

# PHARMACOLOGY OF CANNABINOID RECEPTORS

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## ABSTRACT

Two subtypes of cannabinoid receptors, CB1 and CB2, have been described to date, although future investigations may elucidate other receptors. The actions of cannabimimetic agents via CB1 receptors in brain are mediated by  $G_{i/o}$  to inhibit adenylyl cyclase and  $Ca^{2+}$  channels. Little is known about signal transduction mechanisms utilized by CB2 receptors. Three classes of agonist ligands regulate cannabinoid receptors: cannabinoid, aminoalkylindole, and eicosanoid derivatives. Cannabinoid receptors produce analgesia and modify cognition, memory, locomotor activity, and endocrine functions in mammals.

## CANNABINOID RECEPTOR SUBTYPES

$\Delta^9$ -Tetrahydrocannabinol ( $\Delta^9$ -THC) is considered to be the predominant compound in preparations of *Cannabis sativa* (marihuana, hashish, bhang) responsible for the CNS effects in humans (1). The recognized CNS responses to these preparations include alterations in cognition and memory, euphoria, and sedation. Potential therapeutic applications of *Cannabis* preparations that are of either historical or contemporary interest include analgesia, attenuation of the nausea and vomiting of cancer chemotherapy, appetite stimulation, decreased intestinal motility of diarrhea, decreased bronchial constriction of asthma, decreased intraocular pressure of glaucoma, antirheumatic and antipyretic actions, and treatment of convulsant disorders. These effects have been reviewed recently (2-5).

Animal behaviors, including drug discrimination, that have been developed for the study of cannabimimetic drugs have been reviewed (6–8). In rodents, low doses of  $\Delta^9$ -THC produce hypermotility and hyperreactivity, and higher doses evoke hypomotility, hypothermia, and a rigid immobility (catalepsy). Antinociception can be observed using a variety of methods in rodents. A static ataxia in dogs is unique to cannabimimetic compounds.

## CB1

The first identified cannabinoid receptor subtype, CB1, was cloned and demonstrated to have an amino acid sequence consistent with a tertiary structure typical of the seven transmembrane-spanning proteins that are coupled to G proteins (9–11). In addition to being found in the CNS, mRNA for CB1 has also been identified in testes (10). The CNS responses to cannabinoid compounds are believed to be mediated exclusively by CB1, inasmuch as CB2 transcripts could not be found in brain tissue by either Northern analysis or in situ hybridization studies (12). CB1 transduces signals in response to CNS-active constituents of *Cannabis sativa* as well as synthetic bicyclic and tricyclic cannabinoid analogs, aminoalkylindole, and eicosanoid cannabimimetic compounds. CB1 is coupled to  $G_i$  to inhibit adenylate cyclase activity and to a pertussis-sensitive G protein to regulate  $Ca^{2+}$  currents. Several recent reviews have described the pharmacology, biochemistry, and CNS distribution of this receptor subtype (2, 13–16).

## CB2

The second cannabinoid-binding seven-transmembrane spanning receptor, CB2, exhibits 68% identity to CB1 within the helical regions, and 44% identity throughout the total protein (12). The CB2 clone was derived from a human promyelocytic leukemia cell HL60 cDNA library. The rat or human CB2 clones were able to hybridize with mRNA in undifferentiated HL60 cells and in HL60 cells that had been differentiated into granulocytes or macrophages. Probes also hybridized with a splenic macrophage/monocyte preparation but not to splenic T cells. Northern blots detected CB2 mRNA from spleen, but not from mature blood neutrophils, thymus, liver, brain, lung, or kidney, indicating that the distribution is distinct from that of CB1. Probes derived from CB1 have identified a signal in splenocytes and mononucleocytes after reverse transcription–PCR amplification (17, 18). Presently unclear are which cell types express CB1 or CB2, and the relative abundance of each subtype within immune or other tissues.

[ $^3$ H]CP-55940 binding observed in membranes from CB2-transfected COS cells demonstrated the cellular production and membrane localization of the expressed protein (12). [ $^3$ H]CP-55940 binding has also been found in membranes from the myeloid cell line U937 (17), a report consistent with the

myeloid localization of CB2 proposed by Munro and colleagues (12). However, Lynn & Herkenham (19), having localized [ $^3\text{H}$ ]CP-55940 binding to B lymphocyte-enriched areas, specifically the marginal zone of the spleen, cortex of the lymph nodes, and nodular corona of Peyer's patches, have hypothesized a B lymphocytic origin of the binding sites. Heterogeneous spleen cell suspensions have also been shown to bind [ $^3\text{H}$ ]CP-55940 (18). It is not readily apparent whether [ $^3\text{H}$ ]CP-55940 binding is associated with CB1 or CB2 in the latter studies.

### *Non-Receptor-Mediated Effects of Cannabinoid Compounds*

As discussed in reviews by Martin (20), Pertwee (21), and Howlett (22), certain *in vitro* effects of cannabinoid drugs may not be mediated by a receptor mechanism. Criteria applied to define receptor-mediated effects include the correlation of the pharmacology with an *in vivo* cannabinoid biological response, enantiomeric specificity for cannabimimetic compounds, and appearance of the effect at drug concentrations consistent with expected concentrations at the site of action during an *in vivo* response.

The ability of lipophilic cannabinoid compounds to interact with biological membranes in a very specific manner has been reviewed (23). This property may be responsible for altered responses of membrane-associated enzymes and proteins, and perhaps for associated cellular effects *in vitro*. Using a series of cannabinoid compounds having a wide range of potencies *in vivo*, researchers demonstrated that the property of lipophilicity (determined by reverse-phase high-performance liquid chromatography) did not correlate with biological activity in mouse behaviors attributable to CB1 (24).

## STRUCTURE-ACTIVITY RELATIONSHIPS FOR CANNABIMIMETIC AGONISTS

### *Cannabinoid Pharmacology*

A large number of cannabinoid agonists, active and inactive metabolites, and related structures are available, and the activities in typical animal models of over 300 such compounds have been compiled by Razdan (8) in a comprehensive review. These studies included the tachycardia and subjective rating of human experience with cannabinoid compounds, behaviors observed in monkeys, static ataxia measured in dogs, and various behaviors determined in rodents. The most extensive structure-activity relationship investigations of cannabinoid compounds *in vivo* have been performed by Martin and coworkers, who used a multiparameter mouse model comprising spontaneous activity in an open field, rectal temperature, antinociception determined by tail-flick latency, and the ring stand test for catalepsy (7). Structure-activity relationship

studies in vitro have been performed by measuring inhibition of adenylate cyclase in the N18TG2 neuroblastoma cell model (13–15, 22).

With respect to a series of the naturally occurring cannabinoid compounds and their derivatives, some generalizations can be made regarding biological activity (see Figure 1).

1. Enantioselectivity can be demonstrated for  $\Delta^9$ -THC and its congeners. The (–)-*trans* isomers of  $\Delta^9$ -THC and  $\Delta^8$ -THC were more potent than the (+)-*trans* (or (+)-*cis*) isomers in tests of monkey behavior; dog static ataxia; and mouse hypothermia, analgesia, and spontaneous activity (25–28). Stereochemical selectivity could be demonstrated both in vivo and in vitro using the pure enantiomers of 11-OH- $\Delta^8$ -THC-dimethylheptyl. In studies using the Martin multiparameter mouse model, the (–)-enantiomer exhibited  $ED_{50}$  values between 4 and 21  $\mu\text{g kg}^{-1}$ , whereas the (+)-enantiomer was inactive at 30 mg  $\text{kg}^{-1}$  (29). Static ataxia in the dog was pronounced at 10  $\mu\text{g kg}^{-1}$  of the (–)-enantiomer but could not be discerned at 1 mg  $\text{kg}^{-1}$  of the (+)-enantiomer (29). Similarly, enantioselectivity was noted in the ability of this pair to bind to brain membrane cannabinoid receptors and to inhibit cyclic AMP production in neuroblastoma cells and membranes (30).

