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Cellular Effects of Cannabinoids*, †

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I. Introduction

CANNABINOIDS produce prominent effects on the central nervous system that have provided marihuana with the dubious distinction of being one of the leading drugs of abuse in current times. The alarm concerning the resurgence of recreational use of marihuana in the 1960s ignited an intensive research effort aimed at understanding all pharmacological aspects of the cannabinoids. This research endeavor has been successful in that the effects of cannabinoids on numerous organ systems, most importantly the brain, have been characterized. Recent efforts have been devoted to identifying potential adverse effects on health that may result from long-term use, much in the same way as for alcohol abuse. One of the most glaring deficiencies in our knowledge of cannabinoid pharmacology is that regarding mechanism of action. While much information has been gathered regarding both biochemical actions and effects of cannabinoids, association of these events into plausible cause-effect relationships has not been forthcoming.

There are numerous factors that may have contributed to this slow progression of understanding. Clearly, pharmacological advances occur more rapidly when the appropriate research tools are in existence beforehand. One problem with cannabinoids is that they represent a unique pharmacological class of compounds. While there are similarities between cannabinoids and other classes of centrally acting drugs, none is sufficiently similar as to allow their use as probes for cannabinoid mechanistic studies. An additional complication with cannabinoids is that they almost invariably alter any system in which they are examined. Traditionally, assignment of "meaningfulness" to pharmacological and biochemical data is based upon the responsiveness of the system to the drug.

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Even this rather straightforward concept is complicated by the physiocochemical characteristics of the cannabinoids. The extreme lipophilicity of these compounds dictates that they be dissolved in hydrophobic solvents for either in vivo or in vitro studies. In addition, they are neutral compounds which precludes formation of water-soluble salts. These properties of cannabinoids disallow an estimate of the concentration of agent at its site of action and hence compromise assessment of responsiveness.

The objective of this review is to critically evaluate our present knowledge regarding possible mechanisms of cannabinoid action at the cellular level. The focus of this discussion will be directed toward the central nervous system, although some peripheral actions will be included. The primary goal is to identify biochemical processes that appear most likely to be involved in the myriad of cannabinoid effects. Further evaluation of the appropriate biochemical system will be essential for understanding the mechanism of cannabinoid action.

II. Interaction with Membrane Components

A. Nonspecific Interactions

Cannabinoids would be expected to have very high lipid/aqueous partition coefficients due to their carbocyclic backbone. Indeed, several investigators have reported that Δ^9-THC‡ will partition to a much greater degree in either octanol or membranes than in aqueous solutions. Gill and Jones (97) reported an octanol/saline partition coefficient of approximately 6000 for Δ^9-THC, while Roth and Williams (244) calculated an octanol/water partition coefficient of about 60,000. Seeman et al. (250a) found a synaptosomal membrane/buffer partition coefficient of approximately 380 which greatly contrasted with that of Roth and Williams (244) who reported a synaptosomal membrane/buffer partition coefficient of 12,477. Regardless of the apparent conflict in the above experiments, it is certain that cannabinoids have a much higher affinity for biomembranes than for aqueous medium. As a result, there has been considerable speculation that many of Δ^9-THC's central effects result from general CNS depression caused by nonspecific neuronal membrane perturbation. Numerous studies have been carried out with artificial membrane systems as well as with intact biomembranes in order to determine whether cannabinoids produce membrane disturbances.

The artificial model systems have been employed in an effort to distinguish between action at the protein and lipid bilayer of membranes. Suspensions of lecithin and cholesterol, prepared ultrasonically, have been shown to consist of spherical vesicles (liposomes) stacked together in biomolecular layers to produce a structure very similar to the lipid phase of cell membranes. Lawrence and Gill (98, 172) used these ultrasonically dispersed lecithin/cholesterol vesicles to find that cannabinoids reduced the order within these bilayers, an effect considered to be consistent with fluidization of membranes. They concluded that the molecular perturbation of liposomes produced by Δ^9-THC was qualitatively similar to that produced by general anesthetics (172). These investigators also pointed out that the disordering of the liposome bilayer produced by Δ^9-THC is far less than that produced by general anesthetics and therefore would not be expected to produce anesthesia. On the other hand, they demonstrated that the nonanesthetic alcohols, hexadecanol and tetradecane, produced effects similar to Δ^9-THC in mice as measured by the ring immobility test. They concluded that Δ^9-THC was able to exert slight perturbation of membranes which was sufficient to account for its behavioral effects but insufficient for producing anesthesia. Efforts were also undertaken to demonstrate that the degree of fluidity induced by a number of cannabinoids would correlate well with their psychoactivity. (−)-Δ^9-THC was more potent than (+)-Δ^9-THC and less potent than either 11-hydroxy-Δ^9-THC or the dimethylheptyl analog of Δ^9-THC. CBD and CBN stabilized the bilayers, an effect opposite to that of Δ^9-THC. This latter observation is interesting given the fact that CBN has weak, THC-like psychoactivity in man (226). In addition, it would be expected that CBD and CBN would have antagonistic activity since their action on membrane fluidity is opposite to that of Δ^9-THC, yet they lack biological antagonistic activity.

The findings of Lawrence and Gill (172) were extended by Tamir and Lichtenberg (261) who demonstrated that cholesterol was essential for the expression of Δ^9-THC-induced changes in egg phosphatidylcholine vesicles. They suggested that the effects of Δ^9-THC resulted from an alteration of the physical properties of the lipid membranes matrix through a cholesterol-dependent mechanism. They concluded that cannabinoids exert their psychotropic effects through this mechanism based upon the correlation between psychoactivity and perturbation of membranes for several compounds. Unfortunately, close scrutiny of this correlation reveals some discrepancies. (−)-Δ^9-THC and its dimethylheptyl analog were most effective in fluidizing membranes, while (−)-Δ^8-THC was somewhat less effective—observations consistent with psychoactivity. However, the dimethylheptyl analog of

‡ Abbreviations used are: ADTN, ^{3}H-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene; cAMP, adenosine 3′,5′-monophosphate; CBC, cannabicyclol; CBCr, cannabichromene; CBD, cannadidiol; CBG, cannabigerol; CBN, cannabinol; CHAPS, [3-(3-cholamidopropyl)dimethylammomia]-1-propane-sulfonate; CNS, central nervous system; DHA, ^{3}H-dihydroalprenolol; DMHP, Δ^{6a-10a}-dimethylheptylpyran; ED_{50}, concentration which, in a dose-response experiment, induces 50% inhibition of cell proliferation; ETYA, 5,8,11,14-eicosatetraynoic acid; GABA, gamma-aminobutyric acid; HETE, 12-monohydroxyeicosatetraenoic acid; HPETE, 12-hydroperoxyeicosatetraenoic acid; ^{3}H-tdr, ^{3}H-thymidine desoxyriboside; IC_{50}, 50% inhibitory concentration; PDBU, phorbol-12,13-dibutyrate; PG, prostaglandins; PMA, phorbol myristate acetate; REM, rapid eye movement; SAR, structure-activity relationships; Δ^9-THC, Δ^9-tetrahydrocannabinol; TMA-THC, 5′-trimethylammonium-Δ^8-tetrahydrocannabinol phenolate; TRH, thyrotropin releasing hormone; TX, thromboxanes.

bezoxocin-4-one and (−)-Δ^8-THC were equally effective in fluidizing the membranes, but (−)-Δ^8-THC was more effective in altering behavior of monkeys. Nabilone, CBD, and the dimethylheptyl analog of (+)-Δ^9-THC produced an effect on membranes opposite to that of the above compounds. While CBD and the dimethylheptyl analog of (+)-Δ^9-THC are devoid of behavioral activity, nabilone is quite effective in producing euphoria in humans (176). These anomalies weaken the argument that membrane perturbation is responsible for psychoactivity.

Bach et al. (9) investigated the interaction of CBD and Δ^9-THC on artificial phospholipid membranes generated from either lecithin or phosphatidyl serine. CBD and Δ^9-THC decreased the electrical resistance of these phospholipid membranes, an effect that would be expected to result in altered ion permeability.

As for intact membranes, Seeman et al. (250a) examined the binding characteristics of Δ^9-THC to synaptosomal membranes prepared from guinea pig brains and found that a concentration of 10 μM resulted in a membrane/buffer partition coefficient of 380. According to the Meyer-Overton rule for local anesthesia, an anesthetizing membrane concentration must be in the order of 30 mmol/kg dry membrane. They found that the membrane anesthetizing concentration of Δ^9-THC was only 3.8 mmol/kg dry membrane weight which was one-tenth of that necessary to satisfy the Meyer-Overton rule of anesthesia.

Bloom and Hillard (30, 120) have also examined the effects of cannabinoids on mouse synaptic plasma membranes. They found that 10 μM concentrations of Δ^9-THC, 11-OH-Δ^9-THC, and CBN produced an increase in membrane fluidity, whereas CBD did not. At 30 μM, Δ^9-THC, CBD, and CBN decreased membrane fluidity, whereas 11-OH-Δ^9-THC produced a further increase. CBD and 11-OH-Δ^9-THC produced similar effects in total lipid extract of mouse synaptic plasma membranes which led them to conclude that membrane proteins were not essential for these effects.

Partitioning studies have also been conducted with nonneural tissue in an attempt to evaluate cannabinoid-induced membrane disordering. Leuschner et al. (179a) and Wing et al. (281a) determined that the concentration of Δ^9-THC was 10 μM in membrane protein of erythrocytes from Δ^9-THC-treated mice. This concentration also produced a decrease in membrane order comparable to that produced by 30 mM concentrations of general anesthetics. Based upon this information, these investigators rejected the theory of volume occupancy as a mode of cannabinoid action, postulating instead a more specific interaction with the lipid, protein, or lipid-protein phases. Wing et al. (281a) suggested that a specific interaction of this nature could result in a conformational change that could alter the overall lipid-protein interactions in membranes. They also suggested that similar effects could occur in brain due to the presence of membrane proteins in brain that are immunologically related to erythrocyte proteins (13, 14). However, direct evidence for these postulated cannabinoid-induced conformational changes in brain is lacking.

B. Specific Interactions

While the foregoing discussion focused on some of the attempts to explain cannabinoid effects as emanating from nonspecific perturbations of membranes, evidence was also presented which suggested that membrane disorder resulted from a specific interaction with the lipid or protein component of membranes. It is interesting that several studies have supported a stereospecific component of Δ^9-THC's effects on membrane order. It has generally been assumed that structural modifications of a molecule have little effect on membrane perturbation and hence anesthetic potency, since a wide variety of molecules, including inert gases, exhibit anesthetic properties. Kendig et al. (161) demonstrated that both (+)- and (−)-isomers of halothane depressed synaptic transmission in isolated cervical sympathetic ganglion, an effect which was taken as an indication of anesthetic potency. The two halothane isomers also did not differ in their ability to increase membrane fluidity as measured in sonicated phosphatidylcholine vesicles. These authors concluded that these findings were consistent with the thesis that membrane perturbation produces anesthesia that is independent of stereospecific molecular configuration. However, it would seem that the membrane perturbation observed with Δ^9-THC is distinct from that produced by anesthetics. Lawrence and Gill (171) argue that the chirality (asymmetry) of the membranes (due to the presence of large quantities of cholesterol and lecithin) is an important factor with molecules larger than halothane. The chirality of membranes might favor interaction with one isomer of a drug. Indeed, they point out that the enantiomers of hexobarbital produce different degrees of CNS depression in the rat (272).

There are numerous components of membrane with which cannabinoids can interact. There are some suggestions that cholesterol may be involved in cannabinoid actions on membrane integrity (36, 171, 220) as well as evidence that Δ^9-THC treatment will lead to decreased concentrations of cholesterol in rat brain (248). The interactions of Δ^9-THC with various phospholipids have been studied in model membranes which led Bruggemann and Melchior (36) to suggest that cannabinoids could form phospholipid complexes which in turn may be modulated by the concentration of cholesterol. There have also been reports that smoking cannabis will alter the concentrations of phospholipids in platelets (148), erythrocytes (147), plasma (166), and in leukocytes (166). However, the significance of these cannabinoid-induced changes remains to be established, particularly as they relate to the cannabinoid mechanism of action. Even though all of the consequences of membrane perturba-

tion are not known, it would be reasonable to expect effects on membrane-associated enzymes.

III. Alterations in Enzymes

A. Adenosine Triphosphatase

Alterations in ATPase activity have implications for almost every biological process due to the energy production afforded by the hydrolysis of ATP. Unlike cannabinoid effects on most other enzyme systems, there is general agreement as to the effects of Δ^9-THC and related compounds on ATPases. In most instances, cannabinoids have been shown to inhibit all ATPases, regardless of the tissue or species, at approximately 50 μM concentration (table 1). In selected cases, 1–10 μM concentrations have effectively inhibited the ATPases. There appears to be little specificity of this inhibition with regard to either the type of ATPase or the cannabinoid. There is clearly lack of correlation between ATPase inhibition and psychoactivity (61, 95, 116, 218).

