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# Some Structural Requirements for Inhibition of High-Affinity Synaptosomal Serotonin Uptake by Cannabinoids

KENNETH M. JOHNSON, WILLIAM L. DEWEY, AND LOUIS S. HARRIS

*Department of Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298*

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SUMMARY

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The effect of $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) and 18 of its metabolites and analogues on the high-affinity uptake of [$^3$H]serotonin into a synaptosome-enriched homogenate of rat forebrain has been determined *in vitro*. Each of the cannabinoids which inhibited [$^3$H]serotonin accumulation did so in a dose-responsive manner. Although some of these compounds do not possess typical $\Delta^9$-THC or marijuana-like effects in laboratory animals or humans, each of the cannabinoids tested, with one exception, inhibited the uptake of serotonin at the concentrations used. A positional activity requirement for the phenolic hydroxyl group was demonstrated by the increased $IC_{50}$ values for the abnormal analogues of $\Delta^8$-THC and cannabidiol relative to their parent compounds. $\Delta^8$-THC and cannabinol were slightly more active than $\Delta^9$-THC, implying that the orientation of protons at the C-8 position may be important for activity. Pseudoequatorial hydroxylation of C-8 resulted in diminished activity, while pseudoaxial hydroxylation of C-8 resulted in little change. In addition, equatorial hydroxylation of C-9 diminished activity relative to axial hydroxylation of C-9. It was also found that hydroxylation of C-9 increased the $IC_{50}$ almost 3-fold relative to the C-9 methylated compound. Finally, it was determined that nonpolar substitution at C-11 diminished the activity only slightly compared to the reduction obtained by hydroxylation of C-11.

## INTRODUCTION

(−)-*trans*-$\Delta^9$-Tetrahydrocannabinol has been reported to alter several physiological functions, such as sleep (1, 2), temperature regulation (3, 4), and predatory aggression (5–7), that are mediated, at least in part, by serotonin. Several authors have reported that relatively high doses of $\Delta^9$-THC[1] elevated endogenous brain 5-HT levels (8–10), while lower doses of $\Delta^9$-THC have been observed to increase (11), decrease (12), or have no effect on brain 5-HT concentrations (6, 13).

Sofia *et al.* (9) reported that $\Delta^9$-THC treatment subsequently retarded the rate of reserpine-induced 5-HT depletion, and suggested an alteration of the vesicular membrane surrounding stored 5-HT to account for the effects of $\Delta^9$-THC on 5-HT concentration. In a subsequent report (14) $\Delta^9$-THC was shown to inhibit the uptake of

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[1] The abbreviations used are: THC, tetrahydrocannabinol; 5-HT, serotonin (5-hydroxytryptamine); HHC, hexahydrocannabinol; CBD, cannabidiol; CBN, cannabinol; PVP, polyvinylpyrrolidone; DMSO, dimethyl sulfoxide.

[$^3$H]5-HT into a synaptosomal preparation. In order to determine the importance of this effect for other pharmacological activity, as well as determining something of the physicochemical characteristics of the receptor involved in this action of $\Delta^9$-THC, we have examined the effects of $\Delta^9$-THC and several of its metabolites and congeners on the high-affinity synaptosomal accumulation of [$^3$H]5-HT.

## METHODS AND MATERIALS

Adult male Sprague-Dawley rats (200–300 g) were used in all experiments. The rats were decapitated between 1:00 and 3:00 p.m. Their brains were removed, and the forebrain was dissected from the brain stem by a section passing just anterior to the superior colliculi and exiting at the anterior border of the mammillary bodies.

