MONOAMINE OXIDASE AND CANNABIS: SPECIES, TISSUE, AND DRUG SELECTIVITY

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I. INTRODUCTION

The interaction of cannabis components with monoamine oxidase (EC 1.4.3.4. amine: O_2 oxidoreductase, MAO) has been studied for several reasons. First, MAO plays a key role in the metabolism of monoamines in brain tissue (1). Secondly, interaction of cannabinoids with biological membranes is a paramount feature of their effect (2-8). Finally, since MAO can be isolated from both brain and liver tissues using similar means, it would be possible to discern tissue selectivity with respect to the drug effect.

Monoamine oxidase is believed to be an enzyme with multiple activities. Whether or not these activities are a result of the existence of multiple forms of the enzyme is still under debate (9-12). Nevertheless, the effect of inhibitors on these activities is a major component of this debate. The ability of different MAO inhibitors to exert selective inhibition usually depends on the substrate specificity (13, 14). Moreover, MAO preparations from different species (human, pig, beef, rat) tissues (brain, liver, kidney) give rise to differences in substrate specificity and inhibitor selectivity (11). Hence, the involvement of MAO with the psychoactivity of cannabis, or with any other of its effects, has to be tested using more than one source of the enzyme and more than one substrate.

Four different sources for MAO were used in the present study; human brain and liver and procine brain and liver. A variety of substrates was used to follow the two distinct

activities of MAO. Type A activity is believed to be responsible for the deamination of 5-hydroxytryptamine (5-HT, serotonin). Type B activity deaminates 2-phenylethylamine (PEA) and benzylamine (BA). Since MAO is a lipoprotein containing some 20 moles of phospholipids per mole of protein (1),we examined the hypothesis that these phospholipids mayfunction as the site of interaction with the lipophylic cannabinoids.

Various cannabinoids including delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) and a crude extract of cannabis were used and compared in this study.

II. METHODS

A. Preparation of Mitochondria

Mitochondria were prepared according to Tipton (15) with the exception that the mitochondria (protein concentration of 10 mg/ml) were stored frozen in sucrose 0.25 M, pH 7.6.

B. Solubilization of Monoamine Oxidase

The mitochondrial preparation (stored frozen overnight) was allowed to thaw at room temperature, and the suspension was then sonicated in a sonifier 100 W at an amplitude of 8 microns peak to peak, for 15 min. The sonicate was then centrifuged at 100,000 g for 120 min. Temperature was kept under 4°C through all the treatments. The supernatant (soluble MAO) was decanted and used for the assay of enzyme activity.

C. Assay of MAO Activity

Either spectrophotometric (16) or radiochemical (17) determination of the deamination products of the different substrates was used.

D. Effects of Cannabinoids

Aliquots of ethanolic stock solutions of cannabis extract and the various cannabinoids were mixed with the assay buffer before adding the MAO preparation. Ethanol was included in the control tubes at the same final concentration (0.33% v/v). Following a preincubation period of 30 min at 37°C, theenzymatic reaction was initiated by adding the substrate.

E. Chloroformic Extraction of Soluble MAO Preparation

To a given volume of soluble MAO preparation, THC or CBD were added to a final concentration of about 30-60 mcg per mg protein. In the controls ethanol replaced THC or CBD. After an incubation of 30 min at room temperature, the mixture was extracted with 3 volumes of cold chloroform by vigorous mixing for 5 min. Following centrifugation at 10,000 g for 20 min the upper aqueous layer was collected, a stream of N_2 was passed over it to assure the removal of chloroform, and then the preparation was assayed for enzyme activity. The extraction procedure must take place no later than 24 hr after the sonication step.

F. Preparation of Liposomes

Liposomes were prepared according to Alhanaty and Livne (7) in KPi buffer, 50 mM, pH 7.4 using various phospholipids.

G. Cannabis Extract

Cannabis (hashish) was extracted twice with petroleum ether (1 g in a total of 100 ml). The extract included about 25% of the hashish solid materials. Stock solutions were prepared in ethanol.

H. Fractionation and Isolation of Cannabinoids from Cannabis Extract

Silca gel (GF) preparative thin layer chromatography (TLC) plates were used with toluene: chloroform: methanol (100: 10:1, by volume) as a solvent system. The R_f values of the different fractions were determined by using UV light. To assure that cannabinoids were actually detected, the following procedure was used: Fractions were scraped from the preparative TLC plate and were re-extracted from the silica gel with chloroform: methanol (10: 1, by volume). Samples of these re-extracted fractions were applied to a silca gel (GF) analytical TLC plate, and developed with the same solvent system as above. After drying, the plate was sprayed with ethanolic solution of Black K Salt (1 mg/ml) (18). Purple spots, if apparent on the TLC plate, indicate the presence of cannabinoids.