B. Adenylate Cyclase and Phosphodiesterase

The studies summarized in table 2 show that the effects of cannabinoids on enzymes involved in turnover of cAMP are dependent upon the tissue, dose, or concentration of cannabinoid, as well as the cannabinoid being studied. The numerous dependent variables, when coupled with reported biphasic responses produced by can-

TABLE 1
Effects of cannabinoids on adenosine triphosphatase

Organ or cell type	Cannabinoid	Concentration or dose	Remarks	Ref.
Rat brain	Δ^9-THC	1–2 mg/kg	NaK-ATPase from synaptic membranes was inhibited 20 min after injection.	175
Rat brain	Δ^9-THC, CBD	1–1000 μM	CBD was 3 times more potent than Δ^9-THC in depressing Mg-ATPase from synaptic vesicles. Δ^9-THC at 10 μM produced 43% inhibition.	95
Mouse brain	Δ^9-THC, CBD	50 μM	Both inhibited NaK-ATPase and Mg-ATPase by 40%.	116
Mouse brain	Δ^9-THC	1–40 μM	NaK- and MgCa-ATPases were inhibited in all subcellular fractions. Mg-ATPase was stimulated in the crude mitochondrial and synaptosomal fractions but inhibited in the microsomal fraction.	29
Rat brain	Δ^9-THC	3 μM	Inhibited NaK-ATPase by 50%. Also inhibited electric eel NaK-ATPase activity.	264
Mouse cardiac microsomes	Δ^9-THC, CBD, DMHP	1–100 μM	CBD > Δ^9-THC > DMHP in inhibiting microsomal Ca-ATPase. Only CBD inhibited at 1 μM. The authors noted that this order of potency did not correspond to that for decreasing heart rate and blood pressure. Concluded that cannabinoid activity is related to a membrane interaction.	61
Rat ileum microsomes	Δ^9-THC	10 μM	Completely abolished NaK-ATPase activity and decreased Ca-ATPase activity by 60%. Concluded that this effect was likely due to a general action of THC on membranes.	170
Mouse brain microsomes	Δ^9-THC	10 mg/kg	Increased ATPase activity by 27% 15 min after treatment. Suggested that increased ATPase activity would stimulate neurotransmitter reuptake which could account for sedative properties of cannabinoids.	135
Rat brain	Δ^9-THC	3 μM	Δ^9-THC inhibition (49%) of NaK-ATPase was potentiated by addition of cardiolipids.	265
Rat brain synaptosomes	Δ^9-THC, CBN, 11-OH-Δ^9-THC, olivetol	8–30 μM	Δ^9-THC (16 μM) inhibited NaK- and Mg-ATPase by ≈50%. CBN and Δ^9-THC were equipotent, and 11-OH-Δ^9-THC was only one-half as active. Olivetol was inactive.	218

TABLE 2

Effects of cannabinoids on adenylate cyclase activity and cAMP levels

Organ or cell type	Cannabinoid	Concentration or dose	Remarks	Ref.
Rat heart homogenates	Δ^9-THC, Δ^8-THC	200 μM	Δ^9-THC (but not Δ^8-THC) decreased adenylate cyclase activity which led to decreased cAMP levels.	181
WI-38 fibroblasts	Δ^9-THC	0.32–16 μM	Δ^9-THC anatagonized the PGE_1- and epinephrine-induced elevation in cAMP levels.	159
Human fibroblasts	Δ^9-THC, CBD, CBN, CBC	0.2–22 μM	Δ^9-THC reduced cAMP accumulation in response to prostaglandins and catecholamines. However, long-term incubation resulted in increased accumulation of cAMP. The effects of CBD were similar to those of Δ^9-THC, whereas CBN and CBC provided mixed results. Authors suggested that at low concentrations Δ^9-THC might act as a weak agonist of cAMP accumulation and as an antagonist at high concentrations.	160
Mouse brain	Δ^9-THC	0.1–10 mg/kg i.p.	Δ^9-THC produced a biphasic effect on cAMP levels in whole brain and brain areas. Doses of 0.1–1.0 mg/kg caused a 50–160% increase in levels, whereas doses of 2–10 mg/kg decreased cAMP levels 30–60%. Concluded that this biphasic effect of Δ^9-THC correlated with effects on biogenic amines, temperature regulations, and behavior.	79
Rat brain areas	Δ^9-THC, Δ^8-THC	10 mg/kg i.v.	Δ^9-THC had no effect on cAMP levels in any brain area. Δ^8-THC increased cAMP levels in midbrain only. Δ^8-THC also decreased both adenylate cyclase and phosphodiesterase activity in midbrain. Δ^9-THC was not studied in these brain areas.	8
Mouse brain	Δ^9-THC, 11-OH-Δ^9-THC, CBN, CBD, levonantradol, dextronantradol	1–100 μM	All of the compounds, except dextronantradol, increased adenylate cyclase activity at 30 μM. This effect appeared to result from increased prostaglandin production. Lack of correlation with psychoactivity suggests that these cannabinoid actions are involved in effects other than behavior.	118a
Mouse brain	Δ^9-THC, 11-OH-Δ^9-THC, CBN	0.25–10 mg/kg	Δ^9-THC (0.25 mg/kg) stimulated adenylate cyclase activity by ≈20%. CBN (10 mg/kg) was without effect. None of the cannabinoids (212 μM) decreased phosphodiesterase activity.	80
Neuroblastoma cells	Δ^9-THC, levonantradol		Both compounds decreased prostacyclin-induced accumulation of cAMP as a result of decreased adenylate cyclase activity. Phosphodiesterase activity was unaffected.	130
Neuroblastoma cells	Δ^9-THC, Δ^8-THC, CBN, CBD, levonantradol, dextronantradol, desacetyllevonantradol	1 μM	Adenylate cyclase activity was inhibited by Δ^8- and Δ^9-THC, levonantradol, and desacetyllevonantradol. This inhibition did not involve the prostanoid, muscarinic, $alpha_2$-adrenergic, or opiate receptors. A reversal of the cannabinoid inhibition was observed between 3 and 10 μM.	132
Neuroblastoma cells	Δ^9-THC, desacetyllevonantradol	0.002–2.0 μM	At 2 μM, Δ^9-THC and desacetyllevonantradol produced a 21 and 48% inhibition of adenylate cyclase, respectively. The characteristics of this inhibition were similar to that of muscarinic cholinergic agents which led to the suggestion that the cannabinoid inhibition was receptor mediated.	131

nabinoids, make it difficult to ascertain the role of cAMP in cannabinoid action. Any alterations in cAMP levels most likely result from either stimulation or inhibition of cyclic nucleotide adenylate cyclase, since cannabinoids do not appear to alter phosphodiesterase activity. Δ^9-THC and related compounds have been reported to stimulate adenylate cyclase activity in brain (80, 118a), which is consistent with the reported elevation in cAMP levels following low doses of Δ^9-THC (79). However, there is not total agreement regarding the effect of cannabinoids on cAMP levels. Askew and Ho (8) reported no change in brain concentrations of cAMP by Δ^9-THC, whereas Dolby and Kleinsmith (79) found an elevation in cAMP levels following low doses of Δ^9-THC and a decrease after high doses of Δ^9-THC.

There are reports that low concentrations of cannabinoids decrease adenylate cyclase activity in neuroblastoma cells, whereas higher concentrations stimulate activity (130, 131). Howlett and Fleming (132) have suggested that inhibition of adenylate cyclase by low concentrations of cannabinoids represents a pharmacologically specific, receptor-mediated event, whereas the effects produced by higher concentrations of cannabinoids result from a nonspecific membrane perturbation. Cannabinoid inhibition at low concentrations was similar to that produced by muscarinic agents in that both were Mg^{2+} and GTP dependent (131).

C. Miscellaneous Enzymes

There are numerous enzymes, several of which are presented in table 3, that are inhibited by cannabinoids. The purpose of compiling this list is to demonstrate the diversity of enzymes that can be influenced by the cannabinoids. The question arises as to how cannabinoids alter the activity of all of these enzymes. While there have been no apparent attempts to explore such a general aspect of the cannabinoids, its ubiquitous nature implies a nonspecific mechanism, such as membrane perturbation.

IV. Macromolecular Metabolism

The in vitro studies concerning the biological activities of cannabinoids on macromolecular metabolism (e.g., DNA, RNA, protein) were based on results from in vivo experiments utilizing normal and neoplastic cells. In general, the in vitro experiments demonstrate that certain cannabinoids produce a marked concentration-related inhibition of one or more steps involved in macromolecular metabolism. Only a brief description of these findings will be discussed here because this area was reviewed recently by Munson and Fehr (208).

A brief synopsis of the in vitro studies of cannabinoid effects on macromolecular events is presented in table 4. It is apparent that cannabinoids can inhibit macromolecular events in a wide variety of cells and tissues. The concentrations of cannabinoids used in these studies were in the μM range. A limited number of studies have dealt with the biological activities of various cannabinoid derivatives. These studies demonstrated that changes on the pyran ring (as in the case of CBD), or the lack of a free phenolic hydroxyl group, to name a few, decreased or abolished biological activity (50, 234). However, these studies concerning structure-activity relationships strongly suggest that the macromolecular events described in these types of studies are not related to the central nervous system activities observed in vivo, since behaviorally inactive cannabinoids, such as CBN and abnormal Δ^8-THC, were shown to be quite active in altering macromolecular synthesis (50).

Specific aspects of macromolecular metabolism have been studied in vitro yielding complex results. Cannabinoids have been shown to inhibit DNA, RNA, and protein synthesis in specific ways depending upon the system under examination. Results from Blevins' laboratory (23–25) demonstrated that cannabinoids selectively inhibited semiconservative DNA synthesis while not affecting DNA repair processes. Studies in Lewis lung adenocarcinoma cells indicated that cannabinoids which inhibit ^{3}H-tdr incorporation into DNA do not alter substrate availability. This would suggest that plasma membrane effects are not involved in this response. In other experimental systems, cannabinoids have been shown to alter the cellular uptake and distribution of certain macromolecular precursors (48–51, 84, 260) and steroids (49).

In addition to cannabinoids exhibiting wide biological responses, it is possible that certain aspects of their activity may be regulated by the cell itself. Two studies support this contention in that Δ^9-THC exhibited selective toxicity with respect to bone marrow and Lewis lung adenocarcinoma (276) and neuroblastoma and glioma cells (84). In the latter study an apparent explanation for this selectivity was attributed to the restrictive uptake of ^{3}H-Δ^9-THC by glioma cells in which cannabinoid exposure did not effect cell growth or macromolecular synthesis. In the sensitive cell (i.e., neuroblastoma), both the uptake and subcellular distribution of ^{3}H-Δ^9-THC, macromolecular synthesis, and cell growth were entirely consistent with the nucleus representing a significant biological target.

There are reports in other cellular systems (for review, see ref. 208) which demonstrate a myriad of cellular responses. Certain of these responses are clearly associated with the lipid-partitioning properties of cannabinoids at the plasma membrane. With respect to the macromolecular events reported here, membrane partitioning does not appear to be playing a major role. The precise biochemical targets associated with cannabinoid activities are at present unresolved.

V. Alterations in Prostaglandin Synthesis

A. In vitro effects

The ability of cannabinoids to interfere with prostaglandin biosynthesis has been demonstrated in several

TABLE 3

Effects of cannabinoids on miscellaneous enzymes

Enzyme	Cannabinoid	Concentration or dose	Remarks	Ref.
β-Glucuronidase	Δ^9-THC	10–50 mg/kg s.c.	Reduced activity in rat testis, epididymis, and prostate which may interfere with reproduction.	56
α-Glucosidase	Δ^9-THC	10–50 mg/kg s.c.	Reduced activity in rat testis, epididymis, and prostate which may interfere with reproduction.	56
Acid Phosphatase	Δ^9-THC	10–50 mg/kg s.c.	Reduced activity in rat testis, epididymis, and prostate which may interfere with reproduction.	56
Fructose-6-phosphatase	Δ^9-THC	10–50 mg/kg s.c.	Reduced activity in rat testis, epididymis, and prostate which may interfere with reproduction.	56
Esterase isozymes	Δ^9-THC, CBD	2 mg/kg i.p., 10 days	Both compounds deceased testicular weight by 77%. They also decreased the quantity of esterase isozymes which led authors to suggest involvement with steroidogenesis.	102
Tyrosine aminotransferase activity	Δ^9-THC, Δ^8-THC	3–30 mg/kg p.o.	Δ^8-THC was more effective than Δ^9-THC in reducing enzyme activity in mice treated twice weekly for 8 or 12 wk.	285
Lysophophatidylcholine acyltransferase	Δ^9-THC		Δ^9-THC inhibited acylCoA:lysophosphatidylcholine acyltransferase in mouse lymphocytes and synaptosomes with $K_i = 0.35$ μM. Suggested that psychoactivity may involve inhibition of this enzyme.	107
Lysophophatidylcholine acyltransferase	Δ^9-THC	15–70 mg/kg i.v.	Δ^9-THC inhibited synaptosomal and spleen lymphocyte enzyme activity by 44 and 24%, respectively, at the highest dose, and less than 20% at the lowest dose.	133
Lysophophatidylcholine acyltransferase	Δ^9-THC, Δ^8-THC, CBN, CBG, 11-OH-Δ^9-THC		Δ^9-THC was 100 times more potent than Δ^8-THC and 11-OH-Δ^9-THC which were, in turn, 10 times more potent than CBN and CBG in inhibiting enzyme activity in mouse brain synaptosomes. Concluded that cannabinoids inhibited this enzyme at concentrations below those necessary for acting as lipid-soluble anesthetics.	108
Lysolecithin acyltransferase	Δ^9-THC	0.1–10 μM	Δ^9-THC abolished concanavalin A-stimulated acyltransferase activity as well as basal activity in lymphocyte membranes.	109
Cholesterol esterase	Δ^9-THC	3.2–16 μM	Blocked activity of esterase from rat adrenals and ovary. Concluded that this inhibition could lead to decreased hormone production.	41
Cholesterol esterase	Δ^9-THC, CBN, 11-OH-Δ^9-THC	3.2–32 μM	Δ^9-THC at concentration of 16 μM produced ≈ 50% inibition of enzyme activity. CBN and Δ^9-THC were equiactive, and 11-OH-Δ^9-THC was less active. Concluded that cholesterol esterase probably was not altered rapidly enough to account for psychoactivity.	42
5′-Nucleotidase	Δ^9-THC, CBN, (+)- and (−)-Δ^8-THC, CBD, DMHP, CBG	10–30 μM	All of the compounds inhibited lymphocytic 5′-nucleotidase by 50% in the concentration range of 15–30 μM.	245
NADP-oxidase	Δ^9-THC	10 μM	Inhibited brain enzyme activity 50–73%, depending upon the brain area. Heart enzyme activity was inhibited 69%.	12

TABLE 4
In vitro model of cannabinoid effects on macromolecular metabolism

Cells/tissue	Response	Ref.
Bone marrow	Decreased	51
Lymphocytes	Decreased	208
Neuroblastoma	Decreased	49
C6 glioma	No change	49
Hepatoma	Decreased	141, 273
Protozoa	Decreased	234
Adrenal cortex	Decreased	273
Rat brain	Decreased	138
Testicular	Decreased	136–139
Carcinoma	Decreased	50–51, 90, 276

different organs or cell types as illustrated in table 5. It appears that cannabinoids affect the biosynthesis of prostaglandins in a complex fashion (scheme 1). Not too surprisingly, the effects of cannabinoids are dependent upon their concentrations as well as the tissue being examined. In general, cannabinoids stimulate phospholipase A_2 which results in increased production and release of arachidonic acid (37, 38, 277). There was a resultant increased formation of several PGs in lung fibroblasts (39, 40) and HeLa cells (37) but a decreased synthesis in seminal vesicles (43, 45, 46, 259) and in rat brain (238). The decreased synthesis appeared to be due to inhibition of cyclooxygenase, much like that produced by the nonsteroidal antiinflammatory agents.

