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THE EFFECT OF HASHISH COMPOUNDS ON PHOSPHOLIPID PHASE TRANSITION

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Summary

The interaction of hashish compounds, Δ^1-tetrahydrocannabinol and cannabidiol, with dipalmitoyl phosphatidylcholine was investigated using differential scanning calorimetry. Both drugs affect the transition of dipalmitoyl phosphatidylcholine from the gel to liquid crystalline state, decreasing both the melting temperature and the enthalpy of melting. At a drug to dipalmitoyl phosphatidylcholine ratio of approx. 1:5, two peaks appear in the transition profile, suggesting a phase separation in the drug dipalmitoyl phosphatidylcholine mixture.

In addition to the psychomimetic activity exhibited by hashish extracts, hashish compounds (cannabinoids) have been shown to affect a variety of parameters of biological and artificial phospholipid membranes.

The psychomimetic effect is mainly due to the activity of Δ^1-tetrahydrocannabinol [1], while in interaction with membraneous systems leading to changes in membrane permeability and inhibition of membrane functions, both Δ^1-tetrahydrocannabinol and cannabidiol have a similar potency. Cannabidiol is a nonpsychoactive hashish component comprising 40% of the cannabis extracts [2]. It is the precursor in the synthesis and probably also in the biogenesis of Δ^1-tetrahydrocannabinol [1].

Cannabinoids are lipid-soluble neutral compounds with a very high membrane/aqueous solution partition coefficient [3]. As such they can insert into lipid bilayer regions of synthetic and biological membranes and probably cause perturbation of ordered phospholipid regions.

Both hashish compounds, Δ^1-tetrahydrocannabinol and cannabidiol show the well-documented biphasic interaction pattern of lipid-soluble compounds with membranes, i.e. at low concentrations they act as membrane stabilizers and at high concentrations as membrane labilizers [4,5]. Membrane stabilization against hypoosmotic lysis could stem from a fluidizing effect on membrane phospholipids enabling the membrane to accommo-

date a larger volume within its boundaries. At high drug concentrations the compounds probably have a disordering effect on membrane lipids in a way leading to damage to the membrane permeability barrier.

Indeed, permeability changes observed in lipid bilayer membranes [6], damage to organelles (mitochondria [7], lysosomes [5]) and cytotoxicity towards macrophages [8] may all reflect initial drug-induced reorganization of lipids leading to impairment of the membrane-permeability barrier.

Drug-induced disorganization of the lipid environment of membrane enzymes could possibly explain also the inhibition of $(Na^+ + K^+)$ATPase activity by hashish compounds (ref. 9 and Herschkovitch, M., Raz, A. and Goldman, R., unpublished).

To probe the changes in the physical state of phospholipids that might be induced by hashish compounds we have studied the influence of Δ^1-tetrahydrocannabinol and cannabidiol on the electrical resistance of planar lipid bilayer membranes [6]. We now present evidence that both Δ^1-tetra hydrocannabinol and cannabidiol can affect the transition of phospholipids from crystalline gel to liquid crystalline state as revealed by differential scanning calorimetry measurements.

L-β,γ-Dipalmitoyl-α-lecithin puriss grade, was purchased from Fluka (Buchs, Switzerland). Dipalmitoyl phosphatidylcholine was freshly dissolved in distilled chloroform before each experiment. Δ^1-Tetrahydrocannabinol and cannabidiol were obtained from Makor Chemicals, Jerusalem, Israel. Stock solutions of the drugs (5 mg/ml in spectroscopic grade ethanol) were kept at −18°C. Mixtures of dipalmitoyl phosphatidylcholine and cannabinoids at the desired molar ratios were prepared. The solvents were evaporated by a stream of N_2 and high vacuum (2 h) and the material was dispersed in 0.2 ml of $1 \cdot 10^{-2}$ M NaCl in $5 \cdot 10^{-4}$ M Tris · HCl buffer pH 7.4, and incubated with shaking for 2 h at 48°C. The samples were concentrated about ten fold by evaporation of water with a stream of N_2, and transferred to aluminum pans which were subsequently sealed. The same amount of ethanol as that present initially at the drug: dipalmitoyl phosphatidylcholine ratios was added to dipalmitoyl phosphatidylcholine as a control.

