FURTHER STUDIES OF THE INTERACTION OF DELTA-9-TETRAHYDROCANNABINOL WITH THE BETA-ADRENERGIC RECEPTOR*

Cecilia J. Hillard
Alan S. Bloom

Department of Pharmacology and Toxicology
Medical College of Wisconsin
Milwaukee, Wisconsin

I. INTRODUCTION

The ability of delta-9-tetrahydrocannabinol (THC) to influence the structure and function of biological membranes is well documented. The partition coefficient of THC into crude brain synaptosomal membranes is on the order of 12,000, indicating that THC has an extremely high affinity for the membrane (1). Lawrence and Gill demonstrated that THC dissolved in and increased the fluidity of spin-labeled liposomes, which are synthetic phospholipid bilayers (2). Furthermore, they found that the less potent (+)-stereoisomer of THC produced a smaller change and that cannabidiol and cannabinol, cannabinoids devoid of THC-like psychoactivity, decreased membrane fluidity.

THC can exert a wide range of effects on membrane associated systems as well. For example, THC has been shown to affect neurotransmitter uptake systems (3,4), membrane bound enzyme systems (5-7) and glucose efflux (8). These findings suggest that a mechanism of THC action could be a primary alteration in the physical properties of the phospholipid bilayer of the membrane (e.g., increased fluidity) which secondarily effects the functioning of membrane associated macromolecules.

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To further investigate this hypothesis, we have begun to study the effects of THC on the beta-adrenergic receptor in membranes from mouse cerebral cortex. Our initial studies demonstrated that the psychoactive cannabinoids THC and 11-OH-delta-9-THC, when administered *in vitro*, biphasically increased the binding of the beta-adrenergic ligand ^{3}H-dihydroalprenolol (^{3}H-DHA) to mouse cerebral cortical membranes (9). We found that cannabidiol, a cannabinoid devoid of THC-like psychoactive properties, did not affect ^{3}H-DHA binding. Results within this limited range of cannabinoids suggestthat the increase in ^{3}H-DHA binding is specific for the psychoactive cannabinoids. These results provide another example of THC-induced changes in a membrane-bound entity.

We now report further studies of the interaction of *in vitro* THC and cerebral cortical beta-adrenergic receptors. We have extended the analysis of the effect of THC on ^{3}H-DHA binding and have investigated the effects of THC on agonist binding as well. These results allow further insight into the mechanism of THC-induced alterations in the beta-adrenergic receptor system.

II. MATERIALS AND METHODS

Delta-9-Tetrahydrocannabinol (generously supplied by NIDA) was administered using an Emulphor (GAF, New York, NY)-ethanol-buffer vehicle in 0.5:0.5:9 proportion (10). Sotalol hydrochloride was a gift from Ayerst Laboratories (New York, NY). All other drugs were obtained from the usual commercial sources.

The beta-adrenergic binding assay was carried out using a modification of the method of Alexander et al. (11). Male ICR outbred mice (ARS, Madison, WI) weighing 20-30 g were used in all studies. After sacrifice, cerebral cortices were removed, pooled and homogenized in 10 volumes of cold 0.25 M sucrose buffer containing 5 mM Tris-HCl (pH 7.4) and 1 mM $MgCl_2$. The homogenate was centrifuged at 800 x g for 20 min; the resulting supernatant was recentrifuged at 14,000 x g for 14 min. The final pellet was resuspended in cold 75 mM Tris HCl (pH 7.4) buffer containing 25 mM $MgCl_2$ to yield a final protein concentration of approximately 0.5 mg/ml. The total incubation volume was 1.0 ml; 10 mcM dl-propranolol was added to half the incubates to account for nonspecific binding. After preincubation of tissue with drugs at 37°C, ^{3}H-DHA (30-60 Ci/mmol, Amersham, Arlington Heights, IL) was added and the incubation was continued for 15 min. Bound and free ^{3}H-DHA were separated using filtration under reduced pressure through

Whatman GF/B glass fiber filters. Specific binding was calculated by subtracting the amount of ^{3}H-DHA bound in the presence of dl-propranolol (non-specific binding) from the amount of ^{3}H-DHA bound in the absence of dl-propranolol. The protein content of each homogenate was estimated using the Bio-rad (Bio-Rad Laboratories, Richmond, CA) dye-binding method of Bradford (12).

