

Structure-Activity Relationships of Indole- and Pyrrole-Derived Cannabinoids¹

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ABSTRACT

Early molecular modeling studies with Δ^9 -tetrahydrocannabinol (Δ^9 -THC) reported that three discrete regions which interact with brain cannabinoid (CB1) receptors corresponded to the C-9 position of the cyclohexene ring, the phenolic hydroxyl and the carbon side chain at the C3 position. Although the location of these attachment points for aminoalkylindoles is less clear, the naphthalene ring, the carbonyl group and the morpholinoethyl group have been suggested as probable sites. In this study, a series of indole- and pyrrole-derived cannabinoids was developed, in which the morpholinoethyl group was replaced with another cyclic structure or with a carbon chain that more directly corresponded to the side chain of Δ^9 -THC and were tested for CB1 binding affinity and in a battery of *in vivo* tests, including hypomotility, antinociception, hypothermia and cat-

alepsy in mice and discriminative stimulus effects in rats. Receptor affinity and potency of these novel cannabinoids were related to the length of the carbon chain. Short side chains resulted in inactive compounds, whereas chains with 4 to 6 carbons produced optimal *in vitro* and *in vivo* activity. Pyrrole-derived cannabinoids were consistently less potent than were the corresponding indole derivatives and showed pronounced separation of activity, in that potencies for hypomotility and antinociception were severalfold higher than potencies for hypothermia and ring immobility. These results suggest that, whereas the site of the morpholinoethyl group in these cannabinoids seems crucial for attachment to CB1 receptors, the exact structural constraints on this part of the molecule are not as strict as previously thought.

WIN 55,212, the prototypic aminoalkylindole cannabinoid, is related structurally to pravadoline, a novel cyclooxygenase inhibitor originally developed as an alternative to nonsteroidal anti-inflammatory drugs (Haubrich *et al.*, 1990). Although pravadoline is a weak anti-inflammatory agent, it possesses potent antinociceptive activity that apparently is unrelated to its inhibition of cyclooxygenase or to opioid mechanisms. WIN 55,212 shares these antinociceptive effects with pravadoline (Compton *et al.*, 1992). Because WIN 55,212 and related aminoalkylindoles bind to brain cannabinoid receptors (CB1), it has been suggested that these drugs produce their antinociceptive effects *via* cannabinoid mechanisms (D'Ambra *et al.*, 1992). Indeed, these drugs produce a profile of behavioral effects that resemble those of Δ^9 -THC and other classical and bicyclic cannabinoids, including suppression of spontaneous activity, antinociception, decreased rectal temperature and ring immobility in mice (Compton *et al.*, 1992) and cannabimimetic discriminative stimulus effects in rats and rhesus monkeys (Pério *et al.*, 1996; Wiley *et*

al., 1995a, b). Further, the pharmacological effects of Δ^9 -THC and WIN 55,212 are blocked by the cannabinoid antagonist, SR 141716A (Pério *et al.*, 1996; Rinaldi-Carmona *et al.*, 1994; Wiley *et al.*, 1995b), and chronic administration results in cross-tolerance to the hypomotility, hypothermia, antinociceptive and cataleptic effects of these structurally distinct cannabinoids (Fan *et al.*, 1994; Pertwee *et al.*, 1993).

Given the structural diversity of classical, bicyclic, anandamide and aminoalkylindole cannabinoids, it is difficult to imagine how these classes of drugs might bind to an identical receptor. Enantiomer selectivity has been demonstrated in structure-activity relationship studies of classical and bicyclic cannabinoids (Martin *et al.*, 1981), which suggests that a minimum of three sites of attachment are required for receptor binding and activation. The original three-point attachment model proposed the following sites for Δ^9 -THC and similar classical tricyclic and bicyclic cannabinoids: (1) the C-9 position of the cyclohexene ring, (2) a phenolic hydroxyl and (3) a nonpolar side chain at the C3 position (Binder and Franke, 1982; Edery *et al.*, 1971; Razdan, 1986; Thomas *et al.*, 1991). Although the discovery of anandamide (Devane *et al.*, 1992) and increased recognition of the importance of the

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ABBREVIATIONS: DD, drug discrimination; MPE, maximal possible antinociceptive effect; RI, ring immobility; RT, rectal temperature; SA, spontaneous activity; Δ^9 -THC, Δ^9 -tetrahydrocannabinol; BSA, bovine serum albumin; EDTA, ethylenediaminetetraacetic acid.

geometry of the C-9 substituent (*e.g.*, Reggio *et al.*, 1989), as well as subsequent findings (Huffman *et al.*, 1996; Martin *et al.*, 1995), have eroded the validity of these specific putative sites of attachment, the model still serves as an excellent template for making structural comparisons between classical cannabinoids and aminoalkylindoles. Huffman *et al.* (1994) suggested that the structure of the aminoalkylindole cannabinoids might conform to a three-point attachment model with points of attachment at the naphthalene ring at the C7 position, the carbonyl group and the morpholinoethyl group (fig. 1). Eissenstat *et al.* (1995) proposed that the morpholinoethyl group or another cyclic structure was required for binding and cannabimimetic activity of aminoalkylindoles; however, classical cannabinoids and anandamide do not possess such a cyclic structure but rather have a carbon side chain at this location.

In the present study, a series of indole- and pyrrole-derived cannabinoids were developed in which a carbon chain of varying lengths was substituted for the morpholinoethyl group. For purposes of comparison, selected compounds with substitution of a saturated or unsaturated cyclic structure for the morpholinoethyl group of WIN 55,212 were synthesized, as were several compounds in which the carbon chain contained at least one double bond. All compounds were tested *in vitro* for displacement of CP 55,940 binding and, whenever solubility allowed, they were tested *in vivo* in procedures in which cannabinoids produce a characteristic profile of effects in mice (Martin *et al.*, 1991). Selected compounds also were tested in rat cannabinoid discrimination procedures. Cannabinoid discrimination represents an animal model of the subjective effects of this class of compounds in humans (Balster and Prescott, 1992). In addition, for classical cannabinoids, potencies in these *in vivo* procedures with mice and rats show strong positive correlations with binding affinity at CB1 receptors (Compton *et al.*, 1993). Synthesis procedures and preliminary pharmacological data for some of these compounds have been reported previously (Huffman *et al.*, 1994; Lainton *et al.*, 1995).

