 1970) and a digital thermometer (Fisher Scientific, Pittsburgh, PA) were used to measure antinociception and rectal temperature, respectively. The ring immobility device (described by Pertwee, 1972) consisted of an elevated metal ring (diameter = 5.5 cm, height = 16 cm) attached to a wooden stand.

2.3. Drugs

Δ^9-Tetrahydrocannabinol (National Institute on Drug Abuse, Rockville, MD), SR 141716A (National Institute on Drug Abuse), and clozapine (Research Biochemicals International, Natick, MA) were mixed in a vehicle of absolute ethanol, Emulphor-620 (Rhone-Poulenc, Princeton, NJ), and saline in a ratio of 1:1:18. Thioridazine HCl (RBI), chlorpromazine HCl (RBI), desipramine HCl (Sigma, St. Louis, MO), sodium pentobarbital (NIDA), amphetamine HCl (NIDA), morphine sulfate (NIDA), (−)-scopolamine HBr (RBI), apomorphine HCl (RBI), and quinpirole (Sigma) were dissolved in saline. Pimozide (RBI) was mixed in a solution of 1% Tween 80 and distilled water. Haloperidol (McNeil Pharmaceutical, Spring House, PA) was prepared by adding saline to commercially available 5 mg/ml stock solution containing 1.8 mg methyl paraben, 0.2 mg propylparaben, and lactic acid. A stock solution of diazepam, 5 mg/ml, (Schein Pharmaceutical, Port Washington, NY) was also purchased commercially. Lower doses were obtained by dilution with a mixture of ethanol, propylene glycol, and saline (1:4:5 volume ratio). Ethanol (100%; Aaper Alcohol and Chemical., Shelbyville, KY) was diluted to concentration with distilled water. Δ^9-Tetrahydrocannabinol was administered to the mice intravenously in the tail vein or intraperitoneally. Other drugs were injected intraperitoneally. All drugs were injected at a volume of 0.1 ml/10 g. Drug (and corresponding vehicle) injections occurred at the following times before placement in activity chambers: 45 min for desipramine; 30 min for chlorpromazine, haloperidol, thioridazine, pimozide, clozapine, and intraperitoneal Δ^9-tetrahydrocannabinol; 20 min for ethanol and morphine; 15 min for diazepam and amphetamine; 10 min for pentobarbital; 30 min for scopolamine; 5 min for intravenous Δ^9-tetrahydrocannabinol. Preinjection times were based upon previous work with these drugs in our lab and/or surveys of the scientific literature. For antagonist tests, SR 141716A was injected intravenously 10 min before administration of the second drug, and quinpirole and apomorphine were injected intraperitoneally immediately before injection with the second drug.

2.4. Procedure

Prior to testing in the behavioral procedures, mice were acclimated to the experimental setting (ambient temperature 22–24 °C) overnight in groups of five. Preinjection control values were determined for rectal temperature and tail-flick latency (in seconds). During the test session, each mouse

was tested in all four procedures (spontaneous activity, tail flick, rectal temperature, and ring immobility). After the postinjection interval specified above (see Drugs), mice were placed in individual activity chambers, and spontaneous activity was measured for 10 min. Activity was measured as total number of interruptions of 16 photocell beams per chamber during the 10-min test and expressed as percentage inhibition of activity of the vehicle group. Tail-flick latency was measured 5 min after removal from the activity chambers. Maximum tail-flick latency of 10 s was used. Antinociception was calculated as percent of maximum possible effect {% MPE=[(test − control latency)/(10 − control)] × 100}. Control latencies typically ranged from 1.5 to 4.0 s. Rectal temperature was measured at 10 min after tail flick, and values were expressed as the difference between control temperature (before injection) and temperatures following drug administration (°C). In order to measure rectal temperature, the probe was inserted 2 cm into the rectum. Ten minutes after measurement of rectal temperature, mice were placed on the ring immobility apparatus for 5 min. During placement on the ring immobility apparatus, the total amount of time (in seconds) that the mouse remained motionless was measured. This value was divided by 300 s and multiplied by 100 to obtain a percent immobility rating. The criterion for ring immobility was the absence of all voluntary movement, including snout and whisker movement. If a mouse fell or escaped from the ring apparatus during testing, it was immediately placed back on the ring. If it fell more than five times before 150 s had elapsed, data were omitted from analysis. If it fell more than five times but 150 s had passed, percentage immobility scores reflected a decreased maximum duration of testing. Different mice were tested for each dose of each compound.

