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Differential affinity of charged local anesthetics to solid-gel and liquid-crystalline states of dimyristoylphosphatidic acid vesicle membranes

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Cationic local anesthetics decreased the transition temperature of the anionic phospholipid (dimyristoylphosphatidic acid, DMPA) vesicles. The counterion concentration changes the electrical double layer effect, and affects the magnitude of temperature depression caused by anesthetics. From the counterion effect on the transition-temperature depression, the partition coefficients of cationic local anesthetics to liquid-crystalline and solid-gel DMPA membranes were separately estimated. The differences in the partition coefficients between solid-gel and liquid-crystalline membranes correlated to the nerve blocking potencies. There are at least two states in the nerve membranes: resting state at higher temperature and excited state at lower temperature. We speculate that the resting state corresponds to the liquid-crystalline state, and the excited state to the solid-gel state. The difference in the partition coefficients to the resting and excited states is the cause of local anesthesia.

Introduction

Decrease of the main phase-transition temperature by additives is often analyzed by the freezing point depression according to the van't Hoff model [1–6]. The model assumes complete exclusion of additives from the solid-gel membranes. Anesthetic molecules, however, partition into solid-gel membranes as well as liquid-crystalline membranes [7,8].

By maintaining non-zero solubility of local anesthetics into solid-gel membranes, we [9] estimated partition coefficients separately to solid-gel and liquid-crystalline membranes from the depression of the transition temperature. We further explained the mechanism of widening of the temperature zone of the transition in the presence of additives. In this study [9], zwitterionic phospholipid (dipalmitoylphosphatidylcholine, DPPC) was the membrane lipid and uncharged local anesthetics were the ligand. Partition coefficients were obtained from the change in the transition temperature by varying the phospholipid concentrations. At physiological conditions, however, most local anesthetics are positively charged, and cell membranes are negatively charged. The present study analyzes interaction of cationic local anesthetics with anionic lipid membranes.

Negative surface potential of anionic phospholipids forms the electric double layer around the vesicles, and the cationic anesthetics are condensed in the double layer [10]. This condensation facilitates the interaction of cationic anesthetics with anionic membranes [11,12]. Interaction of cationic anesthetics with neutral membranes is almost negligible [1], except at very high anesthetic concentrations [2,12]. The counterion concentration changes the electric double layer effect, and affects the magnitude of the transition-temperature depression caused by cationic anesthetics.

In this communication, the partition coefficients of cationic local anesthetics to solid-gel and liquid-crystalline states of the anionic phospholipid (dimyristoylphosphatidic acid, DMPA) are separately estimated from the effect of counterion concentration on the transition-temperature depression. Biological significance of the difference in the partition coefficient to solid-gel and liquid-crystalline membranes will be discussed.

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Materials and Methods

1. Chemicals

Synthetic dimyristoylphospatidic acid (DMPA), dibucaine-HCl, tetracaine-HCl, and procaine-HCl were obtained from Sigma. Lidocaine-HCl was a gift from Astra (Westbourough, MA). All other chemicals were in the reagent grade. Water was triply distilled, once from alkaline potassium permanganate solution.

2. Vesicle preparation

DMPA was dissolved in a chloroform/methanol mixture (6 : 4, v/v) and the solvent was removed in a rotary evaporator under the flow of nitrogen gas. Lipids were further dried under vacuum for one day. The dried lipids were suspended in an apppropriate amount of NaCl solutions by a vortex mixer until homogeneous suspension was obtained. The obtained multilamellar suspension was sonicated in a cuphorn of a Branson Sonifier Model 185 (Danbury, CT) at above the transition temperature for 15 minutes. The final suspension was consisted of 0.2 mM DMPA, 20 to 200 mM NaCl, and 5 mM Bis-Tris-HCl buffer (pH 5.9). The presence of buffer did not affect the transition behavior.