A particularly bothersome aspect of the cannabinoid effect on prostaglandin synthesis is the apparent lack of structural specificity. Most, if not all, of the other effects of cannabinoids are dependent upon structural requirements. Concern regarding lack of structural specificity is magnified when the concentration at which Δ^9-THC produces its effects on prostaglandin synthesis is considered. Concentrations in the 1 μM range may have relevance to some of the cannabinoid effects, but those that occur at concentrations greater than 50 μM are difficult to interpret. It is likely that, in the event these high concentrations of cannabinoids are achieved in vivo, the ensuing alterations in prostaglandin synthesis could contribute to the overall toxicity of the cannabinoids.

B. In vivo effects

The notion that cannabinoids produce their psychoactive effects by alterations in prostaglandin synthesis is based largely upon the evidence summarized in table 6. These studies show either directly or indirectly that cannabinoids stimulate the release of prostaglandins (44, 66, 85, 146, 158) which would be consistent with the in vitro findings of cannabinoid stimulation of phospholipase A_2 (37–40, 277) and inconsistent with inhibition of cyclooxygenase (43, 45, 46, 259). However, there is evidence that cannabinoids do not alter concentrations of prostaglandins in whole brain and actually decrease the levels in hypothalamus (65). The in vitro data suggest that a decrease would occur in whole brain (129, 238). The doses used in many of the in vivo experiments were less than 10 mg/kg, which are reasonable for purposes of correlation with pharmacological effects.

VI. Interactions with Receptors

A. Putative Cannabinoid Receptors

Evidence for the possible existence of cannabinoid receptors has emerged slowly over the past several years. Some, but not all, of the criteria for receptors have been established for the cannabinoids. Typically, pharmacological evidence for the receptor is obtained in vivo or in situ followed by characterization of its in vitro binding properties. Two of the most fundamental aspects of drug-receptor interactions appear to be structure-activity relationships of the agonist and the existence of specific antagonists. Credence for the receptor is enhanced by numerous other factors, such as its isolation and characterization, identification of the biochemical mechanisms that are associated with it, elucidation of its endogenous ligand, etc.

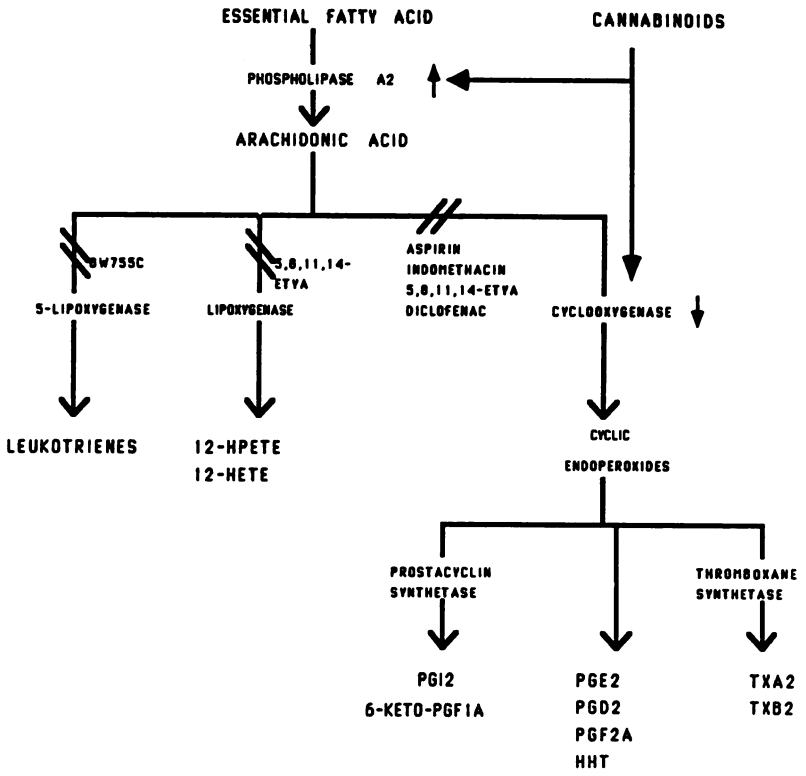
1. Structure-activity relationships. It has been clearly established that modest structural modifications of the THC molecule can result in profound changes in cannabinoid behavioral activity, as discussed by Razdan elsewhere in these reviews. Therefore, only a summary of some of the structural requirements for cannabinoid activity, as they relate to possible receptor function, will be presented here. Alterations in any one of the three rings or in the side chain of THC will change its behavioral activity. It was known from the pioneering work of Adams (2) that the position of the double bond in the A ring (fig. 1) was important for central activity. It has been firmly established that optimum activity is obtained when the double bond is in the 9–10 position and that saturation of the A ring reduces activity (200). Removal of the 9-methyl group entirely (192) or addition of a methyl, methoxy, amino, or acetamido group to the 9-methyl group attenuates behavioral activity (192, 279). Of course, it has been reported that hydroxylation of the 9-methyl results in either no change or an increase in potency (59, 177, 227). Replacement of the 9-methyl with a hydroxyl group enhances potency (281). It has generally been assumed that an intact B ring is essential for activity due to the fact that cannabidiol lacks psychoactivity (125, 226). However, recent data question the necessity of an intact benzopyran. Melvin et al. (202) synthesized a derivative of 9-*nor*-9-beta hydroxy-hexahydrocannabinol that lacked the B ring entirely and had a dimethylheptyl side chain at the 3 position. The behavioral activity of this analog proved to be qualitatively similar to that of Δ^9-THC. This analog was also more potent than Δ^9-THC (275). The spatial relationship between the A and B rings appears to be critical. In the naturally occurring psychoactive cannabinoids, the A and B rings are fused through a *trans* junction, while synthetic *cis* analogs lack central effects (190, 200, 268). Alterations in the gem dimethyls on the B ring, such as

TABLE 5
In vitro effects of cannabinoids on prostaglandins

Organ or cell type	Cannabinoid	Concentration (μM)	Remarks	Ref.
Seminal vesicles	Δ^9-THC	9.6	26% inhibition of PGE_2 synthesis from arachadonic acid. Same concentration of indomethacin produced 93% inhibition.	45
Seminal vesicle microsomes	Δ^9-THC, CBG	320	Eugenol and other essential oil components were more potent than Δ^9-THC in inhibiting conversion of 8, 11, 14-eicosatrienoic acid to PGE_1	46
Seminal vesicle microsomes	Δ^9-THC + other cannabinoids	320	No correlation between psychoactivity and inhibition of PGE_1 formation. Olivetol was most active.	43
Seminal vesicle microsomes	Δ^9-THC, CBN, CBD, and pyrolysis products of CBD	1–110	All inhibited conversion of 8, 11, 14-eicosatrienoic acid to PGs. The pyrolysis products were 10–100× more potent than Δ^9-THC (ID_{50} = 110 μM).	259
HeLa cells	Δ^9-THC, CBN, CBCr, CBC, Δ^9-THC metabolites, eugenol, olivetol, etc.	0.2–160	Cells were labeled with ^{14}C-arachidonic acid. Cannabinoids produced little increase in arachidonate release at < 160 μM. Δ^9-THC (1.6 μM) increased formation of PGF and PGE. No correlation between psychoactivity and stimulation of arachidonate release and PG production.	37
Human platelets, neuroblastoma cells	Δ^9-THC, CBD	10–80	Δ^9-THC and CBD stimulated phospholipase A_2 in platelets labeled with ^{14}C-arachidonate. CBD was 1.5 times more active than Δ^9-THC. Δ^9-THC inhibited thromboxane synthetase and PG lipoxygenase. Authors concluded that these effects unrelated to psychoactivity.	277
Mouse Leydig cells, human lung fibroblasts, HeLa cells	Δ^9-THC, 11-OH-THC, CBCr, 6-OH-THC, 11-OH-CBN, CBN	3.2–32	All stimulated arachidonate release. Stimulated phospholipase A_2 which was not correlated with psychoactivity.	38
Lung fibroblasts	Δ^9-THC, CBN, CBD	3.2–160	All three compounds stimulated production of PGE_2. This effect was blocked by aspirin and by mepacrine (phospholipase inhibitor).	40
Human lung	Δ^9-THC, CBD, CBC, CBG, CBCr, CBN	8	Stimulated synthesis of PGE_2. CBD was most potent. Correlation of fibroblasts with psychoactivity was poor.	39
Rat brain	Δ^9-THC	140	Inhibited release of PGF_{2alpha} from synaptosomal fraction. Potency ≈ to that of benzocain, tetracain, and dibucain but 1000× less than that of indomethacin.	238
Rat brain	Δ^9-THC	0.1–10	Δ^9-THC was more potent than indomethacin in inhibition of PGE_1 synthesis by striatal synaptosomes.	129

replacement with a ketone, also reduce central activity (183, 193). Expansion of the B ring to a 7-membered ring eliminates some of the central effects (193).

As far as the C ring is concerned, Mechoulam and Edery (200) indicated in their early review that behavioral activity was dependent upon the presence of a free or esterified phenolic hydroxyl group. A morpholinobutyrate of Δ^9-THC has been prepared, and its behavioral activity is thought to be due to hydrolysis in vivo to Δ^9-THC (289). Mechoulam and Edery (200) also noted that activity was eliminated by the addition of electronegative groups at positions 2 and 4. Even hydroxylation at position 2 eliminates activity (193). It has also been demonstrated that transposing the phenolic hydroxyl and the side chain also results in loss of activity (1). Adams (2) first demonstrated the importance of the alkyl group by



SCHEME 1. Biosynthesis of arachidonic acid products. BW755C [3-amino-1-(3-trifluoromethylphenyl)-2-pyrazoline] is an inhibitor of leukotriene synthesis. 5,8,11,14-Eicosatetraynoic acid (ETYA) is a false substrate for the lipoxygenase responsible for formation of 12-hydroperoxyeicosatetraenoic acid (HPETE) and 12-monohydroxyeicosatetraenoic acid (HETE). Aspirin and other compounds depicted in the scheme block the fatty acid cyclooxygenase which forms cyclic endoperoxide derivatives which are ultimately converted to prostaglandins (PG), 17-carbon hydroxy acid (HHT), and thromboxanes (TX).

showing in the Δ^{6a-10a}THC series that increasing the length of the side chain increased activity until a maximum was reached with *n*-hexyl. Branching of the side chain has an unusually profound effect on central activity, the most notable example being DMHP which is Δ^{6a-10a}THC with a dimethylheptyl side chain. Hardman et al. (112) have shown that this cannabinoid is more potent than Δ^9-THC in addition to having a longer duration of action. Hydroxylation occurs in all positions of the side chain in vivo by several species (3). In the Δ^8-THC series, hydroxylation at the 5′ position did not alter behavioral activity, hydroxylation at the 3′ position increased activity at least 2-fold, and hydroxylation at 2′ and 1′ decreased activity approximately 5- and 20-fold, respectively (3). Similar results have been found for 3′-hydroxy in the Δ^9-THC series (111). More importantly, introduction of a hydroxy in the 3′ position results in a chiral center. The behavioral activity appears to reside in the S isomer rather than the R isomer (194). This latter observation suggests that the conformation of the side chain is important for activity.

The question arises as to whether alterations in centrally mediated cannabinoid effects arising from these structural modifications are due to changes in receptor interactions or to differences in penetration, distribution, and elimination from the central nervous system. There is evidence that the biodisposition of some of the analogs may differ from that of Δ^9-THC which could account for the modification in activity. It is now clear that the increased activity of 11-OH-Δ^9-THC is due, at least in part, to a decrease in plasma-protein binding and concomitant increase in brain penetrability. Schou et al. (250) showed that, when Δ^9-THC was administered into the carotid artery of mice, its ability to penetrate brain was diminished when plasma was used as the vehicle, whereas the penetrability of 11-OH-Δ^9-THC was essentially the same regardless of whether the vehicle was saline or plasma. These findings suggested that the greater potency of 11-OH-Δ^9-THC was due to greater brain penetrability as a result of its lesser affinity for plasma proteins. Perez-Reyes et al. (225) provided direct evidence for this assumption by administering equivalent doses of 11-OH-Δ^9-THC and Δ^9-THC to mice. The brain levels of 11-OH-Δ^9-THC were almost 5 times higher than those of Δ^9-THC. In contrast to the notion that 11-OH-Δ^9-THC is equipotent with or more potent than Δ^9-THC (59, 177, 227), it appears 11-OH-Δ^9-THC may be less potent when brain concentrations are considered (225). The findings of Gough and Olley (104) are consistent with this suggestion in that intrastriatal injections of Δ^9-THC were more effective than those of 11-OH-Δ^9-THC in producing catalepsy in rats.