I. Data Presented

Each of the experiments was repeated at least four times in duplicate, yielding identical patterns. Duplicates agreed within 5% experimental error, and representative experiments are shown.

III. RESULTS

A. Effects of Cannabinoids and Hashish Extract on Pig Brain MAO

MAO activity of pig brain mitochondria was inhibited by THC and hashish (cannabis) extract. Fig. 1 shows that the inhibition was dependent on preincubation with the cannabinoids. In contrast, CBD was ineffective even after one hour

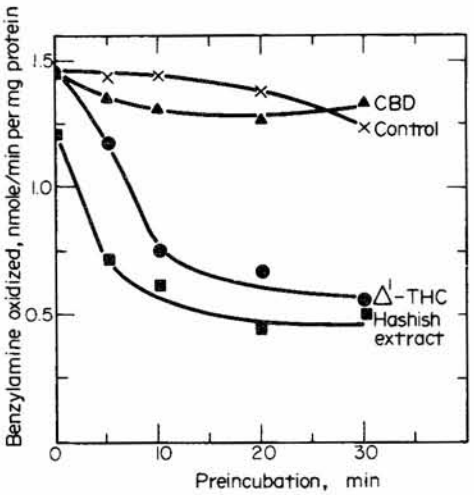

FIGURE 1. The inhibition of pig brain MAO activity by THC and hashish extract as affected by preincubation with the cannabinoids. Aliquots of the mitochondrial suspension (1 mg protein in 3 ml KPi buffer, pH 7.4) were incubated at 37°C with one of the following drugs (in mcg/mg protein): THC, 94; CBD, 94; hashish extract, 19.

of incubation with the mitochondrial preparation. The extent of inhibition of pig brain MAO by THC or by the cannabis extract was dose-dependent (Fig. 2), the extract beingabout ten times more potent than THC.

Unlike THC and the Extract, CBD was essentially ineffective at a wide concentration range (Fig. 2). If administered concomitantly, CBD is known to interfere with the effects of THC in man (19) and in experimental animals (20). CBD is able to counteract in vitro the inhibitory effect of THC on MAO of pig brain mitochondria (Fig. 3). CBD also partially diminishes the inhibitory effect of the extract. If CBD is added at the onset of the reaction, following the preincubation period, it no longer interferes with the inhibitory effect of THC. When CBD is added during the pretreatment with the THC the interference is proportional to the duration of the concomitant incubation.

When other MAO substrates were used, the activity of pig brain mitochondrial MAO was inhibited also by THC and hashish extract (Fig. 4). Again, CBD, was found to be ineffective. Different degrees of inhibition with THC were found for the

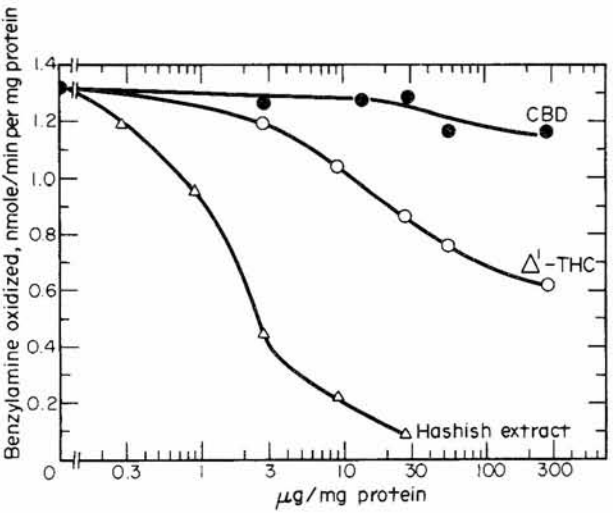

FIGURE 2. Effect of THC, CBD and hashish extract of pig brain MAO activity. Preincubation time: 30 min.

different substrates, ranging from 80% inhibition when dopamine was the substrate to about 25% when tryptamine was used. The extract was inhibitory at a much lower dose and its effect varied as well according to the substrate tested. Phenylcyclopropylamine (SKF-385), a known competitive MAO inhibitor, was used for comparison at a final concentration of 3.3 mcM.

