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Alcohol interaction with high entropy states of macromolecules: critical temperature hypothesis for anesthesia cutoff

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Nerve excitation generates heat and decreases the entropy (review by Ritchie and Keynes (1985) Q. Rev. Biophys. 18, 451–476). The data suggest the existence of at least two thermodynamically identifiable states: resting and excited, with a thermotropic transition between the two. We envision that nerve excitation is a transition between the two states of the excitation machinery consisting of proteins and lipids, rather than the sodium channel protein alone. Presumably, both proteins and lipids change their conformation at excitation. We proposed (Kaminoh et al. (1991) Ann. N.Y. Acad. Sci. 625, 315–317) that anesthesia occurs when compounds have a higher affinity to the resting state than to the excited state of excitable membranes, and that there is a critical temperature above which the affinity to the excited state becomes greater than to the resting state. When the temperature exceeds this critical level, compounds lose their anesthetic potency. We used thermotropic phase-transition of macromolecules as a model for the excitation process. Anesthetic alcohols decreased the main transition temperature of dipalmitoylphosphatidylcholine (DPPC) membranes and also the temperature of the α-helix to β-sheet transition of poly(L-lysine). The affinity of alcohols to the high- and low-temperature states of the DPPC membranes were separately estimated. The difference in the affinity of *n*-alcohols to the liquid (high-temperature) and solid (low-temperature) states correlated with their anesthetic potency. It is not the total number of bound anesthetic molecules that determines the anesthesia, rather, the difference in the affinity between the higher and lower entropy states determines the effects. The critical temperatures of the long-chain alcohols were found to be lower than those of the short-chain alcohols. Cutoff occurs when the critical temperature of long-chain alcohols is below the physiological temperature, such that the anesthetic potency is not manifested in the experimental temperature range.

Introduction

In a homologous series of alcohols, elongation of the carbon chain is accompanied by an increase in anesthetic potency in parallel with an increase in the oil/water partition coefficients. The anesthetic potency, however, disappears when the carbon chain exceeds a certain length, despite increases in the oil solubility: a phenomenon known as cutoff. The cutoff length for *n*-alcohols is 12 to 13 carbon atoms [1–3], and 8 to 10 for *n*-alkanes [4].

Short-chain alcohols (with anesthetic potency) decrease the temperature in the main transition of phospholipid membranes between the solid (rippled, $P_{\beta'}$) and liquid (L_α) states. According to the elongation of the alcohol chain, the potency of alcohols to decrease the transition temperature increases, but when the carbon chain exceeds a certain length, they start to elevate the transition temperature. The switch from depression to elevation coincides with the cutoff length [5–8]. Thus, a correlation of anesthetic potency with the phase transition of lipid membranes [9–11] is to be expected.

Miller and co-workers [2,12] proposed that the perturbation of the membrane structure is essential for pharmacological efficacy, and that the cutoff is caused by the loss of the perturbation effects. Franks and Lieb [13], on the other hand, proposed that anesthetic alcohols bind to specific receptor sites on proteins, where the receptor site has a size limit for accepting the ligand. They [13] contended that cutoff occurs because the non-anesthetic long-chain alcohols are too large to be accommodated into the putative receptor. Chiou et al. [14] reported that the hydrogen bond-breaking activ-

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ity of *n*-alcohols dehydrating the membrane surface increases with the elongation of the carbon chain, but disappears at the cutoff chain length.

The switch from depression to elevation of the phase transition temperature in lipid vesicle membranes indicates that anesthetic alcohols interact preferentially with the liquid phase, whereas non-anesthetic alcohols interact with the solid phase. This does not mean that the change in the main-transition temperature solely determines the anesthetic potency. Lipid membrane polymorphism is well known, and DPPC (dipalmitoylphosphatidylcholine) membrane transforms from solid (L_β) to tilted ($L_{\beta'}$), rippled ($P_{\beta'}$), and liquid (L_α) as the temperature is increased. What is important is the recognition of highly cooperative two systems in equilibrium: the higher temperature structure is more random (higher entropy) than the lower. Anesthetics stabilize the higher entropy structure. This is not limited to lipid membranes. Protein secondary structures also undergo thermal phase transition between α-helix and β-sheet and anesthetics favor the β-sheet structure. The difference between the two phases in the anesthetic affinities determines the anesthetic potency [16]. We [15,16] derived a method to calculate the affinities of anesthetics to solid and liquid membranes separately from their effect on the transition temperature.

This study used the main transition of DPPC membranes as a model for the equilibrium of two states to analyze the mechanisms of cutoff. The effect of 1-butanol on the thermotropic phase-transition between α-helix and β-sheet in a homopolymer peptide poly(L-lysine) is included to show that the alcohol effects on the macromolecular structure are not limited to lipid bilayers.

Materials and Methods

1. Chemicals

Synthetic DPPC, poly(L-lysine · HCl) (nominal molecular weight: 17500, determined by low-angle laser light scattering method), and *n*-alcohols (1-butanol, 1-hexanol) were obtained from Sigma; 1-octanol from Fluka; and 1-decanol, 1-dodecanol, 1-tridecanol, and 1-tetradecanol from Kodak. Water was treated by a water purifier (Sybron/Barnstead, Boston, MA) consisting of two mixed-bed ion-exchanger columns, an activated carbon column, and an ultrafilter.