Methods

Subjects. Adult male Sprague-Dawley rats (290–350 g), obtained from Charles River (Wilmington, MA), were housed individually. Male ICR mice (25–32 g), obtained 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-hr light-dark cycle (lights on at 7 A.M.). Rats were maintained within the indicated

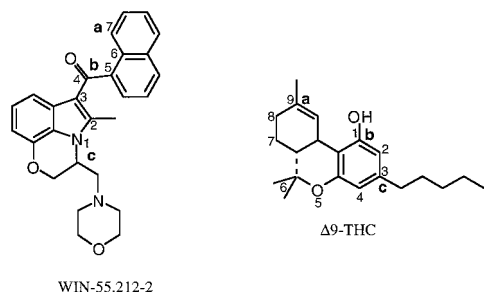


Fig. 1. Chemical structures of WIN 55,212-2 and Δ^9 -THC with presumed three points of attachment marked with letters for each compound, respectively: (a) naphthalene ring and cyclohexene ring, (b) carbonyl group and phenolic hydroxyl and (c) morpholinoethyl group and carbon side chain at C3.

weight range by restricted postsession feeding. Rodents were drug naive at the beginning of the study. Separate mice were used for testing each drug dose in the *in vivo* behavioral procedures. Brain tissue for binding studies was obtained from male Sprague-Dawley rats (150–200 g) obtained from Dominion Laboratories (Dublin, VA), which were maintained on a 14:10 hr light/dark cycle and received food and water *ad libitum*.

Apparatus. Measurement of spontaneous activity in mice occurred in standard activity chambers interfaced with a Digiscan Animal Activity Monitor (Omnitech Electronics, Inc., Columbus, OH). A standard tail-flick apparatus (described by Dewey *et al.*, 1970) and a telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH) 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.

Drug discrimination training and testing used standard operant conditioning chambers (Lafayette Instruments Co., Lafayette, IN) housed in sound-attenuated cubicles. A pellet dispenser delivered 45-mg BIO SERV (Frenchtown, NJ) food pellets to a cup located between two response levers mounted on the front wall of the chamber. Fan motors provided ventilation and masking noise for each chamber. Four-watt houselights were located above each lever; both were illuminated during training and testing sessions.

Drugs. Δ^9 -THC (National Institute on Drug Abuse, Rockville, MD) and CP 55,940 (Pfizer, Groton, CT) were suspended in a vehicle of absolute ethanol, Emulphor-620 (Rhone-Poulenc, Inc., Princeton, NJ) and saline in a ratio of 1:1:18. Novel indole- and pyrrole-derived cannabinoids were synthesized in our laboratories (Clemson University, Clemson, SC) and also were mixed in a 1:1:18 vehicle. In mice, drugs were administered *i.v.* in the tail vein at a volume of 0.1 ml/10g. In rats, all drugs were administered *i.p.* at a volume of 1 ml/kg.

Membrane preparation and binding. [³H]CP 55,940 ($K_D = 690$ nM) binding to P₂ membranes was conducted as described elsewhere (Compton *et al.*, 1993), except whole brain (rather than cortex only) was used. The assays were performed in triplicate, and the results represent the combined data from three individual experiments. Detailed information on the membrane preparation and binding assay are provided below.

The methods for tissue preparation were those described by Devane *et al.* (1988). After decapitation and the rapid removal of the brain, the cortex was dissected free with use of visual landmarks following reflection of cortical material from the midline. The cortex was immersed in 30 ml of ice-cold centrifugation solution (320 mM sucrose, 2 mM TrisEDTA, 5 mM MgCl₂). The process was repeated until the cortices of five rats were combined. The cortical material was homogenized with a Kontes Potter-Elvehjem glass-Teflon grinding system (Fisher Scientific, Springfield, NJ). The homogenate was centrifuged at 1600 × *g* for 10 min, and the resulting pellet was termed P₁. The supernatant was saved and combined with the two subsequent supernatants obtained from washing of the P₁ pellet. The combined supernatant fractions were centrifuged at 39,000 × *g* for 15 min, resulting in the P₂ pellet. This pellet was resuspended in 50 ml of buffer A (50 mM TrisHCl, 2 mM TrisEDTA, 5 mM MgCl₂, pH 7.0), incubated for 10 min at 37°C, then centrifuged at 23,000 × *g* for 10 min. The P₂ membrane was resuspended in 50 ml of buffer A, incubated again except at 30°C for 40 min, then centrifuged at 11,000 × *g* for 15 min. The final wash-treated P₂ pellet was resuspended in assay buffer B (50 mM TrisHCl, 1 mM TrisEDTA, 3 mM MgCl₂, pH 7.4) to a protein concentration of approximately 2 mg/ml. The membrane preparation was divided into four equal aliquots and quickly frozen in a bath solution of dry ice and 2-methylbutane (Sigma Chemical Co., St. Louis, MO), then stored at –80°C for no more than 2 weeks. Before performing a binding assay an aliquot of frozen membrane was thawed rapidly and protein values were determined by the method of Bradford (1976) with Coomassie brilliant

blue dye (Bio-Rad, Richmond, CA) and BSA standards (fatty acid free, Sigma Chemical Co., St. Louis, MO) prepared in assay buffer.

The methods for ligand binding were essentially those described by Devane *et al.* (1988), except that the assay described here is a filtration assay. Binding was initiated by the addition of 150 μg of P_2 membrane to test tubes containing [^3H]CP 55,940 (79 Ci/mmol), a cannabinoid analog (for displacement studies) and a sufficient quantity of buffer C (50 mM TrisHCl, 1 mM TrisEDTA, 3 mM MgCl_2 , 5 mg/ml BSA) to bring the total incubation volume to 1 ml. The concentration of [^3H]CP 55,940 in displacement studies was 1 nM. Nonspecific binding was determined by the addition of 1 μM unlabeled CP 55,940. CP 55,940 and all cannabinoid analogs were prepared by suspension in buffer C from a 1 mg/ml ethanolic stock.

After incubation at 30°C for 1 hr, binding was terminated by addition of 2 ml of ice-cold buffer D and vacuum filtration through pretreated filters in a 12-well sampling manifold (Millipore, Bedford, MA). Reaction vessels were washed once with 2 ml of ice-cold buffer D (50 mM TrisHCl, 1 mg/ml BSA), and the filters were washed twice with 4 ml of ice-cold buffer D. Filters were placed into 20-ml plastic scintillation vials (Packard, Downer Grove, IL) with 1 ml of distilled water and 10 ml of Budget-Solve (RPI Corp., Mount Prospect, IL). After shaking for 1 hr the quantity of radioactivity present was determined by liquid scintillation spectrometry.