2.5. Data analysis

In most instances for all active drugs, maximal possible effects (100%) served as estimates for the maximal effects for each percentage measure and −6 °C was used as maximal change in rectal temperature. Exceptions were ethanol (where −12 °C was used as the maximal temperature change) and Δ^9-tetrahydrocannabinol (where 60% was used as maximal ring immobility). These exceptions were based upon observation of maximal obtained effects for each drug and, in the case of Δ^9-tetrahydrocannabinol, also upon data from numerous previous studies with classical cannabinoid compounds (Compton et al., 1993; Martin et al., 1991). ED_{50} was defined as the dose at which half maximal effect occurred. For compounds that were active in one or more test, ED_{50}s were calculated separately using least-squares linear regression on the linear part of the dose–effect curve for each measure in the mouse tetrad, plotted against $\log_{10}$ transformation of the dose. In order to evaluate effects in SR 141716A antagonism tests, separate two-way analyses of variance (9 drugs × 2 antagonist doses) were performed for each dependent measure. Tukey post hoc tests ($\alpha = 0.05$) were used to analyze differences revealed by the analyses of variance.

3. Results

As expected, Δ^9-tetrahydrocannabinol was active in all four tests (Fig. 1): it produced hypomobility, antinociception, hypothermia, and catalepsy. Activity was observed following intravenous and intraperitoneal injection, although potency was reduced 6–27-fold with intraperitoneal administration. Maximal effects approximated those obtained from numerous previous studies with this drug (see Data analysis). ED_{50}s for intravenous and intraperitoneal Δ^9-tetrahydrocannabinol were similar across all four measures (about twofold difference) (Table 1).

The patterns of effects produced by diazepam and ethanol differed from those of Δ^9-tetrahydrocannabinol. Diazepam suppressed spontaneous activity and produced antinociception and catalepsy; however, it did not produce clear hypothermia as did Δ^9-tetrahydrocannabinol (Fig. 2). While a maximum decrease in body temperature of 5.5 °C was observed with diazepam, body temperature also dropped substantially following injection with the diazepam vehicle (1:4:5 ratio of ethanol, propylene glycol, and distilled water). Further, hypothermia observed following diazepam injection was not dose dependent. In contrast, ethanol produced a dose-dependent decrease in body temperature with a maximal decrease of −12 °C (approximately twice that typically observed with high concentrations of Δ^9-tetrahydrocannabinol) (Fig. 2). Both diazepam and ethanol suppressed spontaneous activity and induced antinociception. ED_{50}s for antinociception and catalepsy were approximately equal for diazepam (Table 1). An ED_{50} for suppression of spontaneous activity could not be calculated due to restricted magnitude of effects over the dose range for which suppression was observed; however, it was estimated to be between 0.3 and 1 mg/kg (similar to those obtained for antinociception and catalepsy). ED_{50}s for ethanol-induced suppression of spontaneous activity, antinociception, and hypothermia ranged from 1.2 to 2.1 g/kg, with overlapping confidence intervals. Unlike diazepam or Δ^9-tetrahydrocannabinol, however, ethanol did not induce catalepsy at lower concentrations. At the highest (5.6 g/kg) concentration, mice were too inebriated to remain on the ring; hence, catalepsy could not be evaluated.