Synaptosomal uptake activity was measured essentially by the method of Coyle and Snyder (15). The forebrain was homogenized with a Thomas grinding vessel and Teflon pestle (0.004–0.006-inch clearance) in 9 volumes of ice-cold 0.32 M sucrose through which 5% $CO_2$–95% $O_2$ had been bubbled for 15 min. The homogenate was centrifuged at 1000 × $g$ for 10 min at 4°. After gently stirring the supernatant to obtain a uniform suspension of synaptosomes, a 0.2-ml aliquot was added to a flask containing 3.8 ml of Krebs-Henseleit bicarbonate medium (pH 7.4) with glucose (11 mM), half-strength calcium (1.3 mM), ascorbic acid (0.2 mg/ml), disodium EDTA (0.05 mg/ml), and pargyline (125 $\mu$M), an inhibitor of monoamine oxidase.

Fifty microliters of the cannabinoid or its vehicle (20% polyvinylpyrrolidone in 0.9% NaCl) were added to the reaction flasks. Following a 10-min preliminary incubation at 37° under 5% $CO_2$–95% $O_2$ in a Dubnoff metabolic shaker, the radioactive serotonin (50 $\mu$l) was added (50 nM) and the incubation was continued for 5 min, a time at which the uptake of 5-HT in this system was still linear. The reaction was terminated by adding 4 ml of ice-cold 0.9% NaCl and placing the flasks in an ice bath. The samples were transferred to cold centrifuge tubes and centrifuged at 20,000 × $g$ for 20 min at 4°. The supernatant was aspirated, and the pellet was washed by resuspension in ice-cold 0.9% NaCl and recentrifuged at 20,000 × $g$. After the surface of the pellet had been rinsed with 0.9% NaCl, the pellet was solubilized in 0.7 ml of "NCS" tissue solubilizer (Amersham/Searle). The radioactivity of 0.5-ml aliquots was then estimated in a scintillation fluid containing 5 g of 2,5-diphenyloxazole and 100 mg of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene per liter of toluene by liquid scintillation spectrometry.[2]

At least three concentrations of each compound, along with a vehicle control, were incubated in triplicate at 37° as well as in ice (2°). The means of each of the triplicates after incubation at 2° were subtracted from each of the respective individual points obtained after incubation at 37°. These values (now corrected for passive diffusion) were converted to percentage of control and plotted against log micromolar concentration. $IC_{50}$ values (the concentration required to inhibit uptake by 50%) were obtained from linear regression analysis, and the significance of this correlation was determined by $t$-test (16). In two cases 50% inhibition was not obtained at the highest concentration used, and so the $IC_{50}$ values were obtained by extrapolation. The 95% confidence intervals about the $IC_{50}$ as well as about the slope of the linear regression line were also determined (16). In addition, one-way analysis of variance was performed on each compound tested, and significance of effect across all concentrations was verified using the $F$-test.

The highly hydrophobic character of the cannabinoids necessitates the use of a suspending agent such as PVP to prevent total precipitation in aqueous medium. In order to determine the amount of $\Delta^9$-THC remaining in solution after addition of the PVP–$\Delta^9$-THC suspension to the medium, [$^3$H]$\Delta^9$-THC in PVP was added to Krebs-Henseleit buffer in a final concentration of 100 $\mu$M (the final concentration of PVP was 0.37%). After thorough mixing of the suspension an aliquot was taken for estimation of radioactivity, and the rest was centrifuged at 40,000 × $g$ for 60 min. After the radioactivity had been determined in

[2] The authors gratefully acknowledge the expert technical assistance rendered by Andrea Leontire.

an aliquot of the supernatant, the solubility of $\Delta^9$-THC was estimated by dividing the concentration of [$^3$H]$\Delta^9$-THC found in the supernatant by the concentration in the original suspension. This technique has previously been reported for determining $\Delta^9$-THC solubility in a DMSO suspension (17).