Assay conditions were performed in triplicate, and the results represent the combined data of three to six independent experiments. All assays were performed in siliconized test tubes, which were prepared by air drying (12 hr) the inverted borosilicate tubes after two rinses with a 0.1% solution of AquaSil (Pierce, Rockford, IL). The GF/C glassfiber filters (2.4 cm, Baxter, McGaw Park, IL) were immersed before use in a 0.1% solution of pH 7.4 polyethyleneimine (Sigma Chemical Co., St. Louis, MO) for at least 6 hr.

Mouse behavioral procedures. Before testing in the behavioral procedures, mice were acclimated to the experimental setting (ambient temperature, 22–24°C) overnight. Preinjection control values were determined for rectal temperature and tail-flick latency (in seconds). Five minutes after *i.v.* injection with drug or vehicle, 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 percent inhibition of activity of the vehicle group. Tail-flick latency was measured at 20 min postinjection. Maximum latency of 10 sec was used. Antinociception was calculated as percent of maximum possible effect [%MPE = [(test – control latency)/(10 – control)] \times 100]. Control latencies typically ranged from 1.5 to 4.0 sec. At 1.5 hr postinjection, each mouse was placed on the ring immobility apparatus for 5 min, during which the total amount of time (in seconds) that the mouse remained motionless was measured. This value was divided by 300 sec 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. Rectal temperature was expressed as the difference between control temperature (before injection) and temperatures after drug administration ($\Delta^\circ\text{C}$). During the course of this extended study, the ring immobility test was discontinued and the time at which rectal temperature was measured was changed. For compounds that were tested in the ring immobility assay, rectal temperature was measured at 60 min postinjection; for compounds that were not tested in this procedure, rectal temperature was measured at 30 min postinjection. Different mice ($n = 5\text{--}6/\text{dose}$) were tested for each dose of each compound. Each mouse was tested in each of the three or four procedures.

Rat drug discrimination procedure. Two groups of rats were trained to press one lever after injection with $\Delta^9\text{-THC}$ (3 mg/kg; $n = 10$) or CP 55,940 (0.1 mg/kg; $n = 8$) and to press another lever after administration of vehicle to obtain food reinforcement under a fixed-ratio 10 (FR-10) schedule of food reinforcement. The position of the reinforced (correct) lever was determined by the type of injection the rat received on a given day. A response on the incorrect lever reset

the ratio requirement on the correct lever. The schedule of daily injections for each rat was administered in a double-alternation sequence of drug and vehicle. Both groups of rats were injected and returned to their home cages for 30 min until the start of the experimental session. Acquisition training occurred during 15-min sessions 5 days a week (Monday through Friday) until the rats had met three criteria during 10 consecutive sessions: (1) first completed FR-10 on the correct lever; (2) percentage of correct-lever responding $\geq 80\%$; and (3) response rate ≥ 0.5 responses/sec.

Substitution tests with novel indole- and pyrrole-derived cannabinoids (CP 55,940 and $\Delta^9\text{-THC}$ groups, respectively) were conducted on Tuesdays and Fridays with continued training during sessions on Mondays, Wednesdays and Thursdays. During test sessions, consecutive responses on either lever delivered reinforcement according to a FR-10 schedule. To be tested, rats must have met the three acquisition criteria (see above) during at least one of the vehicle training sessions and at least one of the drug training sessions occurring within the week before testing. Test sessions lasted 15 min. All rats were tested with their training drug ($\Delta^9\text{-THC}$ or CP 55,940) before being tested with any of the other compounds. Control tests with vehicle and $\Delta^9\text{-THC}$ (3 mg/kg) or CP 55,940 (0.1 mg/kg) were conducted before each dose-effect curve determination. A within-subjects design was used to test all indole (CP 55,940 group)- and pyrrole ($\Delta^9\text{-THC}$ group)-derived cannabinoids, such that each rat received all doses of each test compound presented in ascending order.

Data analysis. Based on data obtained from numerous previous studies with cannabinoids, maximal cannabinoid effects in each procedure were estimated as follows: 90% inhibition of spontaneous activity, 100% MPE in the tail-flick procedure, -6°C change in rectal temperature and 60% ring immobility. ED_{50} values were defined as the dose at which half-maximal effect occurred. For drugs that produced one or more cannabinoid effect, ED_{50} values were calculated separately by 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.

For each rat drug discrimination test session, percentage of responses on the drug lever and response rate (responses/sec) were calculated. When appropriate, ED_{50} values (with 95% confidence intervals) were calculated separately for each drug by least-squares linear regression on the linear part of the dose-effect curves (Tallarida and Murray, 1987) for percentage of drug-lever responding, plotted against \log_{10} transformation of the dose. Because rats that responded 10 times or less during a test session did not press either lever a sufficient number of times to earn a reinforcer, their lever selection data were excluded from data analysis. For the purposes of potency comparison, potencies were expressed as micromoles per kilogram for both rat and mice data.

Pearson product-moment correlation coefficients (with associated significance tests) were calculated between binding affinity (expressed as $\log K_i$) and *in vivo* potency for each measure (expressed as $\log \text{ED}_{50}$ in micromoles per kilogram) for all cannabinoid compounds that bound to the CB1 receptor with a K_i less than 10,000 nM. In addition, multiple linear regression was used to calculate the overall degree of relationship between binding affinity and potency in the mouse tetrad measures.

Results

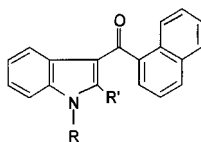
Binding affinities. Tables 1 and 2 contain binding affinities for the indole series with and without a methyl at the 2-position of the indole and the pyrrole series (all without a methyl at the 2-position of the pyrrole). In each series, manipulation of the length of the carbon chain resulted in an inverted U-shaped function for binding affinities. In all three series, substitution of a methyl group for the morpholinoethyl substituent produced a compound that did not bind to CB1 receptors. In both indole series, binding affinity steadily

TABLE 1

Binding affinity and *in vivo* potency of Δ^9 -THC, WIN 55,212-2 and indole-derived cannabinoids

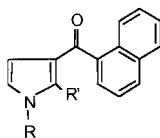
For all tables, "no max" indicates that the compound produced only slightly greater than 50% of the presumed maximal effect. "~dose" indicates an estimated ED₅₀ because the dose-effect curve was not linear. ">dose" indicates that 50% activity was not achieved at this dose, which was the highest dose of the compound that was tested. "NT" = not tested. All ED₅₀ values are expressed as micromoles per kilogram. SA, suppression of spontaneous activity; MPE, % maximum possible antinociceptive effect in the tail-flick assay; RT, rectal temperature; RI, ring immobility; rat DD, drug discrimination in rats.

