Pharmacology Biochemistry & Behavior, Vol. 40, pp. 471–478. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Behavioral, Biochemical, and Molecular Modeling Evaluations of Cannabinoid Analogs

BILLY R. MARTIN,*[1] DAVID R. COMPTON,* BRIAN F. THOMAS,* WILLIAM R. PRESCOTT,* PATRICK J. LITTLE,* RAJ K. RAZDAN,† M. ROSS JOHNSON,‡ LAWRENCE S. MELVIN,§ RAPHAEL MECHOULAM¶ AND SUSAN J. WARD#

**Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298*
†Organix, Inc., Woburn, MA 01801
‡Glaxo Inc., Research Triangle Park, NC 27709
§Pfizer Inc., Groton, CT 06340
¶Hebrew University of Jerusalem, Israel
#Sterling Research Group, Sterling Drug Inc., Rensselaer, NY 12144

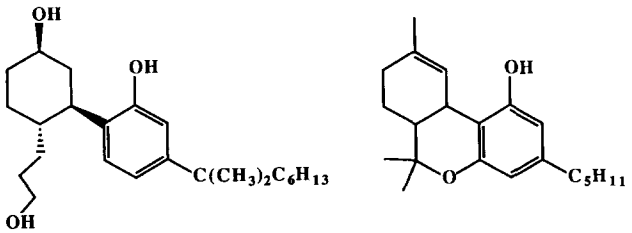
MARTIN, B. R., D. R. COMPTON, B. F. THOMAS, W. R. PRESCOTT, P. J. LITTLE, R. K. RAZDAN, M. R. JOHNSON, L. S. MELVIN, R. MECHOULAM AND S. J. WARD. *Behavioral, biochemical, and molecular modeling evaluations of cannabinoid analogs*. PHARMACOL BIOCHEM BEHAV **40**(3) 471–478, 1991.—Numerous cannabinoids have been synthesized that are extremely potent in all of the behavioral assays conducted in our laboratory. An important feature in increasing potency has been the substitution of a dimethylheptyl (DMH) side chain for the pentyl side chain. Our previous studies have shown that (−)-11-OH-Δ^8-THC-dimethylheptyl was 80–1150 times more potent than Δ^9-THC. Stereospecificity was demonstrated by its (+)-enantiomer which was more than 1400–7500 times less potent. A related series of DMH cannabinoid analogs has recently been synthesized and preliminary evaluations reported here. (−)-11-OH-Δ^9-THC-DMH was found to be equipotent with (−)-11-OH-Δ^8-THC-DMH. The aldehyde (−)-11-oxo-Δ^9-THC-DMH was 15–50 times more potent than Δ^9-THC. Surprisingly, (−)-11-carboxy-Δ^9-THC-DMH was also active, being slightly more potent than Δ^9-THC. In the bicyclic cannabinoid series, the length and bulk of the side chain were found to be equally important. Aminoalkylindoles, which are structurally dissimilar from classical cannabinoids, have been found to exhibit a pharmacological profile similar to Δ^9-THC. Though not extremely potent in vivo, they appear to represent an entirely new approach to studying the actions of the cannabinoids. The structural diversity and wide-ranging potencies of the analogs described herein provide the opportunity to develop a pharmacophore for the cannabinoids using molecular modeling techniques.

Cannabinoid pharmacological profile Dimethylheptyl side chain Bicyclic analogs Aminoalkylindole analogs Molecular modeling Ligand binding

THE cannabinoids produce a complex pattern of behavioral effects which are unique to this class of compounds. Our efforts have been directed toward the design of analogs that may serve as probes for identifying the mechanisms responsible for the behavioral effects of the cannabinoids. We have hypothesized that cannabinoids produce their myriad behavioral effects by altering numerous neurochemical systems. Therefore, one of our objectives has been to develop analogs which have a specific cannabinoid behavioral effect rather than the composite of effects.

It has also been our belief that at least some of the behavioral effects are mediated through a specific cannabinoid receptor, which has led us to search for a cannabinoid antagonist, though no discussion of these data are included here. The relatively recent identification of a cannabinoid binding site has provided the first direct evidence for the long sought after receptor (5). The discovery of this site was accomplished by the use of ^{3}H-CP-55,940 (depicted in Fig. 1), which was subsequently used to determine site distribution within various species in autoradiographic studies (8). The autoradiographic studies contributed to the identification of a previously cloned G protein-linked receptor possessing a similar distribution pattern, and the structure and functional expression of this clone have now been accomplished (18). While there has been considerable excitement regarding the further characterization of this binding site, questions have arisen as to what degree CP-55,940 binding truly represents the site responsible for the behavioral effects of Δ^9-THC. Part of this concern has arisen from the structure of CP-55,940. The two-dimensional depiction of CP-55,940 (Fig. 1) underscores its structural deviation from Δ^9-THC, and thus it has always been referred to as a nonclassical cannabinoid. Additionally, although the acute profile of activity generally resembles that of Δ^9-THC, there are minor differences, and cross-tolerance of CP-

[1]Requests for reprints should be addressed to Billy R. Martin, Ph.D., Box 613 MCV Station, Departments of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0613.



CP 55,940 Δ^9-THC

FIG. 1. Structures of CP-55,940 and Δ^9- THC.

55,940 to Δ^9-THC still remains to be demonstrated.

Although the evaluations of CP-55,940 in production of analgesia (11) and on adenylate cyclase activity (9), as well as its binding characteristics (5,8) and the recent receptor cloning (18) all support the premise that CP-55,940 is acting at a site responsible for the behavioral effects of Δ^9-THC, one of the goals of our laboratory is to more fully characterize pharmacological similarities and differences between CP-55,940 and Δ^9-THC. We have evaluated CP-55,940 (15) and a series of related bicyclic analogs (2) in a battery of behavioral assays designed to distinguish cannabinoids from other classes of drugs. Additionally, these and other cannabinoids are being evaluated in our ^{3}H-CP-55,940 binding assay to determine whether one or more particular pharmacological effects can be correlated to binding affinity. Additionally, as an alternative approach, a radiolabeled ligand with a more typical cannabinoid structure has been synthesized.

