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ANTAGONISM BETWEEN HIGH PRESSURE AND ANESTHETICS IN THE THERMAL PHASE-TRANSITION OF DIPALMITOYL PHOSPHATIDYLCHOLINE BILAYER

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Summary

The antagonizing action of hydrostatic pressure against anesthesia is well known. The present study was undertaken to quantitate the effects of hydrostatic pressure and anesthetics upon the phase-transition temperature of dipalmitoyl phosphatidylcholine vesicles. The drugs used to anesthetize the phospholipid vesicles included an inhalation anesthetic, halothane, a dissociable local anesthetic, lidocaine and an undissociable local anesthetic, benzyl alcohol. All anesthetics decreased the phase-transition temperature dose-dependently. In the case of lidocaine, the depression was pH dependent and only uncharged molecules were effective. The application of hydrostatic pressure increased the phase-transition temperature both in the presence and the absence of anesthetics. The temperature-pressure relationship was linear over the entire pressure range studied up to 340 bars. Through the use of Clapeyron-Clausius equation, the volume change accompanying the phase-transition of the membrane was calculated to be 27.0 cm^3/mol. Although the anesthetics decreased the phase-transition temperature, the molar volume change accompanying the phase-transition was not altered. The anesthetics displaced the temperature-pressure lines parallel to each other. The mole fraction of the anesthetics in the liquid crystalline membrane, calculated from the van't Hoff equation, was independent of pressure. This implies that pressure does not displace the anesthetics from the liquid membrane, and the partition of these agents remains constant. The volume change of the anesthetized phospholipid membranes is entirely dependent upon the phase-transition and not on the space occupied by the anesthetics.

Introduction

It is now well recognized that anesthetics dilate, fluidize and disorder cell membranes [1,2]. It is also known that the actions of anesthetics are usually antagonized by a pressure of 100—150 bars [2—6], although there have been several negative reports with dissociable local anesthetics [7—9].

Phospholipid membranes exist in a closely-packed crystalline gel phase and a loosely-packed liquid crystalline phase. The transition between the two states is highly cooperative and follows quasi-first-order kinetics, especially with zwitterionic phosphatidylcholines. Phase-transitions of phospholipid membranes have been a subject of several recent reviews [10—13].

There have been several reports about the elevation of the phase-transition temperature of phospholipid membranes by high pressures [14—17], and about its depression by anesthetics [18—21]. However, there have been few detailed reports of the combined effects of pressure and anesthetics upon the phase-transition of phospholipid bilayers except the one by Trudell et al. [20] and the recent report by MacDonald [22].

The abrupt changes in membrane properties accompanying the phase-transition can be measured in several ways. One of the methods utilizes the abrupt change of the turbidity of the phospholipid vesicle suspension, clear in the liquid crystalline state and turbid in the gel state, which can be monitored by light absorbance [18] or scattering [19]. It is our intention here to quantitate the effects of hydrostatic pressure in the reversal of the anesthetic-depressed phase-transition temperature in dipalmitoyl phosphatidylcholine bilayer vesicles.

Methods and Materials

Synthetic dipalmitoyl phosphatidylcholine (1,2-dihexadecanoyl-*sn*-glycero-3-phosphorylcholine) was obtained from Calbiochem. Its purity was checked by thin-layer chromatography using chloroform/methanol/water as a solvent and was found to show a single spot. Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) and lidocaine (*N*,*N*-diethyl-*N'*-(2,6-dimethylphenyl)glycinamide) were gifts from Ayerst Labs. (New York, N.Y.) and Astra Pharmaceutical Co. (Worcester, Mass.), respectively. All other chemicals were reagent grade. Water was purified either by triple distillation as previously reported [23] or by passage through ion-exchanger plus activated charcoal columns and an ultrafilter. Both methods appeared to produce high quality water. The absence of surface active impurities was ascertained by measuring the dynamic surface tension of water by the Wilhelmy plate method with a Cahn Electrobalance (Paramount, Calif.) equipped with a dynamic surface tension device as previously reported [23].

The phospholipid vesicles were prepared by suspending dipalmitoyl phosphatidylcholine in water using ultrasonic irradiation at temperatures several degrees above the phase-transition until optical clarity was achieved.