Compound	K _i nM	SA	MPE	RT	RI	Rat DD
Δ^9 -THC	41	0.92	2.7	2.5	NT	1.8
WIN 55,212-2	24	0.19	1.4	1.5	NT	NT



R	R'	K _i nM	SA	MPE	RT	RI	Rat DD
<i>n</i> -Methyl	CH ₃	>10,000	NT	NT	NT	NT	NT
<i>n</i> -Ethyl	CH ₃	1180 ± 44	NT	NT	NT	NT	NT
<i>n</i> -Propyl	CH ₃	164 ± 22	18.7	84.7	99.1	87.2	45.9
<i>n</i> -Butyl	CH ₃	22 ± 1.5	2.6	0.23	4.1	12.9	7.3
<i>n</i> -Pentyl	CH ₃	9.5 ± 4.5	0.70	0.25	4.3	1.9	<8.5
<i>n</i> -Hexyl	CH ₃	48 ± 13	<2.7	9.5	17.1	16.0	19.8
<i>n</i> -Heptyl	CH ₃	>10,000	117	>261	>261	>261	>261
<i>n</i> -Methyl	H	>10,000	>35.1	>35.1	>35.1	NT	NT
<i>n</i> -Ethyl	H	1390 ± 123	NT	NT	NT	NT	NT
<i>n</i> -Propyl	H	1050 ± 55	NT	NT	NT	NT	NT
<i>n</i> -Butyl	H	8.9 ± 1.8	0.34	1.3	3.3	NT	NT
<i>n</i> -Pentyl	H	9 ± 5	0.44	~0.09	1.7	3.9	NT
<i>n</i> -Hexyl	H	9.8 ± 2	0.96	0.73	1.5	NT	NT
<i>n</i> -Heptyl	H	128 ± 17	56.9	17.6	>81.3	NT	NT

TABLE 2

Binding affinity and *in vivo* potency of nonmethylated pyrrole-derived cannabinoids^a

R	R'	K _i nM	SA	MPE	RT	RI	Rat DD
<i>n</i> -Methyl	H	>10,000	106	No max	53.3	NT	NT
<i>n</i> -Ethyl	H	>10,000	84.2	No max	77.2	NT	NT
<i>n</i> -Propyl	H	>10,000	85.9	81.8	90.1	NT	NT
<i>n</i> -Butyl	H	666 ± 77	No max	No max	>108.3	NT	NT
<i>n</i> -Pentyl	H	87 ± 3	3.6	1.2	78.8	98.9	16.1
<i>n</i> -Hexyl	H	399 ± 109	8.8	9.6	62.8	67.8	22.3
<i>n</i> -Heptyl	H	309 ± 11	11.0	9.7	52.1	No max	NT

^a See legend to table 1.

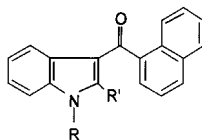
increased with the addition of each carbon until maximum affinity was demonstrated for the 2-methyl-*n*-pentyl indole and the nonmethylated *n*-butyl, *n*-pentyl and *n*-hexyl indoles, each of which had approximately 2.5 times greater receptor affinity than WIN 55,212-2 and 4 times greater affinity than Δ^9 -THC (table 1). Similarly, optimal affinity for the pyrrole series was observed for the *n*-pentyl pyrrole (table 2); however, the affinity of this compound for the CB1 receptor was 2 and 3.6 times less than affinities of WIN 55,212-2 and Δ^9 -THC, respectively. With only one exception, affinities of the pyrrole compounds for the CB1 receptor were consistently lower than the comparable compounds in both series of indoles. The exception is that *n*-heptyl pyrrole showed weak affinity for the CB1 receptor, whereas the 2-methyl-*n*-heptyl indole did not bind to this receptor.

Table 3 shows the results of manipulation of the placement of a double bond in the carbon chain of selected methylated and nonmethylated indoles. Addition of a double bond by substitution of an allyl group for propyl, *E*-2-pentenyl or 4-pentenyl for the pentyl of the methylated and nonmethylated indoles resulted in compounds that had at least 3-fold less affinity for the CB1 receptor than the corresponding parent compound in the indole series. Substitution of another cyclic structure for morpholinoethyl group resulted in compounds that had 2- to 52-fold less affinity for the CB1 receptor than did WIN 55,212-2 (table 3).

Structure-activity relationship in mice. Δ^9 -THC and WIN 55,212-2 produced a characteristic cannabinoid profile of *in vivo* effects in mice which included suppression of spontaneous activity, antinociception and hypothermia. Whereas

TABLE 3

Binding affinity and *in vivo* potency of indole-derived cannabinoids with placement of a double bond on the alkyl chain or substitution of a cyclic structure for the morpholinoethyl group^a



R	R'	K_i	SA	MPE	RT	RI	Rat DD
		<i>nM</i>					
(<i>E</i>)-2-Pentenyl	CH ₃	340 ± 184	1.7	2.8	32.4	30.4	NT
4-Pentenyl	CH ₃	38 ± 13	3.5	0.34	5.6	9.9	NT
Allyl	CH ₃	4518 ± 187	Inactive	Inactive	Inactive	Inactive	NT
(<i>E</i>)-2-Pentenyl	H	58 ± 14	No max	3.1	Inactive	NT	NT
4-Pentenyl	H	43 ± 21	0.88	0.15	2.4	2.1	NT
2-Phenylethyl	CH ₃	1250 ± 250	No max	No max	No max	No max	NT
Cyclohexylethyl	CH ₃	46 ± 13	55.4	58.7	69.7	118	NT
Cyclopropylmethyl	CH ₃	140 ± 44	6.5	7.1	36.9	83.1	NT

^a See legend to table 1.

each drug produced antinociceptive and hypothermic effects with similar potencies across measures, both drugs were more potent at decreasing spontaneous activity than they were at producing the other two effects (table 1); however, greater separation of locomotor and antinociceptive/hypothermic effects was obtained with WIN 55,212-2 than with Δ^9 -THC (7-fold *vs.* 3-fold difference, respectively). Consistent with its higher binding affinity at CB1 receptors, WIN 55,212-2 was more potent than Δ^9 -THC in all three procedures.

