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THERMOTROPIC PROPERTIES OF CALF-BRAIN LIPIDS INTERACTING WITH DRUGS—II*

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Abstract—The influence of different drugs on the thermotropic properties of calf-brain lipids was investigated. Tetracaine, ethanol and ketamine caused a downward shift in the temperature of the maximum in the heat flow, with almost no change in the enthalpy of melting of the lipids. However, cannabinoids caused a decrease in the enthalpy of melting, accompanied by a minor shift in the melting temperature. The effect of cholesterol on drug interactions was also investigated.

We have recently shown [1] that total calf-brain lipids undergo melting in a wide range of temperatures (10–50°). The effect of local anesthetics, dibucaine and tetracaine, on the thermotropic behaviour of the lipids was investigated, indicating that the drugs modify the melting profile, thus changing the ratio between the crystalline and the liquid crystalline lipid.

We have now extended this work by employing other anesthetic drugs, such as ethanol, hashish components and ketamine, and have investigated their interaction with the total calf-brain lipids.

Ethanol is regarded as a general anesthetic, known to block nerve conduction [2] at high concentrations. When interacting with membranes, it causes an increase in fluidity, as revealed by electron paramagnetic resonance measurements [3], and also influences membrane transport processes in the brain [4].

Δ^1-Tetrahydrocannabinol, the major psychoactive component of marihuana [5], acts also as a local anesthetic [6]. Previously, it was shown by us that cannabinoids affect the thermotropic properties of model [7] and biological membranes [8].

Ketamine is a general anesthetic, that causes an increase in fluidity of membrane lipids [9, 10], and affects the activity of membrane enzymes [10, 11].

In view of the above-mentioned properties of these drugs, it was of interest to investigate their interaction with brain lipids, attempting to gain more information on their mechanism of action. We have also further pursued the study of the role of cholesterol in the lipid–anesthetics interactions.

MATERIALS AND METHODS

The brain lipids were isolated from calf brain, as described previously [1]. Cholesterol was removed from the lipids by acetone extraction [12]. Tetracaine hydrochloride was purchased from Sigma, St. Louis, MO, U.S.A. Ethyl alcohol for u.v. spectroscopy was a product of Fluka AG, Buchs-Switzerland. Cannabidiol (CBD) was from Makor Chemicals, Jerusalem. Δ^1-Tetrahydrocannabinol (Δ^1-THC) was a gift from Prof. R. Mechoulam, Department of Pharmacy, Hebrew University, Jerusalem. Δ^1-THC was obtained as ethanolic solution, the solvent was driven off by a stream of nitrogen, and the drug was freshly dissolved in chloroform. Ketamine was from Parke–Davis, Munchen, West Germany. Cholesterol was a BDH-product and was recrystallized in the laboratory.

The calorimetric measurements were performed on DuPont 990 instrument, equipped with cell base II, and the calibrated mode was used. All the experiments were performed in 1.5×10^{-1} M NaCl solution, buffered to pH 7.4 with 10^{-2} N Tris–HCl buffer. The samples for the differential scanning calorimetry were prepared in two ways: (i) The lipids were weighed directly into the aluminum pans, and an excess of salt solution or appropriate amounts of the drug dissolved in the salt solution were added (tetracaine, ethanol, ketamine). The pans were sealed and left at 37° for 2 hr. (ii) The lipids dissolved in chloroform:ethanol 2:1 or chloroform only, and the drugs dissolved in chloroform were mixed together and left at room temperature for 1 hr (CBD, Δ^1-THC). The solvents were driven off by a stream of nitrogen, and the material was kept in high vacuum for 3 hr and subsequently transferred into the aluminum pans, to which an excess of salt was added. The calorimetric experiments were performed either on the day of the preparation or after 24 hr.