The calorimetric measurements were performed in the Perkin Elmer DSC 1B apparatus. The apparatus was calibrated with stearic acid and indium, operated at a scanning rate of 8°C/min and a sensitivity scale of 4 mcal/s for full scale response. The experiments were performed at least in triplicates. The heat of transition (ΔH) was calculated from the areas under the curves of the thermograms and from the phospholipid content as assessed by phosphate determination [10] after digestion of the pan with perchloric acid.

The respective structures of Δ^1-tetrahydrocannabinol and cannabidiol are given in Fig.1. Fig.2 represents differential scanning calorimetry thermograms of dipalmitoyl phosphatidylcholine and of dipalmitoyl phosphatidylcholine/cannabinoid mixtures. At low ratios of the cannabinoids to dipalmitoyl phosphatidylcholine (1:20) the pretransition peak of dipalmitoyl phosphatidylcholine disappears with a concomitant small decrease of the melting temperature. At higher ratios of cannabinoids to dipalmitoyl phosphatidylcholine the

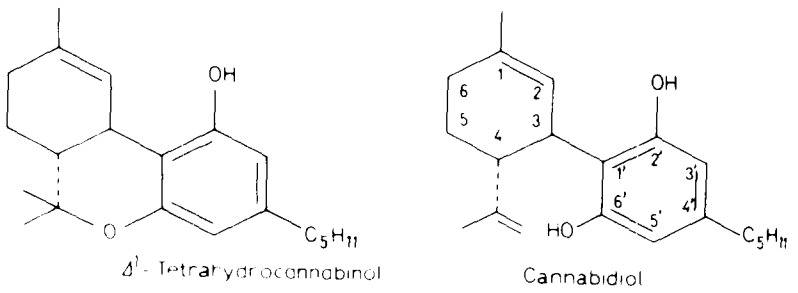

Fig.1. The structure of Δ^1-tetrahydrocannabinol and cannabidiol.

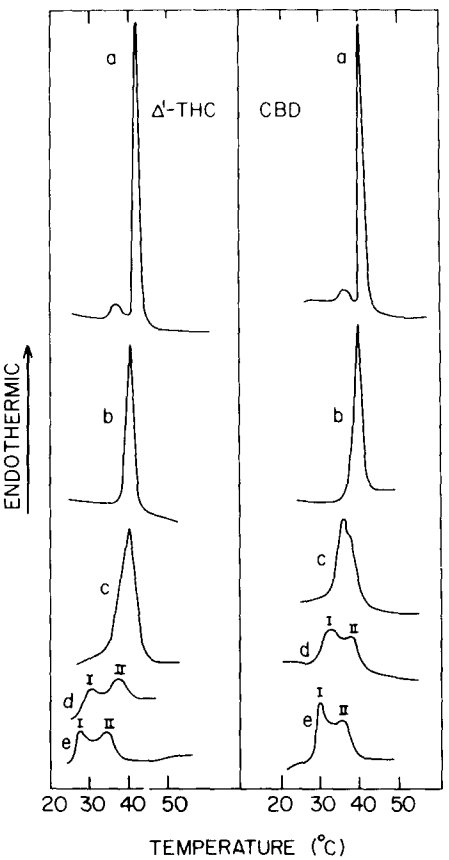

Fig.2. The effect of Δ^1-tetrahydrocannabinol (Δ^1-THC) and cannabidiol (CBD) on the differential scanning calorimetry thermograms of dipalmitoyl phosphatidylcholine (DPL).

