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PRELIMINARY COMMUNICATION

THERMOTROPIC PROPERTIES OF BRAIN LIPIDS IN THE PRESENCE AND ABSENCE OF LOCAL ANESTHETICS

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The mode of action of local anesthetics is still not fully understood, in spite of numerous *in vivo* and *in vitro* studies. Lee [1] suggested that sodium channels in the nerve membranes are surrounded by lipid in the crystalline state and that local anesthetics cause a transition of this lipid from crystalline to liquid-crystalline state, producing a conformational change of the channel protein resulting in a decrease of the sodium conductance. This model is based on experiments showing that local anesthetics increase the fluidity and decrease the melting temperature of vesicles prepared from synthetic lipids or brain phosphatidyl serine [2-7].

By employing differential scanning calorimetry we found that brain lipids undergo melting in a wide range of temperatures (10°C-50°C). This system is especially suitable for investigating the effect of different drugs that may modify the melting properties of the lipids. In this communication we describe the thermotropic properties of calf brain lipids and the effect of the local anesthetics - dibucaine and tetracaine - on these properties.

MATERIALS & METHODS

The brain lipids were isolated from fresh calf brain, as described by Hakomori *et al.* [8]. Fraction 1 (the lower phase) contains the total brain lipids minus the gangliosides. This fraction was further separated by chromatography yielding fraction 2 which contains only phospholipids but not cholesterol or neutral glycolipids. The cholesterol content of the lipids was determined [9].

Tetracaine hydrochloride was purchased from Sigma, St. Louis, Mo., and dibucaine hydrochloride came from K & K Laboratories, Plainview, N.Y..

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 $1.5 \cdot 10^{-1}$ N NaCl solution, buffered to pH 7.4 with 10^{-2} N *Tris*·HCl buffer. The experiments for differential scanning calorimetry were prepared in two ways: (i) The lipids were weighed directly into the aluminum pans and excess of salt solution or appropriate amounts of the drug dissolved in the salt solution were added. The pans were sealed and left at 37°C for 2 hr. (ii) The lipids dissolved in chloroform: ethanol (2:1) and the drugs dissolved in ethanol were mixed together and left at room temperature for 1-hr. The solvents were driven off by a stream of nitrogen, the material was kept at 0.5 Torr for 3 hr and subsequently transferred into the pans and an excess of salt was added. The samples were run either on the day of the preparation or after 24-48 hr. No difference in the results was detected after the first heating scan.

RESULTS & DISCUSSION

Brain lipids undergo melting in a wide range of temperatures, 10°C-50°C, with a maximum in the endothermic heat flow at 35°C (Fig. 1A). The heat of melting of these lipids is 1.7 milical/mg lipid. As found by the analysis and in agreement with literature data [10], the bovine brain lipids used in our experiments contain 16 percent cholesterol.

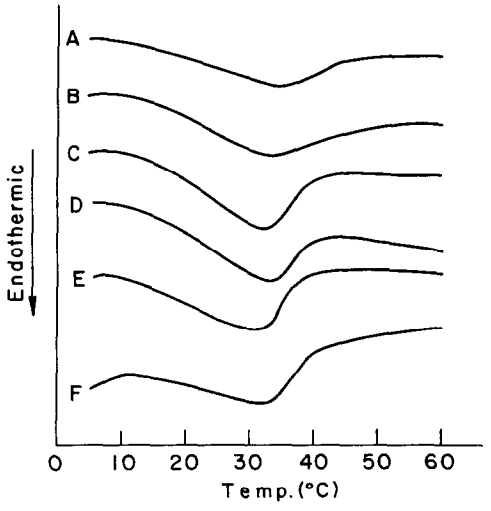

Fig. 1. Thermograms of total brain lipids interacting with local anesthetics.

	mg lipid	molar ratio lipid/drug [a]
A -	1.6	-
B -	1.8	10:1 (tetracaine)
C -	3.0	3.6:1 (")
D -	2.2	2.5:1 (") [b]
E -	2.5	2:1 (") [b]
F -	2.2	2.8:1 (dibucaine)

