Biochimica et Biophysica Acta, 946 (1988) 215–220
Elsevier

BBA 74224

Depression of phase-transition temperature by anesthetics: nonzero solid membrane binding

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(Received 4 July 1988)

Key words: Phospholipid vesicle; Phase transition temperature; Local anesthetic; Partition coefficient; Solid-gel membrane binding

The anesthetic-induced depression of the main phase-transition temperature of phospholipid membranes is often analyzed according to the van't Hoff model on the freezing point depression. In this procedure, zero interaction between anesthetics and solid-gel membranes is assumed. Nevertheless, anesthetics bind to solid-gel membranes to a significant degree. It is necessary to analyze the difference in the anesthetic binding between the liquid-crystal and solid-gel membranes to probe the anesthetic action on the lipid membranes. This article describes a theory to estimate the anesthetic binding to each state at the phase-transition temperature. The equations derived here reveal the relation between the partition coefficients of anesthetics and the anesthetic effects on the transition characters: the change in the transition temperature, and the broadening of transition. The theory revealed that the width of transition temperature is determined primarily by the membrane/buffer partition coefficients of anesthetics. Our previous data on the local anesthetic action on the transition temperature of the dipalmitoylphosphatidylcholine vesicle membrane (Ueda, I., Tashiro, C. and Arakawa, K. (1977) Anesthesiology 46, 327–332) are analyzed by this method. The numerical values for the partition of local anesthetics into the liquid-crystal and solid-gel dipalmitoyl-phosphatidylcholine vesicle membranes at the phase-transition temperature are: procaine $8.0 \cdot 10^3$ and $4.7 \cdot 10^3$, lidocaine, $3.7 \cdot 10^3$ and $2.3 \cdot 10^3$, bupivacaine $4.1 \cdot 10^4$ and $2.6 \cdot 10^4$, and tetracaine $7.3 \cdot 10^4$ and $4.7 \cdot 10^4$, respectively.

Introduction

Anesthetics decrease the main phase-transition temperature of phospholipid membranes, and the magnitude of the temperature decrease correlates with the anesthetic potency. The order-disorder change in the membrane state from the solid-gel to liquid-crystal is considered to be a basic mechanism that leads to anesthesia. Because the depression the phase-transition temperature is similar to the freezing point depression by impurities, Hill [1] devised a method to estimate the membrane-buffer partition coefficients of anesthetics according to the van't Hoff model. A number of reports appeared on the membrane-buffer partition coefficients of anesthetics by applying similar procedures [2–6]. The model assumes that anesthetic molecules are completely excluded from solid-gel membranes.

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The anesthetic binding to solid-gel membranes, however, is not negligible. With radioactive halothane, Simon et al. [7] reported that the solid-gel membrane binding is about 30% of the liquid-crystal membrane binding. With tetracaine, we found that the solid-gel membrane binding was about 57% of the liquid-crystal membrane binding (unpublished). Yokono et al. [8] demonstrated that anesthetics at low concentrations bind to the interfacial polar head region of solid-gel membranes without transforming them into liquid-crystal state.

The depression of the transition temperature is a function of the anesthetic binding to the solid-gel and liquid-crystal membranes. To analyze the anesthetic effect on the phase transition, it is necessary to estimate the partition coefficient for each state of the membrane at the transition temperature. Although Lee [9] estimated the partition of alkanols between solid-gel and liquid-crystal membranes, separate estimates for the membrane-buffer partition coefficients for each membrane state at the transition temperature have never been reported to our knowledge.

We [10] have derived an equation that combines the partition of anesthetics between solid-gel versus liquid-crystal membranes and the partition between membrane versus aqueous phases. In this communication, we shall extend the above equation to resolve the data on the transition temperature depression into two membrane/buffer partition coefficients for solid-gel and liquid-crystal membranes at the transition temperature.

Theory

The transition temperature of phospholipid membranes in the presence of low concentrations of impurities, such as anesthetics, T_t^A, is expressed as [10]

$$\frac{\Delta H^\circ}{R}\left(\frac{1}{T_t^\circ}-\frac{1}{T_t^A}\right)=\ln\frac{1-X_A^l}{1-X_A^g} \tag{1}$$

where Δ_A^g and X_A^l are the mole fractions of anesthetic molecules in the solid-gel and liquid-crystal membranes, ΔH° is the excess enthalpy associated with the phase transition, R is the gas constant, and T_t^0 is the transition temperature in the absence of anesthetics. From Eqn. 1,