Our strategy (Fig. 2) in evaluating newly synthesized analogs for cannabinoid effects is to first determine their ability to produce hypoactivity, hypothermia, antinociception (tail-flick response) and catalepsy (ring immobility) in mice. Subsequently, these analogs are evaluated in rats trained to discriminate between Δ^9-THC (3 mg/kg, IP) and vehicle, as well as for their ability to produce catalepsy in these same animals. Analogs which are weakly potent or fail to produce pharmacological effects are subsequently tested for possible antagonistic properties by pretreating animals with the inactive analog 10 min before administration of Δ^9-THC.

Another goal of our laboratory is to derive a pharmacophore for cannabinoid activity, so the three-dimensional structures of cannabinoid analogs were generated using computer molecular modeling techniques. The three-dimensional structures of cannabinoid analogs have been analyzed by comparative molecular field analysis in order to elucidate quantitative structure-activity relationships (QSAR). The comparison of the steric, electrostatic, and lipophilic properties of structurally diverse compounds to their in vivo and in vitro activities has defined a pharmacophore that can accommodate both classical and nonclassical cannabinoids. This approach allows the prediction of the behavioral potency and binding affinity of unknowns which may serve to facilitate future drug design.

Our efforts have been directed toward the design of analogs that may serve as probes (agonists or antagonists) for identifying the mechanisms responsible for the behavioral effects of the cannabinoids. The behavioral evaluation of novel compounds continues in an effort to more fully characterize pharmacological similarities and differences between aminoalkylindoles, bicyclic cannabinoid analogs (such as CP-55,940) and Δ^9-THC. Biochemical evaluation includes receptor binding analysis with ^{3}H-CP-55,940 and a novel radiolabeled ligand, ^{3}H-(−)-11-OH-Δ^9-THC-DMH. This research is partially aimed at determining the degree to which the binding site reported with CP-55,940 represents a receptor responsible for the behavioral effects of Δ^9-THC.

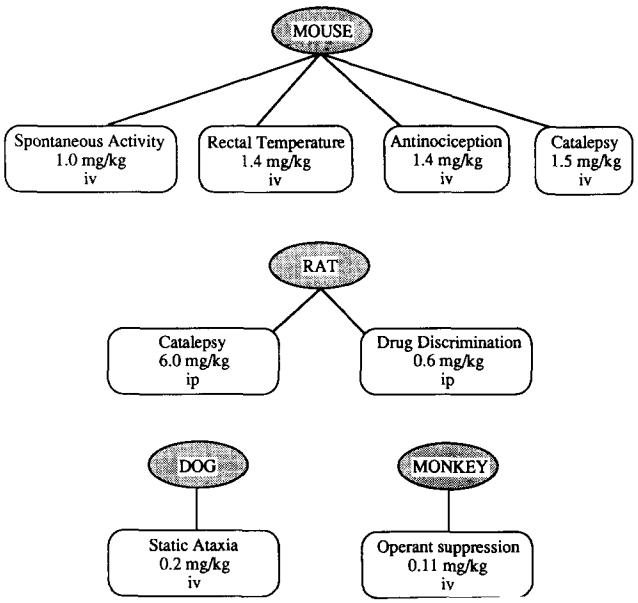


FIG. 2. Pharmacological profile of Δ^9-THC. The ED_{50} values of Δ^9-THC are indicated along with the route of administration.

METHOD

Materials

For in vivo evaluations, male ICR mice (22–30 g) and Sprague-Dawley rats (250–275 g) obtained from Dominion Laboratories (Dublin, VA) were maintained on a 14:10-hour light: dark cycle, and received food and water ad lib. For in vitro ligand binding, male Sprague-Dawley rats weighing 150–200 g were obtained from Dominion Laboratories (Dublin, VA). Δ^8-THC and Δ^9-THC were obtained from the National Institute on Drug Abuse.

Drug Preparation and Administration

The procedure of Olson et al. (21) was used to prepare micellular suspensions suitable for injection, resulting in a final vehicle composition of ethanol:emulphor:saline (1:1:18), which was administered via tail-vein injection (0.1 ml/10 g, IV) to mice or intraperitoneal injection (0.1 ml/100 g, IP) to rats.

Behavioral Evaluations

Locomotor activity (% inhibition), antinociception (tail-flick latency expressed as %MPE), hypothermia (Δ°C), and catalepsy (i.e., ring immobility expressed as % immobility) were evaluated by previously reported methods (3, 4, 15, 16). All of these measures were obtained in the same animal by measuring locomotor activity at 5–15 min, antinociception at 20 min, rectal temperature at 60 min and catalepsy at 90 min after the IV injection. To establish the drug discrimination model in rats, animals were trained to discriminate between vehicle and Δ^9-THC (3 mg/kg, IP) 30 min postinjection. The protocol design was a slight modification (17,23) of the standard two-lever operant

procedure for a FR-10 schedule of food reinforcement (6,10). Catalepsy was measured in rats using ring immobility procedures similar to those for the mouse except the ring diameter was increased from 5.5 to 12.5 cm.

Statistical analysis was performed using ANOVA with Dunnett's *t*-test for comparisons to control and the Scheffe's F test for multiple comparisons. Differences were considered significant at the $p<0.05$ level (two-tailed). The ED_{50} values for agonist activity was determined by unweighted least-squares linear regression of the log dose-probit analysis.

In Vitro Binding Assays

The filtration procedure used for ^{3}H-CP-55,940 binding is a modification of the centrifugation method described by Devane et al. (5). Five rats were decapitated and their cortices rapidly dissected free and homogenized in 30 ml of 0.32 M sucrose which contained 2 mM EDTA and 5 mM $MgCl_2$. The homogenate was centrifuged at $1,600 \times g$ for 10 min, and the supernatant removed. The pellet was washed twice by resuspending in 0.32 M sucrose/2 mM EDTA/5 mM $MgCl_2$ and centrifuging again as described above. The original supernatant was combined with the wash supernatants and centrifuged at $39,000 \times g$ for 15 min. The resulting P_2 pellet was suspended in 50 ml of buffer (50 mM Tris·HCl, pH 7.0, 2 mM EDTA, 5 mM $MgCl_2$) and incubated at 37°C for 10 min before centrifugation at $23,000 \times g$ for 10 min. The P_2 pellet was resuspended in 50 ml of 50 mM Tris·HCl/2 mM EDTA/5 mM $MgCl_2$ and incubated at 30°C for 10 min before centrifugation at $11,000 \times g$ for 15 min. The final pellet was resuspended in 10 ml of 50 mM Tris·HCl (pH 7.4) which contained 1 mM EDTA and 3 mM $MgCl_2$ and then stored at −40°C.