2. The hydrophobic character of the alkyl chain extending from the phenolic ring is important for biological activity. Moderate lengthening or methylation of the 5-carbon chain to the 1,1'-dimethylheptyl or 1,2-dimethylheptyl derivatives of  $\Delta^9$ -THC or  $\Delta^8$ -THC increased biological activity (31–33). In contrast, decreasing the length diminished the biological effects in humans and in animals (34, 35). The length of the  $C_3$  substituent was optimized for an extensive series of derivatives of (–)-9-nor-9 $\beta$ -hydroxyhexahydrocannabinol (HHC) by Johnson et al (36) using antinociception in rodents as the biological measure of cannabimimetic activity.

Hydroxylation along the  $C_3$  alkyl chain of  $\Delta^9$ -THC is a prominent route of metabolism (37–39) and has led to either increased or decreased potency in various animal models (40–43). Hydroxylation at any position along the alkyl chain reduced the potency with which  $\Delta^9$ -THC inhibits adenylate cyclase in N18TG2 membranes (44). Addition of the charged trimethylammonium moiety to the 5' carbon eliminated the spectrum of cannabimimetic biological activities in mice and the characteristic static ataxia in dogs (45).

3. The orientation of the substituent extending from the carbocyclic ring at  $C_9$  is critical for biological activity. Using molecular modeling analyses, Reggio and colleagues (46, 47) determined that the torsion angle of the  $C_{11}$ - $C_9$  bond with respect to the  $C_1$ -O bond was critical for biological activity as defined by behavioral tests in rhesus monkeys. They proposed that a feature common to inactive cannabinoid compounds was the protrusion of the  $C_9$  substituent into the same face of the molecule as the phenolic hydroxyl. Occupancy of this region in space may prohibit activity as the result of steric

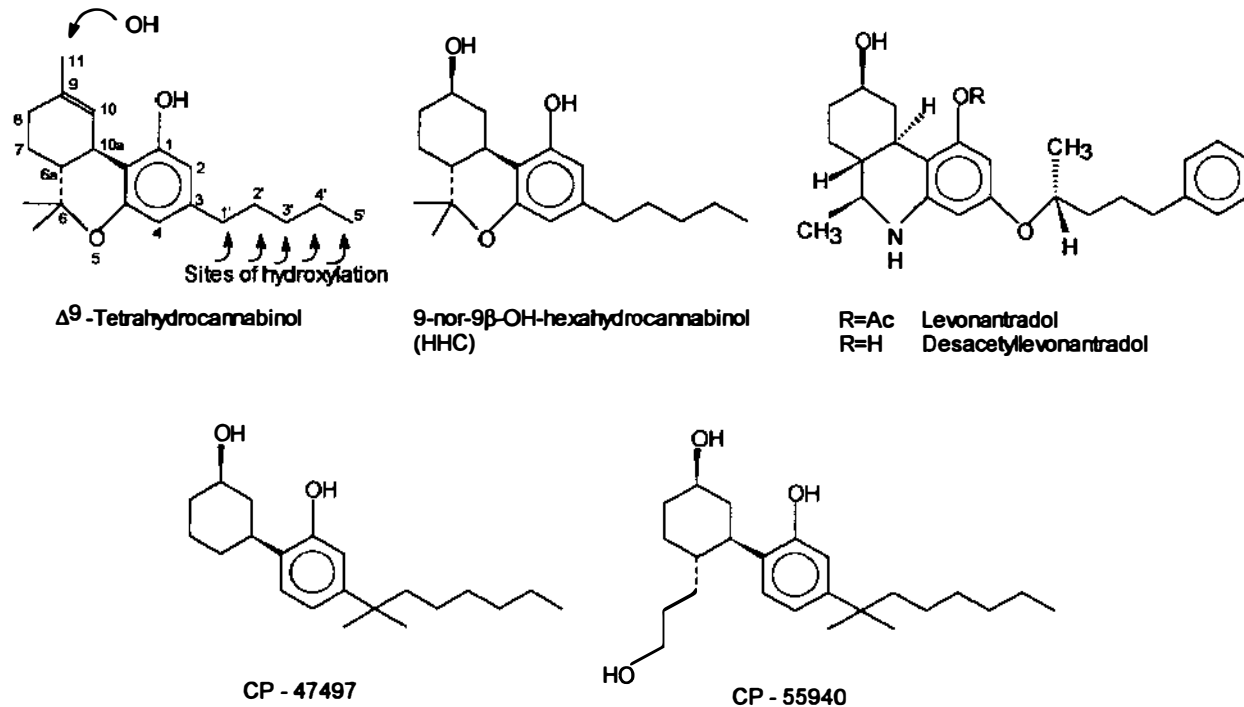


Figure 1 Structures of classical tricyclic and nonclassical bicyclic cannabinoid compounds.

hindrance with some portion of the receptor molecule. Cannabidiol is inactive in humans (48, 49), animal models (6, 28, 33, 41, 50), or CB1-mediated inhibition of adenylate cyclase (44), perhaps owing to an inability to achieve the critical orientation of the carbocyclic ring methyl substituent. Cannabidiol exhibited poor affinity ( $K_i \approx 1\text{--}5 \mu\text{M}$ ) for CB1 in brain membranes (51–52).

4. Hydroxylation of the  $C_9$  substituent on the carbocyclic ring increases biological potency. Hydroxylation at  $C_{11}$  is a metabolic modification of  $\Delta^9$ -THC and  $\Delta^8$ -THC in humans and animals (37–39, 53, 54) that results in an approximately ten-fold increase in biological potency (35, 37, 53–60). Hydroxylation of this position on the carbon extending from the aryl ring of cannabinol converts this relatively inactive compound to one having a potency nearly that of  $\Delta^9$ -THC in the dog static ataxia model (41) and in inhibition of N18TG2 adenylate cyclase (44).

5. The phenolic hydroxyl is an important constituent for cannabimimetic activity. The original observations describing the importance of the phenolic hydroxyl were in the monkey behavior test. In that test, acetylated esters of  $\Delta^9$ -THC and  $\Delta^8$ -THC exhibited less activity than the parent compounds, but methyl ether derivatives were inactive (31, 61). Other complex esters of  $\Delta^9$ -THC exhibited cannabimimetic activity, and yet ether derivatives were inactive (see 8). One might assume that ester linkages could be metabolically hydrolyzed to yield an active compound whereas the ether linkages could not. Sulfation and glucuronidation of  $\Delta^8$ -THC resulted in a loss of activity (62). A series of derivatives of HHC was examined for antinociceptive activity. In this series, elimination of the phenolic hydroxyl resulted in a 400-fold loss of potency and replacement of the hydroxyl with an amine resulted in a 150-fold loss of potency (36). Other substituents ( $\text{CH}_3$ ,  $\text{CH}_2\text{OH}$ ,  $\text{COOH}$ ,  $\text{NHSO}_2\text{CH}_3$ ,  $\text{NHCOCH}_3$ ,  $\text{Cl}$ ,  $\text{SH}$ ) were devoid of antinociceptive activity at the greatest dosage tested (36). One might hypothesize that the phenolic hydroxyl would be important for a hydrogen-bonding interaction with the cannabinoid receptor molecule. Reggio et al (63) proposed that the oxygen might behave as a hydrogen acceptor when oriented properly for hydrogen bonding interactions with the receptor.