$$T_t^A=\frac{T_t^0}{1-\frac{R}{\Delta S^\circ}\ln\left(\frac{1-X_A^l}{1-X_A^g}\right)} \tag{2}$$

where ΔS° is the entropy change at the phase transition and

$$\Delta S^\circ=\frac{\Delta H^\circ}{T_t^0} \tag{3}$$

Let $[A]_T$ represent the total anesthetic concentration. When $[A]_T=0$, X_A^l and X_A^g are both zero. From Eqn. 2, the change in the transition temperature by anesthetics at infinite dilution is expressed

$$\left(\frac{dT_t^A}{d[A]_T}\right)_{[A]_T=0}$$

$$=-\frac{RT_t^0}{\Delta S^\circ}\left\{\left(\frac{dX_A^l}{d[A]_T}\right)_{[A]_T=0}-\left(\frac{dX_A^g}{d[A]_T}\right)_{[A]_T=0}\right\} \tag{4}$$

We express the total number of phospholipid molecules by n_M^T, the number of phospholipid molecules in the i-state by n_M^i, the total number of anesthetic molecules by n_A^T, the number of anesthetic molecules in the i-state membrane by n_A^i, the number of anesthetic molecules in the aqueous phase by n_A^w, and the number of water molecules by n_W.

$$n_M^T=n_M^l+n_M^g \tag{5}$$

$$n_A^T=n_A^l+n_A^g+n_A^w \tag{6}$$

The mole fractions of anesthetics in the i-state membrane and in water are

$$X_A^i=\frac{n_A^i}{n_M^i+n_A^i} \tag{7}$$

$$X_A^w=\frac{n_A^w}{n_W+n_A^w} \tag{8}$$

Let P_A^i represent the partition coefficient of anesthetics between the i-state membrane and water, then

$$X_A^i=X_A^w\cdot P_A^i \tag{9}$$

Distribution of local anesthetics to the solid-gel and liquid-crystal phases is estimated under constant temperature, pressure, and lipid concentration. From Eqn. 7,

$$\frac{dX_A^i}{dn_A^T} = \frac{1}{(n_M^i + n_A^i)^2}\left\{(n_M^i + n_A^i)\frac{dn_A^i}{dn_A^T} - n_A^i\left(\frac{dn_M^i}{dn_A^T} + \frac{dn_A^i}{dn_A^T}\right)\right\} \tag{10}$$

When $n_A^T = 0$, $n_A^i = 0$, hence Eqn. 10 becomes

$$\left(\frac{dX_A^i}{dn_A^T}\right)_{n_A^T=0} = \frac{1}{n_M^i}\left(\frac{dn_A^i}{dn_A^T}\right)_{n_A^T=0} \tag{11}$$

Similarly, from Eqn. 8,

$$\left(\frac{dX_A^W}{dn_A^T}\right)_{n_A^T=0} = \frac{1}{n_W}\left(\frac{dn_A^W}{dn_A^T}\right)_{n_A^T=0} \tag{12}$$

Differentiating both sides of Eqn. 9 by n_A^T at $n_A^T = 0$

$$\left(\frac{dX_A^i}{dn_A^T}\right)_{n_A^T=0} = P_A^i\left(\frac{dX_A^W}{dn_A^T}\right)_{n_A^T=0} \tag{13}$$

From Eqns. 11, 12, and 13,

$$\frac{1}{n_M^i}\left(\frac{dn_A^i}{dn_A^T}\right)_{n_A^T=0} = P_A^i\frac{1}{n_W}\left(\frac{dn_A^W}{dn_A^T}\right)_{n_A^T=0} \tag{14}$$

Hence,

$$\left(\frac{dn_A^i}{dn_A^T}\right)_{n_A^T=0} = P_A^i\frac{n_M^i}{n_W}\left(\frac{dn_A^W}{dn_A^T}\right)_{n_A^T=0} \tag{15}$$

Differentiate Eqn. 7 by n_A^T.

$$1 = \left(\frac{dn_A^l}{dn_A^T}\right) + \left(\frac{dn_A^g}{dn_A^T}\right) + \left(\frac{dn_A^W}{dn_A^T}\right) \tag{16}$$

Combining Eqns. 15 and 16 at $n_A^T = 0$, one obtains

$$1 = \left(1 + P_A^l\frac{n_M^i}{n_W} + P_A^g\frac{n_M^g}{n_W}\right)\left(\frac{dn_A^W}{dn_A^T}\right)_{n_A^T=0} \tag{17}$$