Study of the kinetic parameters of the enzyme activity in the presence of THC and increasing concentrations of the different substrates, revealed that the inhibition produced was of noncompetitive type, independent of the substrate used (Fig. 5). This phenomenon suggests that the cannabinoids are not interacting directly with the active site of MAO but rather with a different moiety of the enzyme or the membrane in which it is embedded. If the interaction of THC with MAO takes place through a membranal component, separation of the enzyme from the membrane by means of solubilization should abolish the inhibitory effect of the cannabinoid. If, on the other hand, THC affects MAO activity directly or through a phospholipid moiety of the enzyme itself, then the cannabinoid must also affect the soluble enzyme, although no membrane or membranal particles are present. To solubilize the enzyme,

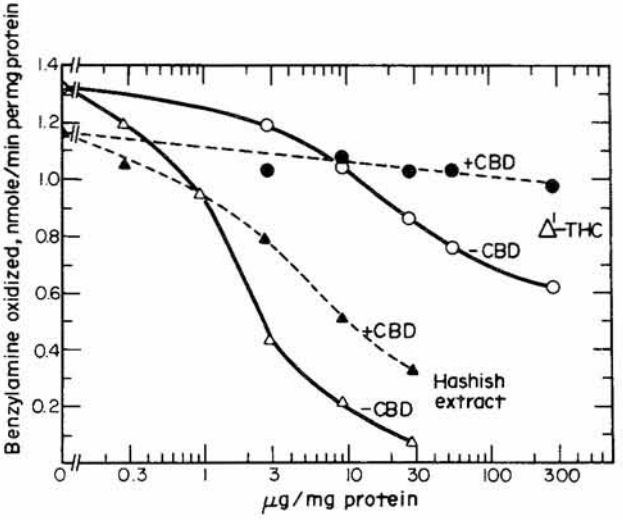

FIGURE 3. Inhibitory effect of THC and hashish extract interfered with by CBD. Preincubation time: 30 min. Where indicated, CBD (135 mcg/mg protein) was added along with the inhibitor.

the technique of Tipton (15) was adopted, mainly because it avoids the use of detergents, which are very difficult to remove after the solubilization. The soluble enzyme thus obtained was found to be inhibited by THC (Table I,Trmnt. I).

Based on this finding it was assumed that the probable lipophylic component through which the cannabinoids interact with the enzyme, is part of, or bound to, the enzyme itself. MAO phospholipids are likely to serve this function and therefore attempts were made to extract the phospholipids from the enzyme. Table I summarizes the various treatments carried out for this purpose. The extraction alone, irrespective of the solvent used (Trmnts. II, III) did not abolish the inhibitory effect of THC.

It has been shown by Sklenovsky et al. that THC causes an increased disruption of firm complexes of phospholipids from

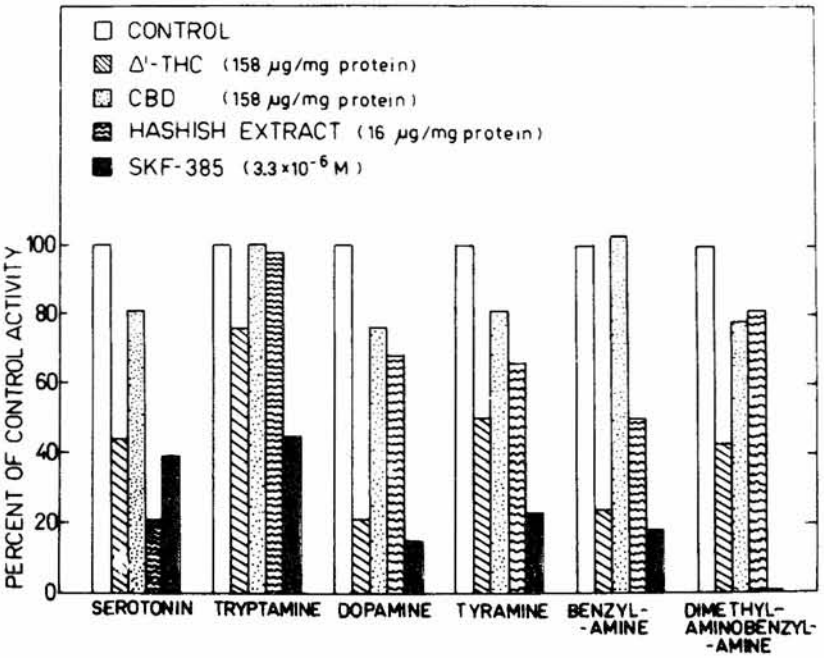

FIGURE 4. The effects of THC, CBD, hashish extract and SKF-385 on pig brain MAO activity in the presence of 1 mM of various substrates (except tryptamine, which was present at 5 mM). The drugs were preincubated with the enzyme preparation for 30 min before adding the substrate. The enzyme activity in the presence of each substrate and in the absence of any inhibitor is 100% of control activity.