A high pressure cell assembly with sapphire windows (American Instrument Co., Silver Springs, Md) was used for the determination of the light absorbance of the bilayer suspension. The temperature-controlled pressure cell has been described in detail previously [24]. The hydrostatic pressure was applied by a hand-operated hydraulic pump.

The cell compartment assembly of a Hitachi Perkin-Elmer spectrophotometer was replaced with the high pressure cell assembly. The spectrophotometer output was recorded together with the temperature signals on an X-Y recorder.

The cuvette temperature was monitored by a thermistor probe inserted into the body of the cell block. The cuvette temperature was raised at a rate of 1.0°C per 2 min by circulating hot water through copper tubing wrapped around the pressure cell.

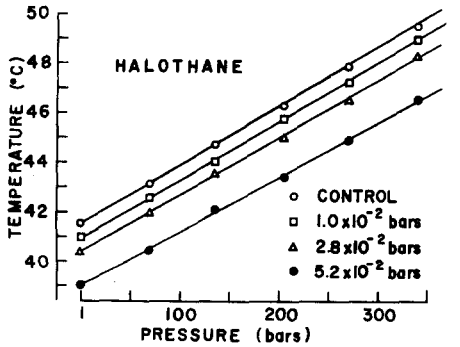
The sudden change of the absorbance accompanying the thermal phase-transition was followed at 560 nm. The midpoint between the beginning of the decrease in absorbance and the point where the absorbance reached its plateau was taken as the phase-transition temperature.

Halothane was vaporized with nitrogen by a copper kettle vaporizer in an anesthesia machine. The anesthetic concentrations were estimated by the ratio of the saturated gas flow through the vaporizer and the flow of the nitrogen diluent. The concentrations were confirmed by gas-chromatography. The vesicle suspension was equilibrated with inhalation anesthetics by bubbling for at least 15 min. Lidocaine and benzyl alcohol were dissolved in distilled water and the solution was mixed with the vesicle suspension. The pH was adjusted with hydrochloric acid or sodium hydroxide and measured by a Corning pH meter and a glass electrode (Corning, N.Y.).

Results

The application of hydrostatic pressure elevated the phase-transition temperature both in the absence and the presence of the anesthetics. Without anesthetics, the phase-transition temperature at ambient pressure was 41.5°C, and it was increased to 49.5°C at 340 bars. When the phase-transition temperature was plotted against the pressure, a straight line was obtained (Fig. 1) with a slope of 0.0224°C/bar.

Addition of halothane depressed the phase-transition temperature in a dose-dependent manner. The changes of halothane concentration, however, did not affect the slope of the temperature-pressure diagram. The dT/dP slopes for halothane tensions of $1 \cdot 10^{-2}$ bars, $2.8 \cdot 10^{-2}$ bars and $5.2 \cdot 10^{-2}$ bars were



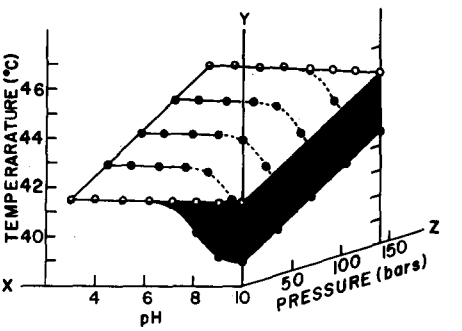
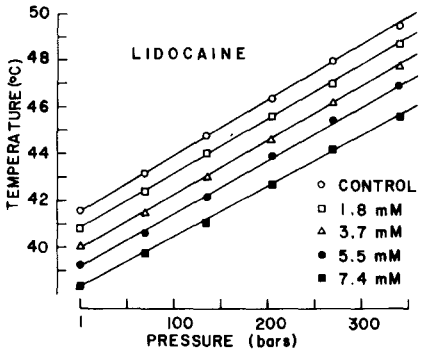


Fig. 1. The temperature vs. pressure diagram for the phase-transition of the dipalmitoyl phosphatidylcholine membrane with halothane at pH 7.0. The partial pressures of halothane are indicated in the figure.

Fig. 2. The relationship of the phase-transition temperature, pH and pressure. The abscissa is pH, the ordinate is temperature (°C) and the Z-axis is pressure (bar). ○, controls; ●, effects of 5.5 mM lidocaine.