The transition of the phospholipid membrane is often broadened in the presence of impurities. We define the degree of transition (α) to be the fraction of the phosopholipid molecule in the liquid-crystal (or, alternatively, solid-gel) state.

$$\alpha = \frac{n_M^l}{n_M^T} \tag{18}$$

Using Eqn. 18, Eqn. 17 is rearranged as

$$\left(\frac{dn_A^W}{dn_A^T}\right)_{n_A^i=0} = \frac{1}{1 + \{\alpha P_A^l + (1-\alpha)P_A^g\}n_M^T/n_W} \tag{19}$$

From Eqns. 12 and 13,

$$\left(\frac{dX_A^i}{dn_A^T}\right)_{n_A^T=0} = \frac{P_A^i}{n_W}\left(\frac{dn_A^W}{dn_A^T}\right)_{n_A^T=0} \tag{20}$$

From Eqns. 19 and 20,

$$\left(\frac{dX_A^i}{dn_A^T}\right)_{n_A^T=0} = \frac{P_A^i}{n_W + \{\alpha P_A^l + (1-\alpha)P_A^g\}n_M^T} \tag{21}$$

By multiplying both side of Eq. 21 with constant, $V \cdot N_A$, where V is the total volume of the system and N_A is Avogadro's number, we obtain

$$\left(\frac{dX_A^i}{d[A]_T}\right)_{[A]_T=0} = \frac{P_A^i}{55.5 + \{\alpha P_A^L + (1-\alpha)P_A^G\}[L]_T} \tag{22}$$

where, $n_A^T/VN_A = [A]_T$, $n_M^T/VN_A = [L]_T$, and $n_W/VN_A = 55.5$.

From Eqns. 4 and 22, the change in the transition temperature, where the degree of transition is α, by anesthetics at infinite dilution is obtained,

$$\left(\frac{dT_t^A}{d[A]_T}\right)_{[A]_T=0} = -\frac{RT_t^0}{\Delta S^\circ}\frac{P_A^l - P_A^g}{55.5 + \{\alpha P_A^l + (1-\alpha)P_A^g\}[L]_T} \tag{23}$$

Eqn. 23 explains two significant effects of impurities on the phase transition of the phospholipid membrane: the change in the transition temperature, and the broadening of the transition. When the partition coefficients of anesthetics into the liquid-crystal membrane is larger than solid-gel membrane ($P_A^l > P_A^g$), the transition temperature decreases. Similary, when $P_A^l < P_A^g$, the transition temperature increases, and when $P_A^l = P_A^g$, the

transition temperature should not change. The case of $P_A^l < P_A^g$ is exemplified by the long-chain alkanols where the transition temperature increases when the alkyl chain length exceeds 10 to 12 carbon atoms. We have proposed a statistical mechanical theory that for the reversal of the transition temperature when the distribution of ligands is reversed [13].

We define the width of transition (W_t) to be the difference between the upper limit of the transition temperature ($T_{t(1)}^A$) where $\alpha = 1$, and the lower limit of the transaction temperature ($T_{t(0)}^A$) where $\alpha = 0$.

$$W_t = T_{t(1)}^A - T_{t(0)}^A \tag{24}$$

Thus, the change in the width of transition by anesthetics at infinite dilution is written,

$$\left(\frac{dW_t}{d[A]_T}\right)_{[A]_T=0} = \frac{RT_t^0}{\Delta S^\circ} \frac{(P_A^L - P_A^g)^2 \cdot [L]_T}{(55.5 + P_A^l \cdot [L]_T)(55.5 + P_A^g \cdot [L]_T)} > 0 \tag{25}$$

This equation shows that the impurities broaden the transition temperature to a certain extent ($dW_t/d[A]_T > 0$). The magnitude of broadening depends on P_A^l and P_A^g.

Data analysis

We have reported [3] that the uncharged forms of local anesthetics, procaine, lidocaine, bupivacaine, and tetracaine linearly decreased the main phase-transition temperature of the dipalmitoylphosphatidylcholine vesicle membrane.

In Table I, the anesthetic-induced depression of the transition temperature, reported in our previous paper [3], is shown at various phospholipid concentrations. The values indicate the rate of the temperature decrease according to the anesthetic concentration, expressed by $dT_t^A/d[A]_T$. The numerical values, however, appear to contradict the clinical potency. This is because the local anesthetic effect upon the phase transition is a combined function of the degree of anesthetic ionization, phospholipid concentration, and the membrane-water partition coefficient. When the local anesthetic concentration was adjusted for the uncharged species at pH 7.4, and the volume ratio between lipid and water was 1 : 50, the potency ratio for the transition temperature depression became procaine 1.0, lidocaine 2.7, bupivacaine 6.3, and tetracaine 5.9 [3]. These values correlate reasonably well with their relative clinical potency in inducing regional or epidural blocks [11].