TABLE 6
In vivo effects of cannabinoids on prostaglandins

Species and/or organ	Cannabinoid	Dose (mg/kg)	Remarks	Ref.
Isolated perfused rabbit kidney and guinea pig lung	Δ^9-THC	16–255 μM	Decreased perfusion pressure and increased urine production in kidney. Increased perfusion presssure in lung. Lung effluent caused contraction of rat stomach fundus strip. These effects were blocked by pretreatment with aspirin. Concluded that Δ^9-THC stimulated the synthesis of PGs.	158
Mouse	Δ^9-THC	0.25–2 p.o.	PGE_1 produced dose-dependent decrease in intestinal motility which was antagonized by Δ^9-THC (0.25 mg/kg) and potentiated by Δ^9-THC (1 mg/kg). PGE_{2alpha} produced non-dose-dependent increase in intestinal motility which was antagonized by Δ^9-THC in dose-dependent fashion. Δ^9-THC decreased in dose-dependent manner PGE_1-induced abdominal stretching. Interpretation was that Δ^9-THC was interacting with PG receptor.	134
Rat	Δ^9-THC	10 s.c.	Stimulated PGE production from estradiol-stimulated uterus. Would have expected decrease due to inhibition of synthetase as reported by Burstein and Raz (45).	145
Mouse	Δ^9-THC	0.6–20 p.o., 0.1–100 i.p.	Oral doses of 0.6–20 mg/kg produced significant catalepsy which was reversed by aspirin and indomethacin. Postulated a role for PGE_2.	85
Mouse	Δ^9-THC	0.25–25	Tested 3 conditions known to alter PGs (fat-free diet, yeast-induced fever, severe cold) for their effect on Δ^9-THC-induced catalepsy. Fat-free diet reduced the Δ^9-THC response, yeast-induced fever increased the Δ^9-THC effect, while cold produced mixed effects. Concluded PGE_2 was involved in Δ^9-THC activity.	86
Rat	Cannabis resin (17% Δ^9-THC)	50–200 i.p.	PGE_1, PGF_{2alpha}, and diclofenac (PG synthesis inhibitor) evaluated for effects on cannabis actions. PGE_1 potentiated Δ^9-THC-induced analgesia, anticonvulsant activity, catalepsy, and cannabis-induced potentiation of hexobarbital hypnosis. PGF_{2alpha} and diclofenac had the opposite effect. Postulated that all of cannabis effects are primarily serotonin mediated and that PG effects are secondary.	18
Mouse	Δ^9-THC, CBN, CBD	50 p.o.	Both cannabinoid and female exposure caused comparable changes in testicular weights and PG production. Cannabinoid-treated males exposed to sexually receptive females had reduced PGE and PGF production in pituitary and testes, whereas cannabinoids elevated PGE levels in female-deprived males	71
Mouse	Δ^9-THC, levonantradol	0.95, 0.012	Both blocked PGE_2-induced diarrhea in mice. X-ray and conformational studies revealed striking structural similarities between levonantradol and PGE_1.	205
Dog anesthetized	Δ^9-THC	0.45 i.v.	Aspirin (50 mg/kg, i.v.) blocked Δ^9-THC-induced hypotension. Postulated that Δ^9-THC stimulates PG release which can be blocked by inhibiting synthesis with aspirin.	44

TABLE 6—Continued

Species and/or organ	Cannabinoid	Dose (mg/kg)	Remarks	Ref.
Rat	Δ^9-THC	2 i.v.	Produced marked behavioral changes and hypothermia but did not alter PGE_2 levels in whole brain. PGE_2 levels were decreased in hypothalamus but not in other brain areas which lead them to postulate this as the cause of the hypothermia.	65
Rat	Δ^9-THC	2 i.v.	Did not alter the amount of PGE_2-like material extracted from the ileum. There was a decrease in this material in the jejunum. Attempted to correlate this change with Δ^9-THC-induced catatonia.	66
Rat	Δ^9-THC	10	Indomethacin and Δ^9-THC potentiated arachidonic acid-induced hypotensive activity in the anesthetized rat.	129

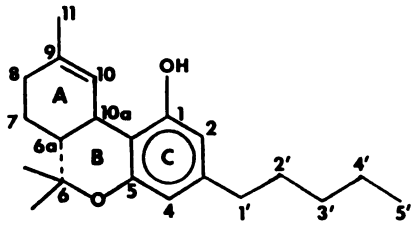


FIG. 1. Structure of Δ^9-THC.

Differences in physicochemical characteristics of Δ^9-THC and its analogs are magnified when different routes of administration are compared. Analgesic studies from our own laboratory have shown that Δ^9-THC is approximately 10 times more potent than several monohydroxylated derivatives following s.c. injection, whereas they are almost equiactive following i.v. injection (187). Ohlsson et al. (216) carried out studies in which they compared the biodisposition of Δ^8-THC to that of metabolites hydroxylated in the side chain. They found that the hydroxylated metabolites penetrated brain more readily than did Δ^8-THC. However, it did not appear that pharmacokinetic features were totally responsible for determining the central activity of these cannabinoid metabolites. For example, 1′-OH- and 4′-OH-Δ^8-THC reached the highest concentrations in brain, but the most potent cannabinoids were Δ^8-THC, 11-OH-Δ^8-THC, and 3′-OH-Δ^8-THC. In addition, the differences in their lipophilicity could not account for their differences in pharmacological potency. Their octanol/water partition coefficients were Δ^8-THC (5000), 1′-OH- and 3′-OH-Δ^8-THC (4000), 2′-OH-Δ^8-THC (2000), 4′-OH-Δ^8-THC (2500), and 5′-OH- and 11-OH-Δ^8-THC (1500), while their rank order for producing catalepsy in mice was 11-OH-Δ^8-THC = 3′-OH-Δ^8-THC > Δ^8-THC > 5′-OH-Δ^8-THC > 4′-OH-Δ^8-THC > 2′-OH-Δ^8-THC > 1′-OH-Δ^8-THC. It was concluded that the differences in the potency of these compounds depended upon inherent pharmacodynamic properties rather than pharmacokinetics. Binder et al. (21) have also shown that the pharmacokinetics of the inactive $\Delta^{(9-11)}$THC are not different from those of Δ^9-THC.

Stereoselectivity is an important criterion for drug-receptor interactions because stereoisomers share the same physicochemical characteristics. In support of this assumption, Jones et al. (143) showed that mouse brain levels of ^{3}H-(+)-Δ^9-THC and ^{3}H-(−)-Δ^9-THC were comparable following administration of similar doses of the respective compounds. It has been firmly established that central cannabinoid activity is stereoselective [see Dewey et al. (77) for a recent review]. Edery et al. (82) showed that (−)-*trans*-Δ^9-THC was at least 20 times more potent than (+)-*trans*-Δ^9-THC in monkeys, while Jones et al. (143) found the (−)-*trans* isomer to be 13 times more potent than the (+)-*trans* isomer in the mouse ring test. Based upon the observations that (−)-*trans*-Δ^9-THC was 6, 9, ~10, and 100 times more effective than (+)-*trans*-Δ^9-THC in producing motor hypoactivity in mice, hypothermia in mice, static ataxia in dogs, and depression of schedule-induced responding in monkeys (190), respectively, it appears that the magnitude of cannabinoid stereoselectivity is dependent upon the species as well as the pharmacological test.

The important question is whether the structure-activity relationships discussed above implicate the involvement of a receptor. There have been some attempts to conceptualize the interaction of the cannabinoid molecule with its biological receptor, but definitive proof is lacking (19–21, 205).

2. Cannabinoid antagonists. As stated previously, an important criterion for a receptor is evidence for a specific antagonist. There are numerous examples of drugs that have been used to reverse or block the effects of Δ^9-THC. However, it is more likely that these studies characterize drug interactions as opposed to specific antagonism. Early comparisons of the pharmacological potency of synthetic Δ^9-THC and marihuana suggested that the activity of the latter was dependent upon factors other

than Δ^9-THC content (33, 52, 54, 92). Karniol and Carlini (154) and Carlini et al. (52) suggested that the CBD in marihuana was interfering with the effects of Δ^9-THC. Subsequently, studies were carried out in humans by Karniol et al. (155) who demonstrated that oral ingestion of comparable doses of CBD and Δ^9-THC resulted in an attenuation of Δ^9-THC-induced tachycardia as well as its psychological effects. However, Bird et al. (22) found no influence of CBD on THC's pharmacological effects after oral administration, while Hollister and Gillespie (125) found Δ^9-THC's effects were slightly intensified when it was coadministered with CBD. Others reported that CBD did not influence Δ^9-THC-induced tachycardia when they were smoked together and that it exerted only minimal effects on THC's psychotropic activity (178). CBD did not alter the pharmacokinetics of orally administered Δ^9-THC in man (4). Animal studies also provided conflicting results. CBD has been shown to block Δ^9-THC-induced catatonia in mice; corneal areflexia, bradycardia, and hypothermia in rabbits; and motor hypoactivity and hypothermia in rats (32, 153). Doses of CBD that were 8 and 50 times greater than those of Δ^9-THC provided a partial reversal of Δ^9-THC food-reinforced operant performance in rats and pigeons, respectively. CBD failed to block THC discrimination in rats in studies carried out by Browne and Weissman (35). Also, studies carried out in our laboratories have not shown CBD to block Δ^9-THC-induced hypothermia or motor hypoactivity in mice (unpublished observations) or analgesia (145). It does not appear that the attenuation of THC's effects by CBD represents specific antagonism for several reasons. (*a*) There is lack of a consensus regarding CBD's antagonistic properties; (*b*) high doses of CBD are required to block most of the THC effects which is usually not the case with specific antagonists; (*c*) CBD does not completely abolish any of the effects of THC.

CBN, another constituent of marihuana, has been reported to block Δ^9-THC potentiation of pentobarbital sleeping time (167). However, Hollister and Gillespie (126) found that CBN exerted no influence on Δ^9-THC's subjective effects in humans, and Musty et al. (209) found that CBN tended to potentiate rather than block THC's effects in man. The latter finding is consistent with the observations of Perez-Reyes et al. (226) that CBN exhibits weak cannabinoid activity in man. Cannabichromene is also a component of marihuana that has been evaluated for synergistic and antagonistic properties, and it was found to have little antagonistic effect on several pharmacological properties of Δ^9-THC in mice (74, 115).

Kudrin and Davydova (169) reported that phenitrone was capable of preventing as well as reversing all the symptoms of hashish intoxication in dogs. However, subsequent studies carried out by numerous investigators failed to show any cannabinoid antagonistic activity of phenitrone in dogs, rats, mice, and pigeons (15, 35, 185, 258).

The CNS depressant effects of Δ^9-THC were blocked by amphetamine in rats (180); conversely, Δ^9-THC enhanced the stimulant properties of amphetamine in mice (94). TRH also has been shown to antagonize decreased locomotor activity induced by Δ^9-THC (17). Δ^9-THC-induced muricide has been blocked by imipramine (91).

There have been several antagonism studies designed to identify neurotransmitter systems that mediate the central effects of the cannabinoids. Although a cholinergic link is implied by some of the pharmacological properties of Δ^9-THC, studies with physostigmine provide a complex picture. Δ^9-THC-induced hyperthermia in rats was attenuated by physostigmine (142), whereas Δ^9-THC augmented physostigmine toxicity in the same species (242). Carlini et al. (53) demonstrated that marihuana-induced aggressiveness in REM-sleep-deprived rats could be blocked by haloperidol and large doses of phenoxybenzamine. However, Jarbe and Ohlin (140) found haloperidol, phenoxybenzamine, and propranolol to be ineffective in blocking the discriminative stimulus effects of Δ^9-THC in rats. The discriminitive stimulus properties of Δ^9-THC were also studied by Browne and Weissman (35) who found that adrenergic, cholinergic, serotonergic, and GABA-ergic agents did not generalize to the THC cue. They also evaluated these agents for possible antagonistic properties, and none was able to block the THC cue.

The only conclusion to be drawn from the foregoing discussion is that there is no single agent that serves as a specific antagonist for the cannabinoids. In most instances, the modest attenuation in THC's effects probably represents an indirect action of the drug being tested as an antagonist. In no case does it appear that these compounds are blocking the effects of THC by interacting with a cannabinoid receptor.

3. Design of a cannabinoid antagonist. The question arises as to whether previous information can provide direction in attempts to develop a specific cannabinoid antagonist (assuming cannabinoid receptors exist). There appear to have been few systematic attempts to synthesize cannabinoid antagonists. The problem is further complicated by the lack of a simple assay that is suitable for evaluation of compounds for antagonism of behavioral activity. It is tempting to look to the opiate field for guidance, particularly in light of the suggestions that cannabinoids and opiates share some common properties (discussed elsewhere in this article). N-Allyl and N-cyclopropyl methyl substituents in naloxone and naltrexone, respectively, account for their opiate antagonistic activity. Hetereocyclic cannabinoids have been synthesized for the purpose of incorporating a nitrogen into THC's carbocyclic structure (173, 174, 222, 283, 288). Numerous substituents have been added to the nitrogen which included both propargyl and allyl. These

compounds exhibited cannabinoid agonistic activity but apparently were not tested for antagonistic activity. Recently some heterocyclic and carbocyclic compounds (fig. 2) were obtained from Dr. Razdan for evaluation of cannabinoid antagonistic activity in our laboratory. Mice were pretreated with an i.v. injection of either 1.0, 6.6, 3.3, 3.3, or 6.6 mg/kg of I, II, III, IV, or V (fig. 3), respectively, 10 min prior to an i.v. injection of Δ^9-THC (3 mg/kg). Either spontaneous activity, rectal temperature, or antinociceptive activity (tail-flick response) was measured as described previously (187, 193). The doses of the analogs were just below those which produce agonistic activity in these tests. All of these compounds failed to attenuate any of the effects of Δ^9-THC. Aminocarbocyclic analogs synthesized by Wilson et al. (279) were found to have weak agonistic activity in rodents and dogs, but they were not tested for antagonistic activity.

