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# DIFFERENTIAL INHIBITION OF MITOCHONDRIAL MONOAMINE OXIDASE FROM BRAIN BY HASHISH COMPONENTS

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**Abstract** Monoamine oxidase (MAO) of porcine brain mitochondria was differentially affected by hashish components: with benzylamine as a substrate, $\Delta^1$-tetrahydrocannabinol ($\Delta^1$-THC) inhibited MAO activity markedly, while cannabidiol (CBD) was essentially innocuous at the same concentrations. When added concomitantly, CBD obviated the inhibitory effect of $\Delta^1$-THC. An extract of hashish was over 10-fold more inhibitory toward MAO than $\Delta^1$-THC on weight basis. A prior incubation of the mitochondrial preparation with the cannabis compounds was required to express the inhibitory effect. Liver mitochondrial MAO was not affected by either $\Delta^1$-THC, CBD or hashish extract, despite a prolonged preincubation period, thus demonstrating tissue selectivity with respect to the cannabinoid effect.

The mode of action of hashish is not known. One of the hypotheses emerging from recent reports associates the influence of hashish constituents to their effects on biogenic amines in the brain. The influence of several cannabis components on biogenic amines in the brain has been examined [1–8] but the results are inconsistent. In view of the complications inherent in *in vivo* studies, it is worthwhile to ascertain the hashish effects on defined preparations from brain. We have chosen to study mitochondrial monoamine oxidase (MAO, E.C. 1.4.3.4. amine:oxidoreductase) for several reasons. First, MAO plays a key role in the metabolism of monoamines in brain tissue [9]. Secondly, the interaction of hashish components with cellular membranes is a paramount feature of their effect [10–14]. Finally, since MAO can be isolated by similar means from both brain and liver mitochondria, it would be possible to discern tissue selectivity with respect to the drug effect. $\Delta^1$-Tetrahydrocannabinol ($\Delta^1$-THC) is a major psychoactive compound, while cannabidiol (CBD) is not active in comparable concentrations [15] and their effect was therefore compared by us. In addition, a petroleum ether extract of hashish was also tested, as a source for other cannabinoids as well.

## MATERIALS AND METHODS

*Preparation of mitochondria.* Porcine brain and liver mitochondria were prepared according to McCauley and Racker [16]. Whole brain (about 100 g) and 100 g of liver were obtained from a nearby slaughter house and kept on ice for about 30 min, until used. During all further operations, the temperature was kept below 5°. The tissues were cut into pieces (3 $cm^3$) and rinsed in 0·25 M sucrose–0·1 M Tris–0·02 M EDTA (pH 7·4) to remove blood. The pieces were homogenized for 45 sec in a Waring Blender in 400 ml of the same medium. The homogenate was centrifuged twice in a Sorvall RC2-B centrifuge at 800 *g* for 10 min and the pellets were discarded. The supernatant was then centrifuged at 12,000 *g* (18,000 *g* for liver mitochondria) for 20 min in the same centrifuge and the precipitate was washed twice more with 100 ml of sucrose–Tris–EDTA and resuspended in 50 ml of the medium. The mitochondrial suspensions were frozen for two days at −18°. To the thawed mitochondria, an additional 50 ml of the sucrose–Tris–EDTA medium were added and the mixture was homogenized in a Teflon–glass Potter–Elvehjem homogenizer and then sedimented at 12,000 *g* (18,000 *g* for liver mitochondria) for 20 min. The pellet was resuspended in 50 mM potassium phosphate buffer, pH 7·4, to give a final protein concentration of 10 mg/ml. This preparation was then used for the assay of MAO activity and cannabinoid effect.

*Assay of MAO activity.* The reaction mixture contained, in a total volume of 3 ml: potassium phosphate buffer, pH 7·4, 50 mM; mitochondrial suspension, 1 mg protein and substrate, 1 mM. The reaction, initiated with the substrate, took place at 37°, commonly for 30 min and was terminated with 1·4% $ZnSO_4$ and 0·03 N NaOH (final concentration). Following centrifugation, the supernatant was analyzed spectrophotometrically (Bausch & Lomb Spectronic 200 UV). When benzylamine was used as a substrate, readings were taken at 250 nm [17]. If tyramine was used as a substrate, the supernatants were first alkalinated (1% KOH, final concentration) and then read at 330 nm. $\epsilon_M$ of the product, $3 \times 10^4$, was determined by simultaneous polarographic [18] and spectrophotometric assay of the enzymatic activity. Protein was determined according to Lowry *et al.* [19].