As shown in Fig. 3, four of the five antipsychotics tested in this study produced dose-dependent effects in all four tests. Clozapine, chlorpromazine, thioridazine, and haloperidol suppressed spontaneous activity, reduced body temperature, and produced antinociception and catalepsy. However, there were also differences in the patterns of behavior produced by the antipsychotics as compared with Δ^9-tetrahydrocannabinol. A clear distinction was observed in the maximal degree of catalepsy produced by Δ^9-tetrahydrocannabinol and these antipsychotic agents. Whereas Δ^9-tetrahy-

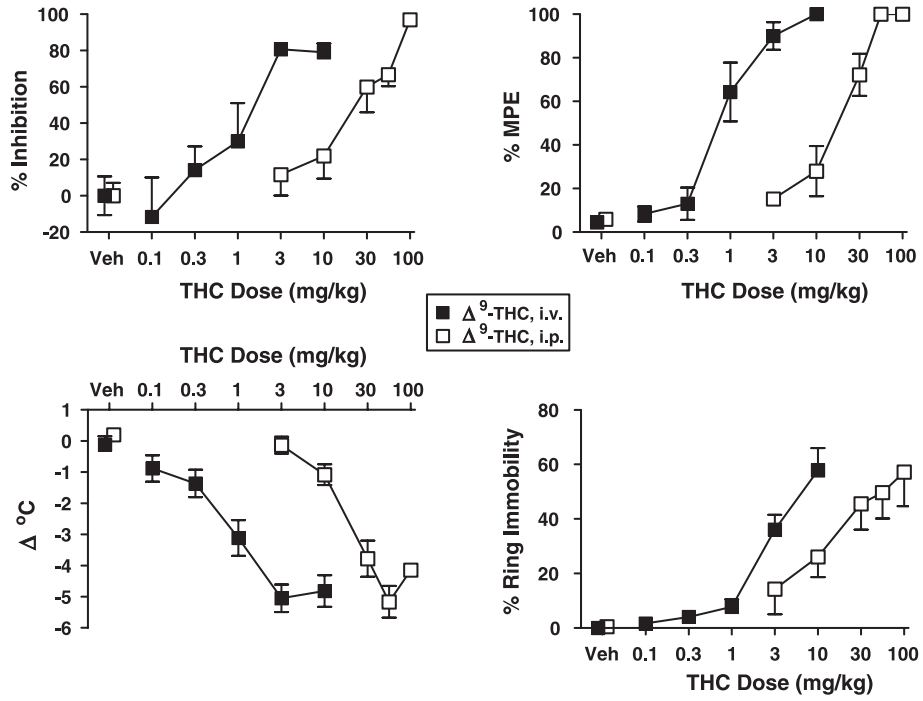

Fig. 1. Effects of Δ^9-tetrahydrocannabinol injected intravenously (■) or intraperitoneally (□) on locomotor activity (top left panel), nociception (top right panel), rectal temperature (bottom left panel), and catalepsy (bottom right panel) in mice. For all figures, locomotor activity was measured as number of photocell beam breaks and was converted to percent inhibition of spontaneous activity after vehicle injection (% inhibition). Antinociception was measured as latency to remove tail from heat source and was converted to percent of maximum possible effect (% MPE). Rectal temperature was measured as change in rectal temperature (°C) from pretest baseline (°C). Catalepsy was defined as percentage of time immobile on a ring apparatus. Each point represents mean (± S.E.M.) of data from 6 mice, except that the vehicle point for intraperitoneal Δ^9-tetrahydrocannabinol includes data for 12 mice.

drocannabinol was only capable of producing 60% catalepsy, the antipsychotics produced nearly 100% immobility. In addition to this difference in efficacy, potencies across the tests varied considerably more than they did for Δ^9-tetrahydrocannabinol (ranging from a maximum of 4-fold difference for thioridazine to over 40-fold difference for haloperidol). In contrast, ED_{50}s for chlorpromazine were similar across tests (less than twofold difference). A fifth antipsychotic pimozide suppressed spontaneous activity and reduced body temperature; however, it showed very little activity (< 30% maximal effect) in the other two tests (Table 2).

Table 2 presents results with selected drugs that were inactive in one or more of the four tests. As might be expected, amphetamine did not exhibit cannabinoid activity in any of the tests; indeed, it dose-dependently increased locomotion (data not shown). Several of the drugs produced effects similar to those produced by psychoactive cannabinoids in only one or two tests: desipramine, pimozide, and pentobarbital decreased locomotion and body temperature; morphine produced pronounced antinociceptive effects and milder hypothermia. Scopolamine stimulated spontaneous activity and was inactive in the other three tests.

