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IMIPRAMINE AND LIPID PHASE TRANSITION IN INNER MITOCHONDRIAL MEMBRANE

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Summary

As ascertained by freeze-fracture electron microscopy, imipramine prevents lateral phase separation from taking place in inner mitochondrial membranes at sub-zero temperatures. Electron spin resonance (ESR) measurements performed on mitochondrial membranes labeled with the *N*-oxyl-4',4'-dimethyloxazolidine derivative of 16-ketostearic acid, show that the spin probe motion is markedly inhibited below 0°C and that 5 mM imipramine attenuates the temperature effect. These results are explained by supposing that imipramine is able to decrease the transition temperature of the inner mitochondrial membrane lipids as it does for simple lipid systems.

Introduction

Several neurotropic drugs such as anesthetics, tranquilizers and antidepressants are able to affect the physical properties of lipids in model membranes. In particular, they increase the mobility of fatty acid chains in lipid bilayers and reduce the temperature of the gel-to-liquid crystal phase transition [1—5]. The goal of our work was to search for similar effects in biological membranes. The drug we have chosen is imipramine, an antidepressant which, according to Cater et al. [1], can markedly decrease the transition temperature of lecithin/water systems. For the biological membranes, we used rat liver mitochondria because the thermotropic phase transition has been described in mitochondrial membranes [6], accompanied with lateral phase separations as being easily observable by freeze-fracture electron microscopy [6].

Materials and Methods

The experiments were performed on mitochondrial fractions from rat livers corresponding to the sum of fractions M and L of de Duve et al. [7]. Freeze-fracture electron microscopy was performed according to the method of Wattiaux-De Coninck et al. [8].

Mitochondrial membranes were spin-labeled with the *N*-oxyl-4′,4′-dimethyl-oxazolidine derivative of 16-ketostearic acid. The probe was purchased from Syva, Palo Alto, CA. 5 μl of a 10 mM stock solution in methanol were introduced into a test-tube and thoroughly evaporated under N_2. The dried lipid film was dispersed in 80 μl of mitochondria suspension (1 g fresh w. per ml) by vortex mixing. The sample was always kept at low temperature (±4°C) to avoid rapid disappearance of the spin label. After homogenization, the labeled suspension was diluted either with 80 μl of 1 mM imidazole, pH 7.2, containing 80% (w/w) ethylene glycol or with 80 μl of imipramine at appropriate concentrations, 1 mM imidazole, pH 7.2, containing 80% (w/w) ethylene glycol.

For the ESR measurements, the membrane suspension was introduced into glass capillaries (0.8 mm inner diameter). The spectra were recorded with a Varian E3 spectrometer equipped with a quartz dewar, nitrogen gas flow and a variable temperature control unit. Temperature precision was within ±1°C.

The spectra were analyzed using the following relationship [9]:

$$\tau = 0.635 \cdot 10^{-9} \times \Delta H_0 \left(\sqrt{\frac{I_0}{I_{-1}}} - \sqrt{\frac{I_0}{I_{+1}}} \right)$$

τ should be considered as an empirical 'motion parameter' which is convenient for estimating the changes of the probe motion. This parameter does not represent a true measurement of the rotational correlation time since the hypothesis of isotropic motion is obviously not fulfilled in a membraneous system. ΔH_0 is the width of the central line (in G) and I_m ($m = +1, 0, -1$) represents the amplitudes of the low-, central- and high-field lines, respectively.

Results and Discussion

Fig. 1A shows the convex fracture face of the inner membrane of rat liver mitochondria maintained at 0°C in the presence of different concentrations of imipramine. Apparently, the presence of the drug does not particularly change the aspect of the membrane after freeze-etching. The face is covered with numerous particles disposed in a relatively homogeneous manner. Fig. 1B illustrates the same face of the membranes, when mitochondria are maintained at −10°C near the lower transition temperature of mitochondrial membrane lipids [6]. In the absence of imipramine, large smooth areas devoid of particles become apparent, illustrating lateral phase separations which occur during thermotropic transition [6]. The same results are obtained in the presence of 0.75 mM imipramine. At higher drug concentrations (5 mM) the smooth areas disappear; particles are dispersed in the convex face as observed in the mitochondria maintained at 0°C. Thus, at such concentrations, imipramine prevents