Extensive structure-activity relationship studies conducted by Pfizer, Inc. led to the development of a model for ligand interaction with the cannabinoid receptor using antinociception as the biological activity (64, 65). A three-point association between the agonist and the cannabinoid receptor was hypothesized to consist of 1. the  $C_3$  alkyl hydrophobic side chain, 2. the carbocyclic ring  $C_9$  equatorial hydroxyl, and 3. the phenolic hydroxyl. This model was tested by the development of CP-47497, a bicyclic structure that possessed the minimal structural features required for analgesia yet could adopt the three-dimensional orientation that was present in the tricyclic HHC after expending only a slight increase in conformational energy above the predicted minimum (66). In five

tests for antinociception in rodents, the potency of CP-47497 was the same as or greater than that of HHC; it was at least five times that of  $\Delta^9$ -THC (67). In the Martin multiparameter mouse model of cannabimimetic activity, CP-47497 exhibited maximal efficacy for all tests and had potency equivalent to or greater than that of  $\Delta^9$ -THC (68). CP-47497 was five times as potent as  $\Delta^9$ -THC in inhibiting N18TG2 neuroblastoma adenylate cyclase (67); it was as potent as  $\Delta^9$ -THC in binding to CB1 in rat brain membranes (69). A two-dimensional nuclear magnetic resonance analysis (performed in the hydrophobic solvent CDCl<sub>3</sub>) coupled with computer molecular modeling was used to define the energetically favored conformation of CP-47497 (70). It was possible to perfectly superimpose this bicyclic structure over the constrained HHC-dimethylheptyl structure. This study defined a chair conformation for the cyclohexyl ring comparable to the previously proposed slightly flattened chair conformation proposed for the carbocyclic ring of  $\Delta^9$ -THC (71). The position of the C<sub>3</sub> alkyl side chain of CP-47497 appeared to be perpendicular to the plane of the phenolic ring structure, and the phenolic hydroxyl was coplanar with the aromatic ring and pointed away from the cyclohexyl ring (70)—a structure comparable to that previously proposed for  $\Delta^9$ -THC (71).

A comprehensive examination of the requirements for binding to CB1 and for producing analgesia was performed using CP-47497 bicyclic analogs synthesized by Pfizer, Inc. (69). Maximal affinity for CB1 and maximal antinociceptive agonist activity were achieved by a C<sub>3</sub> alkyl side chain seven or eight carbons long (69), a length that was also optimal in the Martin multiparameter mouse model (68). Lengths of less than five carbons or more than ten carbons exhibited relatively poor affinity for CB1 and were devoid of biological activity *in vivo* (68, 69). These data would indicate binding of that region of the ligand within a hydrophobic pocket that exists a critical distance away from the other points of ligand-receptor association. Further extension in that region of the ligand beyond that critical distance would be prohibited either by steric hindrance or by exclusion. CB1 affinity analyses suggested that hydrogen bonding interactions of the receptor with the ligand could occur at the phenolic hydroxyl and at the cyclohexyl hydroxyl but that substitution with other functional groups at the cyclohexyl position was permitted (69).

Building from the cyclohexyl ring of CP-47497, alkyl extensions up to four carbons long had little influence on the affinity for CB1 or on analgesic activity. Hydroxyalkyl side chains exhibited optimal binding affinity and antinociceptive activity at lengths of three or four carbons (69). The hydroxypropyl derivative CP-55940 exhibited optimal CB1 binding affinity, being two to five times as potent as CP-47497. This added functionality led to a 100-fold increase in enantiomeric selectivity for CB1 binding, inhibition of adenylate cyclase, and antinociceptive activity (67, 69); enantiomeric selectivity was increased 30-fold for behaviors in the Martin multiparameter mouse model

(68, 72). CP-55940 has been radiolabeled for radioligand binding studies of CB1 (51) and is now one of the most widely used cannabinoid agonists for analyses of cannabinoid receptor pharmacology, physiology, and neuroanatomy.

### *Aminoalkylindole Pharmacology*

Research conducted at Sterling Research Institute demonstrated that a series of analogs of pravadoline, termed aminoalkylindoles (see Figure 2), act as cannabinoid receptor agonists (73, 74). Pravadoline is of interest because while it exhibited potent antinociceptive activity and was able to inhibit brain cyclooxygenase activity, its spectrum of activities deviated from those of classical nonsteroidal anti-inflammatory agents. Pravadoline exhibited no gastrointestinal cytotoxicity and was not anti-inflammatory (75). The analgesic and smooth muscle relaxation actions of pravadoline were not mediated by opioid receptors—naloxone failed to block these effects (75). Pharmacologic profiles using the assays of inhibition of electrically stimulated or neurotransmitter-induced contractions of mouse vas deferens and guinea pig ileum smooth muscle indicated that pravadoline and several analogs did not act via muscarinic cholinergic,  $\alpha$ -adrenergic, 5-HT<sub>2</sub>, 5-HT<sub>3</sub>,  $\mu$ -,  $\kappa$ -, or  $\gamma$ -opioid, P1-purinergeric, neurokinin-1, bradykinin, or PGE<sub>2</sub> receptors (76).

Structurally constrained analogs of pravadoline have been developed that exhibit greater potency as antinociceptive agents but fail to block prostaglandin synthesis (74, 76). In a structure-activity relationship analysis by D'Ambra and colleagues (74), restraining the amine side chain reduced the analog's ability to inhibit cyclooxygenase activity. Activity in the mouse vas deferens assay was reduced by extending the methyl substituent beyond one carbon in length, which

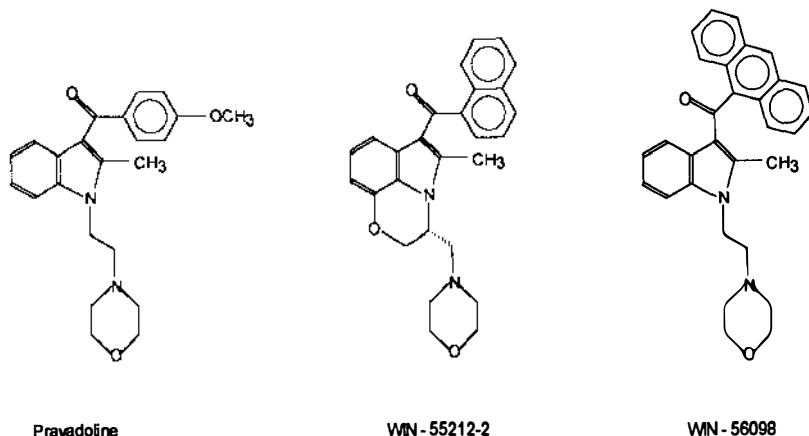


Figure 2 Structures of aminoalkylindole compounds.



contrasts with the requirement for cyclooxygenase inhibition. The racemic mixture of a structurally restricted benzoxazine derivative was three times as potent as pravadoline and exhibited enantioselectivity; the R-(+)-stereoisomer was more than 200 times as potent as the S-(−)-stereoisomer. Replacing the monocyclic 4-methoxybenzoyl group with a bicyclic aryl, as exemplified by the naphthoyl moiety in WIN-55212, increased the potency of the racemate nearly 20 times in both the mouse vas deferens and the antinociception determinations (74, 77). WIN-55212 exhibited enantioselectivity; the  $EC_{50}$  in the mouse vas deferens assay was 0.2 nM for the R-(+)-enantiomer WIN-55212-2, while the S-(−)-enantiomer WIN-55212-3 was inactive at 3  $\mu$ M (74, 77).