3. Transition temperature measurement

The transition temperature was determined by the absorbance methods [13]. A Perkin-Elmer Model 554 UV-visible spectrophotometer (Norwalk, CT) was used to measure the light absorbance of the liposome at 400 nm. The cuvette temperature was scanned at 0.5 K/min by a programmable Perkin-Elmer digital temperature controller and a Peltier heat exchanger. The temperature of the sample was measured with a thermistor probe inserted into the cuvette slightly above the light-path, and monitored by a Digitec thermometer Model 5810 (Dayton, OH) with 0.01 K resolution. The absorbance at 400 nm and the temperature of the sample were recorded on a Heath/Schlumberger *X-Y* recorder Model SR-270 (Benton Harbor, MI). The sample solution was continuously mixed with a direct drive Spectro-Stir (Oreland, PA) during the temperature scan. The phase transition was determined by the sudden change in the absorbance, and the transition temperatures were the mean values of the heating and cooling cycles. The transition temperature obtained by this optical method was in good agreement with that obtained with differential scanning calorimetry (DSC) as previously reported [13].

Results

All tested local anesthetics decreased the transition temperature of DMPA membranes at pH 5.9 linearly to the anesthetic concentration. Fig. 1 shows the effect of NaCl concentration on the dose-dependent depression

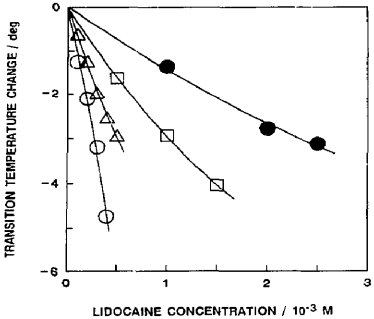

Fig. 1. The dose-dependent depression of transition temperature by lidocaine at pH 5.9, and the effect of NaCl concentrations. NaCl: 200 mM (●), 100 mM (□), 50 mM (△), 20 mM (○). The curves are fitted to $\Delta T = a_1 \cdot [A]_T^2 + a_2 \cdot [A]_T + a_3$ with the least-squares method.

of the transition temperature by lidocaine. The temperature changes (ΔT_t) were fitted to the second-degree equation

$$\Delta T_t = a_1 \cdot [A]_T^2 + a_2 \cdot [A]_T + a_3$$

with the least-squares method. The extrapolated values of the transition-temperature change $(d\Delta T_t/d[A]_T)$ to the zero anesthetic concentrations are summarized in Table I.

In 100 mM NaCl, the transition temperature changes $(d\Delta T_t/d[A]_T)$ were $1.15 \cdot 10^5$ (dibucaine), $4.64 \cdot 10^4$ (tetracaine), $2.82 \cdot 10^3$ (lidocaine), and $8.35 \cdot 10^2$ $K \cdot M^{-1}$ (procaine). The order of the potency to decrease the transition temperature is in good agreement with the order of their nerve blocking potency.

According to the decrease in the NaCl concentrations, the degree of the temperature depression increased for all local anesthetics. The temperature depressions of procaine, lidocaine, tetracaine, and di-

TABLE I

The effect of NaCl concentrations on the transition temperature change caused by the local anesthetics at pH 5.9

The values are extrapolated to zero anesthetic concentrations, units $K \cdot M^{-1}$.

Local anesthetics	NaCl concentration			
	200 mM	100 mM	50 mM	20 mM
Dibucaine	$9.81 \cdot 10^4$	$1.15 \cdot 10^5$	$1.24 \cdot 10^5$	$1.30 \cdot 10^5$
Tetracaine	$3.36 \cdot 10^4$	$4.64 \cdot 10^4$	$7.75 \cdot 10^4$	$1.12 \cdot 10^5$
Lidocaine	$1.61 \cdot 10^3$	$2.82 \cdot 10^3$	$6.25 \cdot 10^3$	$1.14 \cdot 10^4$
Procaine	$4.50 \cdot 10^2$	$8.35 \cdot 10^2$	$1.47 \cdot 10^3$	$3.33 \cdot 10^3$

bucaine in 20 mM NaCl were, respectively, 7.40-, 7.08-, 3.33-, and 1.33-times larger than in 200 mM NaCl. The effect of the NaCl concentration was stronger with less potent anesthetics.

Data analysis

The transition-temperature of phospholipid model membranes in the presence of low concentrations of additives, such as local anesthetics, T_t^A is written [14].

