Effect of Δ⁹-tetrahydrocannabinol on monoamine oxidase activity in bovine eye tissues, in vitro

A. M. Gawienowski,* D. Chatterjee,* P. J. Anderson, D. L. Epstein, and W. M. Grant

An investigation was undertaken to analyze the effects of Δ⁹-tetrahydrocannabinol (THC) on monoamine oxidase (MAO) activity from calf ocular tissues. Ciliary processes, retina, trabecular meshwork, choroid, and iris all demonstrated significant MAO activity in decreasing order of magnitude. THC in concentrations from 10⁻⁸M to 10⁻¹²M stimulated MAO activity in extracts from all five tissues, except for iris at 10⁻⁸M. Maximum stimulation of MAO activity occurred at the 1 × 10⁻¹²M level for all tissues. Retina, followed next by trabecular meshwork and then ciliary processes, exhibited the largest increase in MAO activity at 10⁻¹⁰M THC. This latter effect could possibly be related to the influence of THC on intraocular pressure. (INVEST OPHTHALMOL VIS SCI 22:482-485, 1982.)

Key words: monoamine oxidase, Δ⁹-tetrahydrocannabinol, calf eye tissues, intraocular pressure

Marijuana and its psychoactive ingredient, Δ⁹-tetrahydrocannabinol (THC), have been observed to lower intraocular pressure (IOP) when given either topically or systematically. Hepler et al.¹ demonstrated a substantial decrease in IOP of humans after exposure to marijuana smoke. Both topical and intravenous administration of THC have been observed to decrease IOP in rabbits.²⁻³

Green and Bowman⁴ suggested that THC may produce its ocular effects by acting through the adrenergic receptors in the eye. Alternatively, THC may act on the adrenergic system through the enzyme monoamine oxidase (E.C. 1.4.3.4.) (MAO). Banerjee et al.⁴ demonstrated in the rat a dose-dependent stimulation of MAO activity with intraperitoneal injections of THC. In related studies, MAO activity was found to be significantly increased in hypothalamic tissue, blood platelets, and heart mitochondria after THC administration both in vivo and in vitro.⁴⁻⁵ MAO activity in ocular tissues has been measured by Krishna et al.,⁶ Shanthaveerappa and Bourne,⁷ Mustakallio,⁸ and Waltman and Sears,⁹ but investigations of the influence of THC on MAO in the eye have not been reported.

We therefore decided to investigate the effect of THC on MAO from eye tissues in order to gain information that hopefully may illumin-
nate the effect of THC on IOP. In this study, calf eye tissues (ciliary processes, iris, choroid, retina, and trabecular meshwork) were assayed for MAO activity, and comparisons were made with the activity measured after treatment with THC in vitro.

Materials and methods

Bovine eyes obtained from the abattoir were dissected to separate ciliary process, trabecular meshwork, choroid, retina, and iris. The tissues were frozen on Dry Ice, pooled, and stored at −80°C. Each batch of ciliary processes and trabecular meshwork typically contained tissue from 20 eyes; each batch of choroid, retina, and iris, tissue from three to five eyes.

Mitochondria were isolated by mincing the tissues and homogenizing in 3 vol of 0.4M sucrose containing 5 × 10⁻⁴M sodium EDTA at pH 7 with a Polytron homogenizer. The homogenized tissues were diluted to 10 vol and centrifuged in a Sorvall Model RC 5 refrigerated centrifuge at 3000 × g for 3.5 min. The supernatants were centrifuged at 35,000 × g for 15 min, and the pellets were then resuspended and washed twice. The final pellets were suspended in 2 vol of homogenizing buffer.

Protein in each mitochondrial preparation was estimated by the method of Bradford, with bovine serum albumin used as a standard.

A modification of the radiochemical assay of Ostuka and Kobayashi and Grosso and Gaweinski was employed to measure MAO activity in the mitochondria from the various tissues. Each assay tube contained 0.5 nmol of 14C-labeled tyramine HCl (sp. act. 50 mCi/mmol; New England Nuclear Corp., Boston, Mass.) and 125 μg of mitochondrial protein in 0.5 ml of 0.1M borate buffer, pH 7.8, containing 10⁻³M disodium EDTA and 10⁻³M aminoguanidine sulfate.