*Effect of cannabinoids.* Aliquots of ethanolic stock solutions of cannabinoids were mixed with 3 ml of

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the assay buffer and the brain mitochondrial suspension were then added, as described above. Ethanol was included in the control at the same final concentration (0·05%, v/v). Similar procedure was employed for liver mitochondria, except that methanol was used as a solvent. Following the preincubation with the cannabinoids (0–60 min at 37°), the reaction was initiated by adding the substrate.

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

*Compounds used.* The smuggled Lebanese hashish, used for extraction, was kindly provided by the Israeli Police. CBD and $\Delta^1$-THC were purchased from Makor Chemical Co., Jerusalem. Benzylamine and tyramine were obtained from Sigma Chemical Co., St. Louis.

*Data presented.* Each of the experiments was repeated at least 6 times, in duplicates, yielding identical patterns. Duplicates agreed within 5% experimental error, and representative experiments are shown.

## RESULTS AND DISCUSSION

MAO activity of brain mitochondria is inhibited by $\Delta^1$-THC and hashish extract. Figure 1 shows that the inhibition is dependent on preincubation with these cannabis compounds. In contrast, CBD was ineffective even after 1 hr of incubation with the mitochondrial preparation. The requirement for a period of preincubation has been established for known MAO inhibitors [20, 21]. Such a requirement may account for the cited conclusion the 'MAO is not affected by THC *in vitro*' [22].

The extent of inhibition of MAO by $\Delta^1$-THC or by the hashish extract is dose-dependent (Fig. 2), the extract being about ten times more potent than $\Delta^1$-THC. $\Delta^1$-THC present in the hashish extract cannot account for the relatively high efficacy of the extract, and studies are in progress to isolate and identify the potent compounds. The inhibition of MAO activity by $\Delta^1$-THC and hashish extract was also examined at a range of benzylamine concentrations (0·1–2·5 mM). Lineweaver Burk plots [23] in the presence and absence of these drugs were linear, exhibiting different slopes and also different intercept points with the ordinate. Such kinetic behaviour is classified as noncompetitive [24]. However, the interpretation of the kinetics is not clear, particularly since we are not dealing with a pure enzyme system.

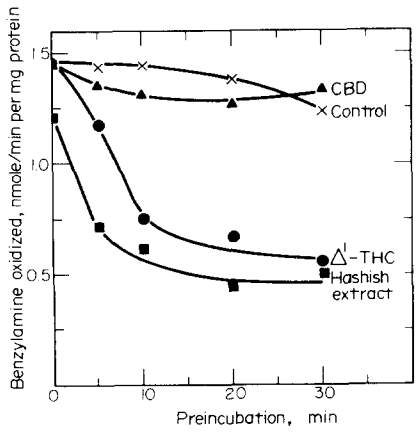


Fig. 1. Inhibition of MAO activity of brain mitochondria by hashish components as affected by preincubation with the drugs. Aliquots of the mitochondrial suspension (1 mg protein in 3 ml KPi, pH 7·4) were incubated at 37° with one of the hashish compounds as follows (μg/mg protein): $\Delta^1$-THC, 94; CBD, 94; hashish extract, 19.

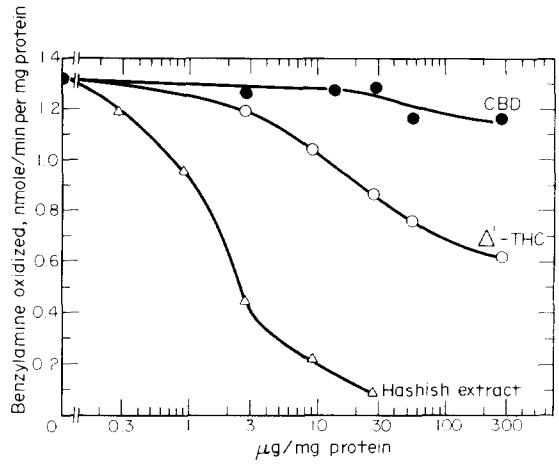


Fig. 2. Effect of hashish compounds on MAO activity. Preincubation time: 30 min.