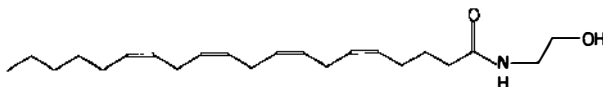
The active aminoalkylindoles exhibit virtually the same spectrum of biological activity as the classical cannabinoid compounds, including antinociceptive activity in rodents and inhibition of the electrically induced twitch response in the mouse vas deferens (75–77). The potency order of several aminoalkylindole analogs in the Martin multiparameter mouse model was consistent with that in the mouse vas deferens assay, with the exception of the test for hypothermia (78). The enantioselectivity ratio for the WIN-55212 pair exceeded 30, and WIN-55212-2 exhibited a potency equal to or greater than that of  $\Delta^9$ -THC for these in vivo tests, with the exception of that for hypothermia. Active aminoalkylindole analogs generalized to  $\Delta^9$ -THC in drug discrimination studies in rats, and WIN-55212-2 was six times as potent as  $\Delta^9$ -THC in this test (78). Active aminoalkylindole agonists produced  $G_i$ -mediated inhibition of adenylate cyclase in rat striatal or cerebellar membranes with a potency order that paralleled their ability to inhibit the electrically stimulated mouse vas deferens contractions (77). The  $EC_{50}$  to inhibit adenylate cyclase for WIN-55212-2 was 0.3  $\mu$ M, and WIN-55212-3 was inactive at 10  $\mu$ M. Cyclic AMP accumulation in cultured rat cerebellar granule cells was also shown to be selective within the WIN-55212 enantiomeric pair (77).

Win-55212-2 was tritium labeled and found to bind specifically to CB1 in rat cerebellar membranes (79). [ $^3$ H]WIN-55212-2-specific binding was displaced by aminoalkylindole analogs in parallel with their potencies in the mouse vas deferens assay. Specific binding was also competitively displaced by  $\Delta^9$ -THC and other CNS-active cannabinoid compounds with potency ratios consistent with their cannabimimetic activities as reported in animals (79). Cannabinoid receptor specificity in the [ $^3$ H]WIN-55212-2 binding site was demonstrated by the failure of ligands from over 20 different neuroreceptor or channel systems to displace binding. Conversely, WIN-55212-2 and other aminoalkylindole analogs failed to displace radioligands selective for at least 20 neuroreceptors (74). Aminoalkylindole analogs were able to displace [ $^3$ H]CP-55940 binding to CB1 with an order of potency that paralleled their ability to inhibit cerebellar adenylate cyclase, inhibit mouse vas deferens contractions, and behave as analgesics in several assays (80).

Within the aminoalkylindole series, WIN-56098 competitively antagonized the effects of pravadoline and WIN-55212-2 in the mouse vas deferens response (77). The potency ratio comparing the presence and absence of 10  $\mu\text{M}$  WIN-56098 was 40 for pravadoline and 100 for WIN-55212-2. By comparison, the potency ratios for  $\Delta^9$ -THC and levonantradol were 6 and 4, respectively, which were significant but not as great as for the aminoalkylindoles. WIN-56098 was also able to shift the curve of inhibition of adenylate cyclase by WIN-55212-2 to the right. However, WIN-56098 failed to antagonize any of the in vivo effects of  $\Delta^9$ -THC in the Martin multiparameter mouse model, including analgesia (78). The relatively low potency of this analog as an antagonist in vitro and its relative selectivity for aminoalkylindole rather than cannabinoid structures suggest that WIN-56098 probably binds to a region of CB1 that would competitively exclude aminoalkylindole agonist-receptor interactions to a greater extent than it would exclude cannabinoid agonist-receptor interactions. The binding of WIN-56098 to CB1 when determined using [ $^3\text{H}$ ]CP-55940 exhibited an  $\text{IC}_{50}$  of greater than 1  $\mu\text{M}$  (AC Howlett, unpublished observations); thus the probability that an adequate concentration of WIN-56098 will reach the sites of action in vivo may be a limiting factor.

### *Eicosanoid Pharmacology*

Arachidonyl ethanolamine amide (Figure 3), generically named anandamide, is an eicosanoid derivative that was initially isolated from porcine brain (81). It was found to displace [ $^3\text{H}$ ]HU-243 from CB1 in membranes from rat brain or rat CB1-transfected CHO cells (81, 82) and to displace [ $^3\text{H}$ ]CP-55940 from CB1 in rat brain membranes or human CB1-transfected L cells (83–85). Arachidonyl ethanolamine amide was independently isolated from calf brain and identified as a regulator of L-type calcium channels (86). Subsequently, ethanolamine amides of homo- $\gamma$ -linolenic and docosatetraenoic acids were identified in organic extracts of porcine brain and found to possess the same affinity for CB1 as did the ethanolamine amide of arachidonic acid (87). Thus, Mechoulam and colleagues (87) proposed that the family of unsaturated fatty acid ethanolamine amides that bind to cannabinoid receptors be referred to collectively as anandamides, and that individual compounds be further iden-



Arachidonyl ethanolamine amide  
[Anandamide (20:4, n - 6)]

Figure 3 Structure of arachidonyl ethanolamine amide.

tified with the parent fatty acid designation following in parentheses—i.e. arachidonyl ethanolamine amide would be termed anandamide (20:4, n – 6).

Arachidonyl ethanolamine amide inhibited mouse vas deferens smooth muscle contraction (81). This eicosanoid behaved as an agonist to attenuate cyclic AMP production in N18TG2 cells and in CHO cells that had been transfected with rat or human CB1 (82, 83). It also inhibited adenylate cyclase activity in membranes from rat cerebellum and striatum as well as N18TG2 cells (84, 85). The attenuation of cyclic AMP accumulation was blocked by pretreatment of the cells with pertussis toxin, and the adenylate cyclase regulation required GTP—a finding consistent with a mediation of the response by  $G_i$  (82, 83, 85). Arachidonyl ethanolamine amide inhibited the  $Ca^{2+}$  current in whole-cell voltage-clamp of the N18 neuroblastoma cells, but this response was less efficacious than that of WIN-55212-2 (55%) (88). Because arachidonyl ethanolamine amide partially antagonized the electrophysiological response to WIN-55212-2, it was proposed to be a partial agonist (88). Arachidonyl ethanolamine amide also appeared to be less effective than cannabinoid or aminoalkylindole agonists in inhibiting adenylate cyclase in some preparations (82, 85). One factor for consideration in the analysis of these studies is that the amide linkage can be cleaved by enzyme(s) present in homogenates of brain and most other tissues (89). This hydrolytic activity could be inhibited by the serine esterase inhibitor phenylmethylsulfonylfluoride (85, 89).

The actions of arachidonyl ethanolamine amide have been examined in rodents and found generally to mimic the effects of  $\Delta^9$ -THC and  $\Delta^8$ -THC (90, 91). In a careful pharmacokinetic analysis using multiple routes of administration, the eicosanoid amide time course was shorter than that of cannabinoid agonists in the Martin multiparameter mouse model (91). Following intravenous administration, maximal efficacy was observed for analgesia, motor hypoactivity, and hypothermia but not for ring immobility. At peak times after intravenous administration,  $EC_{50}$  values for arachidonyl ethanolamine amide were 2–20 times greater than for  $\Delta^9$ -THC in these behavioral determinations (91).