Given that some of the noncannabinoid drugs were active in most of the tetrad tests (e.g., chlorpromazine and haloperidol), we evaluated cannabinoid CB_1 receptor mediation of their effects through antagonism tests with SR 141716A

Table 1
Potencies of drugs active in the tetrad tests[a]

Drug	SA	MPE	RT	RI	Range
Δ^9-THC (i.v.)	1.9 (0.26–14.26)	0.8 (0.71–0.85)	0.9 (0.60–1.47)	1.9 (0.58–6.31)	2.4
Δ^9-THC (i.p.)	20.6 (13.9–30.49)	13.6 (10.61–17.48)	24.1 (17.73–32.76)	11.1 (5.53–22.22)	2.3
Diazepam	ND	0.3 (0.11–0.72)	ND	0.4 (0.17–1.22)	ND
Ethanol	1.7 (1.14–2.50)	1.2 (0.97–1.60)	2.1 (1.57–2.83)	ND	ND
Chlorpromazine	1.5 (0.86–2.56)	2.0 (1.32–3.0)	1.6 (0.98–2.62)	1.7 (1.29–2.32)	1.3
Clozapine	1.1 (0.50–2.50)	4.3 (2.79–6.57)	1.2 (0.56–2.48)	8.7 (7.27–10.38)	7.9
Haloperidol	0.1 (0.04–0.54)	0.6 (0.37–1.13)	4.3 (1.32–OR)	1.0 (0.67–1.41)	43
Thioridazine	1.1 (0.71–1.63)	4.5 (2.46–8.11)	2.4 (1.44–3.98)	4.26 (2.95–6.15)	4.1

ND = not determined; OR = confidence limit out of range; SA=% inhibition of spontaneous activity; MPE = percentage of maximum possible effect in tail-flick test; RT = change in rectal temperature in °C; RI = ring immobility; Range = maximum fold difference in potency across measures obtained by dividing lowest ED_{50} into highest ED_{50}.

[a] Values represent ED_{50}s (± 95% confidence limits) in mg/kg for all drugs except for ethanol where values are expressed in g/kg.

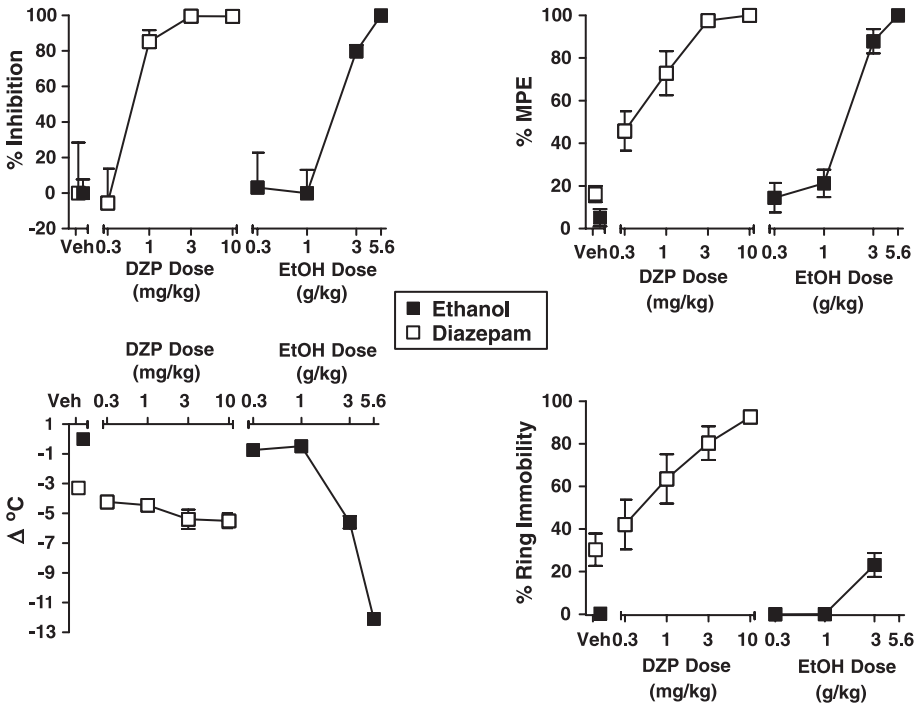

Fig. 2. Effects of diazepam (□) and ethanol (■) on locomotor activity (top left panel), nociception (top right panel), rectal temperature (bottom left panel), and catalepsy (bottom right panel) in mice. Each point represents mean (± S.E.M.) of data from five to six mice.