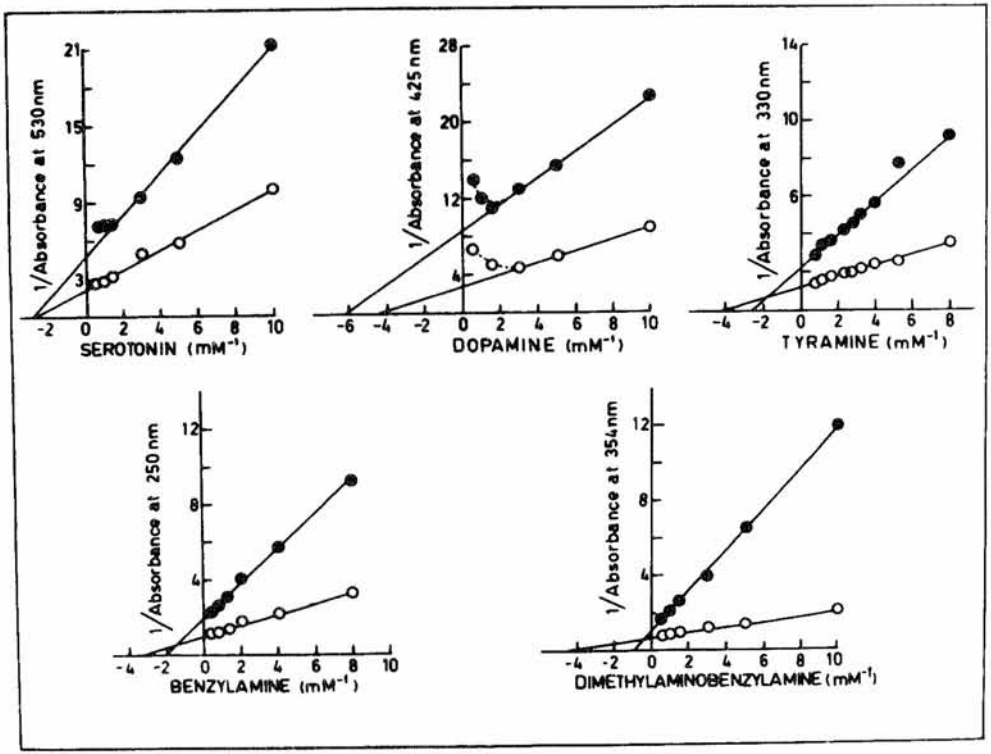

FIGURE 5. Lineweaver-Burk plots of MAO activity with various substrates in the presence (●) or absence (○) of 158 mcg THC per mg protein. Preincubation time: 30 min. Enzyme source: pig brain.

TABLE I. The Effects of Delta-1-THC and CBD on Pig Brain MAO Activity in Soluble Preparation Before and After Various Organic Extraction Procedures

	Treatment	MAO activity[a]		
		Control	THC[b]	CBD
I	None	1.00	35	88
II	2-Butanone extraction[c]	0.76	32	82
III	Chloroformic extraction	0.75	36	86
IV	Chloroformic extraction in the presence of THC	0.75	81	169
V	Chloroformic extraction in the presence of CBD	0.73	100	155

[a]MAO activity in the control: nmoles benzylamine oxidized/min/mg protein. In the presence of the cannabinoids: percent (%) of the control.
[b]The cannabinoid dosage: 50 mcg/mg protein.
[c]The extraction was done according to ref. 26.

brain tissue and thus increases the extractability of these substances (21). Following Treatment IV (Table I), when soluble MAO was preincubated with THC (see methods) and then extracted with chloroform, the inhibitory effect of the cannabinoid was indeed drastically reduced. Since CBD is capable of competing with THC, probably for the same site on the MAO molecule, it might cause the "labilization" of the phospholipid complexes similarly to THC. Treatment V in Table I clearly indicates that this is the case. To determine whether the cannabinoids incubated with the enzyme were extracted into the chloroform phase, tritiated THC and CBD were used in this procedure. The results show that no more than 3% of the cannabinoids remained within the aqueous (enzyme) phase after the chloroformic extraction. Thus a resolution obtained between the MAO and the extractable factor, which is responsible for the enzyme sensitivity to THC. This enabled us to examine the capacity of various phospholipids to restore the sensitivity of MAO to this cannabinoid by adding them, one by one, as liposomes, to the extracted enzyme. As can be seen from Fig. 6, phosphatidylcholine (PPC) reconstituted the inhibitory effect of THC on pig brain mitochondrial MAO activity. Moreover, PPC increased the MAO activity to the level of the untreated (soluble) enzyme and even higher. These effects of PPC were found to be concentration-dependent (Fig. 7). The

optimal concentration was 1.1 mg/mg protein, while lower and higher concentrations were less effective. Other phospholipids examined were much less effective (Fig. 6).

B. Effects of Cannabinoids and Hashish Extract on Pig Liver MAO

MAO activity of pig liver mitochondria is not affected by either THC, CBD or hashish extract (given at doses of 94, 94 and 19 mcg/mg protein, respectively). The lack of effect was independent of the substrate used.