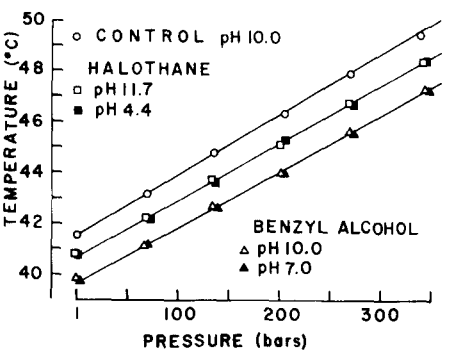


Fig. 3. The temperature vs. pressure diagram for the phase-transition with lidocaine at pH 10.0. The concentrations of lidocaine are indicated in the figure.

Fig. 4. The effect of pH for the phase-transition with 5 mM benzyl alcohol and $1 \cdot 10^{-2}$ bars halothane. The pH values are indicated in the figure. The variation of pH did not show any effects on the drug actions.

parallel to each other and to the control ($dT/dP = 0.0227°C/bar$).

A dissociable local anesthetic, lidocaine, was effective in lowering the phase-transition temperature only in the alkaline range as previously reported [19]. The pH temperature diagram (Fig. 2) closely followed the concentration of uncharged species of this compound calculated from the Henderson-Hasselbach equation.

In the temperature-pressure diagram (Fig. 3), the slope of the lines with 1.8, 3.7 and 5.5 mM lidocaine at pH 10.0 (99.3% of the molecules were uncharged) were virtually parallel with each other, having slopes of dT/dP of 0.0225° C/bar.

The action of uncharged anesthetic, benzyl alcohol, was independent of pH (Fig. 4). At pH values 7.0 and 10.0, 5 mM benzyl alcohol showed the identical degree of the temperature depression. Similarly, $2 \cdot 10^{-2}$ bars halothane depressed the phase-transition temperature to the same degree at either pH 4.4 or 11.7.

Discussion

The present results show that hydrostatic pressure elevates the phase-transition temperature regardless of the presence or the absence of anesthetics.

The pressure-temperature diagram was linear in the pressure range studied (up to 340 bars). This contradicts the isobaric findings of Srinivasan et al. [14] which indicated that the pressure-temperature data obtained by dilatometry were non-linear within 100 bars. However, Liu and Kay [15] later determined this relationship by measuring the pressure-induced isothermal phase-transition and reported it was linear up to 270 bars.

The Clapeyron-Clausius equation is applicable to such a linear relationship.

$$dT/dP = \Delta V/\Delta S$$

where T = phase-transition temperature in degrees Kelvin, P = applied pressure, ΔV = molar volume change, and ΔS = molar entropy change. By using a recent

reported value [25] of ΔS = 29.2 cal/degree per mol, the volume change was calculated to be 27.0 cm^3/mol for the gel to liquid crystalline phase-transition. The calculated value is in good agreement with the dilatometry data reported by Liu and Kay [15]. Trudell et al. [16] also used the Clapeyron-Clausius equation for their pressure data in the absence of anesthetics at 68 and 136 bars by assuming a linear change and reported a similar figure.

The parallel displacement of the straight lines in the pressure-temperature diagram by anesthetics indicates that the ratio of $\Delta V/\Delta S$ is independent of pressure and of the presence of any anesthetics at any concentration.

Jain et al. [21] and Jain and Wu [25] used differential scanning calorimetry and reported that although anesthetics changed the pattern of heat-flow of the phase-transition, the ΔH remained constant. Since for a phase-transition, $\Delta G = 0 = \Delta H - T\Delta S$, and since T changes only over several degrees, ΔS must be essentially constant in the experimental temperature range. This leads us to the conclusion that the change in volume is not affected by the presence of anesthetics.

The entrance of the anesthetic into the membrane promotes the formation of the liquid crystalline state. High pressure or low temperature freezes the liquid membrane into the crystalline gel state with concomitant exclusion of the ligand molecules from the solid domain. The mole fraction of phospholipids (X_a) and anesthetics (X_b) in the liquid crystalline membrane can be computed from the present temperature data according to the van't Hoff equation and by using a reported enthalpy value of 9.2 kcal/mol [25] for the transition:

$$\Delta H/R \cdot (1/T' - 1/T) = \ln X_a$$

$$X_b = 1 - X_a$$

where R = gas constant, T' = phase-transition temperature without anesthetics and T = phase-transition temperature with anesthetics. As shown in Table I, the X_b values were independent of the applied pressure.

