EFFECTS OF CANNABINOIDS ON NEUROTRANSMITTER RECEPTORS IN THE BRAIN*

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I. INTRODUCTION

Although it was first reported over 15 years ago that cannabinoids can affect biogenic amine neurotransmitters (1), the role of these transmitters in the many actions of marijuana is still not well understood. Furthermore, it is evident from reviewing past literature (for review, see 2) that there is not total agreement as to what the effects of various cannabinoids are on dopaminergic, noradrenergic and serotonergic containing neurons. Delta-9-tetrahydrocannabinol (THC) and other behaviorally-active cannabinoids increase the synthesis and turnover of dopamine and norepinephrine in rat and mouse brain while producing little or no change in endogenous levels of catecholamines (3,4). This indicates that THC can increase the utilization of the catecholamine neurotransmitters. The observed increase in synthesis was not a side effect of drug-induced hypothermia (5). Moreover, THC increases the synthesis of dopamine and norepinephrine in a synaptosomal preparation suggesting that cannabinoids can have a direct action on neuronal elements (6).

THC and other cannabinoids can also effect other functional aspects of catecholaminergic neurotransmisson. THC alters the active uptake of biogenic amine neurotransmitters and their precursors into synaptosomes (6-9) and also transmitter release from synaptosomes. In addition, THC can alter the activity of enzymes involved in the synthesis and degrada-

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tion of the catecholamines. Chronic treatment with THC has been reported to increase the activity of tyrosine in rat brain (10). THC has also been reported both to increase (11) or inhibit (12) monoamine oxidase. THC also inhibits the activity of Na^+-K^+-ATPase which is involved in the active reuptake of the monoamine neurotransmitters (8,13).

In contrast to the large number of reports on the effects of cannabinoids on other aspects of dopaminergic and noradrenergic systems, there has been only one published report of cannabinoid effects on receptors for these transmitters. In these studies (14), we observed that THC *in vitro* increased the binding of the beta-adrenergic antagonist, ^{3}H-dihydroalprenolol (DHA) to beta-adrenergic receptors in the mouse cerebral cortex. This effect was also produced by 11-OH-delta-9-THC but not by cannabidiol (CBD). The chronic *in vivo* administrationof THC caused a decrease in the density (B_{max}) of DHA binding sites with no change in their apparent affinity (K_d) for DHA.

In further studies on the interactions of cannabinoids with the beta-receptor (see Hillard and Bloom; this volume) we found that THC affected the binding beta-antagonists but had little effect on beta-agonists. The question was then asked: are these changes specific to the beta-adrenergic receptor or can THC affect other membrane-bound neurotransmitter receptors in the same manner. As part of our attempt to answer that question, we have examined in some detail the effects of cannabinoids on dopamine receptors in the mouse corpus striatum.

There are many studies demonstrating the existence of more than one type or form of receptor for dopamine in the brain (see review by Seeman, 15). Therefore, two different ligands were used in the present study. It is fairly well accepted that there is a binding site that has nanomolar affinity for neuroleptics such as haloperidol and micromolar affinity for dopamine and other agonists. This is often referred to as the D_2 site. Binding to this site was determined using ^{3}H-spiperone, a neuroleptic of the butyrophenone class. Conversely, there are dopamine binding sites that bind dopamine and other agonists with low nanomolar affinity and neuroleptics with high nanomolar or micromolar affinity. Seeman refers to these as D_3 sites (15). Binding to this type of site was examined using the ligand ^{3}H-ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene), as agonist.

II. METHODS

A. Materials

Male ICR outbred, Swiss, albino mice were purchased from ARS/Sprague-Dawley (Madison, WI) and housed grouped with *ad libitum* access to food and water. Cannabinoids were provided by the National Institute on Drug Abuse. Butaclamol was a gift of Ayerst Research Laboratories. ^{3}H-Spiperone and ^{3}H-ADTN were purchased from New England Nuclear Corp. All other chemicals were obtained from standard commercial sources.

In all studies mice were housed in a temperature and humidity controlled room with a 12 hour light-dark cycle. Mice were sacrificed by decapitation and the brains removed. Corpus striata were dissected from the brains by using the method described by Glowinski and Iverson (16) modified for the mouse brain. Washed membranes were prepared from striatal tissue using the methods described below. Protein concentration was determined using a modification of the dye binding method of Bradford (17) from Bio-Rad Laboratories.

