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ASYMMETRIC ANTAGONISTIC EFFECTS OF AN INHALATION ANESTHETIC AND HIGH PRESSURE ON THE PHASE TRANSITION TEMPERATURE OF DIPALMITOYL PHOSPHATIDIC ACID BILAYERS

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Summary

The phase transition temperature (T_t) of dipalmitoyl phosphatidic acid multilamellar liposomes is depressed 10°C by the inhalation anesthetic methoxyflurane at a concentration of 100 mmol/mol lipid. Application of 100 atm of helium pressure to pure phosphatidic acid liposomes increased T_t only 1.5°C. However, application of 100 atm helium pressure to dipalmitoyl phosphatidic acid lipsomes containing 100 mmol methoxyflurane/mol lipid almost completely antagonized the effect of the anesthetic. A non-linear pressure effect is observed. In a previous study, a concentration of 60 mmol methoxyflurane/mol dipalmitoyl phosphatidylcholine depressed T_t only 1.5°C, exhibiting a linear pressure effect. The completely different behavior in the charged membrane is best explained by extrusion of the anesthetic from the lipid phase.

Anesthetic agents have been shown to depress the phase transition temperature (T_t) of pure phospholipid bilayers using the techniques of electron paramagnetic resonance (EPR) [1], fluorescence polarization [2], dilatometry [3] and calorimetry [4,5]. Similarly, application of high-pressure helium has been shown to raise T_t of pure phospholipids using EPR techniques [6]. The effects of inhalation anesthetics and pressure were shown to be antagonistic in these systems: when an amount of pressure sufficient to raise the T_t of dipalmitoyl phosphatidylcholine 2°C was applied to a suspension containing

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sufficient inhalation anesthetic to cause an equivalent depression of T_t, a phase transition curve like that of the unmodified control was obtained. The antagonistic effect appears to be due to a reorganization of the bilayer components around the contained anesthetic rather than to expulsion of the anesthetic molecule from the bilayer [7,8].

The existence of high concentrations of negative phospholipids in nerve membranes [9] and the polypeptide-induced phase separations in phosphatidic-acid bilayers [10] suggested that it would be important to study pressure-anesthetic antagonism on T_t of phosphatidic acid liposomes. Dipalmitoyl phosphatidic acid (Sigma) was tested for purity by thin-layer chomatography. 20 mg dipalmitoyl phosphatidic acid were dispersed in 8 ml of a 300 mM sodium borate buffer (pH 9.0) by sonication with a microtip for 3 min at 55°C. To this dispersion, 7 μl methoxyflurane were added at 55°C in a closed vessel with vortexing to obtain a concentration of 100 mmol/mol phosphatidic acid based on a lipid/water partition coefficient of 20. The resulting suspension was centrifuged at 1000 × g for 5 min to obtain a 0.4 ml pellet of multilamellar liposomes. To this pellet, 30 μl of $3 \cdot 10^{-3}$ M 2,2,5,5,-tetramethylpiperidine-1-oxyl (TEMPO) were added to obtain a final concentration of $2.25 \cdot 10^{-4}$ M. 10 μl of this pellet were injected into a pressurizable 1 mm inner diameter quartz EPR sample cell. This method of handling anesthetic-treated dispersions has been shown not to result in reduction of anesthetic concentration. The spectra were measured on a Varian E-104A EPR with on-line digitized recording and curve-fitting using a DEC PDP-11/03 computer. The transition temperatures were derived from partitioning of the spin probe as described by McConnell et al. [11].