The methylated methyl and ethyl indole derivatives could not be dissolved in the vehicle at concentrations necessary for *in vivo* tests; hence, they were not tested. The 2-methyl-*n*-heptyl indole derivative was tested at doses up to 100 mg/kg (table 1). Although it decreased spontaneous activity at higher concentrations, it was inactive in the rectal temperature and ring immobility procedures at doses up to 100 mg/kg and produced a maximum of only 40 to 57% MPE across a dose range of 30 to 100 mg/kg. In contrast, the methylated *n*-propyl, *n*-butyl, *n*-pentyl and *n*-hexyl indole derivatives produced characteristic cannabinoid effects in the mice on all four measures. For each compound, potencies for hypothermia and ring immobility were lower than potencies for hypomobility and antinociception, although the magnitude of the potency differences was variable across compounds. Rank order potencies for each of the *in vivo* effects corresponded with rank order binding affinities with two exceptions. Although the 2-methyl-*n*-pentyl indole showed 2-fold higher affinity for CB1 receptors than did the 2-methyl-*n*-butyl indole, these two compounds were approximately equipotent in producing antinociception and hypothermia. Second, the 2-methyl-*n*-hexyl indole was more potent at decreasing spontaneous activity than was the 2-methyl-*n*-butyl indole, even though the latter compound exhibited 2-fold higher affinity for CB1 receptors.

In the nonmethylated indole series (table 1), none of the compounds was tested in the ring immobility task, and ethyl and propyl derivatives were not tested in any measure because of their low solubility. Consistent with its lack of binding affinity to CB1 receptors, the nonmethylated methyl indole derivative was inactive in each of the three assays, although it was only soluble up to a dose of 10 mg/kg. The nonmethylated heptyl indole was fully efficacious, but only

moderately potent, in the spontaneous activity and tail-flick procedures, but produced slightly below half-maximal decreases in rectal temperature at doses up to 30 mg/kg. The remaining compounds in this series showed approximately equal affinities for CB1 receptors and each produced potent cannabinoid effects on spontaneous activity, tail flick and rectal temperature. The nonmethylated pentyl indole produced 100% MPE at 1 mg/kg, but the dose-effect curve was not linear; hence, an ED₅₀ could not be calculated but was estimated as <0.03 mg/kg. As with the methylated indole derivatives, active nonmethylated indole derivatives were less potent at decreasing rectal temperature than at producing antinociception and hypomobility.

Despite their lack of binding affinity at CB1 receptors, methyl and ethyl pyrroles were efficacious in the spontaneous activity and rectal temperature assays, but produced only 54 to 55% MPE at a dose of 56 mg/kg in the tail-flick procedure (table 2). The propyl pyrrole, which also did not bind to CB1 receptors, was active in three of the *in vivo* procedures; however, none of these three pyrrole derivatives with the shortest alkyl chains were very potent compared with nonmethylated indole or other pyrrole compounds (table 2). In contrast, the butyl pyrrole showed greater binding affinity, but was not fully efficacious in any procedure. Up to doses of 30 mg/kg, this compound inhibited spontaneous activity and %MPE to a maximum of 69% in a dose-related manner. Similar to corresponding compounds in both indole series, pentyl and hexyl pyrroles were efficacious in all four procedures and were less potent at producing hypothermia and ring immobility. In addition, each of these compounds was less potent at inducing hypomobility and antinociception than were the corresponding nonmethylated indole compounds. However, the reverse was true for the heptyl pyrrole which showed greater potency at decreasing spontaneous activity and rectal temperature and at increasing antinociception than did the methylated and nonmethylated heptyl indoles. At doses up to 30 mg/kg, the nonmethylated heptyl indole increased percent ring immobility only to approximately half-maximal levels.

Substitution of an *E*-3-pentenyl or 4-pentenyl group for the *n*-pentyl group in each indole series resulted in active compounds with less affinity, but greater potency, than corresponding analogs with a saturated substituent. In contrast,

an allyl group in place of the propyl group in the methylated indole series resulted in an inactive compound at doses up to 100 mg/kg.

Substitution of a carbocyclic structure for the morpholinoethyl group of the WIN series resulted in compounds with less affinity and less potency than the parent compound. Substitution of a 2-phenylethyl group produced an inactive compound whereas substitution of a 2-cyclohexylethyl or 1-cyclopropylmethyl group resulted in compounds that were maximally efficacious, but less potent than WIN 55,212-2. As with the indole- and pyrrole-derived compounds, potencies for producing hypothermia and ring immobility were less than potencies for hypomobility and antinociception.

Multiple regression analysis of binding affinity ($Y = \log K_i$) and potency for each measure in the tetrad ($X_{1-4} = \log \text{ED}_{50}$ in $\mu\text{mol/kg}$) confirmed that overall potency at producing the characteristic profile of cannabinoid effects was correlated significantly with binding affinity at CB1 receptors ($r = 0.86$;

$F(4,12) = 8.4$, $P = .002$). Individual correlations between $\log K_i$ and log potency for each measure were 0.65, 0.58, 0.83 and 0.71 for hypomobility, antinociception, hypothermia and ring immobility, respectively ($P < .05$ for all four correlations). Scatterplots for each regression line are presented in figure 2.

Drug discrimination in rats. As expected, CP 55,940 and Δ^9 -THC produced dose-dependent substitution for CP 55,940 (fig. 3, top left panel) with decreases in response rates occurring at higher doses (fig. 3, bottom left panel). Although the indole-derived cannabinoids are structurally different from both classical and bicyclic cannabinoids, four of the five compounds, including the 2-methyl-*n*-propyl and *n*-butyl indoles (fig. 3, left panel) and the 2-methyl-*n*-pentyl and *n*-hexyl indoles (fig. 3, right panel), fully substituted for CP 55,940. With the exception of the 2-methyl-*n*-pentyl indole, substitution was linear and dose-dependent; however, the 2-methyl-*n*-pentyl indole fully substituted at higher doses. Decreases in response rates, if they occurred at all, were seen