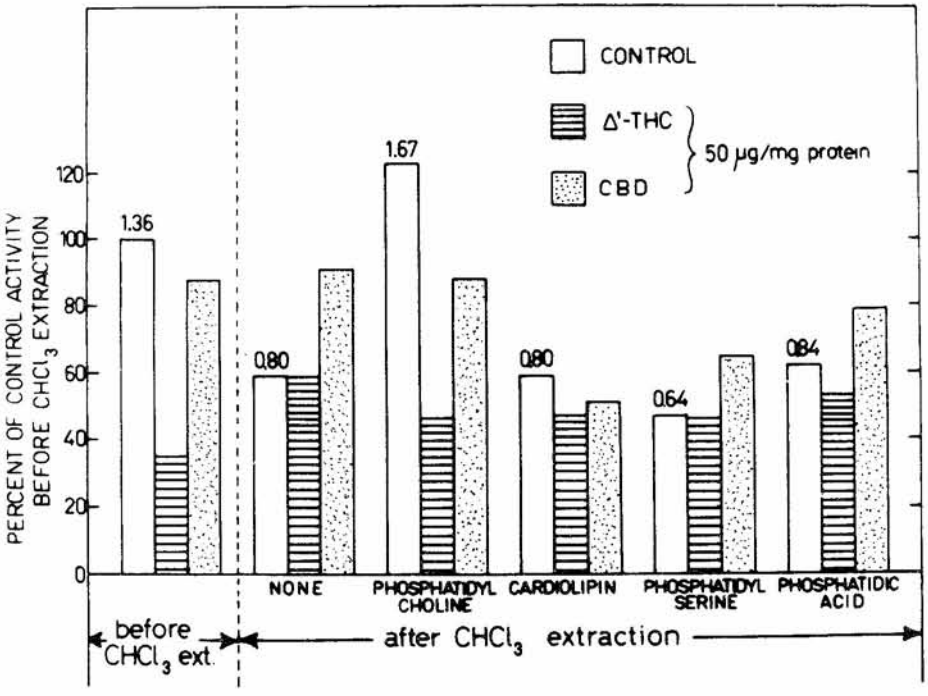

FIGURE 6. The sensitivity of soluble MAO to THC and CBD before and after chloroformic extraction (Treatment V in Table I) and in the presence of 1 mg of various phospholipids per mg of protein added after the extraction. The numbers at the heads of the columns are specific activity of pig brain MAO expressed as nmoles benzylamine oxidized/min/mg protein.

C. Effects of Cannabinoids and Hashish Extract on Human Brain MAO

Unlike pig brain MAO, the human enzyme displayed insensitivity to THC (Figs. 8 and 9, open triangles). The ineffectiveness of this cannabinoid was independent of the substrate used. The cannabis extract on the other hand, inhibited the activity of human brain MAO almost to the same degree it did pig brain MAO. However, the inhibition of the human enzyme by hashish extract was found to be substrate-dependent. Phenylethylamine (PEA) deamination (type B activity) was inhibited to a higher degree than the deamination of 5-hydroxytryptamine (5-HT, type A activity), (Figs. 8 and 9, open circles).

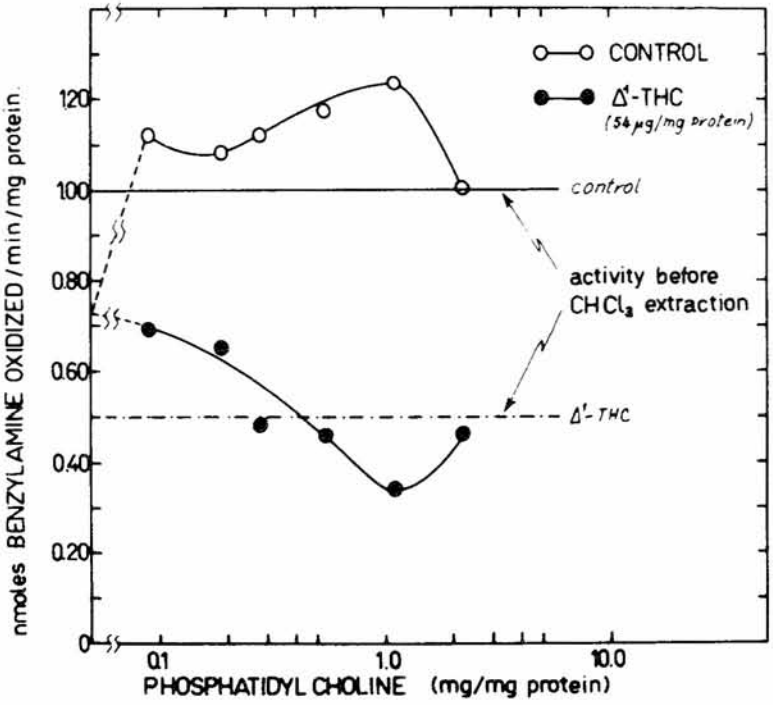

FIGURE 7. The effect of added quantities of phosphatidylcholine on the activity of soluble pig brain MAO, extracted according to Treatment V in Table I, and its sensitivity to THC. Preincubation time: 30 min.