(Table 3). Interactions were significant for analysis of variance on each dependent variable. Post hoc analysis showed that when administered with vehicle, diazepam, chlorpromazine, haloperidol, and thioridazine produced significant activity in each of the four tests (compared to saline levels). Conversely, ethanol (3 g/kg) did not significantly decrease spontaneous activity nor did it induce catalepsy, and clozapine (10 mg/kg) did not significantly decrease spontaneous activity. Saline did not produce cannabinoid effects in any of the tests nor did 3 mg/kg SR 141716A;

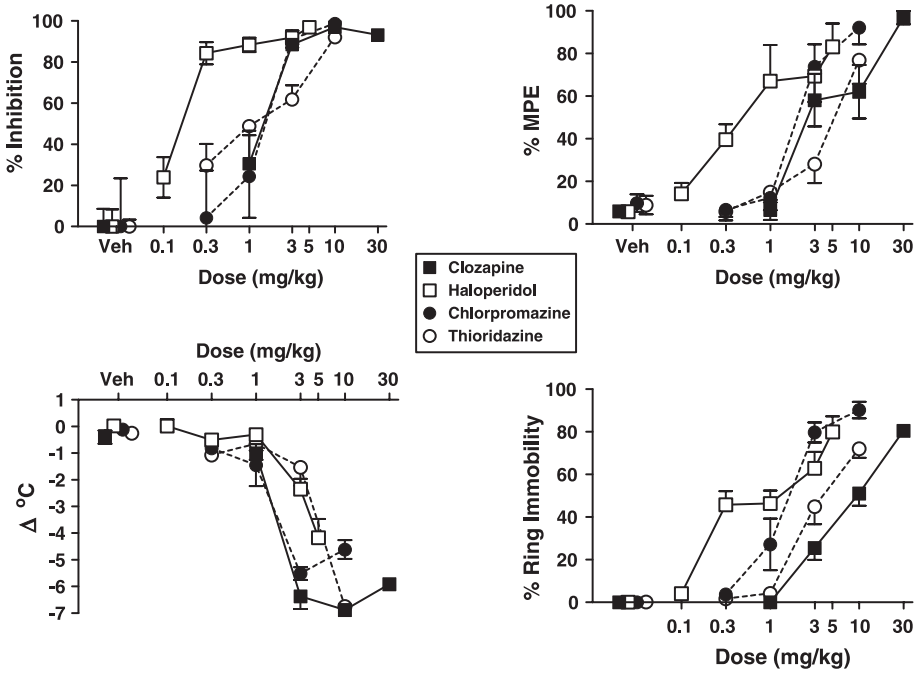

Fig. 3. Effects of haloperidol (□), clozapine (■), chlorpromazine (●), and thioridazine (○) on locomotor activity (top left panel), nociception (top right panel), rectal temperature (bottom left panel), and catalepsy (bottom right panel) in mice. Each point represents mean (± S.E.M.) of data from 6 mice, except the vehicle point for haloperidol which includes data for 12 mice.

Table 2
Evaluation of drugs from several classes in tetrad tests[a]

Drug	Dose range (mg/kg)	SA (± S.E.M.)	MPE (± S.E.M.)	RT (± S.E.M.)	RI (± S.E.M.)
Amphetamine	0.3–3	23 (12.6)	9 (6.2)	−0.8 (0.37)	1 (1.1)
Desipramine	3–60	77 (3.9)	23 (6.8)	−4.1 (0.43)	12 (5.5)
Morphine	0.3–10	24 (12.4)	96 (4.2)	−2.7 (0.41)	14 (5.9)
Pentobarbital	3–30	40 (22.6)	14 (5.7)	−2.6 (0.43)	10 (4.2)
Pimozide	0.1–3	74 (3.4)	28 (5.9)	−4.2 (0.41)	29 (7.0)
Scopolamine	0.3–10	−58[b] (15.1)	10 (3.5)	−1.3 (0.80)	0.3 (0.18)