The radiochemical purity of [*G*-$^3$H]5-hydroxytryptamine creatinine sulfate (500 mCi/mmole, Amersham/Searle) was determined to be 89% by thin-layer chromatography on cellulose in 1-butanol–acetic acid–water (12:3:5). The sources of the cannabinoids were as follows: [$^3$H]$\Delta^9$-THC (30.8 Ci/mole), $\Delta^9$-THC, $\Delta^8$-THC, 11-hydroxy-$\Delta^9$-THC, 11-hydroxy-$\Delta^8$-THC, 8$\alpha$- and 8$\beta$-THC, 8$\alpha$,11-dihydroxy-$\Delta^9$-THC, 8$\beta$,11-dihydroxy-$\Delta^9$-THC, cannabinol, and cannabidiol were received from Dr. Monique Braude of the National Institute on Drug Abuse. 9-*nor*-$\Delta^8$-THC, 11-methyl-$\Delta^8$-THC, 11-methoxy-$\Delta^8$-THC, 11-amino-$\Delta^8$-THC, 9-*nor*-9$\alpha$-hydroxy-HHC, and 9-*nor*-9$\beta$-hydroxy-HHC were received from Drs. Raymond Wilson and Everette May of the National Insitute of Arthritis, Metabolism and Digestive Diseases. The abnormal $\Delta^8$-THC and abnormal cannabidiol were received from Dr. Raj Razdan of Sheehan Institute and Sharps Associates. Figure 1 shows the structures of the cannabinoids used in this study.

## RESULTS

Since many of these cannabinoids were available in limited quantity, the approximate range of concentrations necessary to produce 50% inhibition was estimated by comparing the inhibition produced by each drug at 30 $\mu$M with that produced by 30 $\mu$M $\Delta^9$-THC (18). The relative potencies thus obtained were significantly correlated with the $IC_{50}$ values reported here ($r = 0.73$, $p < 0.001$).

Each of the compounds tested significantly inhibited the high-affinity uptake of [$^3$H]5-HT over the concentration range examined (*F*-test, $p < 0.025$ for 9-*nor*-9$\beta$-hydroxyhexahydrocannabinol; $p < 0.01$ for the other cannabinoids) except for 8$\alpha$,11-dihydroxy-$\Delta^9$-THC, which showed only slight (7.5%), nonsignificant inhibition at the highest concentration tested (90 $\mu$M). In general, the inhibition curves obtained correlated well with the line derived from

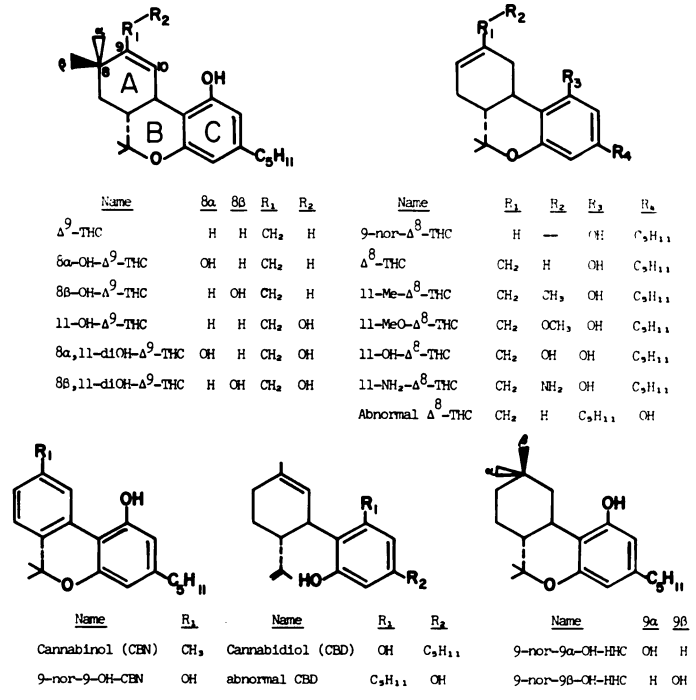


FIG. 1. *Structures of cannabinoids used in this study*

linear regression analysis of the data plotted in log dose-response fashion (mean $r = -0.879 \pm 0.025$) and thus give credence to the method used in determining $IC_{50}$ values.