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

where X_A^g and X_A^l are the mole fractions of additives in the solid-gel and liquid-crystalline membranes, respectively, ΔH^0 is the excess enthalpy associated with the phase transition, R is the gas constant, and T_t^0 is the transition temperature without additives. From Eqn. 1, the transition-temperature change at zero concentration of additives is expressed as

$$\left(\frac{d\Delta T_t}{d[A]_T}\right)_{[A]_T=0}=-\frac{RT_t^0}{\Delta S^0}\left\{\left(\frac{dX_A^l}{d[A]_T}\right)_{[A]_T=0}-\left(\frac{dX_A^g}{d[A]_T}\right)_{[A]_T=0}\right\} \tag{2}$$

where ΔS^0 is the entropy change at the phase transition, and

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

Using the partition coefficients of additives to liquid-crystalline and solid-gel membranes (P_A^l and P_A^g), the change in the transition temperature at zero concentration is derived [9],

$$\left(\frac{d\Delta T_t}{d[A]_T}\right)_{[A]_T=0}=-\frac{RT_t^0}{\Delta S^0}\frac{P_A^l-P_A^g}{55.5+\frac{[L]_T}{2}(P_A^l+P_A^g)} \tag{4}$$

and

$$P_A^l=\frac{X_A^l}{X_A^w} \tag{5}$$

where, X_A^w is the mole fraction of additives in bulk aqueous solution.

A DMPA molecule has two negatively ionizable sites in its head group. Between pH 5 and 7, DMPA molecule has one negative charge [13]. The pK_a values of local anesthetics are between 8 and 9 [15], and more than 99% of the local anesthetics exist as the cationic forms at pH 5.9. Because the present study addresses the problem of the interaction between cationic local anesthetics and anionic phospholipid membranes, the electrostatic term of the surface charge was incorporated into Eqn. 4, as follows.

The partition coefficient of the cationic anesthetics, defined by Eqn. 5, includes the electric double layer effect as well as the hydrophobic interaction, and is an apparent partition coefficient. The intrinsic partition coefficient is independent of the surface potential, and is the activity ratio of anesthetics in the membranes and in the solution near the charged surface. In dilute conditions, the intrinsic partition coefficients of the cationic anesthetics to the i-state membrane ($P_{A\,int}^i$) is

$$P_{A\,int}^i=\frac{X_A^i}{X_A^\zeta} \tag{6}$$

where X_A^ζ is the mole fraction of the charged additives near the membrane surface. According to the Boltzmann distribution law, the relation between X_A^w and X_A^ζ is,

$$X_A^\zeta=X_A^w\cdot\exp\left(-\frac{F\psi_\zeta}{RT}\right) \tag{7}$$

where F is Faraday's constant, and ψ_ζ is surface potential of membrane. Thus, the relation between intrinsic and apparent partition coefficients is

$$P_A^i=P_{A\,int}^i\cdot\exp\left(-\frac{F\psi_\zeta}{RT}\right) \tag{8}$$

According to the Gouy-Chapmann theory, surface potential of charged membranes in a 1:1 electrolyte solution is written as

$$\sigma=(2000\varepsilon\varepsilon_0CRT)^{1/2}\cdot 2\cdot\sinh\left(\frac{F\psi_\zeta}{2RT}\right) \tag{9}$$

where σ is the surface charge density, ε is the relative permittivity of solution, ε_0 is the permittivity of vacuum, and C is the electrolyte concentration expressed by molarity. When the negative surface potential is high enough, Eqn. 9 is arranged as

$$\exp\left(-\frac{F\psi_\zeta}{RT}\right)=\frac{\sigma^2}{2000\varepsilon\varepsilon_0CRT} \tag{10}$$

The equation for the transition temperature change of anionic phospholipid membranes caused by cationic anesthetics at its zero concentration is written as

$$\left(\frac{d\Delta T_t}{d[A]_T}\right)_{[A]_T=0}=-\frac{RT_t^0\left(P_{A\,int}^l\cdot\theta_l-P_{A\,int}^g\cdot\theta_g\right)}{\Delta S^0\left(55.5\cdot C+\frac{[L]_T\cdot\left(P_{A\,int}^l\cdot\theta_l+P_{A\,int}^g\cdot\theta_g\right)}{2}\right)} \tag{11}$$

where

$$\theta_i=\frac{(\sigma_i)^2}{2000\varepsilon\varepsilon_0RT} \tag{12}$$

The ratio of area per phospholipid molecule for solid-gel and liquid-crystalline states was 0.659 for singly charged DMPA [13]. The area of solid-gel membranes is smaller than the area of liquid-crystalline membranes, and the electric double layer effect of solid-gel membranes is 2.30-times larger than that of liquid-crystalline membranes. Subscript, i, of θ and σ indicates that these parameters belong to the i-state membranes.