D. Effects of Cannabinoids and Hashish Extract on Human Liver MAO

THC was innocuous in inhibiting the human liver MAO as it was with the brain enzyme (Figs. 8 and 9, closed triangles). However, the liver MAO displayed high sensitivity to hashish extract, higher than that of the brain enzyme (Figs. 8 and 9, closed circles). This is in contrast to pig liver MAO that was found to be insensitive to cannabinoids.

The potency of the cannabis extract in inhibiting human and pig brain MAO and human liver MAO prompted us to try to isolate the responsible component(s). Shown in Fig. 10 are the R_f values and the abilities of cannabis extract (CE), THC, CBD, and 11 different fractions of cannabinoids isolated from CE, to inhibit human liver MAO activity. When screening tests like the one shown in Fig. 10 were conducted, liver mitochondria and benzylamine (BA) were used for the following reasons: a) Human liver MAO was found to be more sensitive to CE than the brain enzyme. b) The type B activity of the enzyme (deamination of BA or PEA) exhibited higher sensitivity to CE than the type A activity (deamination of 5-HT). c) The

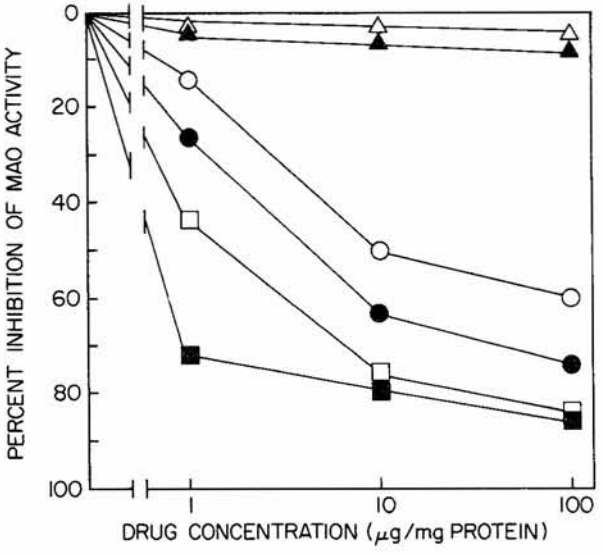

FIGURE 8. Inhibition of human brain (open symbols) and liver (closed symbols) MAO activity by cannabis extract (circles), fraction F_4 (squares), and THC (triangles), using 10 mcM PEA as a substrate.

assay of the enzyme activity with BA is a spectrophotometric one, easier and faster to perform than the radiochemical assay, yet sensitive enough for first screening purposes. Each fraction shown in Fig. 10 including CE, THC and CBD, was tested for its inhibitory effect on MAO at a concentration of 100 mcg/mg protein. As can be seen, fractions F_4, F_5, and F_6 were the most potent as MAO inhibitors. In our study we focused on fraction F_4 only. Upon spraying the TLC plate with Black K Salt solution, this fraction appeared as two purple spots, indicating the presence of two cannabinoids with R_f values of 0.67 and 0.71. These two spots were seen also under UV light and so were all the other cannabinoids. The Black K Salt spraying and the UV light produced identical cannabinoid maps.

The effect of fraction F_4 on both human brain and liver MAO was found to be dose-dependent (Figs. 8 and 9, Squares). Like CE, F_4 was more effective in inhibiting the deamination of PEA (Fig. 8) than the deamination of 5-HT (Fig. 9). Again, like CE, fraction F_4 was found to be more potent against human liver MAO than against the brain enzyme. Moreover, F_4 displayed higher potency than CE in inhibiting MAO activity with

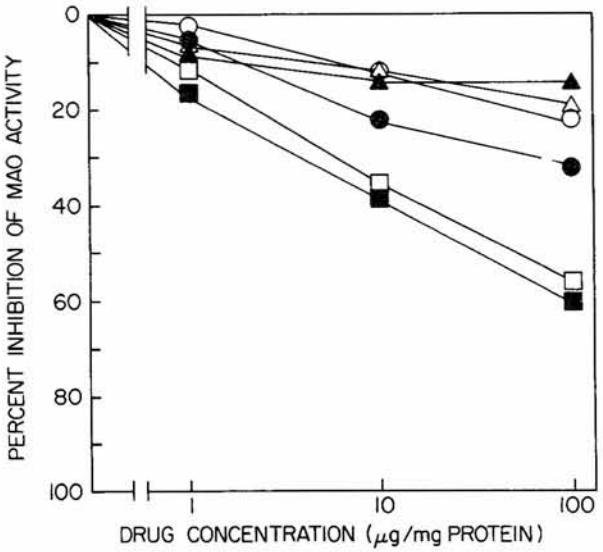

FIGURE 9. Inhibition of human brain (open symbols) and liver (closed symbols) MAO activity by cannabis extract (circles), fraction F_4 (squares), and THC (triangles), using 500 mcg 5-HT as a substrate.

either tissue or substrate; 1 mcg/mg protein of F_4 produced the same degree of inhibition as 10 mcg/mg protein of CE.