Binding data were analyzed using standard methods. The dissociation constant K_D, and binding site density, B_{max}, were estimated from the plot of ^{3}H-DHA bound versus ^{3}H-DHA bound/^{3}H-DHA free using the method of Scatchard (13). The K_D is equal to the negative reciprocal of the slope and B_{max} is equal to the x-intercept; slope and intercept were estimated using linear regression. In competition studies, the IC_{50} values (concentration of cold ligand that reduced ^{3}H-DHA bound by 50%) and 95% confidence intervals were obtained from plots of log concentration of unlabeled ligand versus % ^{3}H-DHA bound using linear regression. Pseudo Hill plots, i.e., log ligand concentration versus log (% ^{3}H-DHA bound/100-% ^{3}H-DHA bound), were also constructed for each ligand. The pseudo Hill coefficient, or slope, was estimated using linear regression.

III. RESULTS

The effect of 10 mcM THC on ^{3}H-DHA binding to the beta-adrenergic receptor of mouse cerebral cortical membranesis shown in Figure 1. In previous studies, we found that the maximum increase in ^{3}H-DHA binding occurred at 10 mcM THC (9). The saturation curves of ^{3}H-DHA binding in the presence of 10 mcM THC or equivalent vehicle show an increase in ^{3}H-DHA binding at all ^{3}H-DHA concentrations with THC treatment. Scatchard analysis of the data is also shown in Figure 1. In the presence of vehicle, the Scatchard plot is linear with a derived K_D (dissociation constant) of 1.86 nM and a B_{max} (binding site density) of 58.6 fmol/mg protein. However, with 10 mcM THC, the Scatchard plot of ^{3}H-DHA binding is shifted to the right and becomes curvilinear.

A curvilinear Scatchard plot is indicative of multiple classes or forms of ^{3}H-DHA binding sites. In Figure 2, a two binding site model was assumed and estimates of the binding parameters were obtained using a curve stripping technique. The low affinity (high K_D) site was assigned parameters close to those obtained in the presence of vehicle alone; the K_D value is 2.5 nM and B_{max} is 70 fmol/mg protein. The high affinity site that results when the low affinity site is "stripped out" has a K_D of 0.4 nM and a B_{max} of 15 fmol/mg