TABLE I

EFFECT OF THE TOTAL DIPALMITOYLPHOSPHATIDYLCHOLINE CONCENTRATION ($[L]_T$) ON THE RATE OF THE TRANSITION-TEMPERATURE DEPRESSION BY LOCAL ANESTHETICS

$[L]_T$ (mM)	$-d\Delta T_t/d[A]_T$ (deg/mM)				
	0.5	1.0	1.5	2.5	5.0
Procaine	1.25	1.21	1.14	1.05	0.850
Lidocaine	0.553	0.542	0.529	0.515	0.448
Bupivacaine	4.69	–	–	2.60	1.56
Tetracaine	6.76	–	–	3.08	1.69

Because the transition temperature was determined by the midpoint of the sudden change in the scattering light intensity, the degree of transition (α) is 1/2. From Eqn. 23, the change in the temperature is,

$$\left(\frac{dT_t^A}{d[A]_T}\right)_{[A]_T=0} = -\frac{RT_t^0}{\Delta S^\circ} \frac{P_A^l - P_A^g}{55.5 + \frac{[L]_T}{2}(P_A^l + P_A^g)} \tag{26}$$

and

$$\frac{1}{\left(\frac{dT_t^A}{d[A]_T}\right)_{[A]_T=0}} = -\frac{\Delta S^\circ \left\{55.5 + \frac{[L]_T}{2}(P_A^l + P_A^g)\right\}}{RT_t^0(P_A^l - P_A^g)} \tag{27}$$

For estimating the partition coefficients (P_A^l and P_A^g), the reciprocal of the rate of temperature change by anesthetics ($dT_t^A/d[A]_T$) is plotted against the phospholipid concentration according to Eqn. 27.

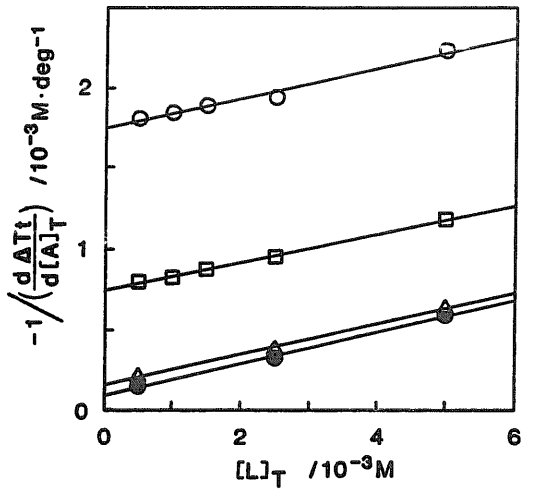

Fig. 1. Effect of the total dipalmitoylphosphatidylcholine concentration on the rate of the depression of the phase-transition temperature by local anesthetics. The data were plotted according to Eqn. 27. Abscissa: phospholipid concentration in mM. Ordinate: reciprocal of $(-d\Delta T_t/d[A]_T$ at infinite dilution of local anesthetics). From the slope and the *Y*-intercept, anesthetic partition coefficients to liquid-crystal and solid-gel membranes are estimated. The values for the slope and *Y*-intercept are shown in Table II. Symbols are: ○, lidocaine; □, procaine; △, bupivacaine; ●, tetracaine.

The relationship between the slope, A, and the *Y*-intercept, B, is expressed by

$$P_A^l + P_A^g = 111 \times \frac{A}{B} \tag{28}$$

and

$$P_A^l - P_A^g = 55.5 \times \frac{\Delta S^\circ}{RT_t^0} \frac{1}{B} \tag{29}$$

TABLE II

THE SLOPE AND *Y*-INTERCEPT OF THE PLOT BETWEEN PHOSPHOLIPID CONCENTRATION (*X*-AXIS) AND THE RECIPROCAL OF THE RATE OF THE TRANSITION TEMPERATURE DEPRESSION BY LOCAL ANESTHETICS (*Y*-AXIS) ACCORDING TO Eqn. 27, AND THE CORRELATION COEFFICIENTS (r)

	Slope (A) (1/deg)	*Y*-intercept (B) (M/deg)	r
Procaine	0.0855	$7.47 \cdot 10^{-4}$	0.9994
Lidocaine	0.0939	$1.75 \cdot 10^{-3}$	0.9908
Bupivacaine	0.0956	$1.58 \cdot 10^{-4}$	0.9988
Tetracaine	0.0988	$9.13 \cdot 10^{-5}$	0.9987

TABLE III

PARTITION COEFFICIENT (MOLE FRACTION) OF LOCAL ANESTHETICS TO THE LIQUID-CRYSTAL (P_A^l) AND SOLID-GEL (P_A^g) DIPALMITOYLPHOSPHATIDYLCHOLINE MEMBRANE.