B. Measurement of 3H-Spiperone Binding

The binding of spiperone to membranes from mouse corpus striatum was measured using a modification of the methods described by Quik and Iversen (18). Tissue was homogenized in 50 vol cold Tris HCl buffer (50 mM, pH 7.7) using a glass and Teflon homogenizer. The homogenate was centrifuged at 40,000 x g for 10 min and the supernatant discarded. The pellet was resuspended in 50 vol of cold Tris buffer and again centrifuged at 40,000 x g for 10 min. The resultant pellet was resuspended in 100 vol of 50 mM (pH 7.7) Tris buffer containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM pargyline and 0.1% ascorbic acid. A 2 ml incubation volume was used and contained about 0.5 mg of membrane protein. Samples were pre-incubated with the appropriate cannabinoid or its Emulphor-ethanol vehicle (19) for 5 min at 37°C. The emulphor vehicle was found to be without significant effect on ^{3}H-spiperone binding over the concentration range used. Incubations were started by the addition of the ^{3}H-spiperone (25-800 pM) and terminated after 10 min by filtration through GF/B glass fiber filters. The filters were then washed twice with 5 ml of the incubation buffer and the bound radioactivity determined. Samples were also incubated with 0.1mcM (+)-butaclamol. Specific binding of ^{3}H-spiroperidol was defined as total binding minus binding in the presence of 0.10 mcM (+)-butaclamol.

C. Measurement of 3H-ADTN Binding

The binding of ^{3}H-ADTN to mouse striatal membranes was determined using a modification of the method described by Creese and Snyder (20). Membranes were prepared as described above, except the final resuspension was in 50 mM Tris (pH 7.1) buffer containing in a 1 ml incubation volume, 1 mM $MnCl_2$ and 0.1% ascorbic acid. Specific binding of ^{3}H-ADTN was determined by subtracting binding in the presence of 4 mcM dopamine from binding in the absence of excess dopamine. Cannabinoids were suspended using a polyvinylpyrrolidone (PVP) vehicle (21) since an emulphor vehicle significantly decreased binding.

D. Data Analysis

All experiments were repeated three or four times. Overall analysis of results was performed using analysis of variance. Treatment comparisons to control group values were performed by using Dunnett's modification of the t-test or the students test (22). K_d and B_{max} values were determined by either the method of Scatchard (23) or directly from the binding data by non-linear regression (24). IC50 values were determined from logit plots of binding competition data and K_i values were estimated by the method of Cheng and Prusoff (25).

III. RESULTS

The effects of several concentrations of THC on the specific binding of ^{3}H-spiperone were examined first. The results of this study are shown in Fig. 1. It can be seen that THC produced a concentration related inhibition of spiperone binding. Significant ($p < 0.05$) was produced by the 10 and 30mcM concentrations. The 30 mcM concentrationreduced binding to 60% of the vehicle control level. 11-OH-delta-9-THC, an active THC metabolite, and cannabidiol (CBD), a cannabinoid without the psychoactive properties of THC, also decreased spiperone binding. Significant ($p < 0.05$) inhibition of binding was produced by 30 mcM concentrations ofeach cannabinoid (Fig. 2). All three cannabinoids decreased specific spiperone binding to the same degree.

In order to further investigate the interaction of THC with spiperone binding, the effects of THC on the equilibrium binding properties were then examined. In this study, spiperone concentrations between 25 and 800 pM were used. Spiperone binding in the presence of emulphor vehicle was saturable (Fig. 3 - inset) and appeared to bind to one class of sites