The 1-hydroxyl group of the cannabinoid compounds is believed to be essential to cannabinoid activity (19). Therefore, when the 1-hydroxyl and the 3-alkyl side chain are exchanged, a reduction in activity is expected. This expectation is confirmed by the data in Table 1A. Both abnormal $\Delta^8$-THC and, particularly, abnormal cannabidiol are less active than their respective parent compounds. It should be noted, however, that an absolute requirement of the phenolic hydroxyl group for activity cannot be inferred from these comparisons, since the position of the alkyl side chain is also altered.

The data in Table 1B show that although the difference is not great, $\Delta^9$-THC is less active than either $\Delta^8$-THC or cannabinol. Since there is no difference in activity between $\Delta^8$-THC and CBN, it appears that minor changes in the electronic and spatial configuration of the A ring can be tolerated without loss of activity. The differences between $\Delta^8$-THC, CBN, and $\Delta^9$-THC must result from the different orientations of the C-8 protons. Both $\Delta^8$-THC and CBN have a single proton coplanar with the A ring, while $\Delta^9$-THC has two protons, one pseudoaxial ($8\beta$) and one pseudoequatorial ($8\alpha$). One or both of these protons may hinder the effective at-

TABLE 1

*Inhibition of synaptosomal uptake of [$^3$H]5-HT by cannabinoids*

| Cannabinoid | $IC_{50}$ (95% confidence limits) | $r$ |
|---|---|---|
| | $\mu M$ | |
| A. Compounds with C-1 and C-3 alterations | | |
| $\Delta^8$-THC | 22.4 (30.7-24.3) | −0.980 |
| Abnormal $\Delta^8$-THC | 32.5 (28.7-36.8) | −0.958 |
| CBD | 9.99 (6.46-15.4) | −0.712 |
| Abnormal CBD | 38.3 (30.7-47.7) | −0.887 |
| B. Compounds with A ring modifications | | |
| $\Delta^9$-THC | 29.3 (24.6-35.0) | −0.927 |
| $\Delta^8$-THC | 22.4 (20.7-24.3) | −0.980 |
| CBN | 21.9 (19.1-25.1) | −0.964 |
| C. Compounds with C-8 hydroxylated | | |
| $\Delta^9$-THC | 29.3 (24.6-35.0) | −0.927 |
| $8\alpha$-OH-$\Delta^9$-THC | 47.5 (40.5-55.9) | −0.696 |
| $8\beta$-OH-$\Delta^9$-THC | 26.1 (21.7-31.4) | −0.895 |
| 11-OH-$\Delta^9$-THC | 62.6 (52.3-74.9) | −0.930 |
| $8\alpha$,11-diOH-$\Delta^9$-THC | 5285.0[a] (755-36967) | −0.731 |
| $8\beta$,11-diOH-$\Delta^9$-THC | 101.0[a] (77.9-131.0) | −0.933 |
| D. Compounds with C-9 hydroxylated | | |
| Cannabinol (CBN) | 21.9 (19.1-25.1) | −0.964 |
| 9-*nor*-9-OH-CBN | 58.5 (49.1-69.8) | −0.922 |
| 9-*nor*-9$\alpha$-OH-HHC | 38.0 (27.3-52.8) | −0.837 |
| 9-*nor*-9$\beta$-OH-HHC | 78.3 (61.3-100.0) | −0.825 |
| E. Compounds with substitutions at C-9 and C-11 | | |
| 9-*nor*-$\Delta^8$-THC | 15.1 (13.2-17.3) | −0.974 |
| $\Delta^8$-THC | 22.4 (20.7-24.3) | −0.980 |
| 11-Me-$\Delta^8$-THC | 39.7 (30.5-51.6) | −0.954 |
| 11-MeO-$\Delta^8$-THC | 17.7 (15.5-20.3) | −0.968 |
| 11-OH-$\Delta^8$-THC | 60.0 (51.8-69.5) | −0.957 |
| 11-$NH_2$-$\Delta^8$-THC | 16.7 (11.8-23.8) | −0.622 |
| $\Delta^9$-THC | 29.3 (24.6-35.0) | −0.927 |
| 11-OH-$\Delta^9$-THC | 62.6 (52.3-74.9) | −0.930 |

[a] Obtained by extrapolation.

tachment of the A ring to its binding site.