The binding assay was performed in silanized glass tubes which contained 100 μl of radiolabeled ligand, 100 μl of competing unlabeled drug (10 μM final concentration), 150 μg of membrane protein (75 μl) and sufficient buffer [50 mM Tris·HCl, pH 7.4, 1 mM EDTA, 3 mM $MgCl_2$ and 5 mg/ml bovine serum albumin (BSA)] to make a final volume of 1 ml. After a 1-h incubation at 30°C, the reaction was terminated by the addition of 2 ml of ice-cold 50 mM Tris·HCl (pH 7.4) buffer containing 1 mg BSA/ml and rapid filtration through polyethyleneimine-treated Whatman GF/C glass-fiber filters. The reaction tube was washed with a 2-ml aliquot of buffer which was then also filtered. The filters were washed with two 4-ml aliquots of ice-cold buffer. The filters were shaken for 60 min in 10 ml of scintillation fluid, and radioactivity quantitated by liquid scintillation spectrometry. Specific binding was defined as the difference between the binding that occurred in the presence and absence of 10 μM unlabeled ligand.

Molecular Modeling of Cannabinoids

In typical structure-activity studies, the definition of alignment rules for the series of compounds under investigation is critical for generating relevant structural comparisons. Development of molecular alignment rules is somewhat arbitrary, and depends in part upon the chemical intuition of the investigator, but these rules should allow inclusion of noncongeneric molecules. The molecular model of Δ^9-THC was initially generated and energy minimized using the TRIPOS molecular mechanics force field. The minimum energy conformation of Δ^9-THC then served as a template for the alignment of all other cannabinoid analogs. The side chains and aromatic rings of all compounds were overlaid to this template molecule, thereby positioning the molecules in similar spatial orientation. The three-dimensional structures of cannabinoid analogs were analyzed by comparative molecular field analysis (SYBYL, Tripos Associates, Inc., St. Louis, MO) in order to elucidate QSAR.

RESULTS AND DISCUSSION

Pharmacological Profile of Δ^9-THC

The cannabinoids are typical of most centrally acting drugs in that they produce numerous complex behaviors rather than a single effect. The production of these multiple behaviors complicates efforts to identify the mechanism(s) of action of the cannabinoids. Ideally, one would like to be able to study an individual behavioral effect of the cannabinoids in the absence of all other pharmacological effects. On the other hand, multiple behavioral effects enhance the likelihood of determining whether another drug is cannabinoid in nature. Over the past decade we have devoted considerable effort in developing procedures for assessing the pharmacological profile of cannabinoids. Our battery of pharmacological evaluations has proven useful in extending the structure-activity relationships, developing analogs with modified pharmacological profiles and in searching for antagonists.

The outline of our evaluation scheme in Fig. 2 provides a summary of the effects of Δ^9-THC in these procedures. The mouse procedure was developed because an IV injection of Δ^9-THC produced all four behavioral effects in the same dose range. When taken individually, these measures are not selective for any particular drug class. However, the composite of these effects is likely to be more selective for the cannabinoids. This straightforward evaluation has proven to be highly predictive of cannabinoids. We are confident in this procedure, especially when analogs structurally related to the cannabinoids are being evaluated. Additionally, drug discrimination has always been accepted as being predictive of the training drug. The ED_{50} of Δ^9-THC in rats trained to discriminate Δ^9-THC from vehicle is 0.6 mg/kg. Cannabinoids also produce catalepsy in rats (ED_{50} 6.0 mg/kg, IP) which occurs concomitantly with response rate suppression. Other behavioral models which have also proven to be indicative of cannabinoid activity are the dog static-ataxia and monkey operant suppression tests.

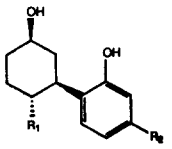
Structure-Activity Relationships of the Nonclassical Bicyclic Cannabinoids

The structure-activity of the the nonclassical cannabinoids has been studied extensively for their antinociceptive properties (12, 20, 26). In the present study, we chose to evaluate the importance of the side chain with regard to its influence on antinociception as well as the other behavioral effects. The data shown in Table 1 represents the average (arithmetic mean) of the potencies in all four of the pharmacological assays in mice. These results demonstrate that the C-4 side chain (R_2) is equally important in the bicyclic structure as it is in the dibenzomorphan structure of Δ^9-THC. Removal of the C-4 side chain ($R_2 = H$; XII) eliminated pharmacological activity. When the 1,1-dimethyl-substituted side chain is four carbon atoms or less in length (Analogs I–III), there is also no pharmacological activity. Increasing the side-chain length to five carbon atoms (Analog V) restores cannabimimetic activity. Optimum cannabimimetic activity was achieved with a 1,1-dimethyl-substituted side chain which was eight carbons in length. A further increase to nine carbons (VIII) decreased the potency to essentially that of Δ^9-THC.