Eqn. 11 indicates that the transition temperature changes ($d\Delta T_t/d[A]_T$) depends on the lipid concentration ($[L]_T$), the transition parameters (ΔS^0, ΔH^0 and T_t^0), the intrinsic partition coefficients to the i-state membranes ($P^i_{A\,int}$), and the electrolyte concentration (C). The lipid concentration ($[L]_T$) was kept constant throughout the study. When the value of $(P^l_{A\,int}\cdot\theta_l + P^g_{A\,int}\cdot\theta_g)$ is large, the effect of the NaCl concentration on the transition temperature depression becomes small (Eqn. 11).

For estimating partition coefficients, the reciprocal $((d\Delta T_t/d[A]_T)^{-1})$ of the transition temperature change, caused by anesthetics, is plotted against the electrolyte concentration (C) in the solution.

$$\frac{1}{\left(\frac{d\Delta T_t}{d[A]_T}\right)_{[A]_T=0}} = -\frac{\Delta S^0\left\{55.5\cdot C + \frac{[L]_T\cdot\left(P^l_{A\,int}\cdot\theta_l + P^g_{A\,int}\cdot\theta_g\right)}{2}\right\}}{RT_t^0\left(P^l_{A\,int}\cdot\theta_l - P^g_{A\,int}\cdot\theta_g\right)} \tag{13}$$

The slope, A, and the Y-intercept, B, are expressed by

$$A = -\frac{55.5\cdot\Delta S^0}{RT_t^0\left(P^l_{A\,int}\cdot\theta_l - P^g_{A\,int}\cdot\theta_g\right)} \tag{14}$$

$$B = -\frac{\Delta S^0[L]_T\cdot\left(P^l_{A\,int}\cdot\theta_l + P^g_{A\,int}\cdot\theta_g\right)}{2RT_t^0\cdot\left(P^l_{A\,int}\cdot\theta_l - P^g_{A\,int}\cdot\theta_g\right)} \tag{15}$$

Following Eqn. 13, reciprocal of the values in Table I was plotted against NaCl concentrations (C) in Figs. 2 and 3. The values for the slope (A) and the Y-intercept (B) are summarized in Table II.

The transition temperature (T_t^0), and the transition enthalpy (ΔH^0) of DMPA membranes between pH 5 and 7 without anesthetics were 324.1 K and 29 kJ·mol^{-1}, respectively [13]. Using these values, apparent partition coefficients of charged local anesthetics in 100 mM NaCl are obtained (Table III).

Copeland and Andersen [16] assumed the area of solid-gel membrane to be 44 Å^2 per one DMPA molecule. The area ratio between solid-gel and liquid-crystalline membranes was 0.659 for singly charged DMPA [13]. This lead to an area of liquid-crystalline of 67 Å^2. The θ_i values were 35 (solid-gel) and 15 M (liquid-crystalline) at 324.1 K, assuming that ε is the permittivity of

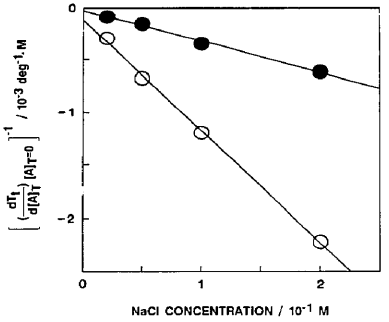

Fig. 2. The effect of the NaCl concentration on the transition temperature change. Symbols: procaine (○), lidocaine (●). The values for the slope (A), and the Y-intercepts (B) are summarized in Table II.

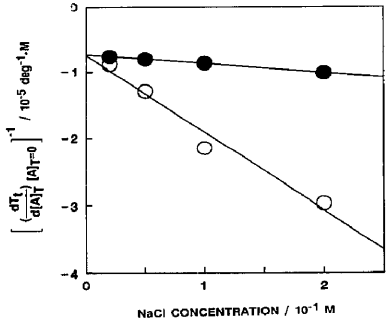

Fig. 3. The effect of the NaCl concentration on the transition temperature change. Symbols: tetracaine (○), dibucaine (●). The values for the slope (A), and the Y-intercepts (B) are summarized in Table II.