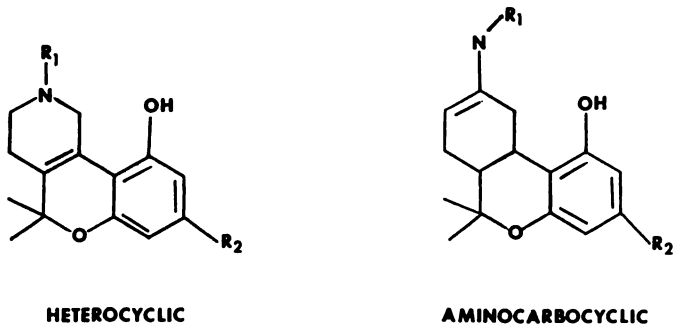


FIG. 2. Heterocyclic and aminocarbocyclic cannabinoid structures.

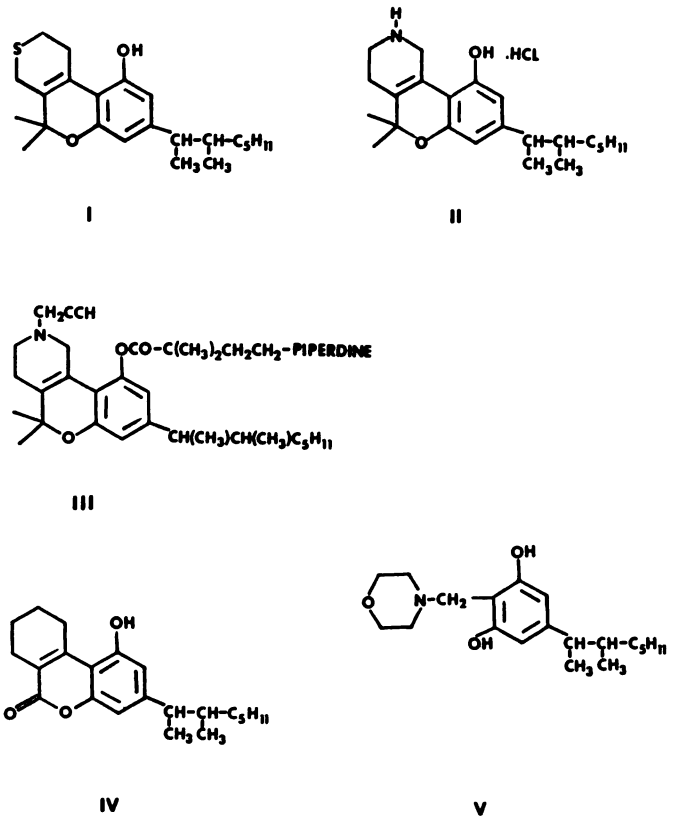
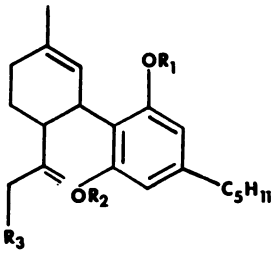


FIG. 3. Heterocyclic and carbocyclic analogs that have been evaluated for cannabinoid antagonistic activity.

In light of the fact that there is no proven formula for development of an antagonist, one is left with the option of mimicking the structural modifications in other series of drugs which resulted in antagonistic activity. The pioneering work of Baker (10) led to alkylating agents that were active-site-directed irreversible enzyme inhibitors. Similar advances in the development of opioid antagonists have come from Portoghese et al. (231, 232), who made fumarate and N-mustard analogs which proved to be long-acting opioid antagonists. While a similar approach might work with cannabinoids, there does not appear to be a logical starting point. In spite of its less-than-impressive antagonistic properties, CBD may be a logical choice for initial structure modification. Recent evidence from our laboratory has shown that CBD modified at position 10 resulted in modest antagonism of THC's analgesic activity (145). The CBD analogs depicted in fig. 4 were tested for antagonism of cannabinoid antinociceptive activity. CBD analogs VI and VII were devoid of antagonistic activity when administered in doses up to 100 and 60 mg/kg, respectively. Analogs VIII, IX, and XI exhibited weak antagonistic activity at doses up to 20 mg/kg. On the other hand, analog X was a potent antagonist in that it produced 67% antagonism of Δ^9-THC's antinociceptive activity at doses as low as 5 mg/kg. Unfortunately, higher doses of this compound could not be tested due to its lethality. While these CBD analogs do not represent potent antagonists, there appears to be some selectivity. It appears that further



Analog	R_1	R_2	R_3
VI	H	H	H
VII	OAc	OAc	C-N$(CH_2CH_2)_2O$
VIII	OAc	OAc	N$[CH(CH_3)_2]_2$
IX	OAc	OAc	N$(CH_3)CH_2C{\equiv}CH$
X	H	H	$NHCH_2CH_3$
XI	H	H	$NHCO(CH_2)_3NH_2$

FIG. 4. Cannabinoid analogs that have been evaluated for cannabinoid antagonistic activity.

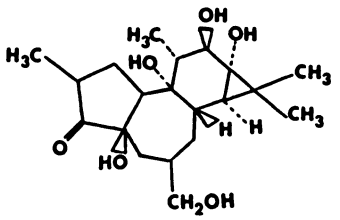
structural modification of CBD, particularly at position 10, would be logical.

In theory, more than one site of attachment is involved in any given ligand-receptor interaction; otherwise, selectivity would be lacking. Clearly, three-point attachment is minimal for stereospecificity. Points of attachment may be thought of as multiple recognition processes which can be subdivided into primary and secondary sites. The primary recognition sites (affector sites) are reflective of the affinity of the ligand for the receptor, while the secondary recognition processes (effector sites) are, at least in part, reflective of the intrinsic activity. With regard to the synthesis of recognition-site-directed alkylating agents, Portoghese and Takemori (233) have discussed the importance of attaching the electrophile to a portion of the ligand that allows interaction with a secondary recognition process (a proximal nucleophile on the receptor) without interference with the primary site of attachment. If one assumes that an agonist must bind to both acceptor and effector sites of a receptor in order to produce a pharmacological effect, then any alteration in the molecule that interferes with binding to the acceptor site will not result in either agonistic or antagonistic activity. Obviously, an antagonist is a compound that has high affinity for the acceptor site but is unable to activate the effector site. As discussed earlier, there are numerous structural modifications that result in loss of cannabinoid agonistic activity; however, it is not known whether these alterations attenuate the interactions with primary or secondary recognition sites, or both. It would appear that an exhaustive search for an antagonist will require modification of all essential functional groups of the cannabinoid molecule to ensure that primary recognition processes remain intact. Of course, another approach to the development of antagonits is the synthesis of photoaffinity labels. Numerous affinity labels have been prepared which have been shown to have affinity for specific binding sites.

4. In vitro binding studies. The physicochemical characteristics of the cannabinoids make it difficult to carry out in vitro studies. Garrett and Hunt (93) have shown that Δ^9-THC has low water solubility (<10 μM) and a high affinity for filter paper, glass, plastic, rubber, etc. In order to add a cannabinoid to any aqueous medium, it is almost always necessary to use a surfactant or an emulsifier which could complicate interpretation of in vitro binding data. Therefore, it is not surprising that in vitro binding studies have been hampered by the large degree of nonspecific, nonsaturable binding of the cannabinoids. However, Harris et al. (114) were able to demonstrate high-affinity, saturable binding of ^{3}H-Δ^8-THC to hepatoma cells in tissue culture. The relevance of this binding has not been established, although there are clear indications of cannabinoid effects on steroidal systems which may be germane to this binding site. One of the most significant aspects of the cannabinoid binding to the cultured hepatoma cells was the demonstration that saturable binding could be measured in an in vitro system. Harris et al. (114) also investigated cannabinoid binding to brain homogenates which obviously would have more relevance to psychoactivity. Unfortunately, the heterogeneous, lipophilic nature of brain tissue precluded the possibility of quantitating saturable binding. A small percentage of binding was displaced by unlabeled Δ^8-THC, and the binding was dependent upon temperature. Roth and Williams (244) studied the binding of radiolabeled Δ^9-THC to both crude and purified rat synaptosomal membranes. The total quantity of bound material was measured following the addition of ^{3}H-Δ^9-THC in the concentration range of 10^{-8}–10^{-6} M. The linear relationship obtained between the quantity of ^{3}H-Δ^9-THC bound and the amount added suggested a single binding site. However, no effort was made to establish the specificity or saturability of this binding site.

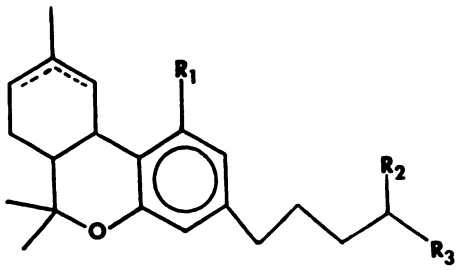
It was clear from the in vitro studies that the ratio of specific to nonspecific binding to brain membranes has to be improved appreciably before specific binding can be studied in a reliable manner. There are several options which can be utilized in order to decrease the nonspecific binding component. One obvious way is to increase the ratio of specific to nonspecific binding sites through some means of sample purification. Attempts in our laboratory to purify specific binding sites by subcellular fraction techniques, column chromatography, etc., have produced disappointing results. The most promising approach may be substitution of a less lipophilic ligand for Δ^8- or Δ^9-THC. A similar approach has been used successfully for the characterization of binding of phorbol esters, one of the most potent classes of tumor promotors (81, 210). It can be seen in fig. 5 that the phorbol esters are carbocyclics, much like cannabinoids, and would therefore be expected to exhibit a high degree of lipophilicity.

Although PMA is more potent than PDBU, the latter compound was utilized for binding studies because of its lower lipophilicity (168). PDBU exhibited substantially less nonspecific binding than PMA which allowed a saturable, specific binding site to be characterized in mouse brain (210). There are numerous ways in which the water solubility of Δ^9-THC can be altered in a fashion similar to that described for the phorbol esters. For example, Zitko et al. (289) made a water-soluble derivative of Δ^9-THC by addition of a morpholinobutyrate at



PHORBOL

FIG. 5. Structure of phorbol.



Analog	R_1	R_2	R_3
XII	morpholinobutyrate	H	$-CH_3$
XIII	$-OPO_3H_2$	H	$-CH_3$
XIV	O^-	H	$-CH_2{+}N(CH_3)_3$
XV	-OH	$-CONH(CH_2)_6NH$-sepharose	$-CH_3$
XVI	-OH	$-CONH(CH_2)_6NH$-sepharose	-H

FIG. 6. Water-soluble derivatives of THC.

the phenolic hydroxyl (XII in fig. 6). This ester retained cannabinoid activity which was probably due to enzymatic hydrolysis to Δ^9-THC. Yoshimura et al. (286) also prepared a water-soluble derivative by addition of a phosphate to the phenolic hydroxyl of Δ^8-THC (XIII). This phosphate, which was not readily hydrolyzed enzymatically, was considerably less potent than Δ^8-THC and had a longer onset of action. Recently, Seltzman et al. (251) synthesized a TMA derivative of Δ^8-THC (XIV) which should be much less lipophilic than the above esters. Nye et al. (215) studied the binding of this radiolabeled ligand to rat neuronal membranes and found a saturable, reversible binding site to which 5′-TMA-Δ^8-THC bound with high affinity (89 nM). This site appeared to be related to some cannabinoid action due to the fact that drugs which act through dopamine, serotonin, catecholamine, histamine, GABA, benzodiazepine, adenosine, and calcium channel receptors did not compete for binding. However, it also appeared that this site was not associated with cannabinoid behavioral effects due to the fact that several inactive or weakly active cannabinoids (for example, cannabinol, cannabidiol, cannabigerol, (+)-THCs, and Δ^{9-11}THC) exhibited a high affinity for binding. Nevertheless, these results lend encouragement to the possible identification of a specific binding site that is involved in the mediation of behavioral effects. A better understanding of the significance of this binding site will become available only after the pharmacological effects of 5′-TMA-THC have been established.

There has been at least one attempt to prepare an affinity column for the isolation of cannabinoid binding sites. Binder et al. (20) synthesized XV and XVI, but details regarding isolation of material that bound to this matrix were not provided. Nye et al. (215) used CHAPS to extract ^{3}H-5′-TMA-THC binding sites from rat cortical membranes which were estimated to be in the 60,000-dalton range by molecular sieve chromatography.

There needs to be continued progress in the area of in vitro binding before the concept of a cannabinoid receptor is to be accepted. It would appear that a combination of all of the above approaches may have to be recruited before a receptor can be characterized.

B. Neurotransmitter Receptors

Despite considerable evidence that cannabinoids alter neurotransmitter function, few studies have been devoted to investigation of Δ^9-THC effects on neurotransmitter receptors. The fact that membrane-associated proteins can be affected by alterations in the physical properties of membranes (184, 246) has led to suggestions that cannabinoid-induced perturbations of membranes may result in modified neurotransmitter receptor functionality (26, 30, 118, 119). Hillard and Bloom (118) have studied the effects of cannabinoids on the binding of DHA, a β-adrenergic antagonist. They reported that Δ^9-THC, at concentrations of 3 and 10 μM, increased the binding affinity of ^{3}H-DHA without altering the number of binding sites. 11-OH-Δ^9-THC produced similar effects while CBD was without effect. They also demonstrated

that Δ^9-THC increased the affinity of other antagonists for ^{3}H-DHA binding but decreased the affinity of agonists for this binding site (119). They concluded that this effect of THC was biologically significant and related to psychoactivity despite the evaluation of only three cannabinoids. While Vachon and Sulkowski (270) reported that propranolol attenuated some of the effects of Δ^9-THC in humans, other investigators have found propranolol to lack both agonistic (140) and antagonistic (35, 140) properties in animals trained to discriminate Δ^9-THC. The finding of Hillard and Bloom (118) that Δ^9-THC decreases the binding of β-adrenergic agonists seems to be incompatible with propranolol blockade of cannabinoid effects.