It does not appear that the cyclohexanol substituent at C-4 (R_1) markedly influences cannabimimetic activity. For example,

TABLE 1

PHARMACOLOGICAL POTENCIES OF THE NONCLASSICAL BICYCLIC CANNABINOID ANALOGS



Analog	R_1	R_2	Relative Potency*
I	H	$-C(CH_3)_2CH_3$	<0.03
II	H	$-C(CH_3)_2CH_2CH_3$	<0.03
III	H	$-C(CH_3)_2(CH_2)_2CH_3$	<0.03
IV	H	$-C(CH_3)_2(CH_2)_3CH_3$	0.13
V	H	$-C(CH_3)_2(CH_2)_4CH_3$	0.61
VI	H	$-C(CH_3)_2(CH_2)_5CH_3$	1.89
VII	H	$-C(CH_3)_2(CH_2)_6CH_3$	7.57
VIII	H	$-C(CH_3)_2(CH_2)_7CH_3$	0.916
IX	H	$-C(CH_3)_2(CH_2)_9CH_3$	0.19
X†	$-CH_3$	$-C(CH_3)_2(CH_2)_5CH_3$	1.96
XI	$-CH_3$	$-C(CH_3)_2(CH_2)_5CH_3$	4.82
XII	$-(CH_2)_3OH$	H	<0.06
CP-55,940	$-(CH_2)_3OH$	$-C(CH_3)_2(CH_2)_5CH_3$	6.64
CP-56,667‡	$-(CH_2)_3OH$	$-C(CH_3)_2(CH_2)_5CH_3$	<0.29
XV	$-(CH_2)_4OH$	$-C(CH_3)_2(CH_2)_5CH_3$	3.03

*Average potency (arithmetic mean) of Δ^9-THC in the mouse behavioral assays divided by that of the analog.

†C1 hydroxy in the axial, rather than equatorial, position.

‡Stereoisomer of CP-55,940.

there is little difference between either a methyl (XI) or a propyl hydroxy (CP-55,940) substitution for a hydrogen (VI). Similarly, a butyl hydroxy derivative (XV) is also active.

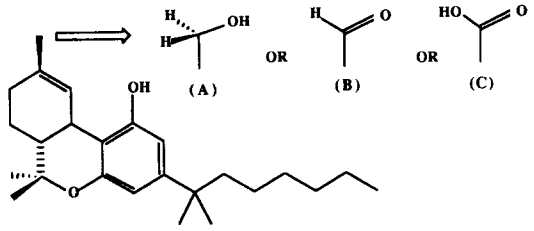
We can conclude from these data that some of these structural modifications, most notably those in the side chain, alter the pharmacological potency without influencing the pharmacological profile. The potency determined in each of the four behavioral evaluations in mice was found to be most easily represented by the average of the ED_{50} values, and a shift in average potency was found to be representative of a similar shift in each of the four evaluations. Similar alterations in the side chain of Δ^9-THC produce comparable changes in pharmacological potency, which supports the contention of a common mechanism for Δ^9-THC and CP-55,940. It is surprising that the substitutions on the C-4 position of the cyclohexanol ring did not exert a greater effect on potency, suggesting this location may be a logical site for future structural modifications when retention of full pharmacological activity is desired.

Pharmacological Activity of the Dimethylheptyl (DMH) Analogs of Δ^8- and Δ^9-THC

Previous evaluations of the enantiomers of 11-OH-Δ^8-THC-DMH have revealed that the (−)-isomer was 79–1143 times more potent than Δ^9-THC (on a mg/kg basis) and that there was a very high degree of stereoselectivity (16,19). Potency comparison to current data on Δ^9-THC (independent dose-response curves generated throughout the year) confirms previous potency data. When potency ratios compared to Δ^9-THC are calculated (on a μmol/kg basis) for each behavior and averaged, the mean

TABLE 2

PHARMACOLOGICAL POTENCY OF DIMETHYLHEPTYL (DMH) ANALOGS OF Δ^8- AND Δ^9-THC



Analog	Relative Potency to Δ^9-THC*
(−)-11-OH-Δ^8-THC-DMH	169
(+)-11-OH-Δ^8-THC-DMH	<0.03
(−)-11-OH-Δ^9-THC-DMH (A)	176
(−)-11-Oxo-Δ^9-THC-DMH (B)	22
(−)-11-nor-9-Carboxy-Δ^9-THC-DMH (C)	2.6

*The potencies (expressed as μmol/kg) of Δ^9-THC and each analog were determined for the four behavioral measures in the mouse. In order to obtain a single relative value, the potency ratios (ED_{50} values) compared to Δ^9-THC were calculated for each individual behavior and the four ratios averaged.

ratio for 11-OH-Δ^8-THC-DMH is 169 times that of Δ^9-THC (Table 2). The properties (potency and stereoselectivity) of this enantiomeric pair make them ideal candidates for further study of the cannabinoid binding site(s). The extremely high potency of 11-OH-Δ^8-THC-DMH is consistent with the profound change in potency that is known to ensue when a DMH side chain is substituted for the five-carbon side chain. Therefore, the potency of (−)-11-OH-Δ^8-THC-DMH was not inconsistent with our present knowledge of cannabinoid structure-activity relationships.

In order to further evaluate the influence of the DMH side chain on cannabinoid potency, DMH analogs of Δ^9-THC were prepared (synthesis to be published elsewhere) which contained either an 11-OH, 11-oxo (aldehyde) or 9-COOH substituent. The summary of these results is presented in Table 2 which reveals that the (−)-11-OH-Δ^9-THC-DMH was 176 times more potent than Δ^9-THC. In addition, the 11-oxo derivative was more potent than Δ^9-THC, although it was considerably less potent than (−)-11-OH-Δ^9-THC-DMH. Not only was it surprising that the C-9 carboxy acid analog was active, but it was actually 2.6 times more potent than Δ^9-THC. Of course, it was almost 70 times less potent than (−)-11-OH- Δ^9-THC-DMH. Additionally, when evaluated in the rat drug discrimination procedure, (−)-11-OH-Δ^9-THC-DMH initially produced response rate suppression, catalepsy and generalization. Yet, at times after injection greater than 24 h this analog produced generalization, but not response rate suppression or catalepsy. Thus this analog was capable of producing a drug cue for periods much longer than that observed for Δ^9-THC, while also producing other CNS effects for much shorter periods.

The relevance of these observations extends beyond merely documenting the influence of the side chain on potency or duration of action. These data actually raise the question of what role the C-11 position plays in cannabinoid action. The importance of the C-11 position has been well documented in the literature devoted to the structural requirements for the cannabinoids. It is quite logical that the C-11 position would be considered one of

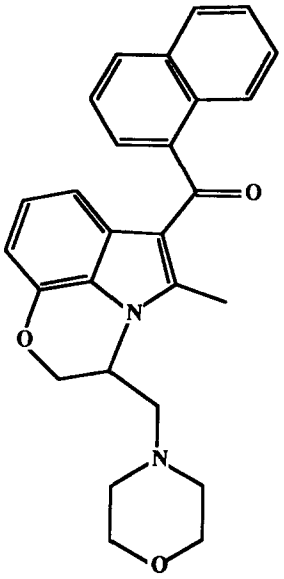


FIG. 3. Structure of WIN-55,212.