TABLE II

The values for the slopes (A), the Y-intercepts (B), and the correlation coefficients (r) of Eqn. 9, calculated from Table I

Local anesthetics	A [a]	B [b]	r
Dibucaine	$1.39\cdot10^{-5}$	$7.37\cdot10^{-6}$	−0.999
Tetracaine	$1.16\cdot10^{-4}$	$7.51\cdot10^{-6}$	−0.985
Lidocaine	$3.01\cdot10^{-3}$	$2.71\cdot10^{-5}$	−0.997
Procaine	$1.06\cdot10^{-2}$	$1.23\cdot10^{-4}$	−0.999

[a] $K^{-1}\cdot$(M of anesthetics)$\cdot$(M of NaCl)$^{-1}$.
[b] $K^{-1}\cdot$(M of anesthetics).

TABLE III

Apparent partition coefficients of cationic local anesthetics to solid-gel and liquid-crystalline states of DMPA at pH 5.9 in 100 mM NaCl, and the differences in the partition coefficients

Values in parenthesis are the intrinsic partition coefficients, assuming that ε is the permittivity of water, and that the areas per lipid molecule are 67 and 44 Å^2 for liquid-crystalline and solid-gel membranes, respectively.

Anesthetics	P_A^l	P_A^g	$P_A^l - P_A^g$
Dibucaine	$2.1 \cdot 10^6$	$8.1 \cdot 10^3$	$1.3 \cdot 10^6$
	$(1.4 \cdot 10^4)$	$(2.3 \cdot 10^3)$	
Tetracaine	$2.6 \cdot 10^5$	$1.0 \cdot 10^5$	$1.6 \cdot 10^5$
	$(1.7 \cdot 10^3)$	$(2.9 \cdot 10^2)$	
Lidocaine	$2.8 \cdot 10^4$	$2.2 \cdot 10^4$	$6.1 \cdot 10^3$
	$(1.9 \cdot 10^2)$	$(6.2 \cdot 10^1)$	
Procaine	$3.3 \cdot 10^4$	$3.1 \cdot 10^4$	$1.7 \cdot 10^3$
	$(2.2 \cdot 10^2)$	$(8.8 \cdot 10^1)$	

water. Intrinsic partition coefficients are shown in Table III in parenthesis.

Discussion

The numerical values of partition coefficients vary according to the unit for solute concentrations [5]. To compare with literature values, the present partition coefficients (mole fraction unit) were converted to molality unit and were listed in Table IV. The intrinsic partition coefficients of the charged forms of tetracaine and procaine agree with the reports on egg phosphatidylcholine (PC) [17] and egg phosphatidylethanolamine (PE) [18] vesicles membranes. The partition coefficients of uncharged local anesthetics (tetracaine, lidocaine, and procaine) to dipalmitoylphosphatidylcholine (DPPC) membranes [9] are 17-(lidocaine, liquid-crystalline) to 140-(tetracaine, solid-gel) times larger than the partition coefficients of the charged forms to DMPA membranes. The partition coefficients of uncharged forms of lidocaine and procaine to organic solvents were 21–126-times larger than charged forms [19]. Thus, the difference in the partition coefficient between charged and uncharged forms to model membranes agrees with that to organic solvents.

TABLE IV

Reported values of partition coefficients of local anesthetics to model membranes

Phospholipid	Dibucaine	Tetracaine	Lidocaine	Procaine
DMPA [a]				
(gel, pH 5.9)	67	8.5	1.8	2.6
(liquid, pH 5.9)	410	50	5.5	6.4
Egg PC [b]				
(pH 5.5)		22	.	2
Egg PE [c]				
(pH 5.5)		46		3.3
DPPC [d]				
(gel, uncharged)		1 200	57	120
(liquid, uncharged)		1 800	91	200

[a] The values were converted from the intrinsic partition coefficient in Table III with the conversion factor of 34.2.
[b] From the Ref. 17.
[c] From the Ref. 18.
[d] The values were converted from Ref. 9 with the conversion factor of 40.6.