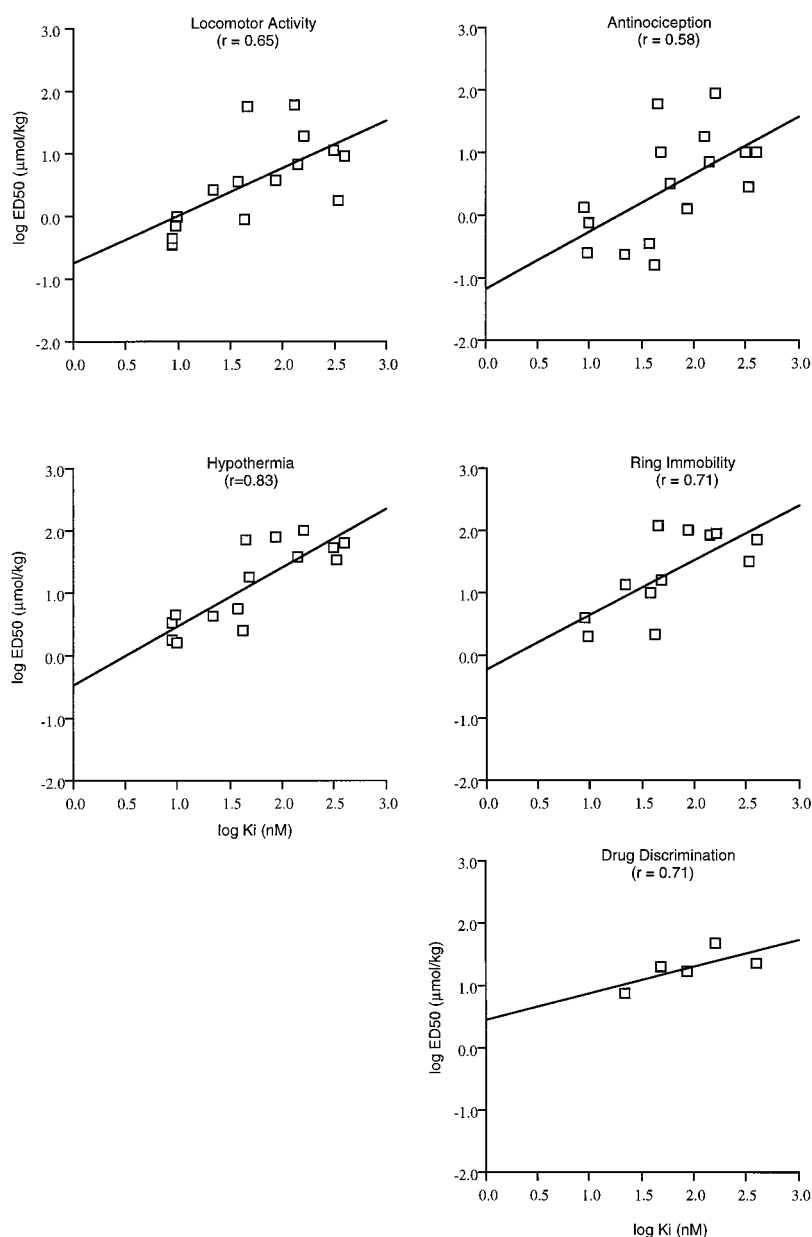


Fig. 2. Scatterplots and regression lines of $\log K_i$ plotted against $\log \text{ED}_{50}$ for each of the five *in vivo* tests (SA, spontaneous activity; MPE, % maximum possible antinociceptive effect; RT, change in rectal temperature; RI, ring immobility; DD, rat drug discrimination).

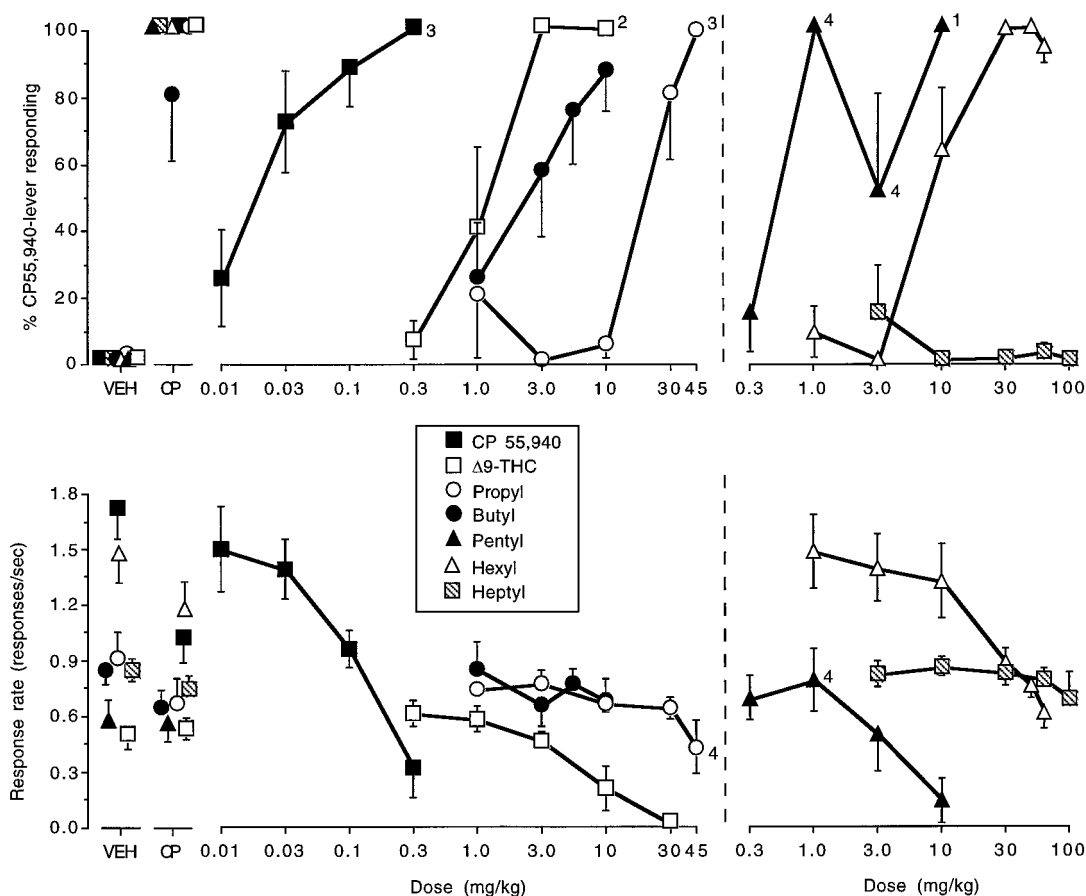


Fig. 3. Effects of CP 55,940, methylated indole-derived cannabinoids and Δ^9 -THC on percentage of CP 55,940-lever responding (upper panels) and response rates (lower panels) in rats trained to discriminate CP 55,940 (0.1 mg/kg) from vehicle. Points above VEH and CP represent the results of control tests with vehicle and 0.1 mg/kg CP 55,940 conducted before each dose-effect curve determination. For all dose-effect curve determinations, each value represents the mean (\pm S.E.M.) of five to eight rats, except as indicated on the graph. ED_{50} values were 0.02 mg/kg for CP 55,940, 1.2 mg/kg for Δ^9 -THC, 15 mg/kg for *n*-propyl, 2.5 for *n*-butyl, <1 mg/kg for *n*-pentyl, 7.3 mg/kg for *n*-hexyl and >100 mg/kg for *n*-heptyl. Values in table 1 are listed in units of micromoles per kilogram.

only at higher doses (fig. 3, bottom panels). In contrast, the 2-methyl-*n*-heptyl indole-derived cannabinoid did not have any effect on percentage of CP 55,940-lever responding or on response rates at doses up to 100 mg/kg (fig. 3, right panels).