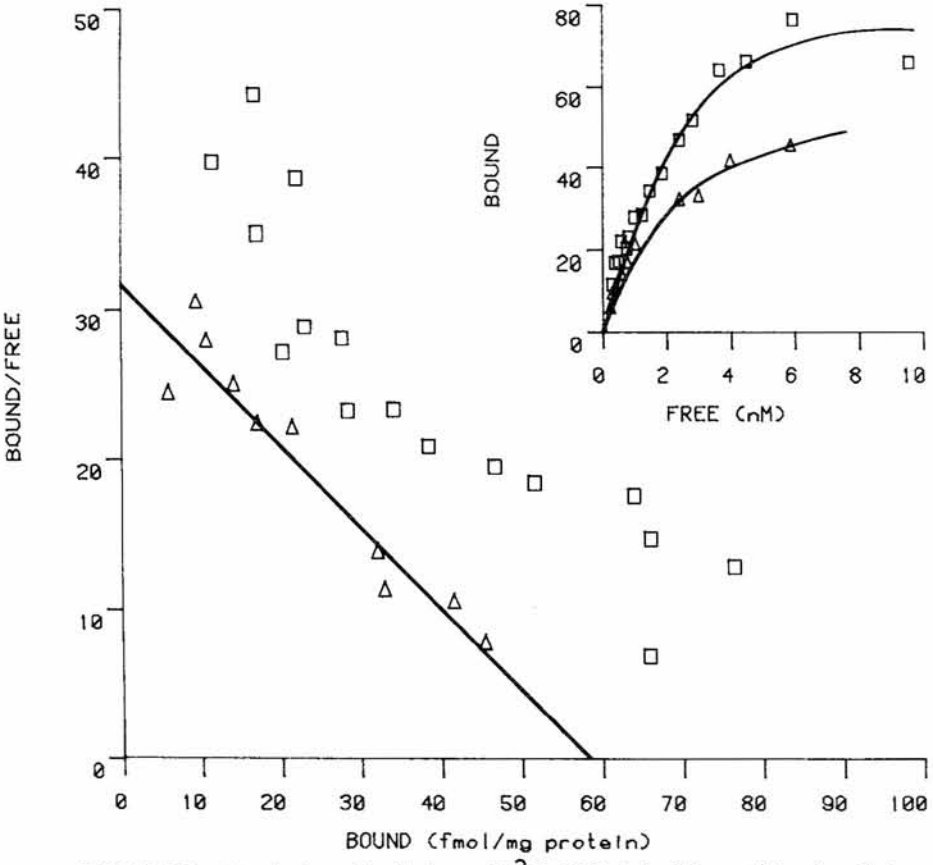

FIGURE 1. Scatchard plots of ^{3}H-DHA binding obtained in the presence of 10 mcM THC (squares) and equivalent vehicle (triangles) are shown. The data points are derived from the saturation curves shown in the inset. The solid line is the best linear fit to vehicle data using linear regression analysis. Inset: Saturation curves of ^{3}H-DHA binding obtained in the presence of 10 mcM THC (squares) and equivalent vehicle (triangles). Each point is the mean of 3-5 experiments.

protein. When these two binding sites are graphically added, the curve shown in Figure 2 is obtained.

Competition studies were carried out in order to determine whether THC produced the same change in binding of both agonists and antagonists to the receptor. In all of the competition studies, ^{3}H-DHA was used at a concentration of 7.0 nM. In Figure 3, the effect of 10 mcM THC on dl-propranolol competition with ^{3}H-DHA is shown. The competition curve is shifted to the left in the presence of THC, corresponding to a change in IC_{50} from 80.4 nM to 36.2 nM (Table I). Pseudo Hill plots

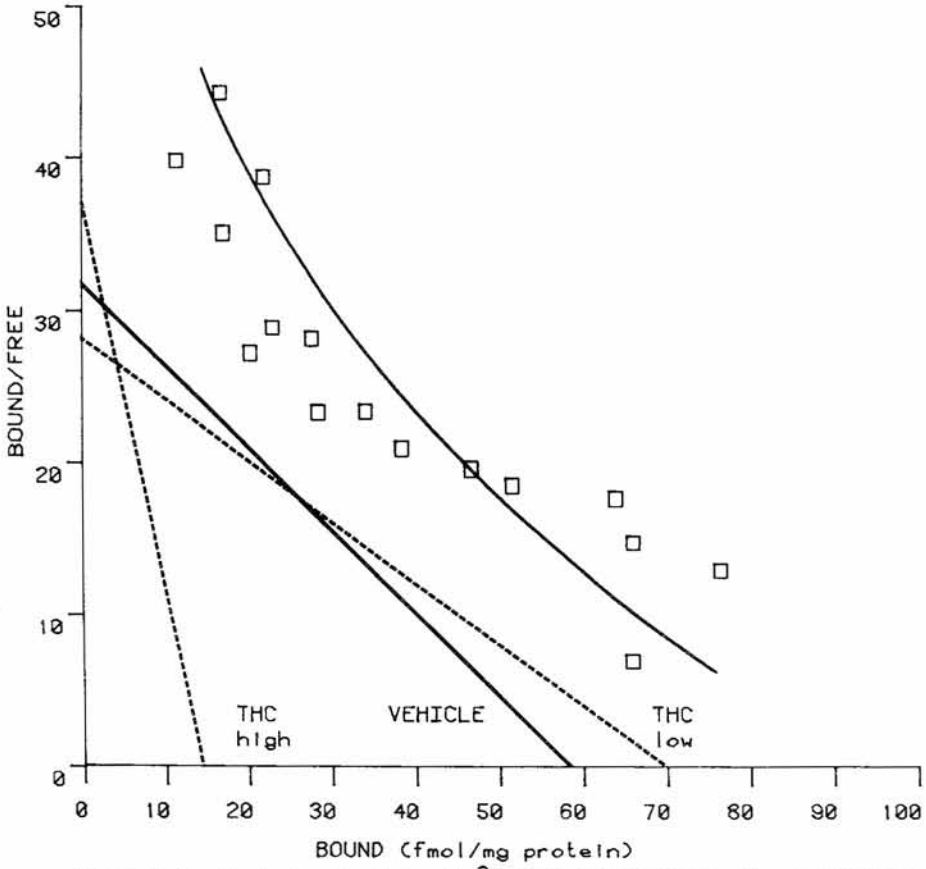

FIGURE 2. Scatchard plot of ^{3}H-DHA binding in the presence of vehicle (solid line) and 10 mcM THC (squares). The high and low affinity sites derived using curve stripping areshown with dashed lines. The Scatchard plot that would be expected from ^{3}H-DHA to these two sites is shown by the curved solid line.