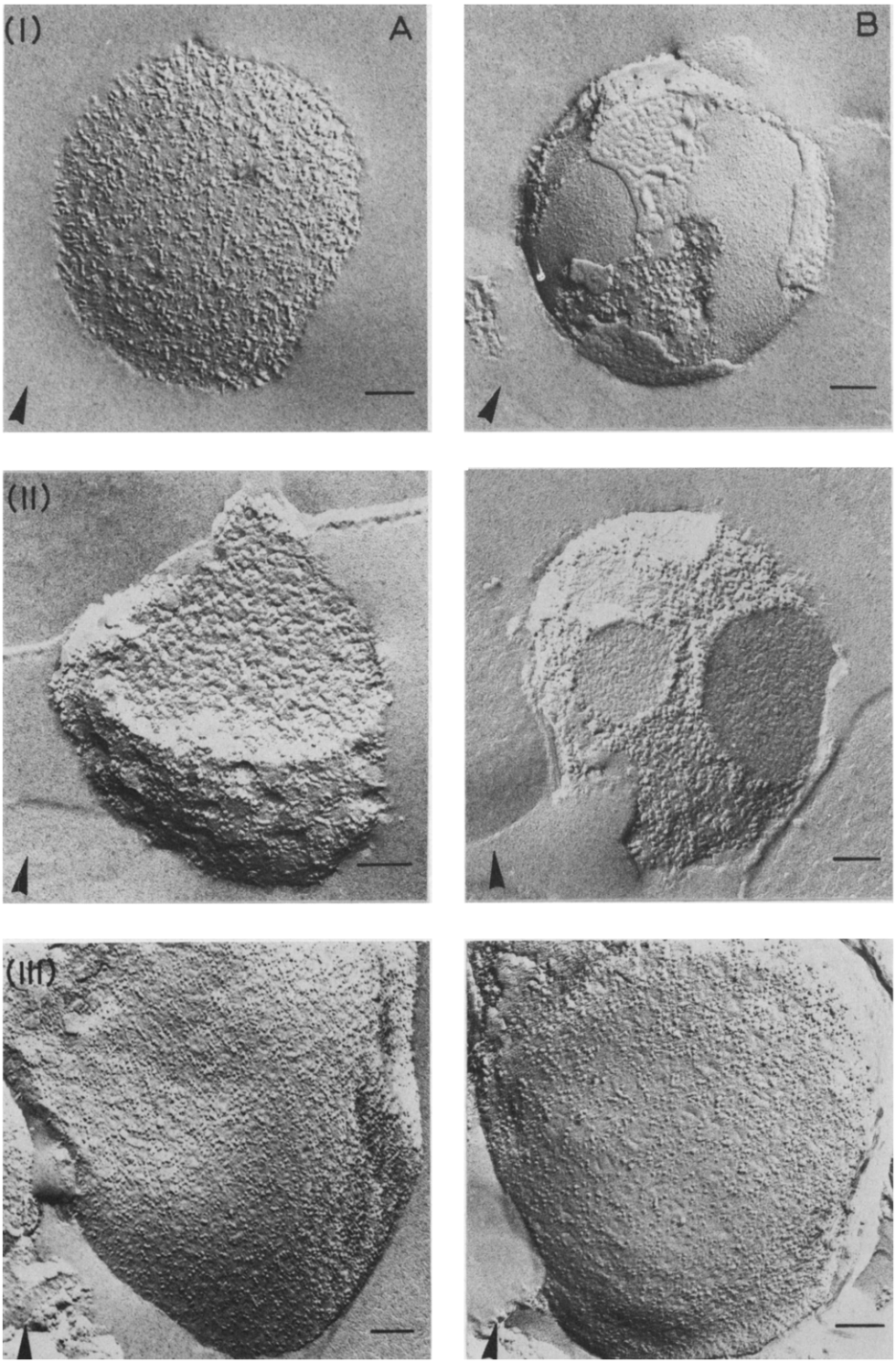

Fig. 1. Convex fracture faces of inner mitochondrial membrane maintained at 0°C (A) or at −10°C (B) for 1 h before freezing. (I) In the absence of imipramine; (II) in the presence of 0.75 mM imipramine; (III) in the presence of 5 mM imipramine. Smooth areas devoid of particles are seen when mitochondria are kept at −10°C. They disappear when 5 mM imipramine is present in the medium.