Some structure-activity relationship studies have been performed using ethanolamine amides of arachidonic and other fatty acids. Modification of the arachidonyl moiety by the reduction of one double bond in dihomog- $\gamma$ -linolenic acid, or by the extension of the fatty acid by two carbon units in docosahexaenoic acid, failed to alter potency for binding to CB1, inhibiting cyclic AMP accumulation and reducing the  $Ca^{2+}$  current in neuroblastoma cells (83, 86). However, the existence of six double bonds in docosahexaenoic acid resulted in a 20-fold loss of CB1 binding potency (83). Reducing the length of the fatty acid to 18 carbons but maintaining three double bonds in  $\gamma$ -linolenic acid reduced CB1 binding potency more than 80-fold. The ethanolamine amide of the fully saturated 16-carbon palmitic acid failed to interact with CB1 at

concentrations as high as 1 mM (83). Thus, unsaturation appears to be critical in the fatty acid moiety, and length appears to be restricted to a narrow deviation from the 20-carbon arachidonyl structure.

Modifications of the amide moiety determined that the amide of arachidonic acid was sufficient to allow binding to CB1; however, the ethanolamine amide appeared to be at least 15 times more potent (83, 84). Maintaining the two-carbon spacing between the hydroxyl and the amide nitrogen but adding a methyl group at either carbon appeared to reduce the potency five- to ten-fold (83). The optimal position for the hydroxyl oxygen appeared to be four atoms from the amide nitrogen, but this modification was only slightly more potent than the three-atom length of arachidonyl ethanolamine amide (84). The potency of the longer butanol and pentanol derivatives was lower by approximately one order of magnitude than that of arachidonyl ethanolamine amide, perhaps indicating steric hindrance with increasing lengths of the polar region. That the terminal hydroxyl group is not necessary for interaction with CB1 is demonstrated by the high-affinity binding to the receptor of the N-(2-butyl) and N-(2-propyl) arachidonylamides (84). Consistent with the steric hindrance hypothesis, the N-benzyl and the N-2-[(N-formyl)aminoethyl] derivatives exhibited potencies comparable to those of the longer hydroxyalkyl derivatives. Although the hydrogen bonding potential of the hydroxyl appears not to be a major source of ligand-receptor interaction, the major loss of affinity in substitution of a primary amine for the hydroxyl may be due to a potential field incompatibility or electrostatic charge repulsion from  $\text{-NH}_3^{(+)}$ .

Arachidonyl ethanolamine amide, a relatively flexible aliphatic amide, could adopt many conformations at its active site, including a "hairpin" structure typical of prostaglandins. Ethanolamine amides of  $\text{PGE}_2$ ,  $\text{PGA}_2$ ,  $\text{PGB}_1$ , and  $\text{PGB}_2$  all failed to bind to CB1 at concentrations as high as 100  $\mu\text{M}$  (84). Previous modeling studies had implicated prostaglandin receptors as potential sites at which cannabinoid compounds might act (92). The  $\text{PGE}_2$   $\text{O}_{11}\text{-O}_{15}$  distance and dihedral angle are comparable to the levonantradol  $\text{O}_1\text{-O}_9$  distance and dihedral angle. The cannabinoid agonist desacetyllevonantradol bound to the  $\text{PGD}_2$  receptor in platelets with an  $\text{IC}_{50}$  similar to that for  $\text{PGE}_1$  (about 30  $\mu\text{M}$ ) (93). The failure of the prostaglandin ethanolamine amides to interact with CB1 suggests either that the "hairpin" conformation as it is constrained by the prostaglandin structure is not appropriate for interaction with the receptor, or that the functionalities on the cyclopentyl ring or the hydroxyl along the fatty acid moiety preclude interaction with the receptor.

### *Other Endogenous Agonists*

Ligands characterized for their binding to neuromodulator receptors were screened for their ability to bind to cannabinoid receptors in brain membranes. Of those tested, all neuromodulator agonists or antagonists failed to compete

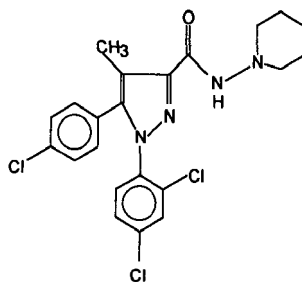
effectively at concentrations at which they would be expected to bind to their identified receptor type (15, 79, 94). Nevertheless, in addition to eicosanoid and other fatty acid derivatives in lipid extracts from brain, other compounds that are endogenous to the brain might also interact with CB1 in a significant manner. Two such leads have been reported (95–97).

Howlett's laboratory followed the premise that an endogenous cannabinoid receptor ligand should have the properties of a neuromodulator being stored in intracellular vesicles. Thus, the ability of increased intracellular  $\text{Ca}^{2+}$  to stimulate release from rat brain slices was examined (95, 96). The  $\text{Ca}^{2+}$  ionophore A23187 released CB1-binding activity in the presence but not in the absence of  $\text{Ca}^{2+}$  in the media (95), a finding consistent with vesicular release. The endogenous CB1-binding activity could also be released in response to a depolarizing stimulus (75 mM  $\text{K}^+$ ) in the presence of extracellular  $\text{Ca}^{2+}$  (96), and this response was reduced by over 50% in the presence of either verapamil or  $\omega$ -conotoxin, blockers of the L-type and N-type  $\text{Ca}^{2+}$  channels, respectively. CB1-binding activity was enhanced by the presence of captopril and thiorphan, peptidase inhibitors that act on angiotensin converting enzyme and enkephalinase. The specificity of the released factor(s) for cannabinoid receptors was corroborated by the ability to compete with both [ $^3\text{H}$ ]CP-55940 and [ $^3\text{H}$ ]WIN-55212-2. Fractions from a semi-purified (reverse-phase high-pressure liquid chromatography) sample of the effluent released from brain slice preparations bound to the cannabinoid receptor and inhibited adenylate cyclase activity (96). The released material was stable to boiling and treatment at acid pH and was able to pass through filters having nominal molecular weight cut-offs of 1000 Da (95). The compound(s) present in the released preparation have elution profiles on reverse-phase high-pressure liquid chromatography that are quite different from those of arachidonyl ethanolamine amide. Of note, captopril and thiorphan failed to increase the apparent affinity of arachidonyl ethanolamine amide in the CB1 binding assay (84).

Preliminary reports from Childer's laboratory have described the isolation of a substance from acid extracts of bovine brain that competes for binding of both [ $^3\text{H}$ ]CP-55940 and [ $^3\text{H}$ ]WIN-55212-2 to CB1 (97). The compound is relatively polar, has an estimated molecular weight between 100 and 500, and appears not to be a peptide. The compound does not have the same chemical or chromatographic profile as arachidonyl ethanolamine amide. These studies suggest the presence of nonlipid substances in the brain that might act as neuromodulatory agonists of CB1.

### *Antagonists*

Studies from Sanofi Recherche elucidated an antagonist ligand, SR141716A (Figure 4), that displayed nanomolar affinity for CB1 but micromolar affinity for CB2 in ligand binding assays (98). SR141716A antagonized the responses



SR141716A

**Figure 4** Structure of SR141716A.

to CP-55940 and WIN-55212-2 in the mouse vas deferens and rat brain adenylate cyclase assays *in vitro*. When orally administered to animals, SR141716A antagonized the hypothermia, ring-immobility, antinociception, and the cannabinoid popcorn effect produced by WIN-55212-2. Thus, this compound appears to be the first of a series of high-potency antagonists for cannabinoid receptors.