Kelusky et al. [20] reported that the electrostatic effect contributed to the partition of the charged local anesthetics to the negatively charged membrane, phosphatidylserine (PS). They mixed the PC and PS to control the surface charge density of the vesicle membranes. In the present study, the surface electrostatic effect was controlled by changing the NaCl concentrations. The partition of the cationic anesthetics to the anionic phospholipid membranes in 100 mM NaCl is estimated to be 350-(solid-gel) or 150- (liquid-crystalline) times higher than to the neutral membranes. We [13] have shown that the condensation in the electric double layer holds in the present NaCl concentration range. Subsequent partition into the membrane occurred by hydrophobic forces.

Ohki [21] estimated that the charge density of squid axon membranes was about 1 unit charge per 180 Å^2. The charge density of the DMPA membranes (1 unit charge about 67 and 44 Å^2) is about 2.5–4-times higher than the nerve membrane. From Eqns. 7 and 10, the electrostatic effect of the nerve membrane on the anesthetic concentration near the membrane surface changes by the quandratic function of the surface charge density. In 100 mM NaCl, the membrane surface concentration of anesthetics in biological systems is estimated to be about 20-times that in the bulk water at 100 mM NaCl.

The order of the nerve blocking potency of local anesthetics is dibucaine > tetracaine > lidocaine > procaine. The partition coefficients (P_A^l) were dibucaine > tetracaine > procaine > lidocaine. The order of lidocaine and procaine was reversed. The order of differences in the apparent partition coefficients between solid-gel and liquid-crystalline membranes ($\Delta P_A = P_A^l - P_A^g$), however, agrees with the clinical potency. Fig. 4 shows an excellent correlation between minimum blocking concentration (MBC) of local anesthetics [22] and ΔP_A.

Several reports [23–28] indicate that at least two states of the nerve membranes exist for the nerve excitation. It has been shown that the nerve excitation (depolarization) is an exothermic process, and the repolarization is an endothermic process [23–26]. Further, the nerve excitation was reported to be accompanied by a decrease in entropy [27]. The membrane potential of the squid giant axon showed the polarized potential at high temperature, and the depolarized potential at low temperature [28]. These results indicate that the resting state (polarized potential) is stable at high temperature,

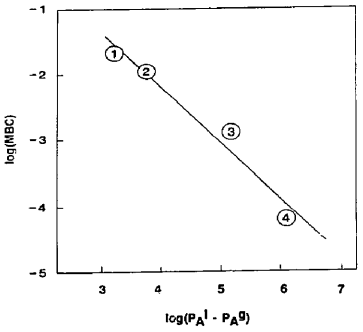

Fig. 4. The correlation between minimum blocking concentrations (MBC) and differences in the apparent partition coefficients between the solid-gel and liquid-crystal membranes ($\Delta P_A = P_A^l - P_A^g$). The data are fitted to $\log(\text{MBC}) = -0.848 \cdot \log(\Delta P_A) + 1.203$ ($r = -0.979$) with the least-squares method. 1, procaine; 2, lidocaine; 3, tetracaine; 4, dibucaine.

and the excited state (depolarized potential) is stable at low temperature.

With phospholipid membranes, the liquid-crystalline state is stable at high temperature, and the process from the liquid-crystalline state to the solid-gel state is exothermic. Thus, the liquid-crystalline state corresponds to the resting state of the nerve membranes, and the solid-gel state corresponds to the excited state. The nerve excitation is not identical with the phase transition of the phospholipid model membranes, but the transition of the phospholipid membranes may serve as a model for the nerve excitation. Recent developments in the single channel measurement confirm the existence of open and closed states in sodium channels. The phase transition of membrane lipid relates to the transition of channel states [29].

Hille [30] proposed three states (R, I, and O states) in the nerve excitation. Local anesthetics increase the ratio of the I state. The resting and excited states are similar to the R and I states of Hille's hypothesis. Anesthetics increase the ratio of resting state membranes. This is equivalent to say that the anesthetics prefer to bind to the resting state, or that the affinity of anesthetics to the resting state is higher than to the excited state.

We speculate that the resting and excited states relate to the nerve excitation, and correlate to liquid-crystalline and solid-gel states of phospholipid model membranes. The difference in the affinity of anesthetics to the resting and excited states causes the local anesthetic action. In a model membrane system, the difference in the partition coefficients to solid-gel and liquid-crystalline states decreases the transition temperature, and correlates to the nerve blocking potency (Fig. 4).

Acknowledgments

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