RESULTS AND DISCUSSION

In Fig. 1, the thermograms of the total brain lipids interacting with ethanol are presented. As seen from the figure and from Table 1, ethanol causes a downward shift of the maximum in the heat flow and shortens the range of melting, shifting it towards physiological range. No change in the enthalpy, due to interaction, was detected. The influence of ethanol on the brain lipids shows similarity to that of tetracaine (ref. 1 and Table 1). Both these drugs are hydrophilic, and tetracaine is also charged at neutral pH's. Due to interaction, tetracaine and ethanol are

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Table 1. The temperature of the middle of the peak, T_m, for different drug-lipid mixtures

Sample	T_m (°C)	T_m
Total lipids	35.0	—
Total lipids: tetracaine (3.6:1)*†	32.5‡	−2.5
Total lipids: tetracaine (2.5:1)*	32.0	−3.0
Total lipids: 5% ethanol	33.0	−2.0
Total lipids: 10% ethanol	32.5	−2.5
Total lipids: CBD (4.3:1)	35.0	—
Total lipids: CBD (2.1:1)	35.0	—
Total lipids: Δ^1-THC (10:1)	35.0	—
Total lipids: Δ^1-THC (4:1)	35.0	—
Total lipids: Δ^1-THC (2:1)	33.5	about −1.5
Total lipids: ketamine (2.6:1)	33.5	about −1.5
Phospholipids	49.0	—
Phospholipids: tetracaine (2.6:1)	43.0	−6.0
Phospholipids: CBD (2.1:1)	45.0	−4.0
Phospholipids: Δ^1-THC (2:1)	44.5	−4.5

* Reference [1].

† Assuming molecular weight for the lipid to be 800, the ratios given are molar ratios.

‡ The temperature is a mean of two or three experiments.

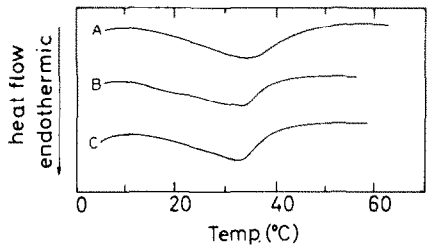
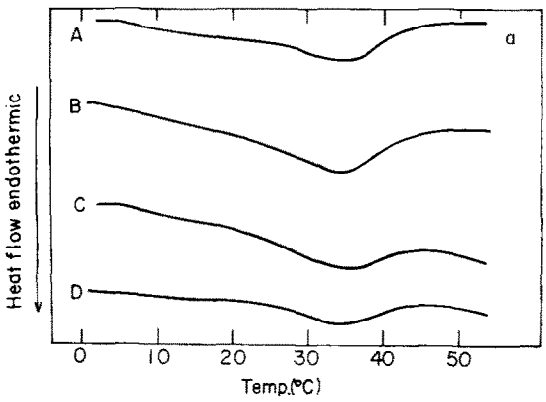


Fig. 1. Thermograms of total brain lipids interacting with ethanol. (A) 3.9 mg lipid. (B) 2.9 mg lipid +5% ethanol solution. (C) 2.9 mg lipid +10% ethanol solution. Scan rate 5°/min. Sensitivity 0.04 milical/sec.inch.

probably located in the head-group region of the lipids, slightly increasing the spacing between the lipid molecules with concomitant decrease in the melting temperature.

Another charged anesthetic, ketamine hydrochloride, was also investigated. This drug is very soluble in water and, hence, has probably very small membrane–aqueous solution partition coefficient. Ketamine hydrochloride only slightly affects the thermotropic properties of the investigated lipids (Table 1), causing a small shift in the melting temperature and a very small decrease in the enthalpy of melting, at high drug to lipid ratio (1:2). These data are in keeping with those of Lenaz *et al.* [9], who had shown that ketamine has a small effect on the fluidity of lipid vesicles and a bigger one on whole membranes, affecting probably more the lipid–protein interactions.

Ketamine hydrochloride is a 'dissociative anesthetic', the action of which *in vivo* differs from that of other anesthetics [13]. However, in this study we have investigated the influence of the various drugs on the lipid phase; thus, we cannot discern between the mechanism of the *in vivo* effect of ketamine, as compared to the other anesthetic drugs.