of the competition data are linear in the presence of both 10 mcM delta-9-THC and vehicle (Figure 3). The pseudo Hill coefficient, however, is lower in the presence of THC; 0.73 compared to 1.10 in the presence of vehicle (Table I).

The effect of 10 mcM THC on the competition of the agonist isoproterenol with ^{3}H-DHA is shown in Figure 4. In contrast to its effect on antagonist binding, THC shifts the isoproterenol competition curve to the right. This shift corresponds to a change in IC_{50} from 0.63 mcM to 1.49 mcM with THC treatment (Table I). The pseudo Hill plots are both linear with a slight decrease in pseudo Hill coefficient in the presence of THC.

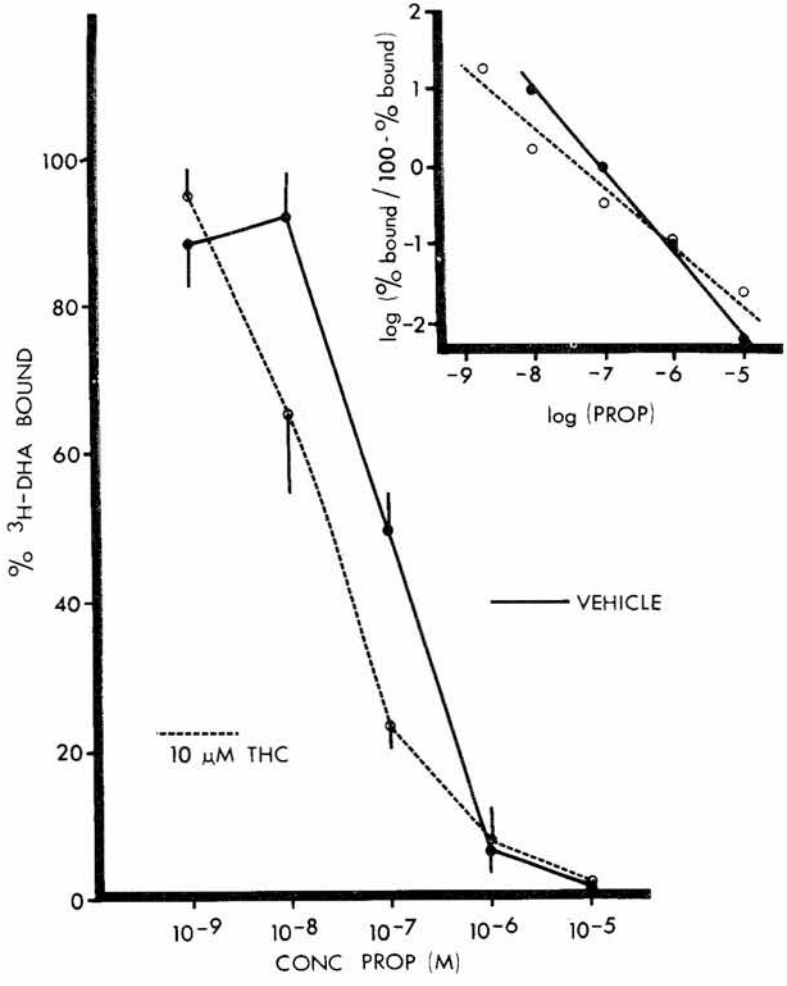

FIGURE 3. The % ^{3}H-DHA bound at various propranolol concentrations in the presence of vehicle (solid circles and line) and 10 mcM THC (open circles and dashed line) are shown. Each point is the mean of 3-5 experiments; vertical lines represent S.E.M. Inset: Pseudo Hill plots of propranolol competition with ^{3}H-DHA in the presence of vehicle and 10 mcM THC (key, as above) are shown. Each point is derived from competition curves shown. Lines are drawn using linear regression analysis.