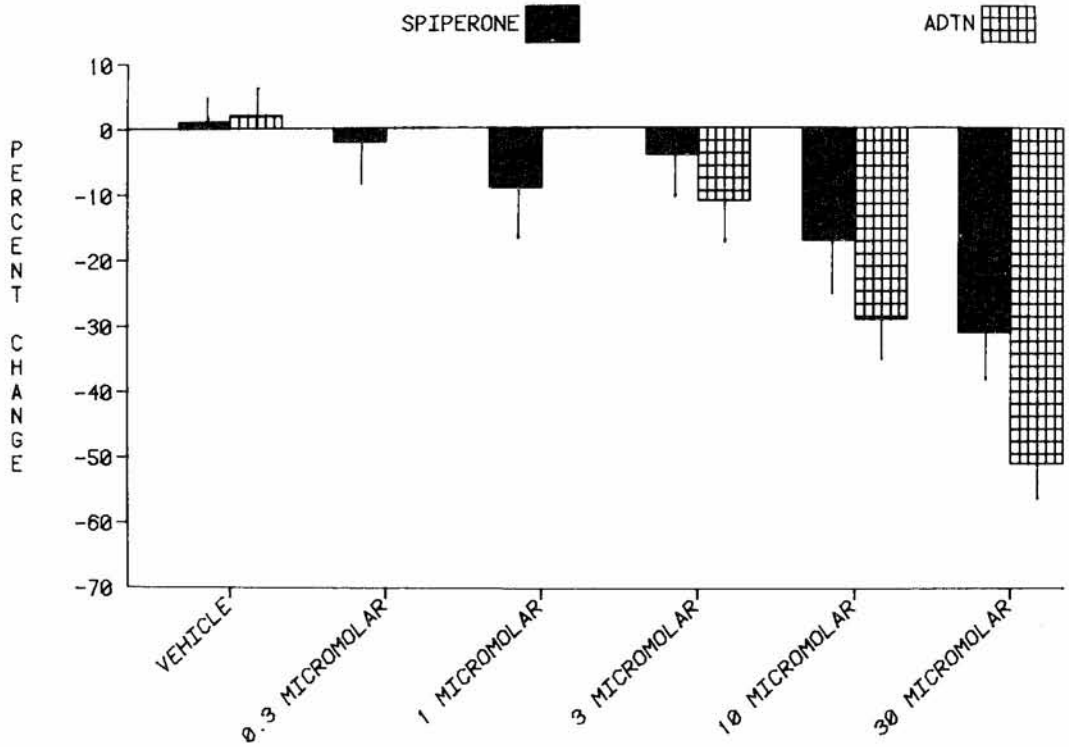

FIGURE 1. Effects of THC on the specific binding of ^{3}H-spiperone and ^{3}H-ADTN to membranes from mouse corpus striatum. Values are expressed as percent change from non-drug control samples and are the mean +/- S.E.M. of 4 to 6 experiments. ^{3}H-spiperone content was 0.3 nM. Binding in control samples was 99.4 +/- 7.4 fmol/mg of protein for spiperone and 75.6 +/- 8.5 fmol/mg of protein for ADTN.

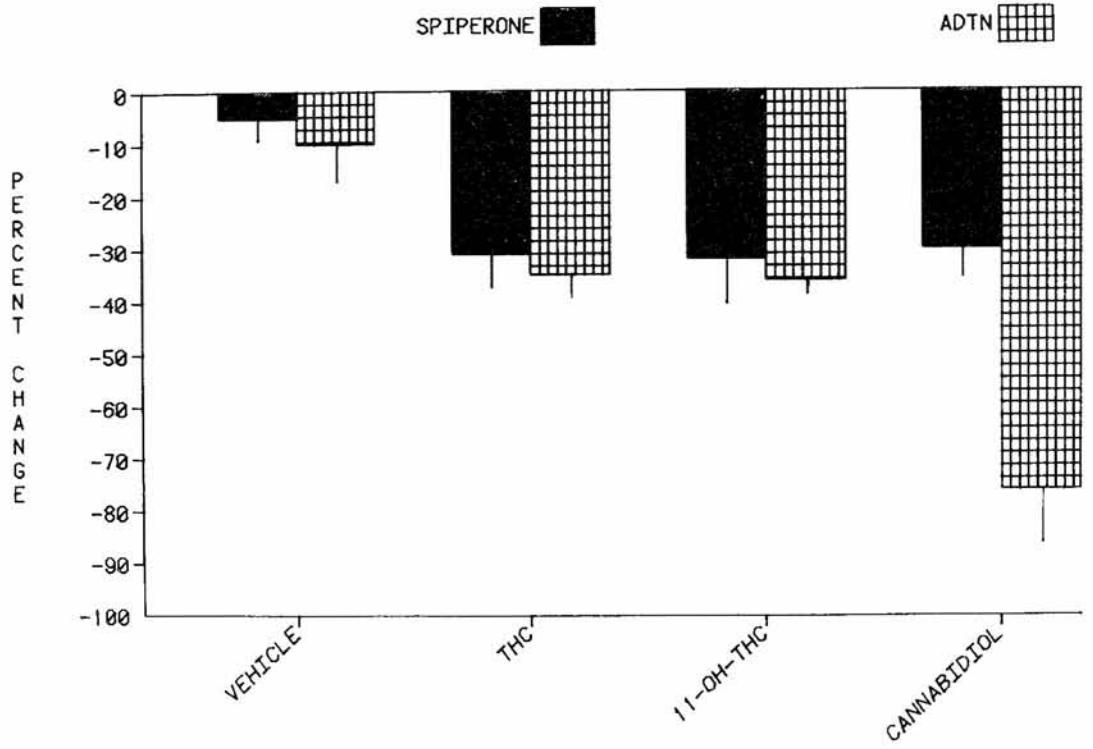

FIGURE 2. Effects of cannabinoids on the specific binding of ^{3}H-spiperone and ^{3}H-ADTN to membranes from mouse corpus striatum. Values are expressed as percent change from non-drug control samples and are the mean +/- S.E.M. of 3 or 4 experiments. Drug concentration was 30 mcM for all cannabinoids. Ligand concentrations were the same as in Fig. 1.