[a] SA = % inhibition of spontaneous activity; MPE = percentage of maximum possible effect in tail-flick test; RT = change in rectal temperature in °C; RI = ring immobility. Maximum drug effect indicated for each test. Values represent means (± S.E.M.) for six mice.
[b] Indicates maximal stimulation. Scopolamine-stimulated locomotor activity at all doses tested.

however, significant stimulation of locomotion was observed with SR 141716A alone. As expected, the effects of intravenous and intraperitoneal Δ^9-tetrahydrocannabinol were significantly attenuated by 3 mg/kg SR 141716A. With the exception of the hypothermic effects of intraperitoneal Δ^9-tetrahydrocannabinol, SR141716A reversed Δ^9-tetrahydrocannabinol-induced effects to vehicle levels. In contrast, SR 141716A failed to completely block activity of any of the other drugs in all four tests, although it significantly reversed effects of some of the drugs in one or two tests. For example, SR 141716A partially attenuated the hypothermia and catalepsy induced by diazepam, the antinociception induced by ethanol and chlorpromazine, and the hypothermic effects of clozapine and haloperidol. Only in the case of the hypothermic effects of haloperidol, however, was this reversal to vehicle levels. In clozapine-treated mice, SR 141716A stimulated motor activity to a similar degree as it did when administered alone.

In order to evaluate the possible effects of dopamine receptor antagonism in the activity of antipsychotics in these tests, we attempted to reverse the effects of 10 mg/kg clozapine and 3 mg/kg chlorpromazine with the nonselective dopamine receptor agonist, apomorphine (0.1 mg/kg), and the dopamine D2-selective receptor agonist, quinpirole (0.01 mg/kg). The effects of neither antipsychotic was altered by either of these drugs (data not shown).

4. Discussion

Consistent with the results of numerous previous studies (e.g., Compton et al., 1993; Martin et al., 1991), Δ^9-tetrahydrocannabinol decreased spontaneous locomotion and produced antinociceptive, hypothermic, and cataleptic effects. When administered intravenously, it was approximately equipotent across tests. Further, maximal effects approximated those obtained previously with tetrahydrocannabinols: 90% inhibition of spontaneous activity, 100% antinociception, 6 °C temperature drop, and 60% ring immobility. Similarly, intraperitoneally administered Δ^9-tetrahydrocannabinol was fully active in all four tests at similar potencies.

Table 3
Evaluation of SR 141716A blockade of drug effects in tetrad tests[a]

Drug	Drug dose	SR141716A	SA	MPE	RT	RI
Saline	–	0	−0.01 (6)	9 (2)	−0.4 (0.2)	0.3 (0.1)
		3	−41 (11)[b]	15 (2)	−0.7 (0.1)	2 (0.7)
Δ^9-THC (i.v.)	3 mg/kg	0	89 (2)	87 (10)	−4.6 (0.5)	55 (5)
		3	−4 (27)[b]	18 (11)[b]	−0.8 (0.3)[b]	1 (1)[b]
Δ^9-THC (i.p.)	56 mg/kg	0	85 (3)	100 (0)	−6.7 (0.4)	47 (6)
		3	−5 (31)[b]	32 (10)[b]	−3.4 (0.5)[b]	8 (6)[b]
Diazepam	3 mg/kg	0	100 (0)	77 (15)	−5.0 (0.4)	75 (8)
		3	89 (5)	68 (16)	−2.5 (0.2)[b]	42 (9)[b]
Ethanol	3 g/kg	0	47 (14)	88 (4)	−3.7 (0.5)	11 (4)
		3	29 (19)	45 (12)[b]	−3.1 (0.5)	7 (5)
Chlorpromazine	3 mg/kg	0	95 (4)	80 (7)	−5.5 (0.4)	64 (6)
		3	86 (2)	42 (10)[b]	−4.8 (0.3)	59 (4)
Clozapine	10 mg/kg	0	47 (18)	71 (10)	−3.9 (0.4)	30 (8)
		3	−56 (46)[b]	52 (11)	−2.4 (0.4)[b]	23 (7)
Haloperidol	3 mg/kg	0	99 (0.9)	92 (8)	−3.4 (0.6)	69 (10)
		3	89 (0.9)	73 (15)	−1.9 (0.4)[b]	77 (9)
Thioridazine	10 mg/kg	0	87 (6)	75 (12)	−4.5 (0.6)	69 (12)
		3	84 (8)	80 (8)	−3.3 (0.3)	57 (10)