In Table I are listed the IC_{50} values and pseudo Hill coefficients for propranolol and isoproterenol as well as several other beta-adrenergic receptor ligands. THC reduced the IC_{50} values for all the antagonists investigated. This occurs regardless of the lipid solubility of the antagonist, practolol and sotalol being relatively lipid insoluble ligands (14). The pseudo Hill coefficients of all the antagonists are reduced by THC. Interestingly, the pseudo Hill coefficients for practolol and sotalol are less than one but still decrease in the presence of THC. All of the antagonist pseudo

TABLE II. Effects of THC On Competition of Agonists and Antagonists with ^{3}H-DHA[a]

Competing Ligand	IC50[b] Vehicle	IC50[b] 10 mcM THC	Pseudo Hill Coefficient[c] Vehicle	Pseudo Hill Coefficient[c] 10 mcM THC
Antagonists:				
Propranolol	80.4 (58.7-110.3)	36.2 (27.8-47.0)	1.10	0.73
Alprenolol	29.4 (18.2-42.6)	8.1 (6.8-9.6)	0.91	0.64
Practolol	21.4 (17.3-26.4)	11.8 (9.9-13.9)	0.76	0.50
Sotalol	1.84 (1.53-2.21)	0.98 (0.93-1.03)	0.54	0.49
Partial Agonist:				
Dichloro-isoproterenol	95.7 (88.3-103.8)	167.0 (141-198)	0.50	0.59
Agonists:				
Isoproterenol	0.63 (0.53-0.75)	1.49 (1.36-1.62)	0.63	0.48
Norepinephrine	7.26 (6.37-8.28)	12.52 (10.2-15.3)	0.47	0.59
Epinephrine	6.17	11.22	0.64	0.49

[a] ^{3}H-DHA concentration was 7.0 nM in all studies.

[b] Units are in nM for antagonists and partial agonist and in mcM for agonists (95% confidence interval).

[c] IC_{50} values and pseudo Hill coefficients were obtained from plots of combined data from 3-5 experiments as outline in the Methods section.

Hill plots were qualitatively similar to that shown for propranolol.

The effects of THC on agonist binding are the opposite of the effects of THC on antagonist binding. The IC_{50} values of all the agonists were increased in the presence of THC. The pseudo Hill coefficients are all less than one in the presence of vehicle and changed slightly and inconsistently in the presence of THC. The partial agonist, dichloroisoproterenol, appears agonist-like, since the IC_{50} is increased and pseudo Hill coefficient is slightly increased in the presence of THC.

IV. DISCUSSION

In these studies we have demonstrated that THC increases the binding of antagonists but not agonists to the beta-adrenergic receptor binding site of cerebral membranes. The increase in antagonist binding is accompanied by a decrease in pseudo Hill coefficient while there is no consistent change in the pseudo Hill coefficients or agonists. The differential effects of THC on agonist and antagonist binding indicate a complex interaction of THC with the beta-adrenergic receptor system.

In a previous report from our laboratory, we demonstrated that THC increased the binding of ^{3}H-DHA to beta-adrenergic receptors (9). In that paper, the slopes of Scatchard plots, constructed from a limited number of ligand concentrations, were steepened in the presence of THC but appeared to remain essentially linear. We concluded that the increase in ^{3}H-DHA binding due to a decrease in K_D (increase in affinity). In the present paper, we have increased the number of ligand concentrations used and still find that THC increases ^{3}H-DHA binding. However, a Scatchard plot of these data is distinctly curvilinear in the presence of THC.

The most likely cause of a nonlinear Scatchard plot is ligand binding to more than one class or form of binding sites, each having a different affinity for the ligand. In Fig. 2, we assumed a two binding site model and solved for the K_D and B_{max} of each site a using graphic curve-stripping method. However, when the two sites are added back together, the resulting curve poorly approximates the actual data. It appears more likely that this Scatchard plot is the result of binding to a range of binding sites with a continuum of K_D values. Therefore, THC changes the character of the ^{3}H-DHA binding sites from a single class of sites to a heterogeneous pool with several, perhaps a continuum of K_D values. This interpretation was also confirmed by our inability to fit this

data to a two site model using nonlinear regression techniques.