The effects of cannabinoids on subpopulations of dopaminergic receptors have also been investigated (26, 30). Δ^9-THC, as well as 11-OH-Δ^9-THC and CBD, decreased the binding of ^{3}H-ADTN, a putative D_3 dopamine agonist. These cannabinoids decreased the B_{max} without altering the affinity of ^{3}H-ADTN which differed from the effects on the β-adrenergic receptor. These same three cannabinoids also decreased the binding of ^{3}H-spiperone, a selective D_2 antagonist, by decreasing its affinity. Δ^9-THC but not CBD increased agonist (dopamine and apomorphine) affinities for the antagonist preferring D_2 binding site, suggesting some selectivity.

It is clear that relatively high concentrations of cannabinoids can cause a modest alteration in neuroreceptor binding. However, these effects do not appear to arise from a general nonspecific phenomenon due to the fact that Δ^9-THC increased the binding affinity of beta-adrenergic antagonists and decreased that of dopamine D_2 antagonists. The pharmacological relevance of these observations can be established by evaluation of other cannabinoids to determine whether structure-activity relationships are consistent with psychoactivity.

C. Steroid Receptors

Steroid hormones have long been recognized as having important influences on mood and affective states. As a consequence, there have been frequent endeavors to understand the actions of steroids on brain cellular function. Brain appears to be a target tissue in that it contains receptors for all five classes of steroids which include estrogens, progestins, androgens, glucocorticoids, and mineralocorticoids (198). Cannabinoids are related to steroids in that they both have similar carbocyclic structures as shown in fig. 7. There is experimental evidence which suggests that cannabinoids can interact with steroid systems, although these findings are not without controversy. In vivo studies in laboratory animals have shown Δ^9-THC to be either antiestrogenic (57), estrogenic (62, 70, 103, 113, 163, 182, 204, 213, 256, 257), or without effect (271). A possible relationship between cannabinoids and sex steroids was greatly intensified by a report that marihuana use resulted in decreased plasma testosterone levels, impotence, and oligospermia in men (164). However, others reported no significant reductions in plasma testosterone levels after marihuana use (69, 203). Studies in laboratory animals have generally shown the cannabinoids to exhibit adverse effects on reproductive function. These effects could arise from any number of actions of cannabinoids which include alterations in gonadotropin output, suppression of estrogen and androgen production, macromolecular synthesis, and target-organ receptor function, to name a few.

There have been efforts to investigate all of the possible mechanisms by which cannabinoids could modify steroid function, including receptor interactions. Rawitch et al. (239) reported that Δ^9-THC was a weak competitor for estrogen binding to rat uterine cytoplasmic receptors. ^{3}H-Estradiol specific binding was reduced 18% by 5 μM Δ^9-THC which was interpreted as being consistent with the antiestrogenic effects of Δ^9-THC on uterine tissue (256, 257) and on testosterone levels (164). Conversely, Okey and Bondy (217) reported that Δ^9-THC did not interfere with ^{3}H-estradiol binding to rat uterine cytosolic receptors. Rather, they found that ^{3}H-estradiol binds to macromolecules in the 8S region, while ^{3}H-Δ^9-THC, at a concentration of 10 nM, binds to nonspecific sites in the 4–5S region. Neither excess Δ^9-THC nor estradiol was able to displace the ^{3}H-Δ^9-THC binding. Smith et al. (253) also found that Δ^9-THC was unable to compete with ^{3}H-estradiol binding to cytosol preparations from rhesus monkey and human uteri. They examined the binding of ^{14}C-THC to these cytosol preparations and found that the binding was not displaced

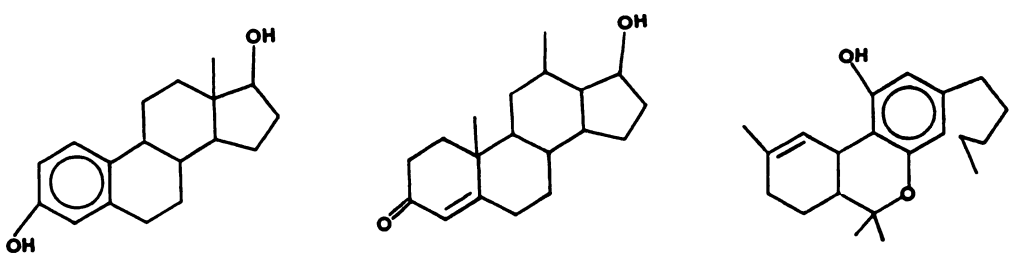


FIG. 7. Comparison of cannabinoid and steroid structures.

by Δ^9-THC, estradiol, progesterone, cortisol, or dihydrotestosterone. An important implication of these studies, as pointed out by Smith et al. (253), is that THC's inability to interact with the estrogen receptors in primate uterus suggests that cannabinoids will not interact with estrogen receptors in the central nervous system. Estrogen receptors in brain and pituitary exhibit properties very similar to those in nonneural target tissue (see ref. 198 for a review).

Of course, it is possible that the antiandrogenic properties of Δ^9-THC discussed above are due to alterations in androgen receptors rather than to antiestrogenic activity. Purohit et al. (237) demonstrated that Δ^9-THC and CBN were capable of blocking the stimulatory effects of testosterone and dihydrotestosterone on the prostate and seminal vesicles in castrated and hypophysectomized rats. These findings prompted this group to investigate the possibility that Δ^9-THC had a direct effect on androgen receptors. They found that both Δ^9-THC and CBN competed for ^{3}H-dihydrotestosterone binding to androgen receptors in rat prostate cytosol (236). Their respective K_i values were 260 and 210 nM. The cannabinoid interaction with the androgen receptor was indeed modest as exemplified by further experiments in which a 1000-fold excess of Δ^9-THC inhibited ^{3}H-dihydrotestosterone binding by only 32%.

While the effect of cannabinoids on estrogen and androgen binding to macromolecules in nonneural target tissues has been investigated, it remains to be established whether Δ^9-THC alters the binding of the other classes of steroids. There is also no direct evidence as to whether the cannabinoids alter steroid binding to brain tissue.

D. Opioid Receptors

Δ^9-THC has been shown to produce effects similar to those of opioids including antinociception (27, 28, 47, 58, 76, 96, 187, 188, 214, 254, 255, 280), hypothermia (47, 280), and dimunition of the morphine abstinence syndrome (121). There are also reports that naloxone precipitates a withdrawal syndrome in rats treated chronically with Δ^9-THC (122, 157, 265a). Cross-tolerance has also been reported to develop between opiates and cannabinoids (27, 156, 199). However, there is controversy as to whether any of these actions of opioids and cannabinoids are mediated through a similar mechanism, particularly a common receptor.

As far as antinociception is concerned, Wilson and May (280) reported that naloxone antagonized the antinociceptive activity of 11-OH-Δ^9-THC, and Bloom et al. (28) found that naloxone partially antagonized antinociception produced by 9-*nor*-9β-hydroxyhexahydrocannabinol, a potent cannabinoid. In similar experiments, Tulunay et al. (265a) found that chlornaltrexamine, a selective long-acting irreversible opioid antagonist, inhibited Δ^9-THC-induced antinociception. However, several other investigators (58, 188, 247) have been unable to block cannabinoid-induced antinociception with opioid antagonists. Tulunay et al. (265a) found that chlornaltrexamine reduced THC hypothermia by approximately 20% in rats.

There is also evidence for and against cannabinoid-opioid cross-tolerance. McMillan et al. (199) and Kaymakcalan (156) reported that chronic treatment with Δ^9-THC decreased the effect of morphine in the mouse tail-flick test which was consistent with cross-tolerance. However, Martin (188) failed to find cross-tolerance between THC and morphine as measured by the tail-flick antinociceptive test. Bloom and Dewey (27) reported asymmetric cross-tolerance in that morphine-tolerant mice were also tolerant to Δ^9-THC, but Δ^9-THC-tolerant mice were not tolerant to morphine as measured by antinociceptive activity. The reverse was found with hypothermia: Δ^9-THC-tolerant mice were tolerant to morphine, but morphine-tolerant mice were not tolerant to Δ^9-THC.

Zaluzny et al. (287) found that Δ^9-THC and morphine produced a very similar degree of suppression of the quasimorphine withdrawal syndrome induced by a phosphodiesterase inhibitor; however, naloxone reversed the effects of morphine but not those of Δ^9-THC.

It appears that most investigators who have studied the interactions of cannabinoids and opioids have emphasized their commonalities rather than their differences. It is probably for this reason that one of the most interesting pharmacological aspects of these two classes of drugs, i.e., their behavioral effects, has largely been ignored. It is certainly clear that the behavioral alterations these two compounds produce in man are distinctly different and thereby suggest different modes of action. Consistent with this notion are the findings of Browne and Weissman (35) and Jarbe and Ohlin (140) that naloxone and naltrexone, respectively, lack the capability to interfere with the subjective effects of Δ^9-THC in rats trained to discriminate Δ^9-THC from vehicle. Naltrexone also failed to block the actions of Δ^9-THC in the chronic spinal dog preparation (96).

There have been a few attempts to identify common molecular events that could serve to unify the actions of cannabinoids and opioids. Bloom and Dewey (27) found that both morphine and Δ^9-THC increased catecholamine synthesis in mouse brain. Morphine produced a greater increase in dopamine synthesis, while Δ^9-THC produced a greater increase in norepinephrine synthesis. An additional difference between morphine and Δ^9-THC was observed with naloxone. It completely blocked morphine-induced synthesis but only partially blocked the Δ^9-THC stimulation of norepinephrine synthesis.

It would not seem likely that cannabinoids interact with opioid receptors due to their insensitivity to opioid antagonists in most test systems. Bloom and Hillard (30) did not find alterations in the binding of ^{3}H-D-ala-D-leu-enkephalin by concentrations of Δ^9-THC up to 40 μM. They did show that Δ^9-THC decreased the in vitro bind-

ing of ^{3}H-naloxone. It was found that the affinity of ^{3}H-naloxone was decreased without a change in number of binding sites. However, this effect of Δ^9-THC only occurred at high concentrations; actually, the IC_{50} value of Δ^9-THC was found to be 20 μM. The authors suggested that this effect of Δ^9-THC on naloxone binding may be the result of a nonspecific perturbation of membrane in which the opioid receptor resides, rather than a specific interaction with the receptor. This certainly appears to be a reasonable argument due to the fact that high concentrations of cannabinoids will alter binding of numerous ligands. The unanswered question is whether these modest changes in receptor binding induced by cannabinoids have any relevance to the pharmacological actions of cannabinoids. In light of the ambiguity regarding the putative opioid-cannabinoid pharmacological commonalities, the weak action of cannabinoids at the mu opioid receptor does not strengthen the notion of opioid-cannabinoid interaction.

VII. Cellular Aspects as Related to Specific Effects

A. Alterations in Behavior

1. Membrane effects. The lipophilicity of cannabinoids has been the primary impetus for comparing their biochemical properties to those of other drugs that affect membrane fluidity (101, 108, 179, 186, 196, 219, 235, 241). However, the point was raised earlier in this review that the physicochemical similarities between cannabinoids and anesthetic agents do not necessarily mean they produce their effects through a common mechanism. Actually, most of the effects of these two classes of compounds on the central nervous system are quite different, which would suggest different mechanisms of action. For example, a distinguishing feature of Δ^9-THC is its lack of anesthetic properties. Paton et al. (223) addressed this issue during a discussion of the lipid solubility of Δ^9-THC. They suggested that Δ^9-THC was a partial anesthetic due to the limited volume fraction that it was capable of occupying. The findings of Seeman et al. (250a) support such a conclusion. While it may be possible that cannabinoids may not be capable of achieving sufficient membrane perturbation to produce anesthesia, if the mechanisms of action of cannabinoids and anesthetics are similar, then lower concentrations of anesthetic agents should mimic the effects of cannabinoids. Indeed, attempts were made by Gill and Lawrence (172) to show that nonanesthetic alcohols were capable of producing cannabis-like catatonia in mice. It would seem reasonable that the generalized depression of the central nervous system that occurs with high doses of cannabinoids could be due to a nonspecific perturbation of membranes. Such an effect may be similar to that which could be produced with almost any anesthetic agent. This depressant effect is in contrast to the unique subjective effects of cannabinoids, i.e., cannabinoid effects not produced by anesthetics. Hence, the problem arises as to which animal model is predictive of cannabinoid subjective effects. This issue is complicated by the fact that cannabinoid subjective effects are just that, a composite of effects. The appearance of these effects is probably dependent upon the dose as well as the animal model. In the dog static ataxia test, lower doses of cannabinoids produce a unique profile of overt behavioral effects, but higher doses produce profound CNS depression which is not readily distinguished from that produced by any CNS depressant. It is our premise that these effects do not necessarily arise from the same action of the cannabinoid. There is reason to believe that the CNS depression results from nonspecific actions of the cannabinoids, such as membrane perturbation, whereas the unique effects of the cannabinoids result from a more specific action.

This latter notion is consistent with the observations made by Pertwee (228). He concluded that neurochemical changes produced by Δ^9-THC at concentrations less than 1 μM appeared to be structure dependent and were not produced by cannabinoids devoid of psychoactivity. In contrast, effects produced by Δ^9-THC at concentrations greater than 1 μM could also be produced by psychoinactive compounds. He suggested that the neurochemical changes produced by low concentrations of Δ^9-THC resulted from interactions with specific receptors which led to cannabinoid behavioral effects.