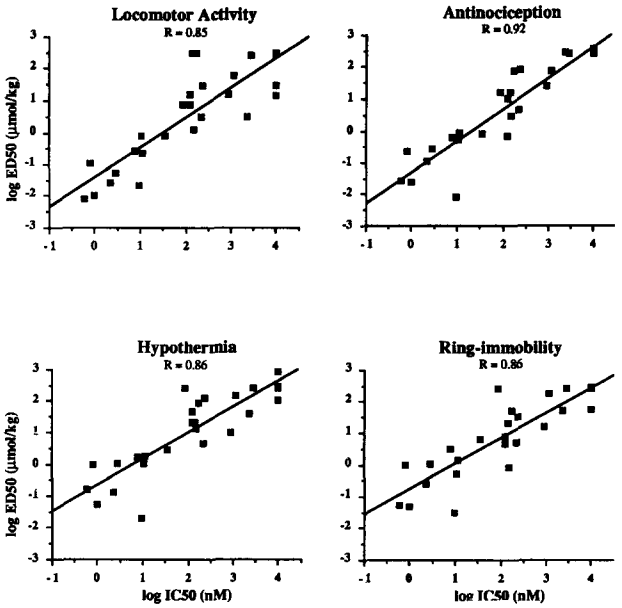


FIG. 4. Correlations between the in vivo and in vitro activities of cannabinoids. The abilities of over 25 cannabinoid analogs to inhibit locomotor activity and produce antinociception (tail-flick latency), hypothermia, and ring immobility (catalepsy) in mice are plotted as the log of their ED_{50}-values (expressed as the μmol/kg dose) versus the log of their IC_{50} values (nM) in the ^{3}H-CP-55,940 ligand binding assay.

the critical sites involved in cannabinoid interaction with a putative receptor. However, it is difficult to conceptualize a ligand-receptor interaction occurring at a critical contact site regardless of whether the substituent is a simple methyl, an alcohol, an aldehyde or a carboxylic acid functional group.

Pharmacological Activity of the Aminoalkylindole (AAI) WIN-55,212

WIN-55,212-2 is a prototypic aminoalkylindole (25), structurally related to pravadoline (7). The ability of WIN-55,212-2 to block nociception does not appear to be related to inhibition of cyclooxygenase, nor can it be explained by opioid mechanisms. Although pravadoline and related AAIs are structurally distinct from Δ^9-THC, results in a cannabinoid ligand binding assay and in an AAI assay utilizing radiolabeled (+)-WIN-55,212 (Fig. 3) as the ligand indicate similar rank potencies and suggest identical binding sites (unpublished observations). Therefore, studies were undertaken to confirm that the AAIs share a common pharmacological profile with Δ^9-THC. The stereoisomers of WIN-55,212 were evaluated in the mouse behavioral assays as well as in the rat drug discrimination procedure. The (+)-isomer exhibited ED_{50}'s of 0.1, 0.4, 12.0 and 1.1 mg/kg in producing hypoactivity, antinociception, hypothermia and ring-immobility, respectively, in mice. The (−)-isomer was inactive up to the highest dose tested (10 mg/kg). The (+)-isomer did generalize from the Δ^9-THC cue in the drug discrimination paradigm despite considerable response rate suppression. It is interesting to note that this enantiomeric pair of drugs represents the first instance where cannabinoid pharmacological activity resides in the (+)-isomer. However, it should also be pointed out that (as with CP-55,940) complete characterization of the AAI class of drugs will also require demonstration of cross-tolerance to Δ^9-THC.

Although the relevance of this structurally diverse class of compounds exhibiting cannabinoid properties remains to be fully established, it is clear that these analogs represent an entirely new approach to the examination of the mechanisms responsible for the effects of Δ^9-THC. In addition, this class of compounds represents an entirely new chemical template for designing cannabinoid agonists and possibly antagonists.

The Correlation Between In Vitro ^{3}H-CP-55,940 Displacement and In Vivo Pharmacological Activity

The ED_{50} values of a series of over 25 cannabinoids have been determined in the four in vivo mouse evaluations (described above) as well as their IC_{50} values for displacing CP-55,940 in the ligand binding assay. Since binding assays do not differentiate between agonists and antagonists, the data obtained with binding assays should always be compared to behavioral activity in order to more clearly evaluate their pharmacological profile. Therefore, compounds devoid of activity (such as Analogs I–III, Table 1) were not included in these correlative studies. The molecular weights of this series of cannabinoids varied from 290 (Analog IV, Table 1) to 554 (compound not shown). Therefore, the in vivo effects of these cannabinoids were expressed as ED_{50} values in μmol/kg units (rather than mg/kg), and in vitro IC_{50} values in nM units. The ED_{50} values of the cannabinoids in each behavioral measure were plotted against the corresponding IC_{50} value for that analog (Fig. 4). The correlation coefficients obtained for these four graphs varied from .85 to .92, which was observed with the antinociceptive measure. Thus there is a reasonably good correlation between the ability of a cannabinoid to bind to the receptor labeled by ^{3}H-CP-55,940 and its ability to produce any one of the behaviors measured. In fact, these evaluations suggest that if any one behavior is produced, then all are produced, and potency can be predicted based upon ligand binding values. Actually, the average ED_{50} value (across all four measures) for each compound may also be plotted against their IC_{50} values, and a similarly good correlation (R = .90 is ob-