[a] SA = % inhibition of spontaneous activity; MPE = percentage of maximum possible effect in tail-flick test; RT = change in rectal temperature in °C; RI = ring immobility. Values represent mean (± S.E.M.). $n = 11–12$ mice for clozapine, chlorpromazine, and ethanol; $n = 36$ for saline; $n = 5–6$ mice for all other drugs.
[b] Indicates $P < 0.05$ compared to vehicle + drug condition.

Noncannabinoid compounds showed distinct differences in comparison to Δ^9-tetrahydrocannabinol. First, and not unexpectedly, many of the noncannabinoid compounds tested in the present study were not active in all four of the mouse tests, although individual drugs sometimes produced one or two of the effects. Drugs that did not produce the complete profile of effects were amphetamine, desipramine, pimozide, pentobarbital, morphine, diazepam, ethanol, and scopolamine. Second, of the classes of drugs tested, antipsychotics and central nervous system depressants tended to show the most consistent activity across tests; however, there were also clear differences between these drugs and Δ^9-tetrahydrocannabinol, as discussed in further detail below.

The pattern of effects produced by central nervous system depressants differed from that of Δ^9-tetrahydrocannabinol primarily in that none of the central nervous system depressants was active in all four tests. Diazepam lacked clear hypothermic effects; ethanol did not induce catalepsy; pentobarbital did not produce antinociception or catalepsy and was not very efficacious in the other two tests. Similarly, previous research with the central nervous system depressants has shown that their pharmacology only partially overlaps that of cannabinoids (Barrett et al., 1995; Wallace et al., 2001; Wiley and Martin, 1999). Further, antagonism studies show that mediation of the shared effects of cannabinoids and central nervous system depressants is through different mechanisms (e.g., Mokler et al., 1986; Pertwee et al., 1988). In addition (and in contrast to results obtained with Δ^9-tetrahydrocannabinol), SR 141716A did not completely block the pharmacological effects of ethanol or diazepam in the tetrad tests of the present study. (SR 141716A partially attenuated the hypothermic effect of diazepam and the antinociceptive effect of ethanol; however, reversal was not complete and did not occur for all effects, as it typically does for cannabinoids.) Together, these results suggest that, although there is some overlap in the pharmacological profile of central nervous system depressants and cannabinoids, their psychoactivity in the tetrad tests are mediated via distinct mechanisms.

Notable differences in the effects of antipsychotics and cannabinoids were also observed. Although four of the five antipsychotics tested (clozapine, haloperidol, chlorpromazine, and thioridazine, but not pimozide) were active in all four tetrad tests, their potencies across tests tended to be more variable than were those of intravenous or intraperitoneal Δ^9-tetrahydrocannabinol. In contrast with aminoalkylindole cannabinoids which show greater potencies for a single effect (suppression of locomotor activity) (Compton et al., 1992; Wiley et al., 1998), antipsychotics were not consistently more or less potent in particular test(s). Second, the magnitude of the antipsychotic-induced cataleptic effect exceeded that produced by cannabinoids (over 90% and 60%, respectively). For thioridazine and chlorpromazine, maximum catalepsy occurred at doses close to the ED_{84} dose for suppression of activity, whereas for clozapine and haloperidol, the ED_{84} dose for suppression of spontaneous activity was close to the ED_{50} dose for catalepsy, reflecting the greater variability across measures obtained with the two latter drugs. Differences in efficacies have also been seen with anandamide and its analogs, but these differences were noted in the magnitude of the hypothermic effect. Although equally efficacious in producing antinociceptive and hypomobility effects, anandamide-like cannabinoids decrease body temperature by a maximum of about 3 °C in contrast to the 6 °C drop seen with classical and indole-derived cannabinoids (Ryan et al., 1997; Seltzman et al., 1997). A final difference between the effects of antipsychotics in the tetrad tests and those of Δ^9-tetrahydrocannabinol was that the antipsychotic-induced effects were not consistently or completely blocked by SR 141716A, although partial attenuation of a couple of individual effects were observed as noted in Results. Overall, these results suggest that antipsychotics did not produce these effects via the same mechanism as did cannabinoids.