Unlike $\Delta^1$-THC and the hashish extract, CBD is essentially innocuous at a wide concentration range (Fig. 2). The marked difference in potency of CBD and $\Delta^1$-THC is of significance since it correlates with the established differential effects of these cannabinoids *in vivo* [15]. Solubility properties of CBD and $\Delta^1$-THC and their partition between membranes and aqueous media are too similar to account for their different *in vitro* effects, particularly in view of the wide concentration range tested. Some specific interaction between the cannabinoids and a membrane component, possibly a lipophylic one, is a likely interpretation. The differential effect as well as the extent of inhibition are greatly diminished if tyramine is used as a substrate, instead of benzylamine (Fig. 3). Possibly, MAO isozymes are present in porcine brain mitochondria, which vary in sensitivity to hashish components. The presence of MAO isozymes in beef

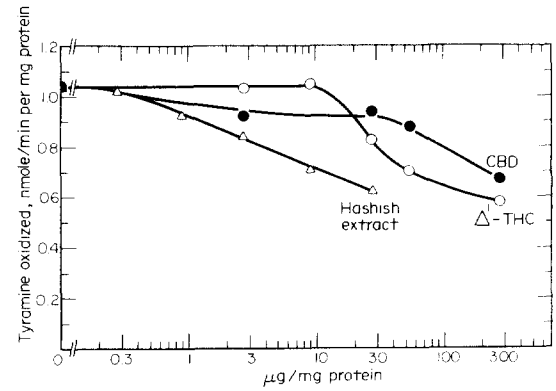


Fig. 3. Effect of hashish compounds on MAO activity, with tyramine (1 mM) as a substrate. Preincubation time: 30 min.

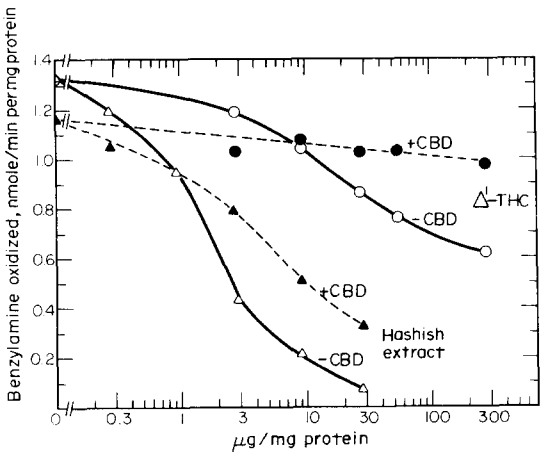


Fig. 4. Inhibitory effect of $\Delta^1$-THC and hashish extract interfered with by CBD. Preincubation time: 30 min. Where indicated, CBD (135 μg/mg protein) was added along with the inhibitor.

and rat brain mitochondria has already been demonstrated [16, 25].

If administered concomitantly, cannabidiol is known to interfere with the effects of $\Delta^1$-THC in man [26], and in experimental animals [27]. The counteraction of CBD, which by itself appear to exert little activity, is clearly of interest, but its mechanism is not known. The phenomenon can be demonstrated *in vitro* as the inhibitory effect of $\Delta^1$-THC on MAO of brain mitochondria is essentially obviated by CBD (Fig. 4). CBD also partly diminishes the inhibitory effect of the hashish extract. If CBD is added at the onset of the reaction, following the preincubation period, it no longer interferes with the inhibitory effect of $\Delta^1$-THC. When CBD is added during the pretreatment with $\Delta^1$-THC, the interference is proportional to the duration of concomitant incubation. On the basis of these data, we are encouraged to pursue experimentally the challenging antagonistic effect of CBD.

The present study is probably the first case in which a differential effect of the cannabinoids is demonstrated in biochemical terms, *in vitro*. Evidently, the MAO system from brain is potentially very useful for studying structure-function relationships of cannabinoids and particularly the phenomena of antagonism and synergism. This conclusion is reinforced by an intriguing tissue selectivity: MAO of porcine liver mitochondria is not affected by either $\Delta^1$-THC, CBD or hashish extract (given in doses of 94, 94 and 19 μg/mg protein, respectively), with either benzylamine or tyramine as a substrate, despite a prolonged preincubation period.

In contemplating the mode of action of hashish in brain tissue, an effect on MAO should be taken into account.

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