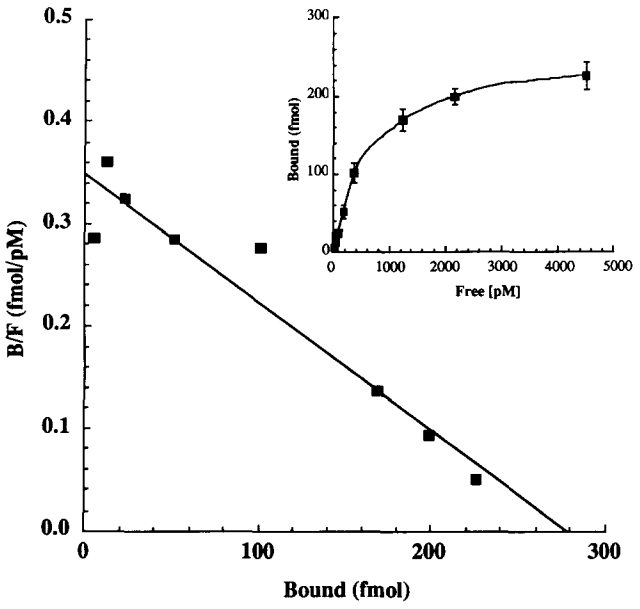


FIG. 5. Binding of ^{3}H-11-OH-Δ^9-THC-DMH to whole brain P_2 membranes.

tained. Therefore, such an average may be used to express the "overall" potency of a cannabinoid in the mouse, and this average used to determine potency ratios relative to the average ED_{50} value of Δ^9-THC (as in Table 1). These data provide further support for the contention that the receptor identified using CP-55,940 is responsible for cannabimimetic activity, though distinction cannot be made between the various behavioral measures.

In Vitro Binding of ^{3}H-11-OH-Δ^9-THC-DMH to Whole Rat Brain Membranes

The synthesis of radiolabeled 11-OH-Δ^9-THC-DMH provided an excellent complement to the approach using ^{3}H-CP-55,940 for studying the cannabinoid binding site. The methods currently being used are simplifications of the filtration assay using ^{3}H-CP-55,940. Preliminary data is being generated using a whole brain P_2 membrane fraction, rather than that of the cortex only. Additionally, all tissue is prepared fresh for each assay, rather than freezing prepared aliquots. The Scatchard analysis of ^{3}H-11-OH-Δ^9-THC-DMH binding (Fig. 5) calculated from the saturation curve (Fig. 5 inset) suggests the presence of a single binding site with a B_{max} of approximately 280 fmol/100 μg protein. The K_D is 1.2 nM, which is nearly ten-times larger than the 133 pM K_D previously reported for the interaction of CP-55,940 to its binding site (5). However, the K_D of 1.2 nM is only twice as large as the K_D obtained in our laboratory of 700 nM. These differences in the K_D's obtained between laboratories may be due to differences in assays, filtration versus centrifugation. Interestingly, the B_{max} obtained with 11-OH-Δ^9-THC-DMH is nearly 50% greater than both the value obtained with CP-55,940 in our laboratory, and that previously reported (5). This difference is observed even when experiments are run concomitantly, whether freshly prepared or frozen tissue is used, or whether whole brain is used rather than cortex alone. Thus the data suggest that 11-OH-Δ^9-THC-DMH is binding to an additional population of binding sites.

Molecular Modeling of Cannabinoids

The three-dimensional structure of Δ^9-THC determined here by molecular modeling and energy minimization is in close agreement with previous structural determinations of this compound by proton magnetic resonance analysis (1) and high resolution NMR spectroscopy (14), as well as with X-ray crystallographic data for Δ^9-tetrahydrocannabinolic acid B (24) and 8β-OH-Δ^9-THC (22). The rotation of the bicyclic ring system in CP-55,940 revealed two energy minima with relatively large energy barriers between these two preferred conformations. This profile of rotational energy is similar to that reported for CP-47,497 using MMI computational methods (13,20). The global energy minimum was determined to be one which placed the oxygen of the hydroxyl group of the cyclohexanol ring 5.1 Å away from the phenolic oxygen. The minimum energy conformations of Δ^9-THC, CP-55,940 and its (+)-isomer (CP-56,667), as well as (+)- and (−)-11-OH-Δ^9-THC-DMH are shown overlaid according to the alignment rules described in the Method section (Fig. 6). Also shown in Fig. 6 (but not aligned) is one minimum energy conformation for WIN-55,212 (other similarly low energy conformations are also obtained via rotation of the morpholino group).

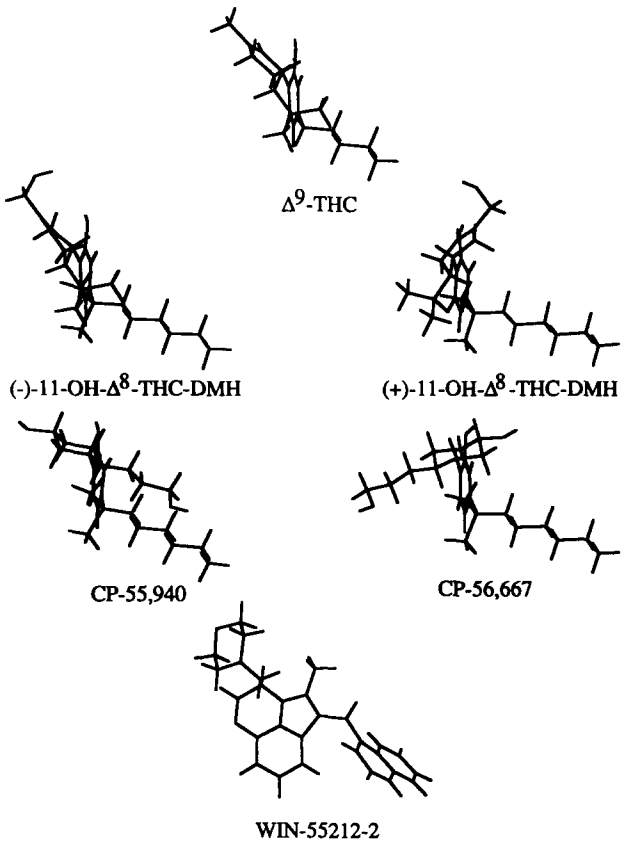


FIG. 6. Minimum energy conformations of cannabinoids. The conformations of these six drugs were minimized as discussed in the Method section. All drugs except WIN-55212 are shown aligned to Δ^9-THC and depicted with the 3-substituent (side chain) projecting out of the page (towards the reader).