Hydroxylation of C-8 can be either pseudoequatorial (8$\alpha$) or pseudoaxial (8$\beta$). It is evident from the C-8 hydroxylation of both $\Delta^9$-THC and 11-hydroxy-$\Delta^9$-THC (Table 1C) that pseudoaxial hydroxylation results in only slight changes while pseudoequatorial hydroxylation results in greatly diminished activity. This implies either that the 8$\alpha$-hydroxyl produces steric hindrance about the C-9 and C-11 positions of the molecule or that the C-8 position itself is important in binding to this receptor site.

Apparently the portion of the receptor which binds the C-9–C-11 region is hydrophobic in nature. This can be inferred from the difference in activity between CBN, which is methylated at C-9, and 9-*nor*-9-hydroxy-CBN, which is hydroxylated at C-9 (Table 1D). Table 1D also shows data which illustrate the importance of the place of the hydroxyl group at C-9 relative to the A ring. An equatorial hydroxyl group at C-9 results in a much less active molecule (9-*nor*-9$\beta$-hydroxy-HHC) than does axial hydroxylation at the same position (9-*nor*-9$\alpha$-hydroxy-HHC).

The physicochemical properties of the C-9–C-11 region of the receptor are illuminated by the data shown in Table 1E. Some reduction in activity can be produced by methylation of both the C-9 of 9-*nor*-$\Delta^8$-THC and the C-11 of $\Delta^8$-THC. That this reduction in activity is produced by the bulkiness of the groups is disputed somewhat by the finding that the addition of a methoxy group to C-11 does not reduce the activity. However, it should be noted that because of the C–O–C bond angle the possible positions of the C-11 *O*-methyl group are radically different from those of the C-11 methyl group. This factor could account for the observed differences between 11-methyl-$\Delta^8$-THC and 11-methoxy-$\Delta^8$-THC. The hydrophobic quality of the receptor in this region is also demonstrated by the great diminution of activity observed as a result of the hydroxylation of the C-11 of both $\Delta^8$-THC and $\Delta^9$-THC. The obvious discrepancy between 11-hydroxy-$\Delta^8$-THC and 11-amino-$\Delta^8$-THC may be a result of repulsion between the 11-hydroxyl and the 1-hydroxyl in one case and hydrogen bonding between the 11-amino and the 1-hydroxyl in the other.

In order to gain an indication of $\Delta^9$-THC availability in the aqueous phase as well as to provide a basis for comparison of the solubility of $\Delta^9$-THC in PVP with that found for $\Delta^9$-THC in a DMSO suspension (17), we determined the solubility of 100 $\mu$M [$^3$H]$\Delta^9$-THC in PVP exactly as described for determining [$^{14}$C]$\Delta^9$-THC solubility in DMSO (17). We found 3.9% ± 0.04 of the [$^3$H]$\Delta^9$-THC in the aqueous 40,000 × *g* supernatant, relative to the amount found in the original suspension. This is greater than the 0.5% found using 100 $\mu$M [$^{14}$C]$\Delta^9$-THC in a DMSO suspension, but roughly equal to the 1.9% using 50 $\mu$M $\Delta^9$-THC in DMSO (17).

## DISCUSSION

Two obvious questions arise from these structure-activity relationships: (a) What is the mechanism of action of the observed inhibition of serotonin uptake, and (b) what is the relationship between these structure-activity correlations and the pharmacological activity of these cannabinoids? In an earlier communication we reported that $\Delta^9$-THC inhibited the high-affinity synaptosomal uptake of [$^3$H]5-HT in a noncompetitive manner (20). This study also showed that $\Delta^9$-THC inhibited the uptake of norepinephrine and dopamine with the same relative affinity. These observations have recently been confirmed (17). We also showed that this effect was rather nonspecific, since leucine accumulation was inhibited to a similar degree, and that the inhibitory capacity of $\Delta^9$-THC for 5-HT uptake was not diminished by treating the rats with reserpine (5 mg/kg intraperitoneally) 18 hr prior to the assay *in vitro* (20). These data imply that the primary locus of uptake inhibition is not the reserpine-sensitive synaptic vesicle, but probably the neuronal membrane itself.