For experiments in which the action of THC on MAO activity was to be studied, a 5 mM stock solution of THC in absolute ethanol was diluted with the borate buffer to give a range of working solutions. Aliquots (0.1 ml) of these working solutions were added to give concentrations of 0, 10⁻¹⁴M, 10⁻¹³M, 10⁻¹²M, 10⁻¹¹M, 10⁻¹⁰M, and 10⁻⁹M THC. The tubes were incubated for 30 min at 37°C in a shaker water bath. The reaction was stopped by adding 0.2 ml of 2M citric acid followed by 5 ml of anisole containing 0.5% Omnifluor (New England Nuclear). The tubes were capped, and the radioactive product was extracted by shaking for 1 min. Extracts cooled to 4°C were centrifuged for 10 min in a clinical centrifuge to separate the phases. Aliquots of 4.5 ml were withdrawn from each tube, mixed with 10 ml of toluene and 0.4% Omnifluor scintillation solution, and counted.

The results were statistically compared with a t test for equal numbers.

Results

Mitochondrial preparations from the five eye tissues all demonstrated MAO activity in decreasing order of magnitude: ciliary processes, retina, trabecular meshwork, choroid, and iris (Table I). In the extracts exposed to THC, a possible biphasic response was noted (Table II). At 1 × 10⁻⁶M THC concentration, very slight inhibition of MAO activity was observed. Stimulation of MAO activity with significance at the 5% or greater level was noted for all five tissues for concentrations of 10⁻⁸M to 10⁻¹²M THC, except for the iris, which was not significantly affected at 10⁻⁸M. Maximum stimulation occurred at the 1 × 10⁻¹²M level for all tissue preparations, and stimulation of MAO was progressively less at the 1 × 10⁻¹⁴M and 1 × 10⁻¹⁶M concentrations of THC. The retina exhibited the largest increase in MAO activity after exposure to THC at 10⁻¹²M concentration, followed next by trabecular meshwork and then ciliary processes.

Discussion

Our study demonstrates that THC stimulates MAO activity in mitochondrial preparations from calf ocular tissues (Table II). The maximum stimulation was found at 1 × 10⁻¹²M THC for the retina, trabecular meshwork, ciliary processes, choroid, and iris. The retina exhibited the largest increase in

<table>
<thead>
<tr>
<th>Tissue</th>
<th>μmol/30 min/μg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciliary process</td>
<td>3.0 × 10⁻⁵ ± 5.0 × 10⁻⁴</td>
</tr>
<tr>
<td>Retina</td>
<td>2.5 × 10⁻³ ± 3.2 × 10⁻⁴</td>
</tr>
<tr>
<td>Trabecular meshwork</td>
<td>2.4 × 10⁻³ ± 3.9 × 10⁻⁴</td>
</tr>
<tr>
<td>Choroid</td>
<td>2.1 × 10⁻³ ± 3.1 × 10⁻⁴</td>
</tr>
<tr>
<td>Iris</td>
<td>1.5 × 10⁻³ ± 2.2 × 10⁻⁴</td>
</tr>
</tbody>
</table>

Values represent average of 6 experiments ± S.E.M.
Table II. Effect of THC on MAO activity in calf eye tissues