2. Membrane versus receptor. A basic tenet regarding the establishment of cause-effect is the relationship between the tissue concentration of drug following an effective dose and the concentration needed to produce a biochemical change. It is generally assumed that receptor activation occurs at drug concentrations far below those which cause membrane perturbation. While it appears to be a relatively straightforward question, there is uncertainty as to what brain concentration of Δ^9-THC is required to produce various cannabinoid effects in laboratory animals and in humans. There have been numerous studies in which concentrations of Δ^9-THC have been measured in brains of laboratory animals during the time of its pharmacological effects (7, 97, 144, 191, 216). In many cases the brain concentrations have been as high as 1 μM. Obviously, it has not been possible to determine what concentrations of Δ^9-THC in the human brain are necessary for producing behavioral effects. However, it may be possible to estimate a concentration range from existing laboratory animal and human data. A review of the literature, regarding the doses of Δ^9-THC that are required to produce different pharmacological effects in humans, revealed a dose range between 2 and 22 mg of Δ^9-THC per cigarette for smoking and 20–90 mg by the oral route. These dose ranges are similar to those provided in recent reviews by Klonoff (162) and Agurell et al. (5). Clearly, individuals smoking marihuana cigarettes containing 10 mg of Δ^9-THC experience a

psychological "high." It has been estimated that only 15–20% of Δ^9-THC is delivered intact in mainstream marihuana smoke (6, 72) which would reduce the dose to a concentration range of 0.4–4.4 mg/person. Using an estimated body weight of 60 kg, the smoking dose would translate into about 7–73 μg/kg. Rosenkrantz (243) has also addressed the issue of human dosage of Δ^9-THC, although his major goal is calculation of animal doses of Δ^9-THC that are relevant to man. He estimated that marihuana smoking resulted in a somewhat higher human dosage range of Δ^9-THC (100–500 μg/kg), because he assumed only 50% of Δ^9-THC was lost during smoking, and a 50-kg mean body weight was used to account for younger users. Extrapolation of this dosage range to animals, using body surface conversion factors, provided estimates of equivalent doses in dogs and monkeys of 0.2–1.0 and 0.3–1.5 mg/kg, respectively. However, these doses ranges are 1–5 and 6–30 times higher than the i.v. dose of Δ^9-THC that produces profound behavioral effects in dogs (192) and monkeys (82). It would therefore seem that the range of 7–73 μg/kg is a more reasonable estimation of human dosage than that calculated by Rosenkrantz (243).

If one were to assume that the pharmacokinetics of Δ^9-THC in humans are similar to those in laboratory animals, then less than 1% of the administered dose (0.4–4.4 mg/person) would be expected to penetrate the brain. The anticipated concentration of Δ^9-THC in the human brain would be expected to be in the range of 10–100 nM with less than 50 nM being necessary to produce a marked psychological "high." A 50 nM concentration would be at least 10 times less than that necessary to produce behavioral disruption in laboratory animals. This difference in brain concentrations between humans and animals is consistent with that observed with most centrally active drugs.

Apparent differences between behaviorally active brain concentrations of Δ^9-THC in laboratory animals and humans, albeit inconclusive, could be due to different mechanisms of action. The argument has been presented that the high brain concentrations of Δ^9-THC that are required to produce behavioral effects in laboratory animals are consistent with those of agents that perturb membranes (120). Of course, establishing the significance of brain concentrations is complicated by several factors. In light of the fact that Δ^9-THC lacks anesthetic potency, Lawrence and Gill (172) proposed that cannabinoids exhibit limited solubility in the lipid phase of membranes and therefore mimic subanesthetic doses of general anesthetics. If cannabinoids do mimic the membrane perturbation of partial anesthetics, then the result would most likely be a general depressant effect rather than a specific behavioral effect inasmuch as general anesthetics have not been reported to produce the cannabinoid psychological "high." Given the highly lipophilic nature of the cannabinoids, it is quite likely that a large proportion of Δ^9-THC in the CNS is located at sites that are irrelevant to its mechanism of action. Parry et al. (221) examined the significance of ligand partitioning into membranes and concluded that partitioning of the ligand between the aqueous phase and the membrane lipid, as well as the location of the receptor binding site with respect to the lipid environment, should be considered when examining ligand-receptor interactions in membranes. This situation apparently occurs with numerous centally acting drugs. For example, the brain concentration of morphine required to produce an antinociceptive ED_{50} is approximately 0.5 μM (224), yet the K_i of morphine for the mu receptor is 1.8 nM (195). Likewise, the high concentrations of cannabinoids found in brains of laboratory animals do not rule out the possibility of receptor interactions. Although it is highly speculative, it may be that multiple mechanisms come into play as a result of the fact that Δ^9-THC accumulates so readily in lipid membranes. High concentrations of Δ^9-THC may be required before sufficient quantities accumulate at a specific site, such as a putative receptor. As the dose increases, sufficient quantities may accumulate in membranes to cause perturbation, resulting in general membrane disordering.

One of the important considerations in studying the mechanism of action of Δ^9-THC has been the use of SAR. While SAR has been the primary stimulus for postulating a putative cannabinoid receptor, it may be that membrane perturbation is also structure dependent. As Goldstein (101) has pointed out, membrane disorder does not exclude specificity. For example, the stereospecificity of anesthetic steroids, chloralose, and long-chain alkenols to disorder membranes suggest that the spatial orientation of hydrogen bonding may be critical for this process. In support of such a notion, Brockerhoff (34) postulated that anesthetics form hydrogen bonds with membrane lipids and thereby restructure the hydrogen belt of the lipid bilayer. He further suggested that this alteration in the hydrogen-bond network would be translated into changes in the hydrogen bonding of the membrane proteins. The degree to which cannabinoids are dependent upon structure for membrane effects has not been firmly established. The major drawback in previous attempts to establish SAR for membrane perturbation is the small number of cannabinoids that have been evaluated. While cannabinoid SAR supports the concept of a specific cannabinoid receptor, a disconcerting element is the apparent lack of greater stereoselectivity (5–100 fold) in some animal models.

There have been attempts to formulate a receptor concept for anesthetics, the success of which would enhance the likelihood of a receptor for the lipid-soluble cannabinoids. Richards et al. (241) suggested that anesthetics, including the aliphatic alcohols, occupy multiple receptor sites in the hydrophobic regions of proteins. However, there is no evidence for direct interaction of

anesthetics with protein receptors. Goldstein (101) pointed out that there is a linear relationship between the concentration of aliphatic alcohols and the magnitude of their effects which contrasts the logarithmic dose-response curve that typically results from drugs binding to a specific receptor.

One approach that has proven successful in the study of mechanism of action is an in vivo or in vitro model that exhibits altered sensitivity to a particular drug. Bradley (33a, 33b) reported that endotoxin-insensitive C3H/HeJ mice were resistant to the lethal actions of Δ^9-THC. An i.p. injection of Δ^9-THC (500 mg/kg) produced 0 and 65% lethality in C3H/HeJ and C3H/HeDUB (endotoxin sensitive) mice, respectively. Subsequent investigations of cannabinoid effects in these strains substantiated the differences in lethality but revealed no differences with regard to Δ^9-THC-induced hypothermia, analgesia, or motor impairment (B. R. Martin, unpublished observations). Although the C3H/HeJ strain does not appear to be less sensitive to cannabinoids as far as behavioral effects are concerned, it does not preclude the possibility that such an insensitive animal strain can be found.

To summarize, there is some evidence to suggest that cannabinoid effects arise from interactions with specific components of membranes which may be either lipid, protein, or some combination of the two. Of course, there are several important weaknessess regarding putative cannabinoid receptors, the most notable of which is the absence of an antagonist.

3. Prostaglandin synthesis. It is not an easy task to identify specific cannabinoid effects that may be mediated via prostaglandins and other arachidonic acid products. The major problem is that the role of prostaglandins in the central nervous system has not been fully elucidated (for a review, see ref. 284). There is, first of all, conflicting evidence as to which prostaglandins are synthesized in nervous tissue (284). PGD_2 is synthesized to a greater extent than the other prostaglandins ($PGF_{2\alpha}$, PGE_2) in rat brain. However, the importance of PGD_2 has been questioned due to its absence in cat and human brain. There is evidence that the thromboxanes and leukotrienes are formed in brain of some species. As for a central role for the prostaglandins, administration of prostaglandins intraventricularly or into specific brain areas results in numerous pharmacological effects (207). PGE_1, PGE_2, and PGE_3 produce sedation, stupor, and catatonia when injected intraventricularly into the cat and sedation in chicks after intravenous injection (127). Rats exhibit a sedative or tranquilizing effect when treated s.c. with PGE_1 or PGE_2 but not with $PGF_{2\alpha}$, PGA_1, and PGA_2 (100). Also, PGD_2 (i.v.) produces sedation and prolongation of pentobarbital sleeping times in mice (124). Arachidonic acid (i.v.) prolongs pentobarbital sleeping time which is inhibited by i.p. pretreatment with indomethacin (123).

The investigations discussed earlier in this review show that there are some central effects that are common to both cannabinoids and prostaglandins and that these effects are blocked by cyclooxygenase inhibitors. It would appear that any actions of cannabinoids on prostaglandins would result in a rather nonspecific central depressant effect. Studies conducted in my laboratory clearly show that indomethacin (10 mg/kg administered i.p. 10 min before i.v. injection of Δ^9-THC, 0.3 mg/kg) is not capable of blocking cannabinoid-induced static ataxia in dogs (unpublished observations). It is clear also that the in vitro cannabinoid effects on prostaglandins do not correspond to in vivo psychoactivity. In order to show that prostaglandins are involved in the expression of behavioral effects unique to Δ^9-THC, studies are needed which demonstrate that prostaglandins are capable of producing behavioral effects that mimic cannabinoid psychoactivity, not just CNS depression. For example, the sedative effects of prostaglandins may be due to nothing more than reduction in blood flow to brain (100). Other studies which might prove to be beneficial would be tolerance and cross-tolerance studies with cannabinoids and prostaglandins.

4. Enzymes. The ubiquity with which cannabinoids affect enzymes does not allow concrete conclusions. The studies reviewed in tables 1 to 3 generally show modest effects on enzymes unless rather high concentrations of cannabinoids are used. While many of the effects of cannabinoids may be mediated via these enzymes, it is presently not clear that psychoactivity is among them. In most instances, too few cannabinoids have been evaluated in order to establish a correlation between psychoactivity and enzyme inhibition.

Alterations in adenylate cyclase have been implicated in mediation of psychoactivity. This evidence stems primarily from that generated in neuroblastoma cell culture. Howlett and coworkers (130–132) have shown that psychoactive cannabinoids (desacetylnantradol > levonantradol > Δ^9-THC > Δ^8-THC > CBN), but not CBD and dextronantradol, inhibited adenylate cyclase at relatively low concentrations. The most intriguing aspect of these findings is the demonstration that the cannabinoid inhibition is similar to that produced by hormone-receptor interactions. Although the structure-activity relationship for adenylate cyclase inhibition in cell culture is consistent with psychoactivity, there is lack of a general consensus among all of the adenylate cyclase studies. Administration of Δ^9-THC results in either no change in brain concentrations of cAMP (8) or in elevation in cAMP levels following low doses of Δ^9-THC (79), effects which are not expected from inhibition of adenylate cyclase. In addition, Hillard and Bloom (118a) have found that adenylate cyclase from mouse cerebral cortical homogenates is inhibited by both psychoactive and nonpsychoactive cannabinoids, which suggests that adenylate cyclase is involved in some action other than a specific cannabinoid behavioral effect. There are obvious differences in these studies (drug concentration, tissue

or cell studied, vehicle, etc.) which could account for lack of agreement and therefore need to be resolved.

B. Analgesic

1. Neurotransmitter receptors. Studies from our laboratory have suggested a catecholaminergic link between morphine and Δ^9-THC in that yohimbine, an alpha$_2$ antagonist, will block the effects of both in the tail-flick procedure (189). However, it is quite possible that opiates and cannabinoids can exert similar effects on catecholamines through different mechanisms. Gilbert (96) suggested that cannabinoids exert their effects through a pathway that is parallel to that of morphine. Such a configuration could account for the cannabinoid insensitivity to opiate antagonists. Yohimbine will block the antinociceptive properties of several classes of drugs in the tail-flick procedure, implying an action at a final common pathway, which would be consistent with distinctive input pathways for opioids and cannabinoids.

2. Opioid receptors. The interrelationships between cannabinoids and opioids have been examined thoroughly. Lack of consistent findings among the studies reviewed earlier, particularly with regard to opioid antagonist blockade of cannabinoid antinociception, does not allow a convincing argument to be made for cannabinoid interaction with opioid receptors. In addition, cannabinoids alter the in vitro binding of opioids only at concentrations that far exceed those of opioid analgesics (30).

3. Prostaglandins. It is generally recognized that prostaglandins are involved in some aspects of the expression of pain. For example, it is thought that prostaglandins amplify pain during the inflammatory process (87). It is therefore reasonable to speculate that prostaglandins can be involved in cannabinoid-induced antinociception in light of their association with pain and the fact that cannabinoids alter prostaglandin synthesis. Milne and Johnson (205) observed striking conformational similarities between PGE_2 and levonantradol, a cannabinoid with potent antinociceptive properties. They postulated that levonantradol interacts with a prostaglandin receptor coupled to adenylate cyclase to inhibit cAMP formation in much the same way that Collier et al. (60) have proposed for morphine. In this manner, cannabinoids and morphine would be capable of sharing certain pharmacological properties, such as antinociception and antidiarrheal activity, without the necessity of cannabinoids binding to an opioid receptor. The ability of prostaglandins to produce diarrhea and emesis in man (67, 83) was noted as further support for this hypothesis in addition to the observations that levonantradol can block prostaglandin-induced diarrhea (205) and numerous emetic stimuli in cats (197) and humans (68, 78). While there are striking similarities between prostaglandins and cannabinoids, there are also some questions as to the extent of their interrelationship. Milne and Johnson (205) were not able to produce either blockade of PGE_2-induced contractions in the guinea pig iluem or inhibition of prostaglandin biosynthesis with levonantradol. Indomethacin has also been shown not to block Δ^9-THC-induced antinociception in the mouse tail-flick procedure (188). In addition, assigning a role for prostaglandins in the mediation of cannabinoid effects based upon an analogy with morphine should be done cautiously. When it is hypothesized that these two classes of compounds produce antinociception via interactions with prostaglandin receptors, then complications arise with attempts to postulate the involvement of prostaglandins in a pharmacological effect that is unique to only one of them. For example, if emesis is a prostaglandin-mediated event, then one would not expect cannabinoids and opioids to produce opposite emetic effects. If prostaglandins are associated with cannabinoid/opioid antinociception and emesis, then it becomes necessary to propose different roles for prostaglandins in the expression of these effects.