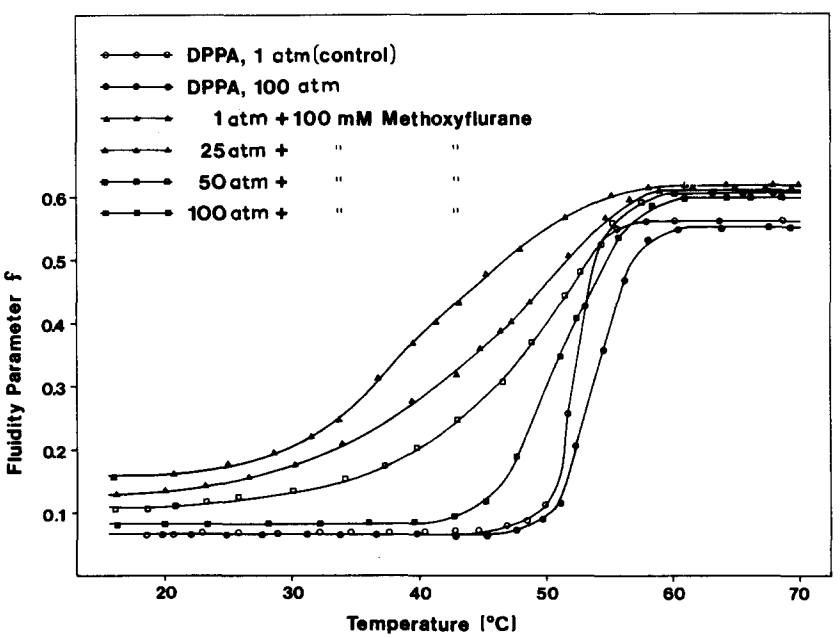

Fig. 1. Pressure-dependent phase transition curves of dipalmitoyl phosphatidic acid bilayers (DPPA) in the presence and absence of the inhalation anesthetic methoxyflurane (100 mmol/mol lipid). Note the asymmetric change in the phase transition temperature with increasing helium pressure.

The effect of pressure on pure dipalmitoyl phosphatidic acid lipsosomes and on liposomes containing 100 mmol methoxyflurane/mol phosphatidic acid is shown in Fig. 1. Application of 100 atm helium pressure to the pure phosphatidic acid dispersion resulted in a 1.5°C increase in T_t. Addition of 100 mmol methoxyflurane under atmospheric pressure leads to a 10° C depression of the T_t. The lipid order-disorder phase transition is broadened, thus demonstrating a loss of cooperativity greater than that observed in previous experiments on uncharged phosphatidylcholine bilayers. Moreover, the fluidity of the membrane is increased below the phase transition temperature as demonstrated by the uptake of the spin label. Application of 100 atm helium pressure on the anesthetic-containing membrane almost completely antagonized the disordering effect of methoxyflurane. The fluidity parameter, F below the lipid phase transition, the phase transition temperature as well as the cooperativity of the transition, approach that of the control at atmospheric pressure without anesthetic (Fig. 1).

Fig. 2 demonstrates the change in T_t of dipalmitoyl phosphatidic acid membranes in the presence and absence of methoxyflurane. The results are compared to earlier results on pure dipalmitoyl phosphatidylcholine membranes and on dipalmitoyl phosphatidylcholine bilayers containing 4 mol% phosphatidic acid from Ref. 1. In the case of pure phosphatidic acid bilayers we observed a small linear 1.5° C increase in T_t by application of 100 atm helium pressure. This change is only 50% of that observed in phosphatidylcholine