In rats trained to discriminate Δ^9 -THC from vehicle, Δ^9 -THC produced dose-dependent substitution with response rate decreases occurring at the higher doses (fig. 4). Similar to the results with the corresponding 2-methyl indoles, pentyl and hexyl pyrrole-derived cannabinoids produced full dose-dependent substitution for Δ^9 -THC without decreasing response rates (fig. 4). Throughout both drug discrimination studies, rats responded predominantly on the injection-appropriate lever during control tests with vehicle, 0.1 mg/kg CP 55,940 and 3 mg/kg Δ^9 -THC (figs. 3 and 4). Rank order potencies were consistent with the rank order of binding affinities of each indole and pyrrole compound (tables 1 and 2, respectively). For drug discrimination, the correlation between $\log K_i$ and $\log ED_{50}$ was 0.71 ($P = .18$) (fig. 2).

Discussion

Molecular modeling studies have suggested that, similar to classical cannabinoids and anandamide, aminoalkylindoles have at least three discrete regions that interact with the CB1 receptor upon binding: (1) the naphthalene

ring (corresponding to the cyclohexene ring of Δ^9 -THC and the polyolefin loop of anandamide); (2) the carbonyl group (corresponding to the phenolic hydroxyl of Δ^9 -THC and the ethanol hydroxyl group of anandamide); and (3) the morpholinoethyl group (corresponding to the carbon side chain at C3 of Δ^9 -THC and the five terminal carbons of anandamide) (Huffman *et al.*, 1994; Thomas *et al.*, 1991, 1996). Previous investigations of the structure-activity relationships of aminoalkylindoles have confirmed the importance of the naphthalene or similar ring structure (e.g., benzofuryl derivatives) at position a (see fig. 1) for *in vitro* and *in vivo* activity (Compton *et al.*, 1992; Eissenstat *et al.*, 1995). All the compounds included in the present study contain this naphthalene ring structure at position a. Manipulation of the carbonyl group of the WIN series has not been examined (position b, fig. 1).

In the present study, the importance of the morpholinoethyl group of aminoalkylindoles was investigated. Eissenstat *et al.* (1995) proposed that, in the aminoalkylindole series, the morpholinoethyl group or another cyclic structure in the same position (position c, fig. 1) is also required for activity. Although indole-derived cannabinoids with substitution of cyclohexylethyl and cyclopropylmethyl, but not phenylethyl, for the morpholinoethyl group of WIN 55,212 retain reasonable CB1 affinity and *in vivo* activity, a cyclic struc-

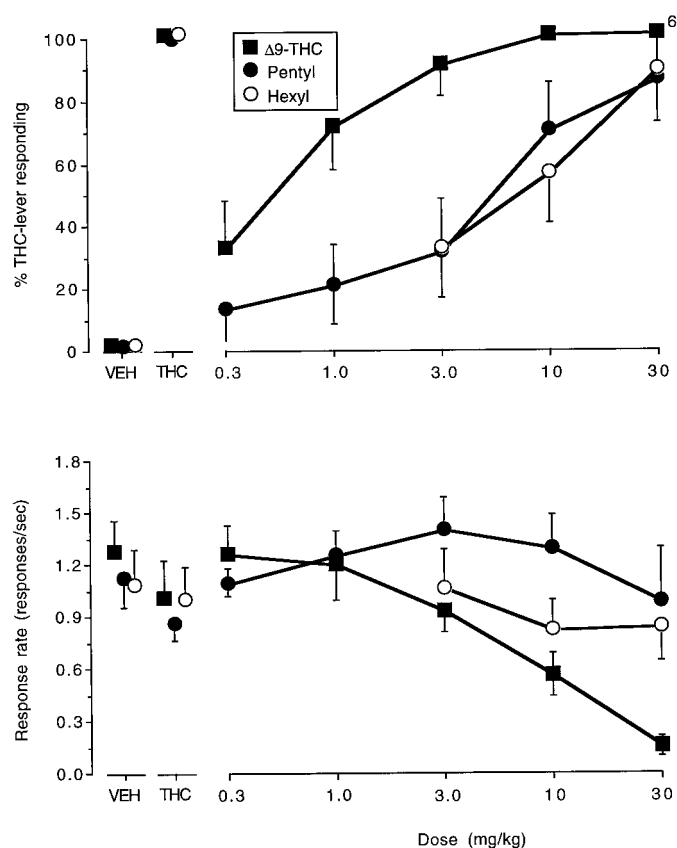


Fig. 4. Effects of Δ^9 -THC and pyrrole-derived cannabinoids on percentage of Δ^9 -THC-lever responding (upper panel) and response rates (lower panel) in rats trained to discriminate Δ^9 -THC (3 mg/kg) from vehicle. Points above VEH and THC represent the results of control tests with vehicle and 3 mg/kg Δ^9 -THC conducted before each dose-effect curve determination. For all dose-effect curve determinations, each value represents the mean (\pm S.E.M.) of seven to ten rats, except as indicated on the graph. ED_{50} values were 0.6 mg/kg for Δ^9 -THC, 4.7 mg/kg for *n*-pentyl and 6.8 mg/kg for *n*-hexyl. Values in table 2 are listed in units of micromoles per kilogram.

ture is unnecessary, in that it may be replaced with an alkyl chain that corresponds more directly in structure to the lipophilic portion of Δ^9 -THC and other classical cannabinoids. As with classical and bicyclic cannabinoids and anandamide (Compton *et al.*, 1993; Ryan *et al.*, 1997; Seltzman *et al.*, 1997), the length of this alkyl chain is important in prediction of binding affinity and *in vivo* potency of both methylated and nonmethylated indoles (table 1). Indoles with short chain lengths (methyl or ethyl) either did not bind to the CB1 receptor or showed only weak cannabinomimetic affinity and activity. Maximal displacement of [³H]CP 55,940 and *in vivo* potency occurred with butyl through hexyl indole derivatives. A similar pattern of length-dependent activity was observed in previous studies in which some of the methylated indoles were tested in Δ^9 -THC discrimination in rhesus monkeys (Wiley *et al.*, 1995a) and in an isolated vas deferens assay in mice (Pertwee *et al.*, 1995). In the indoles lacking a 2-methyl group, the net effect of substitution of hydrogen for the methyl group was to increase the number of carbons needed for maximal binding affinity and potency as compared with corresponding methylated indoles; that is, whereas propyl through hexyl 2-methyl indoles showed reasonable affinity and *in vivo* potency, butyl through heptyl indoles lacking a 2-methyl group showed the greatest activity in the measured

variables. Increasing bulk at the C2 position in aminoalkylindole cannabinoids greatly decreased affinity for CB1 receptors (Eissenstat *et al.*, 1995).

