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Effect of Δ 1-tetrahydrocannabinol* on ATPase activity of rat liver mitochondria

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THE MODE of action of Δ 1-tetrahydrocannabinol, one of the predominant psychoactive components in Cannabis,[1-2] has been the object of intensive study[3-5] after its recent isolation and synthesis.[6] It has been shown[7] that the liver is the major center of accumulation of radioactivity after administration of [^{14}C]-labeled tetrahydrocannabinol and, therefore, it appeared of interest to study the effect of Δ 1-tetrahydrocannabinol (THC) on various parameters in that organ. The work reported below deals with the effect of THC on ATPase activity in rat liver mitochondria.

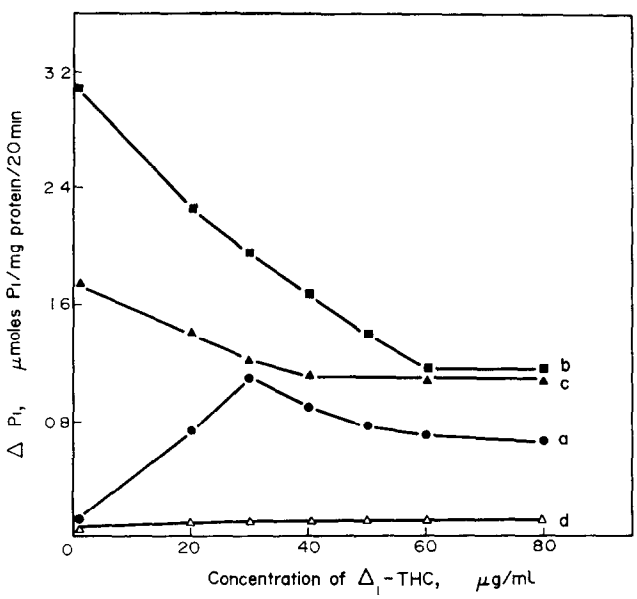

FIG. 1. Effect of Δ 1-THC on ATPase activity. The assay contains in 1 ml: 75 μmoles tris-HCl buffer (pH 7·4); 3·5 μmoles ATP; mitochondrial fraction (0·75 mg protein). a, no addition; b, 0·05 μmoles DNP; c, 0·1 μmoles Ca^{2+}; d, 3 μg oligomycin. In all cases the solutions were made up with sucrose to a final total concentration of 150 μmoles/ml. Temperature 30°. Incubation time 20 min.

* Also designated as Δ 8-tetrahydrocannabinol.

Male albino rats of our own stock, weighing about 150 g each, were used. They were sacrificed and mitochondrial fraction in 0·25 M sucrose was prepared from their livers.[8] To minimize ATPase activity due to aging,[9] the reaction was started within 90 min after sacrifice, by incubating 0·1 ml of mitochondrial fraction (0·75 mg protein) in 1 ml reaction medium that contained: 75 μmoles tris-HCl buffer (pH 7·4); 3·5 μmoles ATP; cations (K^+, Mg^{2+}, Ca^{2+}) at suitable concentrations indicated in the text; the desired quantity of THC; the balance, up to a total of 150 μmoles, was made up with sucrose solution. The incubation was carried out at 30° for 20 min, during which period the reaction rate was found to be constant. The reaction was terminated with 1 ml of 15 per cent trichloracetic acid and the inorganic phosphate was determined immediately thereafter by the method of Fiske and Subbarow.[10] The results were expressed, after duly subtracting the pertinent blank values at 0 min (about 0·1 μmoles P_i per mg protein), as μmoles P_i released per mg protein per 20 min (ΔP_i).

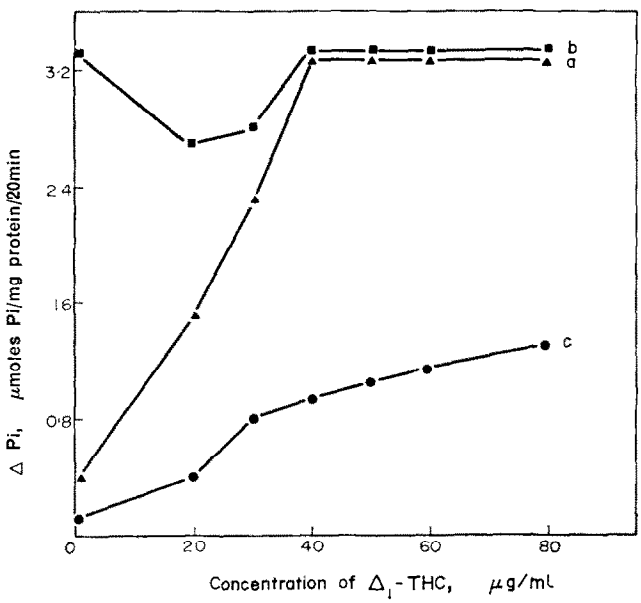

FIG. 2. Effect of Δ 1-THC on ATPase activity in the presence of Mg^{2+}. Conditions as for Fig. 1 except that 2 μmoles Mg^{2+} are added. a, no further additions; b, 0·05 μmoles DNP; c, 3 μg oligomycin.