The apparent nonspecificity mentioned above need not detract from the argument that inhibition of neurotransmitter uptake may be involved in the mechanism of action of $\Delta^9$-THC. For instance, reserpine inhibits both adrenal medulla granular uptake of epinephrine (21) and blood plate-

let uptake of serotonin (22) to the same extent at equimolar concentrations. Also, the endogenous stores of norepinephrine, dopamine, and serotonin are depleted to a similar extent by reserpine, the rate of disappearance being directly related to the turnover times of these amines (23). In addition, reserpine treatment results in an induction of tryptophan hydroxylase (24) and tyrosine hydroxylase (25), both presumably consequent to the depletion of their respective end products, serotonin and the catecholamines. It is clear, then, that specificity need not be a requirement for determining the importance of an observed effect relative to its mechanism of action.

Banerjee *et al.* (17) have recently published data concerning the effects of $\Delta^9$-THC and 12 of its derivatives on the uptake of several putative neurotransmitters, including serotonin. Direct comparisons are possible only with five of the $\Delta^9$-THC analogues. These comparisons reveal the following agreements: (a) $\Delta^8$-THC is significantly more active than $\Delta^9$-THC, (b) 11-hydroxy-$\Delta^8$-THC and 11-hydroxy-$\Delta^9$-THC are similar in potency, and (c) cannabidiol is 2–3 times more potent than $\Delta^9$-THC. The discrepancies are (a) that the C-11 hydroxylated compounds are similar in activity to $\Delta^8$-THC in the report of Banerjee *et al.*, while our data indicate that 11-hydroxy-$\Delta^8$-THC and 11-hydroxy-$\Delta^9$-THC are significantly less active than either $\Delta^8$-THC or $\Delta^9$-THC, and (b) that cannabinol is only moderately active in their study compared to the high activity observed in this study. Possibly these two discrepancies may be due to differing affinities of these compounds for the hypothalamic synaptosomes (used by Banerjee *et al.*) and for the forebrain synaptosomes utilized here.

Unfortunately, the pharmacological profiles of many of the compounds used here are far from complete, making pharmacological correlations tentative, if not impossible. In addition, comparisons between studies *in vivo* and *in vitro* should be tempered by the fact that the amount of drug actually present in the brain *in vivo* is subject to pharmacodynamic considerations not evident *in vitro*. Cannabinol, although inactive in most tests, does display slight anticonvulsant activity (26) as well as have slight non-cannabinoid depressant activity in the dog static ataxia test.[3] Cannabinol, like cannabidiol, was also reported to be active when injected intracerebrally (27). There is increasing evidence that CBD may act as a $\Delta^9$-THC antagonist, implying that CBD and $\Delta^9$-THC may have similar affinities for some pharmacological receptors. For example, CBD has been shown to block the effects of $\Delta^9$-THC on aggression induced by deprivation of rapid-eye-movement sleep (28), on operant behavior parameters (29), and on heart rate, respiration, and rectal temperature (30). In addition, CBD is similar in potency to $\Delta^9$-THC as an anticonvulsant after maximal electroshock treatment (26).

Although the 11-hydroxy metabolites of $\Delta^9$-THC and $\Delta^8$-THC are generally considered to be at least as active as the parent compounds (31–33), there is presumptive evidence that $\Delta^9$-THC is more active than the 11-hydroxy metabolites in producing hypothermic effects (34) and prolongation of barbital sleeping time (35).