The mechanisms through which antipsychotics did produce pharmacological effects in the tetrad tests, however, are unclear. Antagonism of dopamine D2 receptors is one property shared by all of these antipsychotics (Kongsamut et al., 2002; Richelson and Souder, 2000); however, activity in the tetrad tests was not correlated with dopamine D2 receptor affinity. For example, pimozide has greater affinity for dopamine D2 receptors than does clozapine and has approximately equal affinity to thioridazine (Kongsamut et al., 2002; Richelson and Souder, 2000); however, unlike the other antipsychotics, pimozide was not active in all of the tetrad tests. Further, the tetrad effects of clozapine and chlorpromazine were not reversed by the nonselective dopamine receptor agonist, apomorphine, or the dopamine D2 receptor agonist, quinpirole. A second possibility is that antipsychotics may interact with cyclooxygenase or other pathways important in the production of anandamide-induced effects in these tests. Although there is sparse research in this area, a previous study has shown that cyclooxygenase inhibitors inhibit haloperidol-induced catalepsy (Naidu and Kulkarni, 2002; Ross et al., 2002). In addition, trifluoperazine, a phenothiazine antipsychotic similar to chlorpromazine, binds to vanilloid receptors (Szallasi et al., 1996). Vanilloid receptors are ligand-gated ion channels that are activated by heat (Caterina et al., 1997), by capsaicin (an ingredient in foods such as hot chili peppers), and by anandamide (Smart et al., 2000; Zygmunt et al., 1999). Di Marzo et al. (2000a, 2001, 2002) have demonstrated that some vanilloid receptor agonists (with high potency at vanilloid VR1 receptors) and cannabinoid/capsaicin hybrids are active in the tetrad tests and that their effects are not blocked by SR 141716A. Although preliminary, these data suggest that further investigation of antipsychotic modulation of pathways affected by cannabinoids may be worthwhile.

In summary, the tetrad tests are not entirely pharmacologically specific for cannabinoids. Compounds from a

number of different chemical classes produce one or more of the tetrad effects. Certain antipsychotics produce all four effects. While the mechanism(s) through which antipsychotics induce the effects is (are) uncertain, it does not appear to be identical to that through which cannabinoids produce their effects. Notably, SR 141716A, the cannabinoid CB_1 receptor antagonist, reliably antagonizes cannabinoid tetrad effects, but not those of antipsychotics or other drug classes. While the tetrad tests remain useful as a screening battery for determination of structure–activity relationships among cannabinoid ligands that are known to bind to cannabinoid receptors (particularly when combined with SR 141716A reversal and binding data), the present results urge caution in the application of in vivo results to interpretation of receptor mechanisms. In particular, results of the tetrad tests should be scrutinized when making inferences about potential new cannabinoid receptors for the following reasons: (1) the tests are not absolutely specific for cannabinoids, as shown by the present results with antipsychotics as well as findings that vanilloid receptor agonists produce a similar profile of effects and (2) agonists at a novel cannabinoid receptor may or may not produce a similar profile of pharmacological effects as those acting through cannabinoid CB_1 receptors. On the other hand, the results from the tetrad tests can be very informative when there is additional data in support of a receptor mechanism, such as anandamide stimulation of [^{35}S]GTPγS binding in brain membranes from cannabinoid CB_1 receptor knockout mice (Breivogel et al., 2001). Close inspection of the dose–response curves revealed some differences between Δ^9-tetrahydrocannabinol and almost all of the other compounds. Evaluation of one or two doses of a drug in the tetrad could be quite misleading as could elimination of data from two or more of the tests.

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