The QSAR analysis, based on these and 29 additional cannabinoid analogs, defined similar pharmacophores for behavioral activity and binding affinity that were characterized by high r^2 values. The correlation coefficients (all greater than .83) together with the high cross-validated r^2 values (which assess the robustness of the model) are indicative of a model with good predictive value. These findings also indicate that the molecular alignment

rules (from which the resultant pharmacophores are based) are appropriate for determining QSAR within the cannabinoids. However, it remains to be determined whether compounds lacking the structural features necessary for molecular alignment (e.g., WIN-55,212; Fig. 6) can be accommodated within these cannabinoid pharmacophores. The expansion of the model to include such compounds will serve to further refine the QSAR within the cannabinoid class of compounds.

CONCLUSIONS

The in vivo pharmacological data presented here suggest that the fully active cannabinoid drugs may be tentatively categorized according to chemical structure. Each of these groups produces the full spectrum of effects normally observed with Δ^9-THC. Besides the "traditional" or "classical" cannabinoids such as Δ^8-THC or Δ^9-THC, there are fully active bicyclic compounds such as CP-47,497 and CP-55,940 which have often been referred to as "nonclassical" cannabinoids. It now seems appropriate to also consider drugs like WIN-55,212 to be part of an "AAI" category of cannabinoids, since they too seem to produce the full spectrum of effects. Lastly, there may be reason to consider 11-OH-Δ^9-THC-DMH and related analogs as the "DMH" collection of cannabinoids. Again, though all are fully cannabimimetic, there are reasons for considering each set as somewhat unique. 1) The AAI drugs are clearly of different chemical structure, so much so that the necessary alignment for molecular modeling is unclear. Additionally, these compounds appear to be unusually weak in their production of hypothermia. However, it should be noted that these drugs have not been completely characterized as cannabimimetics, since cross-tolerance to Δ^9-THC has not yet been demonstrated. 2) The "nonclassical" bicyclic analogs are also structurally distinct from traditional cannabinoids, though less so than the AAI drugs. Unlike approaches using Δ^8- or Δ^9-THC, a bicyclic analog from this class has been used successfully to label a cannabinoid binding site. Like traditional cannabinoids, the length of the side chain determines potency in the behavioral assays. However, as with AAI drugs, cross-tolerance to Δ^9-THC has not yet been demonstrated. 3) Establishment of the "DMH" subclass is the most difficult tenet to support, since the basic structure of these analogs is that of Δ^9-THC. However, aside from great differences in potency, the pharmacological data suggest a relatively long time-course of action. Unexpectedly, this long time-course does not apply to all behaviors evaluated. Thus the DMH analogs may be useful in distinguishing mechanisms of action between selected behaviors. Additionally, unlike traditional cannabinoids, analogs of this class show an apparent difference in sensitivity to the effects of substituent groups located at the C-11 position. Lastly, an analog from this tentative subclass has been used successfully to label cannabinoid binding sites, and the ligand identifies a larger number of binding sites than that observed with the "nonclassical" analog CP-55,940.

The in vitro binding data provide further support for the hypothesis that the receptor labeled by CP-55,940 is one related to cannabinoid production of analgesia and all other pharmacological effects evaluated here. Similar studies have not been completed using 11-OH-Δ^9-THC-DMH as the ligand. However, differences between these two assays already exist, and further characterization is needed. This alone suggests that multiple binding assays may be necessary to confidently establish correlations between molecular actions and the wide range of behavioral responses produced by cannabinoids. It has been suggested by others that some or all of the behavioral effects of cannabinoids may be produced by receptor-mediated modulation of adenylate cyclase activity. No data is provided concerning this point. However, the strong correlation between binding and behavioral data suggests that further research is needed to conclusively link second messenger systems with in vivo and in vitro receptor binding activities.

The in vitro and in vivo data provide a good foundation for the prospects of developing a useful pharmacophore of cannabimimetic activity with good predictive capabilities. However, the current model needs to be extended to fully incorporate the AAI compounds, which will only be possible by determining the proper molecular alignments. This problem should be alleviated once a sufficient number of previously and newly synthesized AAI congeners have been evaluated for pharmacological activity. Molecular modeling may then be of use in designing cannabinoid agonists possessing only a portion of the spectrum of effects normally produced by Δ^9-THC, or in designing antagonists.

In summary, a behavioral scheme is presented which has proven useful in determining cannabimimetic activity in a variety of structurally distinct compounds. The behavioral and ligand binding data support the hypothesis that in vivo effects are mediated by a receptor, which can be labeled in vitro by multiple ligands. Further investigations into cannabinoid molecular mechanisms of action should be carefully characterized using analogs from each of the "subclasses" presented here. These data and methodologies should prove useful in developing a pharmacophore of cannabinoid activity, in developing analogs with selective actions, in developing antagonists, and in defining the relevance of second messenger systems to in vivo pharmacological activity. Similarly, the potential to evaluate the relevance of the reported (18) receptor clone (as well as other as yet undiscovered receptor clones not coupled to a G protein) is provided by the wide variety of cannabimimetic agents and methods currently available.

ACKNOWLEDGEMENTS

This research was supported by NIDA grants DA-03672 and DA-05488, and the Commonwealth of Virginia Center on Drug Abuse.