IV. DISCUSSION

Biogenic amines have been implicated in the expression of the psychoactivity of cannabis (22-25). Increased levels of biogenic amines and catecholamines were found in experimental animals in vivo after administration of cannabis extract and THC, the psychoactive component of cannabis. Several mechanism may account for these elevated levels: a) Changes in the uptake rates of these compounds into their sites of action, b) changes in their turnover rate, c) changes in their biosynthesis or rate of biotransformation.

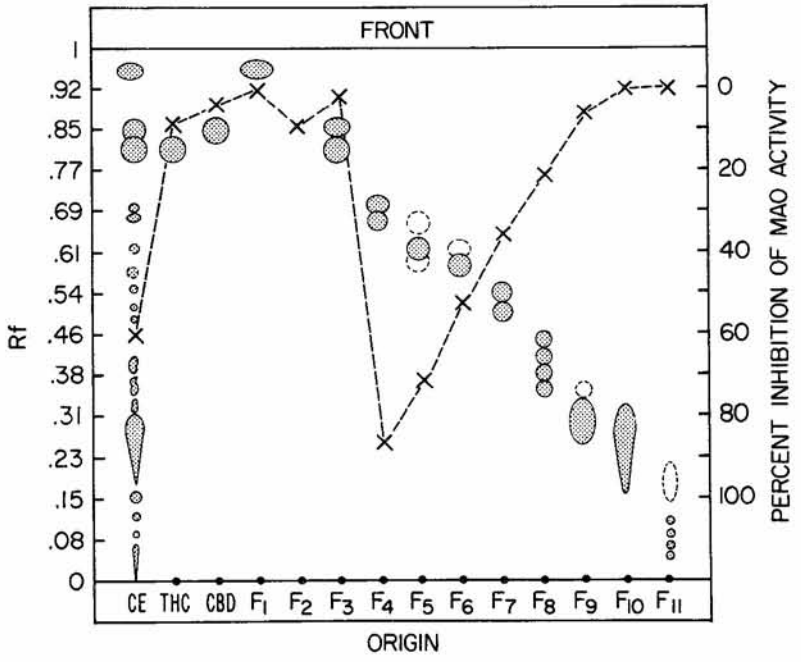

FIGURE 10. TLC fractionation of cannabis extract with toluene:chloroform:methanol (100:10:1, by volume), the R_f values of the different fractions, and the degree of human liver MAO inhibition (X) produced by each fraction (see text for details).

Monoamine oxidase (MAO), an enzyme embedded in the outer mitochondrial membrane, has an important role in the metabolism of endogenous monoamines in the brain (1) and as such may be considered as a possible site for the action of cannabis. The fact that MAO is a lipoprotein makes it even more favorable as a candidate for interaction with the lipophylic cannabinoids. Since we began the study by examining the effects of THC and CBD on pig brain MAO, we were very encouraged to find the pronounced effect of the former on the enzyme activity and the lack of effect of the latter. On the other hand the potency of hashish extract as an MAO inhibitor could not be explained by its content of THC alone (ca. 10%), and one has to assume either a synergistic effect of THC and other cannabinoids or the presence of a much more potent component than THC within this extract. Nevertheless, the tissue selectivity that was displayed by the cannabinoids, inhibiting pig brain MAO without affecting the activity of the liver enzyme, prompted us to continue and investigate the nature of the interaction between the cannabinoids and MAO. The noncompetitive type of inhibition exerted by THC on MAO activity led to the assumption that the interaction of the cannabinoid with the enzyme is not taking place at the active site of MAO. Rather it occurs with a lipophylic moiety of the enzyme or its membranal microenvironment. Solubilizing the enzyme by extracting it from the mitochondrial membrane did not abolish the inhibitory effect of THC. This result indicates that the component, which THC is interacting with, is tightly bound to MAO. We attempted to extract such a component with organic solvents like 2-butanone or chloroform, without success (Table I, Trtmnts. II and III). However, using the chloroform extraction procedure in the presence of THC (Table I, treatment IV), we abolished the inhibitory effect of this cannabinoid. Furthermore, CBD, could also be used instead of THC in the chloroformic extraction to obviated the effect of the latter. CBD has no effect on pig brain MAO, but counteracts the effect of THC on the enzyme (Fig. 3). Using radiolabeled THC and CBD, we showed that 97% of these cannabinoids were extracted into the chloroformic phase (Table I, Trtmnts. IV and V). Postulating that the factor thus extracted is a phospholipid, liposomes were prepared from different phospholipids and were incubated with the extracted enzyme to examine their ability to restore the sensitivity of pig brain MAO to THC. Of the four phospholipids tested, PPC was the only one to reconstitute the inhibitory effect of THC. Moreover, it seems that the ability of this phospholipid is dose related. This phenomenon suggests that the interaction between MAO, PPC and THC is stoichiometric. An optimum concentration of 1.1 mg PPC per 1.0 mg protein was found maximum enzyme activity and inhibition by THC.