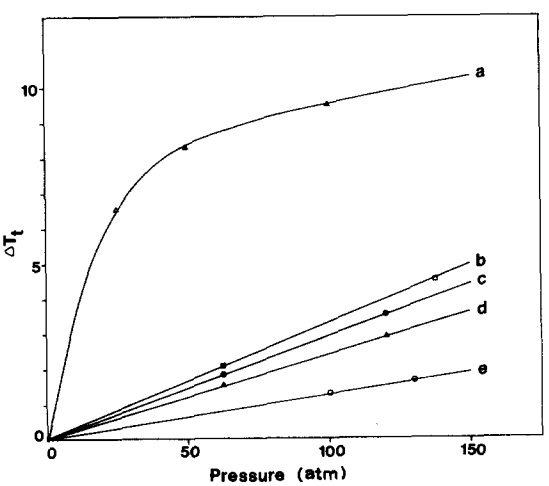

Fig. 2. Change in phase transition temperature of dipalmitoyl phosphatidic acid bilayers in the presence and absence of methoxyflurane. Curves b, c and d are replotted from Ref. 1 for comparison. a; Dipalmitoyl phosphatidic acid bilayers containing 100 mmol methoxyflurane per mol lipid. b; Dimyristoyl phosphatidylcholine bilayers plus methoxyflurane (60 mmol/mol lipid). c; Pure dipalmitoyl phosphatidylcholine bilayers. d; Bilayers of dipalmitoyl phosphatidylcholine containing 5 mol% dipalmitoyl phosphatidic acid. e; Pure dipalmitoyl phosphatidic acid bilayers.

bilayers. An asymmetric antagonistic effect of pressure is observed in anesthetic-containing phosphatidic acid membranes (curve a, Fig. 2). 50 atm pressure raises the anesthetic-depressed phase transition temperature by 8.5°C, there is a further elevation in T_t of only 1.5°C upon increasing the pressure to 100 atm. At $P > 50$ atm, the slope of curve a is about the same as that ob-

served for pure phosphatidic acid membranes (curve e, Fig. 2) over the whole pressure range.

The 10°C depression in the dipalmitoyl phosphatidic acid bilayers caused by methoxyflurane suggests that the high packing density [12] of this surface charged bilayer may make it especially susceptible to anesthetic-induced disorder. The elevation to T_t of pure dipalmitoyl phosphatidic acid bilayers caused by 100 atm of helium pressure is only half of that caused in a similar system of phosphatidylcholine membranes [1]. This indicates that the more condensed charged bilayer has a low compressibility. The result obtained suggests two conclusions: (1) the anesthetic-disordered phosphatidic acid bilayer has a much higher compressibility than the pure one; and (2) the application of pressure to anesthetic-disordered bilayers results in extrusion of the anesthetic in the case of phosphatidic acid but not in phosphatidylcholine membranes.

These conclusions are supported by the following arguments. (1) The change in T_t of the anesthetic-disordered phosphatidic acid bilayer as a function of temperature is asymmetric with a greater effect at low pressure. This non-linearity is best explained as a gradual decrease in the compressibility (dV/dP) of the anesthetic-disordered bilayer as a function of pressure. At high pressure ($P > 50$ atm) the compressibility begins to approach that of the pure phosphatidic acid bilayer indicated by the equivalent slopes of curves a and e in Fig. 2. (2) Application of 100 atm helium pressure to phosphatidic acid membranes containing the anesthetic yields a phase transition curve of about the same transition width, and therefore of the same cooperativity, as that of the pure control.

In previous studies of anesthetic-disordered phosphatidylcholine bilayers, the fluidity measured in the gel phase below T_t was always much higher than that of the control containing pure phosphatidylcholine membranes. Application of increasing pressure did not reduce the fluidity of the anesthetic-disordered gel phase. In contrast, in Fig. 1 it is seen that the fluidity measured at 30°C in the gel phase of dipalmitoyl phosphatidic acid bilayers after addition of 100 mmol methoxyflurane is high, but successive applications of helium pressure cause the fluidity at 30°C to decrease until it approaches that of the control phosphatidic acid bilayers. This could be explained if pressure extruded the anesthetic from the charged liposomes.

Recent evidence has shown that phosphatidic acid membranes interact strongly with polymyxin, a positively charged peptide, leading to an expansion of the membrane and lowering of the lipid phase transition temperature by 30°C. A cooperative phase separation is observed [13]. In this study we have shown that liposome bilayers formed from dipalmitoyl phosphatidic acid are especially sensitive to anesthetic-induced disorder, the disordered phase has a higher dV/dP than the pure phase. In contrast to previous results in other phospholipid systems, the pressure antagonism is best explained by extrusion of the anesthetic from the lipid phase. These findings may be important to understanding anesthetic effects and pressure-anesthetic antagonism in the sodium channel and other proteins that are surrounded by high concentrations of negatively-charged phospholipids [9].

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