### *Pharmacological Studies of CB2*

Very few data are available concerning the pharmacology of CB2. In membranes from COS cells expressing CB2, the cannabinoid [ $^3\text{H}$ ]CP-55940 and the aminoalkylindole [ $^3\text{H}$ ]WIN-55212-2 bound with  $K_d$  values in the 2–4 nM range (12). The order of potency for displacement of one or the other of these ligands was 11-OH- $\Delta^9$ -THC >  $\Delta^9$ -THC = cannabinol > anandamide >> cannabidiol. This pattern generally resembled that for binding to CB1 with the exception that cannabinol bound with about the same affinity ( $K_i \approx 300$  nM) as  $\Delta^9$ -THC in the transfected CB2 receptor system.

Ligand binding studies have been performed using a heterogeneous population of intact mouse spleen cells, but it is not clear whether this reflects CB1 or CB2 binding. Kaminski and coworkers (18) reported binding of [ $^3\text{H}$ ]CP-55940 to intact spleen cells with a  $K_d$  of about 1 nM, but no pharmacological characterization of this binding site was performed. Incubation of heterologous spleen cells with cannabinoid compounds during the five-day sensitization to sheep red blood cells attenuated the development of antibody-forming cells. The pharmacology of this response was not comparable to what would be expected for CB1 because the  $\text{EC}_{50}$  for  $\Delta^9$ -THC was in the range of 10  $\mu\text{M}$ , CP-55940 and 11-OH- $\Delta^8$ -THC-dimethylheptyl ( $\text{EC}_{50}\text{s} \approx 3$   $\mu\text{M}$ ) were not much

more potent than  $\Delta^9$ -THC, and the enantiomeric potency ratio for the 11-OH- $\Delta^8$ -THC-dimethylheptyl pair was only about 5 (18).

Bouaboula and coworkers (17) reported binding of [ $^3$ H]CP-55940 with a  $K_d = 0.1$  nM to membranes from myelomonocytic U937 cells and demonstrated that this binding could be displaced in the nM range by  $\Delta^9$ -THC, CP-55940, and WIN-55225. This displacement pattern differed from that expected for CB1 in that  $\Delta^9$ -THC and CP-55940 exhibited the same apparent affinity for the binding site. However, this laboratory reported the presence of CB1 transcripts, confirmed by restriction-enzyme pattern and sequence analysis, after performing PCR on U937 and other immune-cell types (17). Thus, it is possible that the [ $^3$ H]CP-55940 binding observed in spleen cells, U937, or other immune cells may be to some proportion of both receptor subtypes.

## CANNABINOID RECEPTORS AS G PROTEIN-COUPLED RECEPTORS

### *CB1 Regulation of Adenylate Cyclase via $G_i$*

Given that cannabinoid compounds intercalate into cell membranes and have been reported to alter properties of cellular proteins including G protein-coupled receptors, it is important to demonstrate the distinction between cellular responses directly coupled to cannabinoid receptors and effects that are not receptor mediated. The ability of cannabinoid compounds to inhibit adenylate cyclase in the neuroblastoma N18TG2 cell model is unrelated to influences on membranes or other proteins in the adenylate cyclase pathway (99–101).  $\Delta^9$ -THC,  $\Delta^8$ -THC, and levonantradol as well as its desacetylated derivative produced noncompetitive inhibition of hormone-stimulated adenylate cyclase that reached its maximum at concentrations of 1  $\mu$ M or less (100). Interaction of the cannabinoid compounds with  $\alpha$ 2-adrenergic,  $M_4$ -muscarinic, or  $\delta$ -opioid receptors did not occur because antagonists for those receptors failed to block the cannabinoid response in either the N18TG2 neuroblastoma or the NG108-15 neuroblastoma-glioma hybrid cells (100–102). Other model systems for the study of cannabinoid regulation of adenylate cyclase also exhibit a specific interaction with CB1. Pacheco and coworkers (77) demonstrated cannabimimetic inhibition of basal adenylate cyclase activity in rat cerebellar membranes using potent aminoalkylindole agonists. Neither antagonists for opioid, adrenergic, dopaminergic, and serotonergic receptors nor the cyclooxygenase inhibitor indomethacin were able to block the response.

Biochemical studies demonstrate that  $G_i$  transduces the cannabimimetic inhibition of adenylate cyclase. In membrane preparations from N18TG2 cells (99), desacetyllevonantradol and  $\Delta^9$ -THC reduced adenylate cyclase activity at low mM concentrations of  $Mg^{2+}$ , typical of  $G_i$ . The optimal GTP concentration for inhibition by cannabimimetic compounds was 10  $\mu$ M or greater in

both N18TG2 membranes (99) and rat cerebellar membranes (77). Further, the inhibition of adenylate cyclase was promoted by treatment of the membranes at pH 4.5, which has been demonstrated to decrease the low- $K_m$  GTPase activity and thereby prolong the duration of action of  $G_i$  molecules that have been activated by the receptor (103). Pertussis toxin was shown to deplete functional  $G_{VO}$  proteins in parallel with its ability to prevent the cannabimimetic inhibition of cyclic AMP production in N18TG2 cells and membranes (101) as well as in cerebellar granule cells (104).

CB1 is known to share G proteins and/or effector molecules with other G protein-coupled receptors, as demonstrated by the observation that the maximal response to stimulation of two receptor populations was no greater than that observed owing to stimulation of either one alone. The maximal inhibition of cyclic AMP production obtained with cannabimimetic drugs in cloned N18TG2 cells and membranes was not increased by addition of maximally effective concentrations of muscarinic, adrenergic, or opioid agonists (100, 102). Similarly, Pacheco et al (104) showed that cyclic AMP accumulation was not additively inhibited when either levonantradol or WIN-55212-2 was combined with the GABA<sub>B</sub> agonist baclofen in cerebellar granule cells. This same non-additivity for inhibition of cyclic AMP production was also observed using CNS preparations. Membranes prepared from rat cerebella exhibited a marginally greater maximal inhibition of adenylate cyclase when cannabinoid or aminoalkylindole compounds were combined with the GABA<sub>B</sub> agonist (104). Using rat striatal slices, the interaction of cannabimimetic systems with opioid systems to inhibit cyclic AMP accumulation was neither additive nor synergistic (105). These studies in CNS preparations suggest that these pharmacological receptor types must be colocalized on at least some of the same neurons, and furthermore, that they share a common coupling mechanism.

Multiple  $G_{VO}$  subtypes exist, and it is currently not known which are capable of coupling CB1 to adenylate cyclase or other effectors. The complex behavior of GTP analogs to disrupt the interaction between G proteins and CB1 in detergent solution suggests that this receptor may be associated with more than one G protein, each having a different affinity for GTP analogs (106). Low- $K_m$  GTPase activity stimulated by cannabimimetic drugs has been demonstrated in cerebellar and dentate gyrus membranes (104, 107, 108). In studies of rat cerebellar membranes, the ability of cannabimimetic agonists to stimulate low- $K_m$  GTPase activity was additive with that stimulated by the GABA<sub>B</sub> agonist baclofen (104, 107). This finding suggests either that the pool of  $G_{VO}$  proteins that interacts with CB1 is distinct from that which interacts with the GABA<sub>B</sub> receptors, or that  $G_{VO}$  proteins outnumber the other components of the system. The finding that additivity between pharmacologically distinct receptors is pronounced for regulation of GTPase activity but is minimal or nonexistent for inhibition of adenylate cyclase might suggest that some pool(s) of G proteins are stimulated by the receptors but are not coupled to adenylate cyclase.