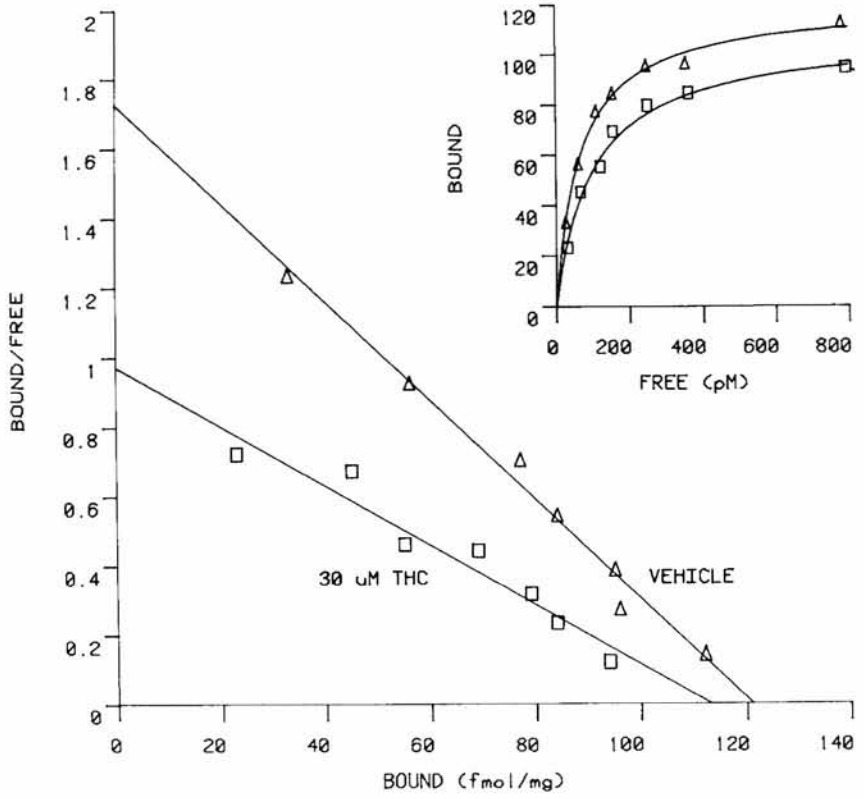

FIGURE 3. Effect of 30 mcM THC on the equilibrium binding properties of ^{3}H-spiperone. Values plotted are the mean of 3 or 4 experiments. Spiperone concentrations were between 50 and 800 pM.

with a K_d of 70 mcM and a B_{max} of 132 pmol/mg of protein. The 30mcM concentration of THC altered the equilibrium binding properties of spiperone. The K_d was increased to 114 pM. This was a statistically significant ($p < 0.05$) 70% decrease in receptor affinity. The Scatchard plot remained linear in the presence of THC. There was also a slight but not statistically significant decrease in the B_{max} parameter. The K_d increase is dependent upon the concentration since a 20 mcM concentration of THC (data not shown) produced an increase of 36% as opposed to a 70% change with the 30 mcM concentration.

Equilibrium binding parameters were also determined in the presence of 30 mcM CBD. The results of this study are shown in Table I. There was a significant increase (260%) in the K_d produced by CBD. There was a slight decrease in the B_{max} that did not reach statistical significance.

The effects of THC and CBD on the binding of dopaminergic agonists and antagonists to spiperone binding sites was then examined. This was accomplished by generating competition curves using dopamine, apomorphine, haloperidol and (+)-butaclamol in the presence of the above mentioned cannabinoids or their vehicle. The effects of a 30 mcM concentration ofTHC are shown in Table II. In vehicle-treated preparations, agonists demonstrated IC_{50} values in the high nanomolar or low micromolar range and antagonists had low nanomolar IC_{50}s. This indicated that spiperone was binding to a site with D_2 (15) properties. IC_{50} values for both dopamine and apomorphine were significantly reduced by the 30 mcM concentration of THC. With K_i values were calculated taking into account the change in spiperone K_d produced by THC, agonist affinities for the D_2 site were enhanced 2 fold by THC.