Because high CB1 affinity was seen with the pentyl indole-derived compound in both indole series, we chose this chain length for the addition of a double bond into the side chain. (*E*)-2-Pentenyl and 4-pentenyl analogs of methylated and nonmethylated pentyl indoles were investigated, as was substitution of an allyl group for the propyl of the corresponding methylated indole. All these indole derivatives with more rigid alkyl chains were less active *in vitro* and *in vivo* than their corresponding parent compounds in the original indole series. These results suggest that, although the ability of this alkyl chain to rotate freely is not necessary for cannabinomimetic activity, it is important in predicting potency of indole-derived cannabinoid compounds. The largest decrease in binding affinity was observed in the allyl and (*E*)-2-pentenyl analogs, although *in vivo* potency was less affected by the latter manipulation. The systematic exploration of the effect of increasing the rigidity of the carbon side chain of classical cannabinoids has not yet been reported.

The benzenoid ring attached to the nitrogen-containing group of the indole portion of aminoalkylindole compounds does not correspond to any of the three hypothesized points of attachment and, theoretically, should be unnecessary for cannabinomimetic activity. In an attempt to test this hypothesis, a series of pyrrole analogs of the nonmethylated indole series was prepared (Lainton *et al.*, 1995). One of the effects of this manipulation was to eliminate receptor binding of compounds with short alkyl chains; hence, whereas ethyl and propyl nonmethylated indoles had measurable binding affinity at CB1 receptors, the corresponding ethyl and propyl pyrrole compounds did not, although they were weakly active in some of the *in vivo* tests. For longer alkyl chains (butyl to heptyl), the pyrrole series showed severely decreased affinity for the CB1 receptor (9–74-fold) and usually a decrease *in vivo* potency, although there were minor exceptions. Similar to both indole series, highest binding affinity and potency was observed for the *n*-pentyl pyrrole compound. Cannabinomimetic pyrroles were approximately equipotent in decreasing locomotor activity and producing antinociception; however, a consistent and pronounced separation of activity was observed between potencies for these two measures and their 5- to 37-fold lower potencies for producing hypothermia and ring immobility. A similar separation of activity was observed with a few of the indole-derived cannabinoids [*e.g.*, 2-methyl-*n*-pentyl and *n*-(*E*)-2-pentenyl indoles and the *n*-cyclopropylmethyl indole], although active compounds from both indole and pyrrole series were fully efficacious in all procedures in most instances (exceptions noted on tables).

Despite the structural diversity of these indole- and pyrrole-derived cannabinoids, overall potency at producing the characteristic profile of cannabinoid effects in mice was significantly correlated with binding affinity at CB1 receptors across all series ($r = 0.86$; $P < .05$). Although the overall correlation between potency in the tetrad measures and binding affinity for the indole- and pyrrole-derived cannabinoids was similar to those found for classical and bicyclic cannabinoids (Compton *et al.*, 1993), individual correlations between binding affinity and potency in single measures were lower for these novel compounds. There are a few possible explanations of this discrepancy. First, although a more compounds were included in cal-

culations of the correlations for traditional cannabinoids, greater structural diversity was represented in the present study, because data for both indole- and pyrrole-derived compounds were included. Second, previous research found differences, as well as similarities, between the pharmacological effects of classical cannabinoids, anandamide analogs and aminoalkylindoles. With methods similar to the present study, Compton *et al.* (1992) demonstrated that, although aminoalkylindole analogs produced a similar profile of *in vivo* pharmacological effects as did Δ^9 -THC, the potencies of aminoalkylindoles for suppression of locomotion were greater than their potencies for affecting the other three measures. In contrast, classical cannabinoids were approximately equipotent in affecting all four measures in the mouse tetrad. This separation of activity also was observed in the present study: WIN 55,212–2 showed a 7-fold difference in potency for hypomotility *versus* potency for antinociception and hypothermia as contrasted with a 3-fold potency difference between these same measures for Δ^9 -THC. Active pyrrole cannabinoids (and some indoles) were also more sedating in the locomotor activity assay than in the rectal temperature and ring immobility assays; however, unlike with aminoalkylindole analogs, pyrroles were equipotent in producing effects on spontaneous activity and nociception. In addition, quantitative differences across these pharmacological measures in the degree of cross-tolerance to WIN 55,212–2 in Δ^9 -THC-treated mice were reported (Fan *et al.*, 1994; Pertwee *et al.*, 1993). In contrast to the differences in potencies across measures that were observed with classical and indole-derived cannabinoids, differences in efficacies were seen with anandamide and its analogs. Although equally efficacious in producing antinociceptive and hypomotility effects, anandamide-like cannabinoids decrease body temperature by a maximum of about -3°C in contrast to the -6°C reduction seen with classical and indole-derived cannabinoids (Ryan *et al.*, 1997; Seltzman *et al.*, 1997). Further, although the pharmacological effects of classical and aminoalkylindole cannabinoids in mice were blocked by the CB1 antagonist, SR141716A, this compound did not block the pharmacological effects of anandamide (Adams *et al.*, 1998). These consistent disparities among the potencies and efficacies of *in vivo* effects of the three major classes of cannabinoids suggest fundamental differences in their actions.

Indeed, differences among the classes of cannabinoids in their molecular interactions with cannabinoid receptors have been demonstrated. Song and Bonner (1996) showed that the active isomer of WIN 55,212 did not require a lysine residue for receptor recognition. In contrast, this lysine residue was required for receptor recognition by classical and bicyclic cannabinoids as well as by the endogenous cannabinoid anandamide. These results suggest at least one unique site of attachment for WIN 55,212–2 that is not shared by other cannabinoids or by the cannabinoid CB1 antagonist, SR 141716A (Petitet *et al.*, 1996). In addition, WIN 55,212–2 has higher affinity for peripheral cannabinoid (CB2) receptors than for CB1 receptors in the brain (Showalter *et al.*, 1996). Because the physiological functions of CB2 receptors are unknown, it is possible that agonist action at these receptors may modulate the pharmacological profile of WIN 55,212–2 and other CB2 selective indoles. Future pharmacological studies should concentrate on further delineation of the functional consequences of structural manipulations of cannabinoids within each class and of the molecular differences in interactions with cannabinoid receptors that may underlie these effects.

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