C. Anticonvulsant

1. Neurotransmission. The cannabinoids have been investigated extensively for their anticonvulsant properties (63, 150). It is interesting that Δ^9-THC also exhibits convulsant activity (151, 266). As far as therapeutic potential is concerned, the emphasis has shifted to CBD because it lacks convulsant activity as well as cannabinoid behavioral effects. Investigation of the anticonvulsant property of cannabinoids has generally focused on the electrophysiological mechanisms rather than on the biochemical mechanisms (267). Turkanis and Karler (267) reported that cannabinoids reduced cortical-evoked responses and spinal monosynaptic reflexes which was consistent with decreased neurotransmission. They also postulated that several possible biochemical mechanisms, including altered neurotransmitter release or transmitter equilibrium potentials or drug-receptor interactions, could be involved. It was argued that the electrophysiological depressant properties of Δ^9-THC were a general central effect because it occurred in every brain area studied in addition to effecting large pools of neurons and in individual neurons in a similar fashion. On the other hand, Turkanis and Karler (267) postulated that Δ^9-THC produces its anticonvulsant effects by altering postsynaptic membrane conductance which implies some degree of specificity. Of course, the convulsant properties of cannabinoids complicate attempts to elucidate their anticonvulsant mechanism of action. Wilkinson (278) concluded from his electrophysiological studies on cortical sensory-evoked activity that Δ^9-THC's effects were dependent on the sensory modality, brain area, dose, etc, which argued for specificity of THC action.

2. Prostaglandin synthesis. Prostaglandins of the E series have long been recognized for their anticonvulsant properties (284). However, there is no direct evidence that alterations in prostaglandin synthesis are responsible for cannabinoid anticonvulsant activity. For example, it would be important if cyclooxygenase inhibitors were

capable of reversing or blocking the anticonvulsant properties of cannabinoids.

3. Cannabinoid receptors. If specific cannabinoid receptors are found to exist, it would not appear that a common receptor mediates both anticonvulsant activity and behavioral effects. Karler et al. (149, 152) reported the following rank-order potency of cannabinoids in blocking maximal electroshock seizures: 11-OH-Δ^9-THC = dimethylheptylpyran 7× > Δ^9-THC ≈ cannabidiol 2× > cannabinol. To reiterate, the major interest in cannabidiol as a potentially useful anticonvulsant agent stems from its lack of behavioral effects. As to whether or not a cannabinoid receptor might be involved, Consroe et al. (64) have established some of the structural requirements for activity which included stereoselectivity. On the other hand, Mechoulam et al. (201) reported that (+)- and (−)-CBD were essentially equiactive in blocking maximal electroconvulsive seizures in mice as were the stereoisomers of the dimethylheptyl analog of CBD. There is clearly not a strong argument for anticonvulsant activity being mediated via a specific cannabinoid receptor.

D. Thermoregulation

1. ATPases. Pertwee (228, 229) has reviewed the literature recently regarding the possible modes by which cannabinoids alter body temperature. It appears that Δ^9-THC-induced hypothermia is centrally mediated due to the fact that it effectively reduces body temperature following either intraventricular injections (89, 99, 110, 249) or administration directly into the hypothalamus (89). There is ample evidence to suggest that central neurotransmitters may be involved in Δ^9-THC-induced hypothermia, either by altering turnover (31, 282) or neuronal uptake (11, 116, 117, 230). There are several possible mechanisms by which cannabinoids could interfere with neurotransmitter uptake and release, one of which could be alterations in membrane-bound ATPase associated with either synaptosomes or synaptic vesicles. In most studies presented in table 1, both psychoactive and psychoinactive cannabinoids inhibited ATPases in brain synaptosomal fractions.

2. Prostaglandins. There has been considerable interest in the possible involvement of prostaglandins in the production of fever (284). However, Wolfe (284) concludes that a causal relationship between endotoxin, prostaglandins, and fever has not been established. It has been shown that intraventricular administration of prostaglandins results in hyperthermia. It may be that Δ^9-THC produces hypothermia by reducing prostaglandin production in the hypothalamus (65). Δ^9-THC has been reported to have antipyretic activity by some investigators (165) but not by others (255).

3. Opioid receptors. There is some evidence which suggests an involvement of opioid receptors in the mediation of Δ^9-THC-induced hypothermia. Tulunay et al. (265a) found that chlornaltrexamine was capable of producing a modest reversal of THC hypothermia in rats. Bloom and Dewey (27) observed unidirectional cross-tolerance between cannabinoid and opioid hypothermia; Δ^9-THC-tolerance mice were tolerant to morphine, but morphine-tolerant mice were not tolerant to Δ^9-THC. Additional studies are needed before a convincing argument can be made for opioid-receptor involvement in cannabinoid influences on thermoregulation.

4. Other. Pertwee (229) has also reviewed the relationship between chemical structure of cannabinoids and their ability to alter body temperature. The structural requirements for producing hypothermia are similar to those necessary for behavioral effects. However, Pertwee (229) points out that many diverse classes of drugs have the ability to lower body temperature so that this cannabinoid action may lack specificity. He also suggests that the doses of Δ^9-THC that are required to reduce body temperature in humans are far in excess of those that are needed for producing psychoactivity. Hence, cannabinoids may produce these two effects through distinctively different mechanisms.

E. Immunosuppression

The immunological effects of cannabis and its constituents have been reviewed recently in detail by Munson and Fehr (208). Therefore, only a brief summary will be included as it relates to the biochemical events described herein. These authors did point out that cellular events associated with the immune system are so interrelated that delineation between a primary site of action and a secondary event is extremely difficult. They concluded that the stereospecificity and dose (or concentration requirements) of the cannabinoid effects on the immune system implicate multiple mechanisms of action.

1. Prostaglandins and leukotrienes. Both groups of compounds are released by all types of insults, and they appear to contribute to the formation of inflammation. Leukotrienes such as LTB_4 are potent chemoattractants for polymorphonuclear leukocytes. Prostaglandins have also been implicated in the control of the immunological response. There is little direct evidence linking prostaglandins to cannabinoid effects on the immune system, although the possiblity has not been ruled out.

2. Membrane and enzyme effects. In general, rather high concentrations of cannabinoids are required to alter the biochemical events associated with the immune system in addition to a lack of pronounced structural requirements for cannabinoid activity. Carchman et al. (51) did report some structural requirements for cannabinoid alteration in macromolecular synthesis which suggested involvement of events more discrete than just membrane partitioning and subsequent perturbation. Munson and Fehr (208) suggest that numerous membrane-bound enzymes may be involved. They cite the cannabinoid inhibition of ATPases which could result in alterations in active transport of essential nutrients into the cell and interfere with phosphorylation of nucleosides. Alterations in the activity of acyltransferases by cannabinoids (table 4) could play a role because these

enzymes are known to be associated with activation of cell-mediated immunity (88).

F. Antiasthmatic

1. Neurotransmitter receptors. Razdan and Howes (240) have reviewed the literature regarding the bronchodilator actions of Δ^9-THC. There are reports that Δ^9-THC is active after either oral administration or inhalation (262, 263, 269). However, little is known regarding the mechanism by which it produces this effect. It appears that the bronchodilator actions of Δ^9-THC are not due to antimuscarinic or β-adrenergic agonistic activity (252).

2. Prostaglandins. The effects of prostaglandins on bronchial and tracheal smooth muscle are complicated, but in general PGFs cause contraction and PGEs produce relaxation (207). Based upon the high content of prostaglandins in lung and the potent bronchoconstrictor effects of PGE_{2alpha}, Howes and Osgood (129) postulated that cannabinoid inhibition of prostaglandin synthesis may be responsible for the bronchodilation. However, supportive data have not been forthcoming. For example, do cannabinoids block prostanoid bronchoconstriction? Also, a comparison of the cannabinoid bronchodilator effects to those of L-640,035 (3-hydroxymethyl-dibenzo[*b,f*]thiepin-5,5-dioxide) and SKF 88046 {N,N′-bis[7-(3-chlorobenzeneaminosulfonyl)-1,2,3,4-tetrahydroisoquinolyl]disulfonylimide}, inhibitors of contractile prostanoids in the lung (55, 274), might be worthwhile.

G. Decrease in Intraocular Pressure

There has been considerable interest in the use of cannabinoids as antiglaucoma agents despite their side effects (psychoactivity, hypotension, etc.) and the lack of topical application (see ref. 211 for a recent review). The research interest arises in part because cannabinoids represent a unique class of antiglaucoma agents. Understanding their mechanism of action could provide valuable insights into the etiology of glaucoma.

1. Prostaglandin synthesis. It has been suggested (129) that cannabinoids alter intraocular pressure by altering prostaglandin synthesis in part because prostaglandins have been reported to increase intraocular pressure (212). In addition, Green and Podos (106) showed that Δ^9-THC attenuated increases in intraocular pressure produced by arachidonic acid in rabbits, an effect which was interpreted as being due to inhibition of prostaglandin synthesis. While there is an interesting casual relationship between cannabinoids and prostaglandins, definitive evidence linking prostaglandins to cannabinoid effects on intraocular pressure is lacking.

2. Neurotransmitter receptors. Alpha- and beta-adrenergic receptors influence aqueous humor dynamics in numerous ways. True outflow is controlled almost exclusively by alpha-adrenergic innervation, while beta-adrenergic stimulation plays a predominant role (alpha-adrenergic plays a minor role) in reducing aqueous humor formation. Green and Kim (105) demonstrated that both alpha- and beta-adrenergic antagonists reduced Δ^9-THC-induced decrease in intraocular pressure by approximately 50%, except for the beta-antagonist sotalol which completely abolished the cannabinoid effect. They concluded that Δ^9-THC was primarily a vasodilator of the efferent blood vessels of the anterior uvea. This vasodilation decreases the capillary pressure within the ciliary body which is responsible for the fall in intraocular pressure. It has not been established whether Δ^9-THC alters cholinergic neurotransmission in the eye. Anticholinesterase inhibitors are effective in reducing intraocular pressure by decreasing resistance to outflow.

VII. Summary

The many studies that have been included in this review suggest that cannabinoids have ubiquitous effects on biological systems. These results also underscore the intensity to which cannabinoids have been studied. While there are numerous reasons for the prodigious amount of cannabinoid research, a major stimulus has been the desire to identify a specific biochemical event or pathway that is responsible for the expression of Δ^9-THC's unique psychoactivity. It is the hope that Δ^9-THC, as with all centrally acting drugs, might serve as an important tool for achieving a better understanding of the central nervous system. As discussed in this review, the psychoactivity of cannabinoids might best be described as a composite of numerous effects. If that is indeed the case, then it would seem logical that these centrally mediated effects do not arise from a single biochemical alteration, but rather from multiple actions. Of course, a major problem arises when one attempts to establish a relationship between cause and effect when multiple mechanisms and effects are involved. An initial approach to reducing the complexity of elucidation of mechanism of action should involve attempts to distinguish those cannabinoid actions which result in specific effects (psychoactivity) from those which produce nonpsychoactive effects (such as general depression).

There are several fundamental principles that can be used to assess specificity, including concentration or dose of the drug that is required to produce a given effect. Low doses of Δ^9-THC are capable of producing the psychoactivity that is unique to cannabinoids, whereas higher doses may produce effects that are both specific and nonspecific for cannabinoids. Unfortunately, establishing this basic tenet for Δ^9-THC has proven to be difficult. It has not been possible to establish the concentration of Δ^9-THC at its site of action that is necessary to produce a given pharmacological effect. While it is a simple matter to measure the concentration of cannabinoids in either a whole tissue or an incubation medium, the hydrophobicity of cannabinoids dramatically affects their affinity for, and hence concentration in, the biochemical components of the tissue. If the concentration of Δ^9-THC could be measured at its site of action, then the relevance of many of its pharmacological effects could be adequately determined.

Two possible mechanisms by which cannabinoids might produce psychoactivity are membrane perturbation and receptor interactions, and indeed, both mechanisms have received considerable attention. It is logical to compare cannabinoids to anesthetics since both are highly lipophilic. It would seem reasonable that effects produced specifically by one class of compounds would not result from an action as general as membrane perturbation. It would seem that high concentrations of cannabinoids would produce a general perturbation of membranes which could account for many of the effects that occur on enzymes as well as neurotransmitter and opioid receptors with high concentrations of cannabinoids. Of course, it is possible that Δ^9-THC could produce any of these effects as a result of a specific membrane perturbation, although there is little evidence to support such a notion. It is tempting to think of receptor-mediated events when unique pharmacological effects occur at reasonably low concentrations. At present, there is insufficient direct evidence to support the notion of cannabinoid receptors.

An understanding of the actions of cannabinoids is crucial for assessing the impact of marihuana abuse on health. The extent to which marihuana abuse adversely affects health is controversial. Those individuals who favor the recreational use of marihuana argue that the occurrence of undesirable effects is slight despite the fact that there is a large population of users. It is probably advantageous that Δ^9-THC has high potency as far as psychoactivity is concerned, which allows for exposure to low concentrations. While the cannabinoids do not appear to be highly toxic, it is disconcerting that they seem to exert some alteration in almost every biological system that has been studied.

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