Δ^1THC			[DPL]	CBD			
(a)	DPL alone		2.4 mg	(a)	DPL alone		2.3 mg
(b)	Δ^1-THC/DPL	1:22 (molar ratio)	2.1 mg	(b)	CBD/DPL	1:20 (molar ratio)	2.2 mg
(c)		1:11	3.8 mg	(c)		1:10	2.5 mg
(d)		1:5	2.4 mg	(d)		1:5	2.0 mg
(e)		1:2.4	2.2 mg	(e)		1:2	2.2 mg

transition curves broaden and the melting temperature further decreases. At a molar ratio of 1:5 an additional peak appears in the transition profile. The second peak could not stem from melting of pure cannabidiol since the melting point of the latter was found to be 68°C. The generation of the second peak might indicate a phase separation occurring at high cannabinoid to dipalmitoyl phosphatidylcholine ratios. This phenomenon could possibly be explained on the basis of the limited solubility of cannabinoids in dipalmitoyl phosphatidylcholine. A further increase in the ratio of cannabinoids to dipalmitoyl phosphatidylcholine does not lead to additional changes in the melting temperatures and the thermotropic profile. Fig.3 shows the dependence of the midpoint melting temperatures on the ratio of the drugs to dipalmitoyl phosphatidylcholine. The maximum shift in the midpoint temperature amounts to about 15°C.

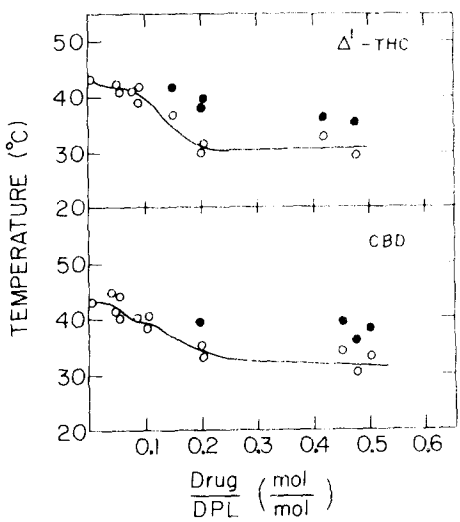

Fig.3. The dependence of the midpoint temperature (T_m) of the phase transition of dipalmitoyl phosphatidylcholine (DPL) on the molar ratio of cannabinoids to phospholipid. ○——○, T_m of the first peak (I): ●——●, T_m of the second peak (II). Δ^1-THC, Δ^1-tetrahydrocannabinol; CBD, cannabidiol.

Table I presents the heats of transition of pure and of dipalmitoyl phosphatidylcholine interacting with Δ^1-tetrahydrocannabinol or cannabidiol. The heat of transition (ΔH) of dipalmitoyl phosphatidylcholine decreases upon interaction with the cannabinoids from a value of 8.6 kcal/mol for pure dipalmitoyl phosphatidylcholine to about 5.4 kcal/mol.

Several reports have appeared dealing with the effect of various drugs on both the transition temperature and enthalpy of the transition of phospholipids. Cater et al. [11] have examined the effect of morphine and tricyclic antidepressant derivatives on the melting behaviour of synthetic phosphatidylcholines. Certain drugs of these series exhibit a shift in transition temperature accompanied by the appearance of additional transition peaks. No appreciable change in the heat of transition could however be detected. On the other

TABLE I

HEATS OF DIPALMITOYL PHOSPHATIDYLCHOLINE (DPL) PHASE TRANSITIONS AT DIFFERENT MOLAR RATIOS OF PHOSPHLIPID TO Δ^1-THC OR CBD

		ΔH (kcal/mol DPL)			ΔH (kcal/mol DPL)
DPL only		8.6			
DPL/Δ^1-THC	22:1	7.5	DPL/CBD	20:1	7.4
DPL/Δ^1-THC	11:1	6.4	DPL/CBD	10:1	5.4
DPL/Δ^1-THC	5:1*	5.2	DPL/CBD	5:1	6.0
DPL/Δ^1-THC	2.4:1	5.4	DPL/CBD	2:1	5.7

*When two peaks appeared in the transition profile, the total area was taken for the calculation.

hand, the interaction of chlorothricin with dipalmitoyl phosphatidylcholine caused a decrease in the heat of transition of dipalmitoyl phosphatidylcholine with almost no effect on the melting temperature [12]. The interaction of a wide range of drugs with dipalmitoyl phosphatidylcholine results in small shifts in the melting temperature [13,14] and in no change in the enthalpy of transition.