The negative effect of  $\text{Na}^+$  on agonist binding to CB1 was first noted in whole-brain P2 preparations using the radiolabeled agonist [ $^3\text{H}$ ]CP-55940 (51) and was also observed in cerebellar membranes using the aminoalkylindole agonist [ $^3\text{H}$ ]WIN-55212-2 (79, 109). However, more careful examination in isolated brain regions revealed that the influence of  $\text{Na}^+$  on the ability of CB1 to couple to  $\text{G}_{\text{I/O}}$  to regulate low- $K_{\text{m}}$  GTPase and adenylate cyclase activities was apparent in the striatum but absent in the cerebellum (109). These findings support the hypothesis that CB1 may interact with different G protein subtypes that have unique sensitivities to  $\text{Na}^+$  in different brain regions or cell types.

### *CB1 Regulation of Ion Channels*

Inhibition of a  $\text{Ca}^{2+}$  channel coupled to CB1 was demonstrated in the differentiated NG108-15 neuroblastoma  $\times$  glioma hybrid cell model (110, 111). Using whole-cell voltage clamp, Caulfield & Brown (110) showed that  $30\text{ }\mu\text{M}$   $\Delta^9\text{-THC}$  and  $1\text{ }\mu\text{M}$  CP-55940 inhibited both peak and end-of-pulse  $\text{Ca}^{2+}$  current by about 40%. Mackie & Hille (111) demonstrated, using a pluronic-nystatin perforated-patch technique, that  $0.1\text{ }\mu\text{M}$  WIN-55212-2 and  $0.1\text{ }\mu\text{M}$  CP-55940 caused a 40% inhibition of the  $\text{Ca}^{2+}$  current. The cannabinoid inhibition of the  $\text{Ca}^{2+}$  current was slow in onset (minutes) and recovery (minutes to hours). Regulation of adenylate cyclase by cannabimimetic compounds was not a factor, because inhibition of the  $\text{Ca}^{2+}$  current was not reversed by addition of the analogs dibutyryl-cyclic AMP and 8-chlorophenylthio-cyclic AMP in the presence of the cyclic nucleotide phosphodiesterase inhibitor isobutylmethylxanthine (111). Coupling via  $\text{G}_{\text{I/O}}$  was demonstrated by the observation that pertussis toxin reduced the response to maximally effective concentrations of  $\Delta^9\text{-THC}$ , CP-55940, and WIN-55212-2 from 40% to less than 10% (110, 111). The  $\text{Ca}^{2+}$  channel was sensitive to  $\omega$ -conotoxin, which reduced the cannabinoid inhibition to less than 10%, implicating the N-type  $\text{Ca}^{2+}$  channel. However, Caulfield & Brown (110) suggested that cannabinoid compounds may also exert other influences on L- or T-type  $\text{Ca}^{2+}$  currents.

Biochemical studies have demonstrated the ability of  $\Delta^9\text{-THC}$  at 10–100 nM to inhibit  $^{45}\text{Ca}^{2+}$  uptake by  $\text{K}^+$ -depolarized synaptosomes prepared from rat cerebellum or brainstem (112). In contrast to the inhibition of depolarization-dependent  $\text{Ca}^{2+}$  levels, Felder et al (113), using the fura-2 technique with CHO cells, demonstrated that micromolar concentrations of the cannabinoid agonists 11-OH- $\Delta^9\text{-THC}$ -dimethylheptyl,  $\Delta^9\text{-THC}$ , and CP-55940 increased intracellular  $\text{Ca}^{2+}$ . This response was not mediated via CB1, however, because identical responses were observed in both control and CB1-transfected CHO cells. Furthermore, the response was not stereospecific. The mechanism is unknown.

Using whole-cell patch-clamp recording from primary cultures of hippocampal cells, Deadwyler and coworkers (108) demonstrated that a voltage-sensitive  $\text{K}^+$  current,  $I_{\text{A}}$ , was modified by cannabinoid compounds whereas other  $\text{K}^+$

currents were not affected. CP-55244, at concentrations surrounding an  $EC_{50} \approx 1 \mu\text{M}$  in the application pipette, decreased the inactivation of  $I_A$  by producing a positive shift in the voltage dependence, and increased the residual  $I_A$  current. WIN-55212-2 ( $2 \mu\text{M}$ ) was as effective as CP-55244, and levonantradol and CP-55940 were only slightly less effective. The opposite enantiomers of these compounds produced no response. The effect of CP-55244 was reversed within minutes of washout. Pretreatment of the cells with pertussis toxin attenuated the effects of cannabimimetic compounds, implicating the involvement of  $G_{i/o}$ . Furthermore, dialysis of cells with the nonhydrolyzable GTP analog GTP- $\gamma$ -S mimicked the effects of cannabinoid drugs, and addition of WIN-55212-2 failed to produce greater effects than those produced by intracellular GTP- $\gamma$ -S. A negative voltage-dependence shift resulted from bath-application of the cyclic AMP analog 8-bromo-cyclic AMP or the adenylate cyclase activator forskolin (114). The response to forskolin was reversed by pressure-injection of WIN-55212-2. These findings are consistent with a mechanism by which the synthesis of cyclic AMP in response to forskolin is inhibited by agonist-stimulated cannabinoid receptors.

### *CB2 Coupling to Effector Systems*

No studies that describe potential signal transduction pathways for the transfected CB2 in CHO cells were reported by Munro et al (12).  $\Delta^9$ -THC at concentrations greater than  $10 \mu\text{M}$  were able to inhibit both forskolin-stimulated cyclic AMP production in spleen cells (115) and NaF-stimulated adenylate cyclase activity in membranes from ML2 human leukemia cells (116). The concentration of  $\Delta^9$ -THC that was required was greater than that required to inhibit cyclic AMP accumulation via CB1 in intact neuroblastoma cells (101, 102). Pretreatment of isolated mouse thymocytes for 10 min with  $\Delta^9$ -THC at  $13 \mu\text{M}$  suppressed the intracellular  $\text{Ca}^{2+}$  release as well as influx of extracellular  $\text{Ca}^{2+}$  in response to Con A stimulation (117). In spite of these intriguing findings, the presence of both CB1 and CB2 in heterogeneous spleen cell populations and various immune cells relegates to the realm of speculation the notion that CB2 is directly coupled to adenylate cyclase or to specific  $\text{Ca}^{2+}$  regulatory mechanisms.

## BIOLOGICAL ACTIONS OF CANNABINOID RECEPTORS

### *Analgesia*

One of the most well-characterized biological actions of cannabimimetic compounds is antinociception (for review, see 64, 65, 118). Data suggesting that the 11-hydroxy metabolites of  $\Delta^9$ -THC and  $\Delta^8$ -THC were more potent in the mouse hot plate test than were the parent compounds (57) led to the development of HHC (see Figure 1) as a prototype cannabinoid analgesic (58, 119).

Research at Pfizer, Inc. (64, 65) extensively examined the structure-activity relationships for analgesia based on HHC, determining that the C-3 alkyl side chain could be optimized by making it longer and that the phenolic hydroxyl was critical for activity. However, because the pyran ring could be modified without extensive loss of potency, the analgesic and antiemetic drug nantradol was developed by replacement of a weakly basic nitrogen for the pyran oxygen and the removal of the axial methyl substituent. The levo enantiomer was approximately two orders of magnitude more potent than the dextro enantiomer (64, 65). This enantioselectivity was subsequently observed in tests of spontaneous activity, hypothermia, and immobility (72) and for regulation of adenylate cyclase *in vitro* (67).