	Partition coefficient	
	liquid-crystal	solid-gel
Procaine	$8.0 \cdot 10^3$	$4.7 \cdot 10^3$
Lidocaine	$3.7 \cdot 10^3$	$2.3 \cdot 10^3$
Bupivacaine	$4.1 \cdot 10^4$	$2.6 \cdot 10^4$
Tetracaine	$7.3 \cdot 10^4$	$4.7 \cdot 10^4$

Because the decrease in the transition temperature was linear to the anesthetic concentration, the left hand side of Eqn. 27 ($d[A]_T/dT_t^A$ at infinite dilution of anesthetic) represents the reciprocal of the value shown in Table I. These values are plotted against the phospholipid concentrations in Fig. 1. Linear relations are evident with all anesthetics. The slope (A) and the *Y*-intercept (B) of these lines together with the correlation coefficients are summarized in Table II.

The main phase-transition temperature, T_t^0, of dipalmitoylphosphatidylcholine membranes is 41.5 °C (314.7 K), and the excess enthalpy, ΔH°, is 36 kJ · mol^{-1} (8.7 kcal · mol^{-1}) [5,10]. From Eqn. 3, $\Delta S^\circ = \Delta H^\circ / T_t^\circ$, hence $\Delta S^\circ / RT_t^\circ = 0.0437$. Using this value, the partition coefficients of local anesthetics between the liquid-crystal and solid-gel membranes and aqueous phase are estimated from Eqn. 27 and are shown in Table III.

Discussion

The present plot between the phospholipid concentrations and the reciprocal of the rate of the change in the transition temperature (Fig. 1) was linear. Although the same procedure was exercised in the previous report [3], we assumed zero partition of anesthetics into the solid-gel membrane. The fitting equation, equivalent to Eqn. 27, was

$$\frac{1}{\left(\frac{dT_t^A}{d[A]_t}\right)_{[A]_t=0}} = -\frac{\Delta S^\circ}{RT_t^\circ}\left(\frac{55.5}{P_A^l} + [L]_T\right) \tag{30}$$

When Eqn. 30 was used to estimate the entropy change in the previous study [3], the value was

about twice the theoretical value of 0.0437 (the slope values, A, in Table II). The cause for this overestimation was our omission of the anesthetic partition into the solid-gel membrane. The results in Table II that all slopes are twice the value of $\Delta S°/RT_t°$ suggest that the anesthetic partition into the solid-gel phase is about 60% of the partition into the liquid-crystal phase.

The reported values of membrane-buffer partition coefficients of local anesthetics scatter depending upon the experimental conditions, such as the concentrations of phospholipids and ligands, state and composition of the membrane, temperature, pH, method of estimation, etc. We cite arbitrarily the following two reports.

By using fluorescence quenching technique, Sikaris and Sawyer [12] reported that the dipalmitoylphosphatidylcholine-water partition coefficients of procaine and tetracaine were 224 and 360, respectively, at pH 9.5 and 20°C. Boulanger et al. [12] estimated the partition coefficient of tetracaine to egg phosphatidylcholine to be 600 at pH 9.5 and 20°C from proton nuclear magnetic spectroscopy. These values are expressed by the ratio of molarity of anesthetics in each phase.

Partition coefficient is defined as the ratio of drug activities between two phases. Hence, the value differs when the expression for the concentration differs, i.e., among molarity, molality, and mole fraction. By the partial molal volume study on distearoyl-, dipalmitoyl-, and dimyristoyl-phosphatidylcholine vesicle membranes, we [5] have reported the conversion factors among partition coefficients expressed by these concentration units. The present data on procaine and tetracaine at low temperature range translate into 110 and 1100, respectively, when expressed in the molarity unit. These values are in reasonable agreement with those reported by other methods.

Acknowledgments

This study was supported by the Medical Research Service of the Veterans Administration, and NIH grants GM25716, GM26950 and GM27670.

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