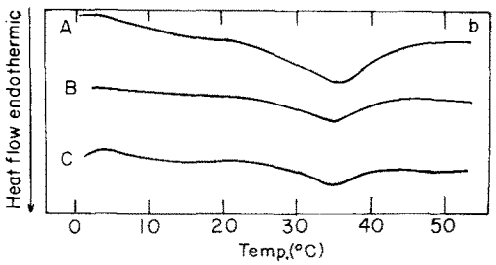


Fig. 2. Thermograms of total brain lipids interacting either (a) with Δ^1-tetrahydrocannabinol (Δ^1-THC) or (b) with cannabidiol (CBD)

mg lipid	ratio lipid/drug (mole/mole)
(a) A—1.4	—
B—1.5	10:1
C—1.8	3.8:1
D—1.7	1.9:1
(b) A—1.4	—
B—1.1	4.3:1
C—1.0	2.1:1

Scan rate 5°/min. Sensitivity 0.02 milical/sec. inch.

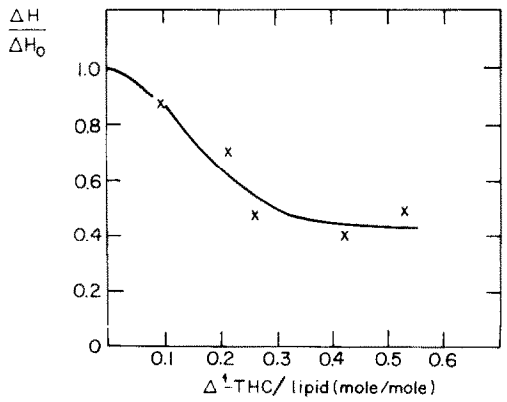


Fig. 3. The ratio of the enthalpy of melting of the total brain lipids, interacting with Δ^1-tetrahydrocannabinol-ΔH, to the enthalpy of melting of the pure lipid-$\Delta H°$, as a function of the molar ratio Δ^1-tetrahydrocannabinol/lipid. Each point is a mean of at least two independent experiments.

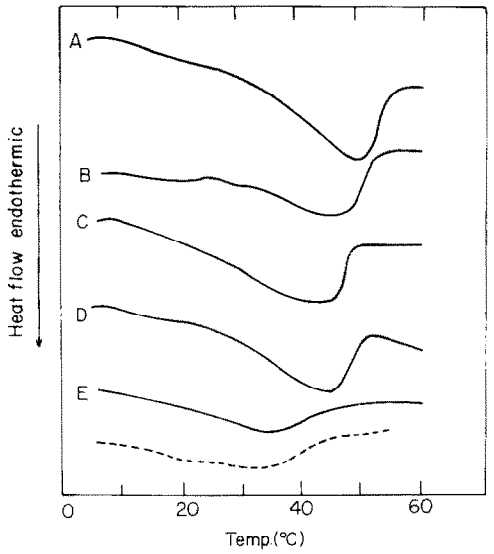


Fig. 4. Thermograms of brain phospholipids interacting either with tetracaine, Δ^1-tetrahydrocannabinol, or with cannabidiol. (A) 1.6 mg phospholipids. (B) 1.3 mg phospholipids: CBD—2:1 (mole:mole). (C) 1.5 mg phospholipids: tetracaine—2.6:1 (mole:mole). (D) 1.3 mg phospholipids: Δ^1-THC—2.2:1 (mole:mole). (E) 1.6 mg total brain lipids. - - - - 2.7 mg phospholipids +15% cholesterol. Scan rate 5°/min. Sensitivity 0.02 milical/sec.inch.

Cannabinoids are lipid-soluble neutral compounds, possessing a very high membrane–aqueous solution partition coefficient [14]. As such, they should have strong influence on the properties of lipids. Figure 2(a) and 2(b) present the thermograms of Δ^1-THC or CBD, interacting with brain lipids. As can be seen from the figures and Table 1, the cannabinoids strongly decrease the enthalpy of melting of the lipid, with almost no change in the temperature of melting. In Fig. 3, the ratio of enthalpy of melting of the interaction products to the enthalpy of pure lipid is given, as a function of the molar ratio Δ^1-THC/lipid. The enthalpy of melting decreases very steeply due to the interaction, reaching a constant value at a molar ratio of about 1:5. Δ^1-THC and CBD have the same effect on the thermotropic properties of lipids, in spite of the fact that Δ^1-THC is both psychoactive and a local anesthetic, whereas CBD is devoid of these properties. This is an agreement with our and other investigators' findings quoted in references [7] and [8], suggesting that the action of Δ^1-THC, manifested *in vivo*, requires probably specific recognition sites. With respect to the interaction with the lipids, the cannabinoids penetrate the lipid core, thus producing a new phase of lower enthalpy of melting. Above a certain concentration, a saturation is reached due to maximal solubility of cannabinoids and no further change in the melting properties is observed.