Nantradol possesses an acetyl functionality on the phenolic hydroxyl to confer greater stability. Both levonantradol and its first-pass metabolite des-acetyllevonantradol produce analgesia in various models of antinociception in rodents (120). In this battery of tests, levonantradol was more than 100 times as potent as  $\Delta^9$ -THC, and yet less tolerance developed with nantradol than with  $\Delta^9$ -THC (64). A controlled evaluation of levonantradol in acute, moderate-to-severe postoperative pain in humans showed significantly more analgesic activity than did placebo (121). Drowsiness was the most frequently reported side effect (40% response); fewer than 10% reported various other mild-to-moderate effects, including dry mouth, dizziness, weird dreams, nervousness, headache, hallucinations, and dysphoria (121).

An extensive structure-activity relationship analysis of the antinociceptive activity by Pfizer, Inc. resulted in the development of a series of nonclassical bicyclic and tricyclic structures having a defined pharmacophore for analgesic activity (reviewed in 64, 65). It was subsequently shown that this activity could be attributed to interaction with the CB1 receptor in brain (67, 69, 122). The potency ratios for this series of bicyclic and novel tricyclic structures correlated with that for changes in spontaneous activity, hypothermia, and immobility (68, 72), suggesting that the same receptor is involved in the modulation of these other behaviors.

The mechanisms of action for cannabinoid antinociception include both spinal and supraspinal actions inasmuch as spinalization of rats partially reduced the antinociceptive effect of systemically administered cannabimimetic compounds (123). Intrathecally administered levonantradol,  $\Delta^9$ -THC, 11-OH- $\Delta^9$ -THC, and CP-55940 induced analgesia, as determined by the tail-flick or hot plate tests in rats or mice (123–125). The opioid antagonist naloxone failed to antagonize the cannabinoid analgesia, indicating that  $\mu$  opioid mechanisms were not critical to this response (124, 125). Antinociceptive responses to CP-55940, levonantradol,  $\Delta^9$ -THC, or  $\Delta^8$ -THC could be attenuated by intrathecal norbinaltorphimine, a  $\kappa$  opioid antagonist, but not by the antagonists naloxone and ICI 174,864 acting at  $\mu$  and  $\delta$  opioid receptors, respectively

(126). Cross-tolerance could be demonstrated for  $\Delta^9$ -THC and  $\kappa$  opioid agonists (127), suggesting that a neuronal pathway involving  $\kappa$  opioid receptors may be distal to or converging with a pathway involving cannabinoid receptors in spinal analgesia. Some synergism must also exist in the mechanisms for  $\mu$  or  $\delta$  opioid analgesia with cannabinoid analgesia because intrathecal pretreatment of mice with sub-effective doses of  $\Delta^9$ -THC or several other cannabimimetic compounds was able to left-shift the dose-response curve for intrathecal morphine or enkephalin[D-Pen<sup>2,5</sup>] (DPDPE) in the tail-flick test (124, 126).

The central actions of cannabimimetic analgesics have been demonstrated by tail-flick analgesia in rats after intraventricular injection of either CP-55940 or WIN-55212-2 under conditions in which tracer amounts of [<sup>3</sup>H]WIN-55212-2 were undetectable in the spinal cord (128). The  $\alpha_2$ -adrenergic antagonist yohimbine, administered intrathecally into the lumbar spinal cord, attenuated the antinociception of systemically administered  $\Delta^9$ -THC, suggesting the involvement of supraspinal noradrenergic descending projections to the spinal cord (129).

### *Cognition and Memory*

The human experience with *Cannabis sativa* (130) includes reported euphoria, tranquility, difficulty in thinking or remembering, rapid flow of thoughts, and dreamy states. Perceptual changes reported include altered perception of visual stimuli, auditory stimuli, and duration. Somatic effects reported include dizziness, dry mouth, paraesthesias, increased body awareness, weakness, incoordination, fatigue, and sleepiness. In controlled studies, humans exposed acutely to cannabimimetic compounds exhibit attention deficits and failure to consolidate short-term memory (131–133).

In chronically instrumented behaving rats, Deadwyler and colleagues (134, 135) demonstrated that  $\Delta^9$ -THC impaired a tone discrimination behavior concurrent with a diminished response of the dentate granule cells to incoming information via the perforant path from the entorhinal cortex. Sophisticated analysis of auditory evoked potentials recorded from the outer molecular layer of the dentate gyrus indicated that  $\Delta^9$ -THC disrupted temporally specific information as it was processed by the hippocampus (136). Recording during a delayed match-to-sample task in rats indicated that the impairment of behavior due to  $\Delta^9$ -THC was associated with a specific decrease in hippocampal cell discharge during the sample phase, a decrease that might account for the cognitive deficits associated with cannabimimetic compounds (137).

### *Locomotor Function*

Several brain regions may be associated with the modulation of locomotor control by cannabimimetic agents. The static ataxia that has been described as

a measure of cannabimimetic activity in larger animals such as the dog (7, 27) may be the result of cannabinoid interactions with the receptors present in the cerebellum. The greatest cannabinoid receptor density in the brain exists within the basal ganglia and particularly within those regions that process motor behaviors and regulate sensorimotor information (138–140). The cannabinoid-induced catalepsy in rodents (141) and the synergistic effects of cannabinoids on immobility in reserpinized rats (142) may be regulated in part at the level of the basal ganglia, as evidenced by stereotaxic implantation of  $\Delta^9$ -THC or levonantradol.

Cannabinoid receptors must be involved in the interactive processing of information within the basal ganglia in concert with several other neuromodulators. The hypokinesia induced by reserpinization of rats could be potentiated by sub-effective doses of  $\Delta^9$ -THC, and this effect could be blocked by nicotinic antagonists and mimicked by nicotinic agonists (142, 143). Immobility in mice in response to  $\Delta^9$ -THC was synergistic with cholinergic stimulation, and this response was attenuated by muscarinic antagonists (144). Pretreatment of mice with benzodiazepines was shown to enhance the cataleptic response to  $\Delta^9$ -THC, suggesting an interaction with GABAergic systems (145, 146).

### *Endocrine Actions*

Cannabinoid compounds exert an influence on the hypothalamic regulation of prolactin and gonadotropin release from the pituitary, and these effects have been reviewed recently (147, 148).  $\Delta^9$ -THC has also been shown to depress thyrotropin release (149) and to stimulate corticotropin release (150, 151).

### *Other Biological Actions*

Cannabinoid compounds decrease body temperature (reviewed in 152), and the structure-activity relationships for this response parallel that for other CB1 receptor-mediated effects (52). Cannabinoid compounds also produce changes in heart rate in humans and animals (153, 154), suppress nausea and vomiting (155), and decrease intraocular pressure (156). Cannabimimetic compounds inhibit neuronally mediated smooth muscle contraction in certain tissues (157), a response that can be described by the same pharmacological profile for agonists as exhibited by CB1 in brain responses (77, 158).

## FUTURE DIRECTIONS

Our understanding of how cannabinoreceptive neurons interact with other neuromodulatory systems in the brain to modify behaviors remains limited. At present, little is known about the cellular localization, signal transduction, and biological function of CB2 receptors. This void in our understanding will no

doubt be filled in the near future. The development of cannabinoid receptor antagonists will be of great utility in the pursuit of physiological effects mediated by cannabinoid receptors. Scientists are now beginning to use the pharmacological tools available and to use anatomical and physiological approaches to study cannabinoid pathways in the brain.

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