IC_{50} values for haloperidol and butaclamol were not significantly changed by THC. Therefore, K_i values for the antagonists were increased, thus, their affinities for the D_2 site decreased, as was the case for spiperone when measuredby direct binding.

TABLE I. Effects of Cannabidiol on the Binding Characteristics of ^{3}H-Spiperone

	Kd pM[a]	B_{max} fmol/mg protein[a]
Vehicle	59 (9)	120 (20)
CBD	154 (13)[b]	100 (11)

[a]Mean (+/- S.E.M.) of 3 to 4 experiments.
[b]$p < 0.05$ when compared to vehicle group.

The effect of CBD on agonist and antagonist binding is shown in Table III. Neither agonist nor antagonist IC_{50} values were significantly affected by a 30 mcM concentration of CBD. Therefore, K_i values for both agonists and antagonists were increased by CBD.

TABLE II. Effects of 30 mcM THC on the Displacement of ^{3}H-Spiperone Binding

	IC_{50}[a]		K_i[a]	
	Vehicle	THC	Vehicle	THC
Agonists:				
Dopamine (mcM)	13.5 (1.5)	4.9 (1.1)[b]	2.5	1.3
Apomorphine (nM)	224 (10)	64 (6)[b]	42.4	17.6
Antagonists:				
Haloperidol (nM)	9.2 (0.5)	8.8 (1.3)	1.7	2.4
(+)-Butaclamol (nM)	1.1 (0.1)	1.1 (0.1)	0.21	0.30

[a]Mean (+/- S.E.M). of 3 to 4 experiments.
[b]$p < 0.05$ compared to vehicle group.

TABLE III. Effects of 30 mcM-CBD on the Displacement of ^{3}-H-Spiperone Binding

	IC_{50}[a]		K_i[a]	
	Vehicle	THC	Vehicle	THC
Agonists:				
Dopamine (mcM)	10.1 (0.8)	8.8 (1.3)	1.6	3.4
Apomorphine (nM)	213 (73)	189 (13)	33.7	64.0
Antagonists:				
Haloperidol (nM)	9.9 (1.1)	10.1 (1.7)	1.6	3.4
(+)-Butaclamol (nM)	1.4 (0.2)	1.6 (0.3)	0.22	0.54

[a]Mean (+/- S.E.M.) of 3 to 4 experiments.

The effects of cannabinoids on the binding of ^{3}H-ADTBN, a dopaminergic agonist were also examined. In these studies PVP was used as the cannabinoid vehicle since emulphor significantly decreased the binding of ^{3}H-ADTN. The specific binding of ^{3}H-ADTN was inhibited by THC in a concentration related manner (Fig. 1). Significant ($p < 0.05$) decreases in binding were caused by the 3, 10 and 30 mcM concentrations. ADTN binding was also decreased by 30 mcM concentrations of 11-OH-delta-9-THC and CBD (Fig. 2). CBD was significantly ($p < 0.05$) more effective than the other cannabinoids. Binding was reduced by 73% with the 30 mcM concentration of CBD.

The effects of THC and CBD on the equilibrium binding properties of ^{3}H-ADTN are shown in Table IV. Unlike their effects on spiperone binding, neither cannabinoid significantly changed the K_d when compared to vehicle controls. However, there were significant decreases in the B_{max} produced by both THC and CBD. B_{max} values were decreased 50% by 20 mcM THC and 65% by 20 mcM CBD. K_d and B_{max} values could not be accurately obtained in the presence of 30 mcM CBD because ofthe large decrease in binding.

IV. DISCUSSION

The results of these studies demonstrate interactions between cannabinoids and dopamine receptors that are complex in their nature. The cannabinoids tested have different effects on D_2 and D_3 type receptors. Furthermore, THC and CBD differentially affected binding to the D_2 receptors. Lastly, the effects of the cannabinoids on binding to dopamine recep-

TABLE IV. Effects of THC and CBD on the Binding Characteristics of ^{3}H-ADTN

Drug	Concentration mcM	K_d (nM)[a] Vehicle	K_d (nM)[a] Drug	B_{max} (fmol/mg-protein)[a] Vehicle	B_{max} (fmol/mg-protein)[a] Drug
THC	20	2.3 (0.4)	2.1 (0.6)	66 (6)	33 (6)[b]
	30	1.9 (0.3)	1.7 (0.1)	71 (18)	25 (8)[b]
CBD	20	2.0 (0.3)	1.5 (0.2)	81 (17)	28 (17)[b]

[a]Mean (+/- S.E.M.) of 3 to 4 experiments.
[b]$p < 0.05$ when compared to vehicle group.

tors were quite different than their effects on binding to beta-adrenergic receptors in the mouse brain (14; Hillard and Bloom, this volume).