Lawrence and Gill [15] studied the effects of Δ^1-tetrahydrocannabinol and other cannibinoids on spin-labelled phosphatidylcholine/cholesterol liposomes. They found that Δ^1-tetrahydrocannabinol fluidized the lipid bilayer whereas cannabidiol decreased the fluidity of the bilayer, and have suggested that the psychoactive cannabinoids may be classified as "partial anaesthetics" producing a perturbation of the membrane structure. They suggest that due to their limited solubility in the lipid phase of cell membranes the cannibinoids are unable to produce the degree of membrane disorder corresponding to clinical anaesthesia.

The studies described in this communication show a remarkable fluidizing effect of both drugs on dipalmitoyl phosphatidylcholine liposomes. Cannabinoids could possibly trigger a localized phase transition of phospholipids at a constant temperature. This might be of relevance for the activity of functional membrane entities such as enzymes and carriers, many of which have been shown to be associated with phospholipids.

No significant difference in the perturbation exerted on a phosphatidylcholine bilayer by Δ^1-tetrahydrocannabinol and cannabidiol was observed. Moreover, our previous studies both on black lipid membranes [6] and on biological membranes (refs. 5 and 8, and Herschkovitch, M., Raz, A. and Goldman, R., unpublished) suggest that both Δ^1-tetrahydrocannabinol and cannabidiol are similarly potent in damaging the permeability of phospholipid and biological membranes. Δ^1-Tetrahydrocannabinol and cannabidiol exhibit also similar cytotoxicity towards macrophages in culture [8].

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References

1 Mechoulam, R. (1970) Science 168, 1159—1166.
2 Grlić, L. (1962) Bull. Narcot. 14, 37—46.

3 Seeman, P., Chau-Wong, M. and Moyyen, S. (1972) Can. J. Physiol. Pharmacol. 50, 1193–1200.
4 Raz, A., Schurr, A. and Livne, A. (1972) Biochim. Biophys. Acta 274, 269–271.
5 Raz, A., Schurr, A., Livne, A. and Goldman, R. (1973) Biochem. Pharmacol. 22, 3129–3131.
6 Bach, D., Raz, A. and Goldman, R. (1976) Biochem. Pharmacol., in press.
7 Bino, T., Chari-Bitron, A. and Shahar, A. (1972) Biochim. Biophys. Acta 288, 195–202.
8 Raz, A. and Goldman, R. (1976) Lab. Invest. 34, 69.
9 Gibermann, E., Chari-Bitron, A., Milo, S. and Gothilf, S. (1974) Experientia 30, 68–69.
10 King, E.J. and Wootton, I.D.P. (1956) Microanalysis in Medicinal Biochemistry, Grune and Stratton, New York.
11 Cater, B.R., Chapman, D., Hawes, S.M. and Saville, J. (1974) Biochim. Biophys. Acta 363, 54–69.
12 Pache, W. and Chapman, D. (1972) Biochim. Biophys. Acta 255, 348–357.
13 Jain, M.K., Wu, N.Y.-M. and Wray, L.V. (1975) Nature 255, 494–495.
14 Papahadjopoulos, D., Jacobson, K., Poste, G. and Shepherd, G. (1975) Biochim. Biophys. Acta 394, 504–519.
15 Lawrence, D.K. and Gill, E.W. (1975) Mol. Pharmacol. 11, 595–602.