This results implies that anesthetics are not squeezed out from the liquid membrane by the applied pressures, and the partition of these agents is uninfluenced. The phase-transition excludes these molecules from the solid domain. Trudell et al. [26] concluded that anesthetic molecules are not displaced from the membrane by high pressure, based on their observation with TEMPO

TABLE I

THE MOLE FRACTION OF LIDOCAINE IN THE LIQUID-CRYSTALLINE PHASE OF THE DIPALMITOYL PHOSPHATIDYLCHOLINE MEMBRANE AND THE EFFECT OF HYDROSTATIC PRESSURE

Lidocaine concentrations (mM)	Applied pressure (bars)					
	1.0	68.0	136.1	204.1	272.1	340.1
1.8	0.037	0.034	0.036	0.036	0.040	0.035
3.7	0.068	0.074	0.080	0.075	0.074	0.077
5.5	0.107	0.108	0.113	0.104	0.106	0.106
7.4	0.144	0.163	0.161	0.152	0.155	0.157

(2,2,6,6-tetramethylpiperidine-1-oxyl) incorporated into the dipalmitoyl phosphatidylcholine membrane. They showed that the spin-probe has some local anesthetic activity and reported that TEMPO remained in the membrane under high pressure. Hsia and Boggs [7], however, reported that the same probe incorporated into biological membranes was displaced to a more hydrophilic site by high pressure. The reason for this discrepancy is unclear.

The depression of the phase-transition temperature by the anesthetics is due to the preferential solubility of these molecules in the liquid crystalline phase. The temperature decrease is caused by the relative decrease of the mole fraction of phospholipid in the membrane.

Since the volume change in the presence of anesthetics was identical to the controls, pressure reversed the anesthetic-induced dilatation of phospholipid membranes entirely by the phase-transition mechanism. It is caused not by the compression of the space occupied by the ligand molecules or by the exclusion of these molecules from the membrane.

Halothane at its partial pressure of $2 \cdot 10^{-2}$ bars depressed the phase-transition temperature by 0.96°C. This depression was antagonized by a hydrostatic pressure of 29.6 bars (Fig. 4). In vivo pressure reversal, however, requires a pressure of about 100—150 bars in order to reverse the effects of the same concentration of halothane. This 3—5-fold difference in antagonizing pressure is intriguing. It may be caused by the difference in the phospholipid composition in natural membranes where lipids are more unsaturated and remain in the liquid crystalline state until higher pressure is applied and presumably the phase-transition of phospholipids may not be the sole mechanism of the pressure reversal of anesthesia.

The results with the dissociable local anesthetic, lidocaine, probably require some comments. The present results show as well as our previous report that the depression of the phase-transition temperature by the dissociable local anesthetics is caused by the uncharged molecules. This indicates two possibilities: (1) Charged molecules partition into the phospholipid membrane without depressing the phase-transition temperature. (2) Charged molecules do not partition into the membrane.

The first possibility appears to be remote in spite of the demonstration by Lee [27] that fatty acids incorporated into the phospholipid membrane did not change or even increased the phase-transition temperature when the alkyl-chain length exceeded 10 carbon atoms. This probably occurs when they form eutectic solutions. Dipalmitoyl phosphatidylcholine and lidocaine are unlike molecules and are not expected to form eutectic solutions.

Our study of surface pressure of dipalmitoyl phosphatidylcholine monolayer (to be reported) showed that the Gibbs surface excess of ionized lidocaine was zero in the presence of the membrane. Similar results were reported by Skou [28] with a number of other dissociable local anesthetics. These results appear to contradict those reported by Eliasz et al. [29] that the charged alkylammonium salts can partition into membranes. The partition, however, is not a simple function of the ionization alone. It is conceivable that slender molecules like alkylamines and bulky local anesthetics with benzene ring attached behave differently for the penetration into highly anisotropic phospholipid membranes.

After the preparation of this manuscript, MacDonald [22] reported the interaction of high pressure and anesthetics upon the phase-transition of dipalmitoyl phosphatidylcholine vesicles measured by dilatometry.

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