The data presented in this paper agree with our previous results obtained with differential scanning calorimetry, where we had shown that either Δ^1-THC or CBD decreases the melting temperature and the enthalpy of melting of synthetic lecithin and of microsomal lipids [7, 8]. In both cases, a saturation with respect to the decrease of enthalpy of melting was found.

In an attempt to evaluate the effect of cholesterol on the thermotropic properties of the brain lipids, and its effect on the lipid-drug interactions, the cholesterol was removed by further fractionation. In the preliminary communication [1], cholesterol removal was achieved by Florisil column fractionation in 1,2-dichloroethane of the acetylated lipids. During this procedure some lipids underwent acetylation, influencing the thermotropic profile. In the present study we have removed the cholesterol from brain lipids by cold acetone extraction [12], without affecting the structure of the phospholipids. The cholesterol-free phospholipids gave the thermogram shown in Fig. 4(a). The enthalpy of melting of the phospholipids is 4 milical/mg, more than twice that of the parent lipids. The total brain lipids used in our experiments contain 16% cholesterol. Addition of cholesterol to the phospholipids, to a final concentration of 15%, produced a thermogram (broken-line curve, Fig. 4), quite similar to that of the native lipids. The shape of the profile and its enthalpy of melting depend very strongly on the percentage of the added cholesterol.

Interaction of the drugs with the phospholipids was investigated. Tetracaine, at a molar ratio of 0.5:1, caused a downward shift in the midpoint melting temperature (Table 1 and Fig. 3), the effect being twice as big as in the case of native or reconstituted lipids. The stronger effect of tetracaine on the phospholipids could be explained by the fact that the lipids are already partially fluidized by their cholesterol, as indicated by their lower ΔH, leading to a smaller degree of disturbance brought about by tetracaine. In analogy, in biological membranes tetracaine would probably have stronger influence on regions possessing lower cholesterol content or entirely devoid of cholesterol.

Cannabinoids interacting with phospholipids give different profiles than those of total lipids. Figure 4 and Table 1 show that Δ^1-THC or CBD, interacting with the phospholipids, cause a big shift in the melting temperature, but do not significantly affect the enthalpy of melting. In the case of interaction with CBD, some splitting of the peak is discerned. These data agree with our previous findings on synthetic

phospholipid-dipalmitoyl lecithin [7], where a decrease of the melting temperature and the appearance of a new phase at higher cannabinoid ratios were detected. However, in the presence of cholesterol, some distortion of the structure of the bilayer occurs and the penetration of cannabinoids is facilitated, as seen from the big decrease in the enthalpy of melting. A possibility exists that a 'complex' lipid-cholesterol–cannabinoid is formed, possessing thermotropic properties different from those of lipid–cannabinoid interaction product. The effect of cholesterol on cannabinoid–lipid interactions was also found by other groups. Pang and Miller [15] claim that, at low concentrations of cholesterol, cannabinol causes an increase in the order parameter of lecithin vesicles, whereas at higher concentrations disordering occurs. Tamir *et al.* [16] claim that the presence of cholesterol is a prerequisite for the interaction with cannabinoids.

We have shown previously [1] that tetracaine causes a downward shift in the melting temperature of the lipids at drug : lipid molar ratio as low as 1 : 60, which is equivalent to the drug concentration of about 3×10^{-3} M. This concentration is similar to those reported for the blocking effect of another positively charged anesthetic—procaine—on impulse conduction of various nerves [17]. The high drug concentrations used in the present study were due to the very concentrated dispersions of lipid employed. In fact, the drug concentration in the membrane phase in physiological studies such as our own, might be entirely different from that given for the aqueous phase in as much as its concentration in the membrane is determined by the lipid/water partition coefficient of the drug.

In conclusion, we have shown that different drugs modify the properties of total calf-brain lipids. The mode of action of drugs depends strongly on the degree of their hydrophobicity, and is also a function of the cholesterol content of the lipids.

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