The effects of THC on the binding of four unlabeled antagonists were assessed using competition studies. The IC_{50} values of all the antagonists are decreased in the presence of THC. The antagonist affinities are apparently increased, although actual K_i values cannot be calculated since an estimate of a single K_D for ^{3}H-DHA in the presence of THC is not easily made.

When agonists are used as the competing ligands, the IC_{50} values are increased in the presence of THC. Since the binding of ^{3}H-DHA itself is increased by THC, it is difficult to determine whether the increase in agonist IC_{50} is due to a real reduction in agonist binding or is a reflection of the increase in ^{3}H-DHA binding with no actual change in agonist binding. In either case, agonist binding is not increased by THC at a concentration which increases antagonist binding.

The slope of pseudo Hill plot of the competing ligand, called the pseudo Hill coefficient, provides an indication of the complexity of the binding process. A pseudo Hill coefficient of less than one will result if the ligand binds to more than one binding site with differing affinities. This reasoning probably explains the low pseudo Hill coefficient for practolol in the presence of vehicle. Practolol has a higher affinity for the beta-1 subtype than for the beta-2 subtype of the beta-adrenergic receptor.

Recent evidence suggests that the low pseudo Hill coefficient observed with agonists results from the formation of a ternary complex between the beta-adrenergic receptor and a guanine nucleotide binding protein (G-protein) within the membrane (15). It is thought that the binding of an agonist to the receptor produces a conformational change in the receptor that allows its association with the G-protein into a ternary complex. Such a conformational change does not occur with antagonist binding. In the absence of GTP, the complex is stable and has a high affinity for the agonist. In the presence of GTP, the ternary complex dissociates and the affinity of the receptor for the agonist decreases. These changes in the receptor are reflected by changes in agonist pseudo Hill coefficients. In the absence of GTP, the majority of agonist binding is to the ternary complex, with high affinity and a low pseudo Hill coefficient. In the presence of GTP, the majority of agonist binding is to the receptor alone having lower affinity and a pseudo Hill coefficient closer to one. Since antagonist binding does not induce the formation of a ternary complex, the pseudo Hill coefficients are approximately one and affinities are the same in the presence and absence of GTP.

The competition studies in these experiments were done in the absence of GTP and agonist pseudo Hill coefficients are less than one in the presence of vehicle. THC treatment appears to have no effect on agonist pseudo Hill coefficient. The pseudo Hill coefficients of the antagonists alprenolol and propranolol are approximately one in the presence of vehicle. Practolol has a low pseudo Hill coefficient for the reason mentioned above. Sotalol, which is reported to be a nonselective antagonist, has a low pseudo Hill coefficient in our study for reasons which are not apparent. In the presence of THC, the pseudo Hill coefficients of the antagonists are all reduced.

The changes in antagonist binding produced by THC, an increase in affinity and decrease in pseudo Hill coefficient, tend to make antagonist binding more agonist-like. It is possible that THC affects the ability of the receptor to discriminate between agonists and antagonists. In the presence of THC, binding of either an agonist or an antagonist has the characteristics of binding to the receptor in a ternary "state". It is possible that THC either promotes the formation of the ternary complex in the absence of any ligand or allows any binding event to result in the change in receptor conformation that is associated with ternary complex formation. To speculate on a mechanism for such a change, it is plausible that THC could act on the membrane to alter the environment of the receptor and G-protein. There is evidence that agents which increase membrane fluidity can affect beta-adrenergic receptor activation (16).

The effects of THC on ^{3}H-DHA binding itself are consistent with this proposal. Binding of ^{3}H-DHA is increased and the Scatchard plot indicates that ^{3}H-DHA binds to more than one form of binding site with THC treatment. It is conceivable that THC could produce a graded change in receptor conformation leading to a spectrum of affinity states. At one end of the spectrum would be the completely unassociated receptor with a low affinity for the ligand and at the other end, the receptor associated with G-protein in a ternary complex having a high affinity for ^{3}H-DHA and other beta-adrenergic ligands.

Although we do not suggest that THC induced changes in binding to beta-adrenergic receptors are a prime cause of the behavioral effect of THC, these data indicate that cannabinoids can have significant effects on the properties of membrane bound entities. These studies offer further support for the hypothesis that the membrane is a primary site of cannabinoid action.

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