THC, 11-OH-delta-9-THC and CBD all inhibited the specific binding of ^{3}H-spiperone to membranes from mouse corpus striatum. Analysis of the binding data indicated that the decrease in binding resulted from a decrease in the apparent affinity (an increase in K_d) of the receptor for ^{3}H-spiperone. The increase in K_d produced by the 30 mcM concentration ofCBD (260%) was greater than the change produced by the same concentrationof THC (70%). The specific binding of the dopaminergic agonist ^{3}H-ADTN was also decreased by all three of the cannabinoids used in these studies. Again, CBD was more effective than THC. However, the decrease in binding appeared to be the result of a decrease in the B_{max} (the theoretical number of binding sites) rather than a change in K_d as was the case for spiperone. Thus it appears that cannabinoids interact differently with D_2 and D_3 receptors. This is not surprising since it appears that D_2 and D_3 sites are separate and distinct molecular entities. The binding of agonist and antagonist ligands differ in their sensitivity to guanyl nucleotides (26) and lesions, and in their sub-cellular and cellular localization (15).

THC, although it decreased the binding of dopaminergic antagonists, appeared to increase the affinity of agonists at ^{3}H-spiperone binding sites. Agonist IC_{50} and K_i values for spiperone binding were reduced by 30 mcM THC. CBD did not produce this effect on agonist binding. It reduced the affinity of both agonists and antagonists. Previous studies indicate that there are differences in the binding of agonists and antagonists to ^{3}H-spiperone binding sites. For example, GTP reduces the agonist inhibition of ^{3}H-spiperidol binding itself. Furthermore, the enhancement of agonist binding argues against the probability that the increased spiperone dissociation constant is the result of competitive antagonism by THC at D_2 binding sites. Rather it seems that THC is altering the properties of the receptor site rendering it less favorable to antagonists and more favorable to agonists. Changes such as this could be the result of changes in the membrane environment that the receptor resides in. The concept that the cell membrane is a primary site of action for THC is supported by the work of Gill and Lawrence (27) who found that THC increased the fluidization and disordering of liposomes, and Bach et al. (28) who demonstrated similar effects using synthetic membranes. Gill and Lawrence reported that this effect was specific for those cannabinoids having marijuana-like behavioral activity since cannabinol and cannabidiol produced effects opposite to those of THC, while 11-OH-delta-9-THC was more active than THC. In other studies THC

has been shown to alter the activity of several membrane associated enzymes such as NADH-oxidase (29,30), acetylcholinesterase (31) and various ATPases (8,13). Similarly, cannabinoids can decrease the high affinity uptake of several neurotransmitters into synaptosomes (7,32). These studies indicate that the interaction of THC with membranes may result in changes in the functional compounds such as enzymes. Thus similar changes in other protein compounds such as receptors are also possible.

The importance of the changes observed in these studies to the psychoactive actions of THC is not clear. Since ^{3}H-spiperone and ^{3}H-ADTN binding are inhibited by both THC and CBD, it seems unlikely that this action is of great significance to psychoactivity. On the other hand, the selective enhancement of agonist binding to D_2 sites by THC but not CBD may be of more significance. The significance of this observation is due more to the differences in the effects of THC and CBD on a membrane bound entity rather than the fact that the entity is a dopamine receptor. Similarly, we have observed that THC but not CBD can enhance binding of antagonist to beta-adrenergic receptors in mouse brain (14). The relative role of any of these receptor systems in the effect of the cannabinoids is as yet unknown.

In summary, the studies described here indicate that THC and other cannabinoids can alter the binding of ligands to several different membrane bound neurotransmitter receptors. Furthermore the effects described are selective for both the cannabinoid and the receptor. Different cannabinoids can affect the same receptor differently; and different receptors can be affected differently by the same cannabinoid. Lastly, since THC is highly associated with synaptic membranes after its in vivo administration (33) and can alter membrane physical properties such as fluidity, it is possible that the effects on receptors are due to cannabinoid induced changes in the membrane environment that neurotransmitter receptors reside in. Thus it is possible that membranes can serve as a selective recognition site for the different cannabinoids.

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