[a] assuming molecular weight of 800 for the lipid

[b] mixed in organic solvents
scanning rate 5°/min
sensitivity 0.02 milical/sec·inch

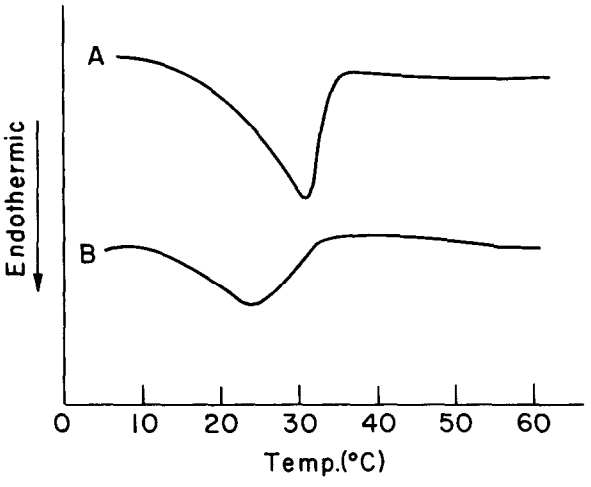

Fig. 2. Thermograms of brain phospholipids interacting with tetracaine.

	mg lipid	molar ratio lipid/drug [a]
A -	2.8	-
B -	1.9	2.4:1

[a] assuming molecular weight of 800 for the lipid
scanning rate 5°/min
sensitivity 0.04 milical/sec·inch

In an attempt to evaluate the effect of cholesterol on the thermotropic properties of brain lipids, the cholesterol and neutral glycolipids were quantitatively removed by further fractionation with 1,2 dichloroethane and 1,2 dichloroethane:methanol (9:1) on a Florisil column. The range of melting of the cholesterol-devoid lipids became narrower; it extended up to ∿35°C and the maximum shifted to lower temperatures, ∿30°C (Fig. 2A). The heat of melting increased to 2.7 milical/mg lipid.

The effect of cholesterol on the broadening of the range of melting of synthetic phospholipids was reported recently [11,12]. The broadening of the range of melting of total lipids,

as compared to cholesterol-devoid fraction, may be explained by the effect of cholesterol on the thermotropic properties.

To our knowledge, only the myelin lipids were investigated by differential scanning calorimetry [13]. The total myelin lipids, which are very rich in cholesterol (~35%) [14], did not exhibit melting around room temperatures, but they do show melting after removal of cholesterol [13].

Interaction of the brain lipids with the local anesthetic-tetracaine causes a downward shift in the temperature of the maximum in the heat flow. At molar ratio lipid:drug ~60:1 the decrease in the temperature is ~1.7°C (not shown) and it becomes larger with the decrease in the lipid:drug ratio (Figs. 1B,C,D,E). At ratios of lipid:drug lower than 4:1, the range of melting also becomes narrower and a small decrease up to 20 percent in the heat of melting of the lipid is detected (Fig. 1E). Compared to tetracaine, interaction with dibucaine gives a smaller shift in the heat flow maximum (Fig. 1F).

To evaluate the influence of cholesterol and neutral glycolipids on the interaction of brain lipids with local anesthetics, the experiments were also carried out on the brain lipid fraction selectively devoid of both components. The results of the experiments are presented in Fig. 2B.

The effect of tetracaine on the brain phospholipids is stronger than on the total lipids (Fig. 1D). The shift in the midpoint temperature is ~6°C, which is twice as big as the shift found in experiments using total lipids. These results suggest the possibility that tetracaine affects the cholesterol or cerebrosides interaction with phospholipids, but at present we cannot assess which component is responsible for this interaction.

To conclude, we have shown that brain lipids undergo melting in a wide range of temperatures and that local anesthetics modify the melting profile changing the ratio between crystalline and liquid crystalline lipid, thus influencing probably the microenvironment of the membrane proteins.

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