REFERENCES

1. Archer, R. A.; Boyd, D. B.; Demarco, P. V.; Tyminski, I. J.; Allinger, N. L. Structural studies of cannabinoids. A theoretical and and proton magnetic resonance analysis. J. Am. Chem. Soc. 92: 5200–5206; 1970.
2. Compton, D. R.; Johnson, M. R.; Melvin, L. S.; Martin, B. R. Pharmacological evaluation of a series of bicyclic cannabinoid analogs: Classification as cannabimimetic agents. J. Pharmacol. Exp. Ther., in press; 1990.
3. Compton, D. R.; Little, P. J.; Martin, B. R.; Gilman, J. W.; Saha, J. K.; Jorapur, V. S.; Sard, H. P.; Razdan, R. K. Synthesis and pharmacological evaluation of amino, azido, and nitrogen mustard analogues of 10-substituted cannabidiol and 11- or 12- substituted Δ^8-tetrahydrocannabinol. J. Med. Chem. 33:1437–1443; 1990.
4. Compton, D. R.; Martin, B. R. Pharmacological evaluation of water soluble cannabinoids and related analogs. Life Sci. 46:1575–1585; 1990.
5. Devane, W. A.; Dysarz, I. F. A.; Johnson, M. R.; Melvin, L. S.; Howlett, A. C. Determination and characterization of a cannabinoid receptor in rat brain. Mol. Pharmacol. 34:605–613; 1988.
6. Ford, R. D.; Balster, R. L.; Dewey, W. L.; Rosecrans, J. A.; Harris, L. S. Discriminative stimulus properties of Δ^9-THC: Generalization to some metabolites and congeners. In: Agurell, S.; Dewey, W. L.; Willette, R. E., eds. The Cannabinoids: Chemical, pharmacological, and therapeutic aspects. New York: Academic Press;

1984:545–561.
7. Haubrich, D. R.; Ward, S. J.; Baizman, E.; Bell, M. R.; Bradford, J.; Ferrari, R.; Miller, M.; Perrone, M.; Pierson, A. K.; Saelens, J. K.; Luttinger, D. Pharmacology of pravadoline: A new analgesic agent. J. Pharm. Exp. Ther., in press; 1990.
8. Herkenham, M.; Lynn, A. B.; Little, M. D.; Johnson, M. R.; Melvin, L. S.; DeCosta, B. R.; Rice, K. C. Cannabinoid receptor localization in the brain. Proc. Natl. Acad. Sci. USA 87:1932–1936; 1990.
9. Howlett, A. C.; Johnson, M. R.; Melvin, L. S.; Milne, G. M. Nonclassical cannabinoid analgetics inhibit adenylate cyclase: Development of a cannabinoid receptor model. Mol. Pharmacol. 33: 297–302; 1988.
10. Jarbe, T. U.; Hiltunen, A. J. Cannabimimetic activity of cannabinol in rats and pigeons. Neuropharmacology 26:219–28; 1987.
11. Johnson, M. R.; Melvin, L. S. The discovery of nonclassical cannabinoid analgetics. In: Mechoulam, R., ed. Cannabinoids as therapeutic agents. Boca Raton, FL: CRC Press, Inc.; 1986:121–144.
12. Johnson, M. R.; Melvin, L. S.; Althus, T. H.; Bindra, J. S.; Harbert, C. A.; Milne, G. M.; Weissman, A. Selective and potent analgetics derived from cannabinoids. J. Clin. Pharmacol. 21:271S–282S; 1981.
13. Johnson, M. R.; Melvin, L. S.; Milne, G. M. Prototype cannabinoid analgetics, prostaglandins and opiates: A search for points of mechanistic interaction. Life Sci. 31:1703–1706; 1982.
14. Kriwacki, R. W.; Makriyannis, A. The confirmational analysis of Δ^9- and $\Delta^{9,11}$-tetrahydrocannabinols in solution using high resolution nuclear magnetic resonance spectroscopy. Mol. Pharmacol. 35: 495–503; 1989.
15. Little, P. J.; Compton, D. R.; Johnson, M. R.; Melvin, L. S.; Martin, B. R. Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. J. Pharmacol. Exp. Ther. 247:1046–1051; 1988.
16. Little, P. J.; Compton, D. R.; Mechoulam, R.; Martin, B. R. Stereochemical effects of 11-OH-dimethylheptyl-Δ^8-tetrahydrocannabinol. Pharmacol. Biochem. Behav. 32:661–666; 1989.
17. Martin, B. R.; Kallman, M. J.; Kaempf, G. F.; Harris, L. S.; Dewey, W. L.; Razdan, R. K. Pharmacological potency of R- and S-3′-hydroxy-delta 9-tetrahydrocannabinol: Additional structural requirement for cannabinoid activity. Pharmacol. Biochem. Behav. 21:61–65; 1984.
18. Matsuda, L. A.; Lolait, S. J.; Brownstein, M. J.; Young, A. C.; Bonner, T. I. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561–564; 1990.
19. Mechoulam, R.; Feigenbaum, J. J.; Lander, N.; Segal, M.; Jarbe, T. U.; Hiltunen, A. J.; Consroe, P. Enantiomeric cannabinoids: Stereospecificity of psychotropic activity. Experientia 44:762–764; 1988.
20. Melvin, L. S.; Johnson, M. R.; Harbert, C. A.; Milne, G. M.; Weissman, A. A cannabinoid derived prototypical analgesic. J. Med. Chem. 27:67–71; 1984.
21. Olson, J. L.; Makhani, M.; Davis, K. H.; Wall, M. E. Preparation of Δ^9-tetrahydro-cannabinol for intravenous injection. J. Pharm. Pharmacol. 25:344; 1973.
22. Ottersen, T.; Rosenqvist, E. The crystal and molecular structure at -165°C of the 1:1 molecular complex formed by 8β-hydroxy-Δ^9-tetrahydrocannabinol and N,N-dimethylformamide. Acta Chem. Scand. [B] 31:749–755; 1977.
23. Reggio, P. H.; Seltzman, H. H.; Compton, D. R.; Prescott, J. W. R.; Martin, B. R. An investigation of the role of the phenolic hydroxyl in cannabinoid activity. Mol. Pharmacol., in press; 1990.
24. Rosenqvist, E.; Ottersen, T. The crystal and molecular structure of Δ^9-tetrahydrocannabinolic acid B. Acta Chem. Scand. [B] 29:379–384; 1975.
25. Ward, S. J.; Childers, S. R.; Pacheco, M. Pravadoline and aminoalkylindole (AAI) analogues: Actions which suggest a receptor interaction. Br. J. Pharmacol. 98(Suppl.):831P; 1989.
26. Weissman, A.; Milne, G. M.; Melvin, L. S. J. Cannabimimetic activity from CP-47,497, a derivative of 3-phenylcyclohexanol. J. Pharmacol. Exp. Ther. 223:516–523; 1982.