Four of the $\Delta^8$-THC derivatives used in this study were examined in our laboratory using the dog static ataxia test. The order of potency for these compounds in this test *in vivo* correlates well with their potency in inhibiting serotonin uptake (9-*nor*-$\Delta^8$-THC $\cong$ $\Delta^8$-THC $>$ 11-methyl-$\Delta^8$-THC $>$ abnormal $\Delta^8$-THC).[3]

In a paradigm in which rats were trained to discriminate $\Delta^9$-THC from 0.9% NaCl, several compounds, including 9-*nor*-$\Delta^8$-THC and 8$\beta$-hydroxy-$\Delta^9$-THC, produced responses appropriate to $\Delta^9$-THC, while 8$\alpha$-hydroxy-$\Delta^9$-THC, 8$\alpha$,11-dihydroxy-$\Delta^9$-THC, and 8$\beta$,11-dihydroxy-$\Delta^9$-THC did not (36). Although these findings are positively correlated with uptake inhibition potency, exceptions were also found; for instance, 9-*nor*-9$\beta$-hydroxy-HHC ($IC_{50}$ = 78 $\mu$M) substituted for $\Delta^9$-THC, while cannabidiol ($IC_{50}$ = 10 $\mu$M) did not (36).

A serious limitation in making structure-activity inferences lies in the possible differential solubilities of various THC analogues tested in this system *in vitro*. Although we have no direct evidence that the

[3] Unpublished observations.

inhibition of serotonin uptake is not a simple reflection of the partition coefficients for these compounds, we have shown significantly different activities for several pairs of cannabinoids which should have the same partition coefficients; i.e., CBD and abnormal CBD, $\Delta^8$-THC and abnormal $\Delta^8$-THC, 8$\alpha$-hydroxy-$\Delta^9$-THC and 8$\beta$-hydroxy-$\Delta^9$-THC, 8$\alpha$,11-dihydroxy-$\Delta^9$-THC and 8$\beta$,11-dihydroxy-$\Delta^9$-THC, and 9-*nor*-9$\alpha$-hydroxy-HHC and 9-*nor*-9$\beta$-hydroxy-HHC are all presumably equally soluble pairs with different inhibitory efficacies. However, this does not rule out the possibility that different cannabinoids may be differentially soluble in PVP suspension, and the results should be viewed accordingly. In this regard it should be noted that Banerjee *et al.* (17) reported that 50 $\mu$M cannabidiol was 5.5 times more soluble than 50 $\mu$M $\Delta^8$-THC and 2.3 times more soluble than 50 $\mu$M $\Delta^9$-THC in a DMSO suspension.

Comparison of the activity of these compounds with some of the clinically used antidepressants [which are approximately 100 times more potent in inhibiting serotonin uptake (37, 38)] should be viewed in light of the fact that only about 4% of $\Delta^9$-THC remains in true solution after the PVP-THC suspension is added to the aqueous buffer. If it is true, as others have suggested (17), that uptake inhibition depends greatly on the amount of THC in solution, then the $IC_{50}$ values reported here would be exaggerated some 25-fold (i.e., $\Delta^9$-THC $IC_{50}$ = 29.3 $\mu$M; 29.3 $\mu$M $\times$ 0.039 = 1.1 $\mu$M), which would make the potency of $\Delta^9$-THC roughly equal to that of imipramine ($IC_{50}$ = 0.5 $\mu$M) in inhibiting serotonin uptake (38).

In finding contradictions to the hypothesis that drugs producing "cannabinoid-like" activity are associated with a high affinity for inhibition of serotonin uptake, we are struck with the thought that, in view of the tremendous diversity of biological activities assigned to the cannabinoids over the last several years, it would be highly unlikely that a single biochemical parameter could predict whether or not a drug will elicit a "cannabinoid-like" response. This is not to say that the effects of $\Delta^9$-THC on neurotransmitter uptake are not involved in its mechanism of action. On the contrary, it seems clear that the "cannabinoid-like" response is a composite of biochemical activities involving the interaction of many neurochemical control mechanisms, and synaptic uptake is one of those mechanisms affected by THC.

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