<table>
<thead>
<tr>
<th>Conc THC (M)</th>
<th>Choroid</th>
<th>Retina</th>
<th>Trabecular mesh</th>
<th>Ciliary process</th>
<th>Iris</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 × 10⁻⁶</td>
<td>0.99 ± 0.008</td>
<td>0.90 ± 0.03</td>
<td>0.99 ± 0.003</td>
<td>0.98 ± 0.001*</td>
<td>0.98 ± 0.001†</td>
</tr>
<tr>
<td>1 × 10⁻⁸</td>
<td>1.05 ± 0.006†</td>
<td>1.04 ± 0.011</td>
<td>1.09 ± 0.06*</td>
<td>1.13 ± 0.011</td>
<td>1.00 ± 0.02</td>
</tr>
<tr>
<td>1 × 10⁻¹⁰</td>
<td>1.06 ± 0.02*</td>
<td>1.13 ± 0.07*</td>
<td>1.19 ± 0.02†</td>
<td>1.14 ± 0.003†</td>
<td>1.09 ± 0.01†</td>
</tr>
<tr>
<td>1 × 10⁻¹²</td>
<td>1.12 ± 0.01†</td>
<td>1.66 ± 0.14†</td>
<td>1.44 ± 0.04†</td>
<td>1.26 ± 0.16*</td>
<td>1.17 ± 0.01†</td>
</tr>
<tr>
<td>1 × 10⁻¹⁴</td>
<td>1.02 ± 0.01</td>
<td>1.47 ± 0.14*</td>
<td>1.03 ± 0.02</td>
<td>1.14 ± 0.05*</td>
<td>0.99 ± 0.01</td>
</tr>
<tr>
<td>1 × 10⁻¹⁶</td>
<td>0.97 ± 0.01</td>
<td>0.96 ± 0.04</td>
<td>0.98 ± 0.01</td>
<td>1.03 ± 0.06</td>
<td>—</td>
</tr>
</tbody>
</table>

Values represent average of 3 experiments ± S.E.M. and are expressed by comparison to control value of 1.

*Significant at p <0.05.
†Significant at p <0.01.

MAO activity in response to THC followed by trabecular meshwork and ciliary processes (Table II). This variation in response possibly may be explained by varied levels of the two isoenzymes (A and B form) of MAO in the different ocular tissues. In general, MAO-A preferentially deaminates 5-hydroxytryptamine and norepinephrine, whereas MAO-B preferentially deaminates phenylethylamine. The major fraction of MAO in the chick retina was found to be type B by Suzuki et al. Determination of the A and B form of MAO present in each ocular tissue and the effect of THC on their kinetic parameters would be of great interest.

Since MAO is associated with membrane components of the mitochondria and since THC is lipophilic, it is possible that the measured increase in MAO activity may be due to the interaction of THC with the mitochondrial membrane, resulting in increased permeability to the corresponding amine substrate. However, it still remains to be determined whether THC affects membrane-bound MAO activity by acting specifically on the membrane or on the enzyme itself.

In studies with human volunteers in vivo, Perez-Reyes et al. found a 33% lowering of IOP at a dose of 51 μg/kg. Green and Bowman obtained a 22% decrease in IOP in rabbits at doses of 3 to 4 μg/kg. In our study the optimal concentration of THC for MAO stimulation was 10⁻¹⁰M. Calculating this as a “dose” based on the amount of mitochondrial protein present in the incubation mixture yields a value of approximately 1 μg/kg mitochondrial protein, which is similar to expected tissue levels in the above in vivo studies. One would have to postulate a partitioning into target tissues of well over 10⁴ to reach levels of THC that would be inhibitory.

Thus the IOP-lowering effect of THC may be associated with a stimulation of MAO in ocular tissues, possibly leading to a reduction in the levels of catecholamines. Although this would not be consistent with the IOP-lowering effect of topical epinephrine it could conceivably be mimicking the action of β-blockers, which are also effective in lowering IOP. On the other hand, the effect on catecholamines could be less significant than the lowering of some other important biogenic amines such as serotonin, dopamine, or tyramine.

It is noteworthy that in our study THC stimulated MAO activity in both the trabecular meshwork and ciliary process preparations. We hope that further studies characterizing the different biogenic amines and MAO isoenzymes present in these two tissues may offer better insight into the mechanisms of the effect of THC on aqueous humor dynamics.

We gratefully acknowledge the assistance of Ms. Ellen Hertzmark who performed the statistical analysis.

REFERENCES

3. Green K and Bowman K: Effect of marihuana and derivatives on aqueous humor dynamics in the rabbit. In Pharmacology of Marijuana, Braude MC and