A schematic model which outlines our proposal for the mode of action of THC in inhibiting pig brain MAO activity is shown in Fig. 11. The dotted squares in this scheme represents the polypeptide chain of MAO, the open circles are phospholipids and the triangle "S" is the substrate. When CBD or THC are added, they interact with the phospholipids which are bound to the enzyme by this interaction (B_2). When soluble MAO (A) is treated with chloroform, to give form D, the phospholipids are not extractable unless the enzyme is preincubated with either CBD or THC. The extracted enzyme (C) is not inhibited by THC. Phospholipids like PPC, could reassociate with the extracted MAO to reconstitute the complex which is sensitive to THC.

One might conclude from these results that MAO is involved in the psychoactivity of THC. Since the enzyme is inhibited by this cannabinoid and not by CBD, such inhibition would result in elevated levels of monoamines. Thus in turn could lead to the known psychotomimetic signs produced by cannabis. However, when the effect of THC was tested on human brain MAO,

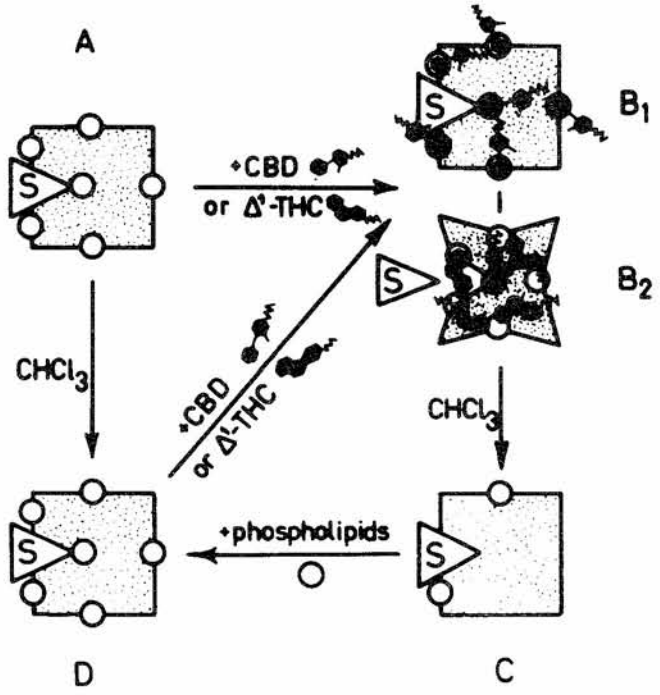

FIGURE 11. Mechanism of inhibition of pig brain MAO activity by THC (see text for details).

none was found. Species selectivity, like tissue selectivity, indicates the existence of differences among different MAO preparation which could lead to different sensitivities to THC. This is especially true if one assumes the presence of different phospholipid moieties with which the cannabinoids are interacting.

However, this is not the case with the cannabis extract which inhibits both pig and human brain MAO, and also liver MAO. Moreover, this extract is ten times more active than THC. Does that mean that the component, or components, in cannabis which is responsible for MAO inhibition, is more specific than THC? Since THC content in the extract cannot account for the strong inhibition of the enzyme activity, the only other explanation is a synergism between THC and other cannabinoids within the extract. The fractionation results (Fig. 10) demonstrate clearly that other cannabinoids, not THC, are responsible for the inhibition of MAO activity produced by cannabis extract. The potential of these components as MAO inhibitors is much greater than that of THC and their specific activity is increasing with the increase in their purity.

Though the differential effect of THC and CBD on pig brain MAO activity alone cannot explain the psychoactivity of the former, the lack of its effect on the human brain enzyme excludes such explanation completely. Nevertheless, the present study emphasizes the importance of species and tissue selectivity in the different effects exerted by THC and other cannabinoids. Moreover, drug selectivity toward different cannabinoids by in vitro preparations is a phenomenon of which we should be aware.

Inasmuch as this study is concerned, the enigma of the psychoactivity of THC is yet to be solved. However, the potential of cannabis as a source of possible therapeutic agents is clear. Fraction F_4 is, undoubtedly, only one of many which contain cannabinoids with even greater selectivity and specificity than THC.

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