ATP disodium salt, purchased from Sigma Chemical Corp. was treated with Dowex 50W-X8H^+ to remove sodium;[11] the pH was then adjusted to the desired value with tris-HCl buffer (pH 7·4). The THC, supplied to us by Dr. R. Mechoulam of the Hebrew University, Jerusalem, was used after dissolution in ethanol. The controls contained ethanol without THC.

The results obtained demonstrate that addition of THC to fresh liver mitochondrial fraction causes a pronounced increase in ATPase activity (Fig. 1 curve a). The maximum effect occurs at about 30 μg THC per ml; it declines gradually towards higher concentrations but, even at 80 μg/ml, ΔP_i still remains well above the control level. Curve a in Fig. 2 shows that addition of 2 μmoles /ml Mg^{2+} markedly enhances ATPase activity (Mg^{2+}-dependent ATPase) and causes the THC concentration of maximum activity to shift from 30 μg/ml to 40 μg/ml. Increase in THC concentration beyond this evel—up to 75 μg/ml—does not cause any further change in ATPase activity. In the whole course of this study, 2 μmoles/ml Mg^{2+} have been employed, since this molarity has proved to be of maximum effectiveness at various THC concentrations. Addition of K^+ (50 μmoles/ml) does not affect the results, both in the presence and absence of Mg^{2+}.

It has been shown[12] that 2,4-dinitrophenol (DNP)-induced ATPase activity is high only in intact mitochondria, and it has become accepted that the structural integrity of mitochondria is reflected by low ATPase activity in the presence of Mg^{2+} and substantial stimulation when DNP is added;[9,13,14] on the other hand, disrupted mitochondria show high Mg^{2+}-dependent ATPase activity which is not enhanced by the addition of DNP;[15] an "intermediate state" of the mitochondria is characterized

by high Mg^{2+}-ATPase activity and low DNP stimulation.[14] On the basis of these reported observations, it seemed of interest to study the effects of THC on ATPase activity in the presence of DNP only and of both Mg^{2+} and DNP.

In the presence of DNP only, addition of THC to liver mitochondria in increasing concentrations (Fig. 1 curve b) results in a gradual decrease of the DNP-induced ATPase activity; beyond a concentration of about 60 μg/ml THC, no further decrease occurs. Comparison of curves a and b in Fig. 1 shows that ΔP_i, in the range of 60–80 μg/ml THC in the presence of DNP, is close to the peak rate measured with THC alone. When both Mg^{2+} and DNP are added, the following findings are obtained (Fig. 2 curve b): At low THC concentrations—up to about 25 μg/ml—a progressive decrease in ATPase activity is observed, analogous to that occurring in the absence of Mg^{2+} (compare Fig. 1 curve b). At higher THC contents, the enhancing effect of the Mg^{2+} starts to preponderate, ATPase activity begins to rise and reaches eventually the curve obtained in the presence of Mg^{2+} alone. The rise of ΔP_i associated with the addition of DNP to Mg^{2+}—i.e. the difference between the ordinates of curves a and b in Fig. 2—decreases continuously with increasing THC concentration (and the concomitantly increasing ATPase activity of curve b), vanishing completely at 40 μg/ml, where the maximum ΔP_i of curve b is reached.

Furthermore, since it has been shown[16] that Ca^{2+} stimulates ATPase activity more strongly in intact mitochondria than in disorganized ones, the action of Ca^{2+} on THC-induced ATPase has been examined. Curve c in Fig. 1 demonstrates that Ca^{2+}-stimulated ATPase activity continuously decreases with rising THC content. At a THC concentration of about 50 μg/ml and above, ΔP_i, in the presence of Ca^{2+}, decreases down to the peak rate measured with THC alone—a behaviour similar to that observed with DNP.

The action of oligomycin, an inhibitor of mitochondrial ATPase,[17] has been examined. Fig. 1 curve d demonstrates that THC-induced ATPase is completely inhibited by oligomycin. When both oligomycin and Mg^{2+} are added, ΔP_i rises monotonously in the whole range of THC concentrations examined (0–80 μg/ml), reaching a value significantly higher than the peak ATPase activity observed in the presence of THC alone (Fig. 2 curve c).

In conclusion it appears that, as reflected by its effects on ATPase activity, THC causes some disorganization of the mitochondria to a degree depending upon its concentration. On the basis of its lipophilic character,[18] it may be suggested that the influence of THC results from its attachment to specific phospholipidic or lipoproteinic binding sites, a possibility which merits further investigation.

Israel Institute for Biological Research,
Ness-Ziona, Israel

A. Chari-Bitron
T. Bino

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