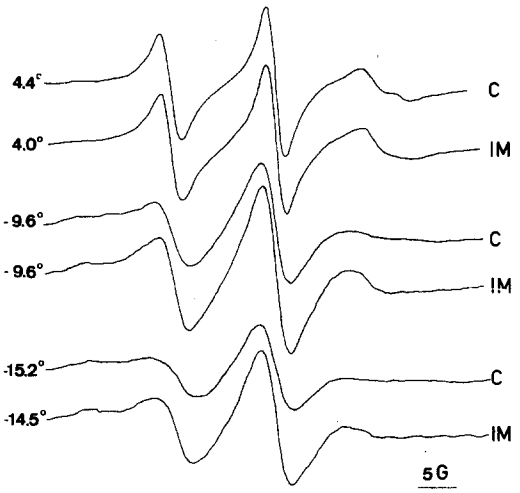

Fig. 2. ESR spectra of inner mitochondrial membranes labeled with 16-ketostearic acid spin label. Effect of 5 mM imipramine at three temperatures. C, control; IM, imipramine. Modulation 1 G. Microwave power 8 mW.

lateral phase separations from taking place in innner mitochondrial membranes at sub-zero temperatures.

Fig. 2 shows the temperature dependence of the ESR spectra of spin-labeled mitochondrial membranes and the effects produced on these spectra by 5 mM imipramine. When imipramine is not used a temperature reduction from +4 to −15°C produces a severe inhibition of the spin probe motion. This is illustrated in Fig. 3 where a plot of the logarithm of the motion parameter, τ, vs. $1/T$ is shown. A sharp increase is observed below 0°C in the case of the control and log τ is about 5-fold higher at −15 than at 0°C. By paralleling these results with the differential scanning calorimetry determinations of Hackenbrock et al. [6], it seems justifiable to explain the spectral modifications as resulting from the lipid phase transition which takes place between 0 and −15°C in the inner mitochondrial membrane lipids whereas the phase transition of the outer membrane lipids occurs at a higher temperature. Imipramine tends to attenuate the temperature effects. Indeed, when the drug is present, the spin probe motion is less hindered (Fig. 2) and in fact, the change of log τ is quasi-linear down to −8°C (Fig. 3). Below −8°C, log τ continues to increase but at −15°C its value does not exceed 3 times the value measured at 0°C. The spectral modifications were found to be insignificant when imipramine was tested at lower concentrations (0.75 and 2.5 mM).

The more plausible explanation of our morphological and ESR observations is that imipramine is able to decrease the transition of the mitochondrial membrane lipids as it does in simple lipid systems [1]; therefore, the presence of proteins in the inner mitochondrial membrane does not qualitatively affect the response of the membrane lipids to the antidepressant. It is to be noted that the imipramine concentration needed to display a significant effect is of the same order of magnitude as that required for related drugs with artificial lipid systems [1—4]. Such a concentration is relatively high; in fact we found that

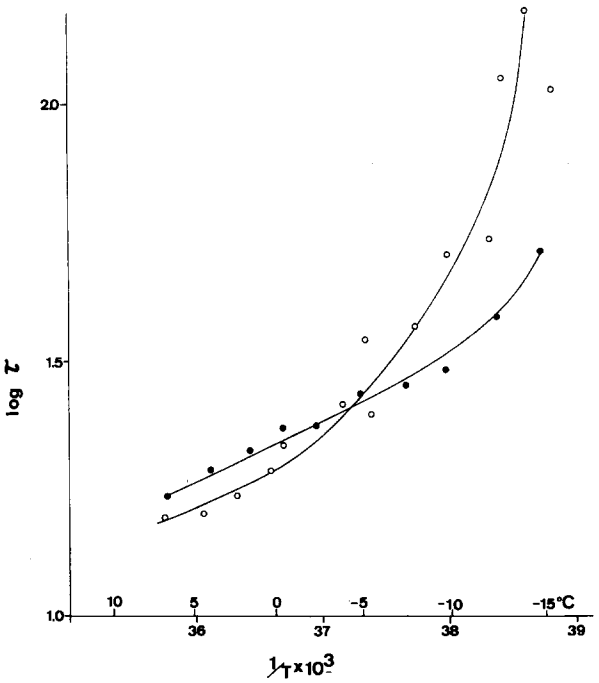

Fig. 3. Arrhenius plot of the motion parameter (τ) of the 16-ketostearic acid spin label in inner mitochondrial membranes in the absence (○) and in the presence (●) of imipramine (5 mM).

it can cause a certain lysis of the mitochondria (unpublished observations). It seems, therefore, difficult to correlate the imipramine effects we have described to the pharmacological effects of the drug on biomembranes. This is in parallel with the observations of Lee [4] and Boggs et al. [2] on anesthetics. These authors have shown that the concentrations of anesthetics able to induce a significant increase in lipid fluidity correspond more closely to those causing lysis of the erythrocytes than those causing anesthesia.

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