2. Vesicle preparation

DPPC was suspended in water by a Vortex mixer until homogeneous suspension was obtained. The multi-lamellar suspension was sonicated in the cuphorn of a Branson Sonifier Model 185 (Danbury, CT) at a temperature above the transition for 20 min. The unilamellar vesicle suspension was aged for a week at 4°C to obtain a relatively homogeneous size distribution [17–19]. Alcohols were added to the multi-lamellar vesicle suspension before ultrasonic irradiation and the added amount was verified by weight with an ultramicrobalance (Perkin-Elmer Autobalance AD-2D, Norwalk, CT) [7].

3. Preparation of poly(L-lysine)

The random-coil solution of poly(L-lysine) was prepared by dissolving it into 100 mM NaCl solution. The stock solution of α-helix was prepared by adjusting the pH of the random-coil solution to 11.6 by adding 0.1 M NaOH, and stored at 4°C. 1-Butanol was added to the stock solution by a microsyringe. The added amount was verified by weight with the ultramicrobalance. The final concentration of poly(L-lysine) was 0.42 wt% (26 mM residue) at pH 11.6.

4. Transition temperature measurement

The transition temperature of the DPPC liposome was determined by a light absorbance method [8,9,16,20–22]. The advantage and disadvantage of the optical method in comparison with differential scanning calorimetry, fluorescence, and electron-spin resonance were discussed in our recent article [8]. A Perkin-Elmer Model 554 UV-visible spectrophotometer (Norwalk, CT) was used to measure the light absorbance of the vesicle suspension at 400 nm. The cuvette temperature was scanned at 0.5 C°/min by a Perkin-Elmer programmable digital temperature controller and a Peltier heat exchanger. The sample temperature was measured with a thermistor probe inserted into the cuvette slightly above the light-path and monitored by a Cole-Palmar thermometer Model 8502–16 (Chicago, IL) with 0.01 C° resolution. The sample solution was continuously mixed with a direct drive stirrer (Spectro-Stir, Oreland, PA) during the temperature scan. The light absorbance and the temperature were recorded by a Nicolet digital oscilloscope Model 310 (Madison, WI) interfaced with an IBM PC. Typical traces of phase transition are shown in Fig. 1. The average of the midpoints in the heating and cooling cycles was chosen as the phase transition temperature.

The thermotropic α-to-β transition of poly(L-lysine) was measured by a MicroCal MC-2 differential scanning calorimeter (Northampton, MA) interfaced with an IBM-AT computer. To prevent the bubble formation in the DSC cell, the sample was degassed under vacuum with stirring for 5 min before injection into the scanning cell. During the preparation and degassing of the sample solution, the temperature was kept at 4°C to avoid the transition from α-helix to β-sheet. The pH was determined by a Radiometer Ion 85 Ion Analyzer (Denmark) and a combination pH electrode (Orion, Cambridge, MA). The DSC cell was pressurized by nitrogen gas at 15 psi. The heating rate was 10 C°/h

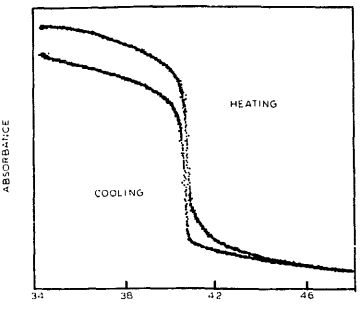

Fig. 1. The main phase transition of dipalmitoylphosphatidylcholine (DPPC) vesicle membranes monitored by optical absorbance. The concentration of DPPC was 1 mM. Ordinate is the light absorbance in an arbitrary unit, and abscissa is the temperature.

between 5°C and 65°C. The DSC spectra were analyzed by MicroCal ORIGIN software.

Results

1. Effects of n-alcohols on the main transition of DPPC membranes

Fig. 2 shows the effect of *n*-alcohols on the main transition temperature of DPPC membranes. 1-Butanol, 1-hexanol and 1-octanol depressed the transition temperature within the experimental concentration range. The effect of 1-decanol was biphasic; depression of the transition temperature at low concentrations, and elevation at high concentrations. 1-Dodecanol did not change the phase transition temperature of DPPC membranes. 1-Tridecanol and 1-tetradecanol elevated the transition temperature. These results were in agreement with our previous reports [7,8].

2. Effect of lipid concentrations

The lipid concentration affected the potency of *n*-alcohols on the transition temperature. The change in the transition temperature ($dT_t/d[A]_T$), with infinite dilution of alcohols at various lipid concentrations, is listed in Table I. The alcohol effects on the transition temperature were stronger when the lipid concentration was lower.

3. Differential scanning calorimetry of poly(L-lysine)

Fig. 3 shows typical differential scanning thermograms of the α-to-β transition of poly(L-lysine) in 100 mM NaCl at pH 11.6 in the presence and absence of 1-butanol. The transition temperature and the excess enthalpy of the α-to-β transition without 1-butanol were 39.8°C and 3.60 kJ per mole residue, respectively. 1-Butanol showed biphasic effects on the transition temperature, T_m. When the 1-butanol concentration was less than 100 mM, the transition temperature decreased. At 100 mM, the transition temperature started to increase (Fig. 4). Despite the biphasic effect on the transition temperature, the excess enthalpy of the α-to-β transition remained constant.

Data analysis and Discussion

1. Membrane / water partition coefficients of n-alcohols

Anesthetics (or any other additives) shift the main transition temperature according to the difference in the partition coefficients of the anesthetics to the liquid (P_A^l) and solid (P_A^g) states of the membrane [15,16]. The partition coefficients of anesthetics to each state can be separately estimated from their effects on the transition temperature (T_t) according to the following equation [15]:

$$\left(\frac{dT_t}{d[A]_T}\right)_{[A]_T\ =\ 0} = -\frac{RT_{to}^2}{\Delta H_o}\cdot\frac{P_A^l - P_A^g}{55.5 + [L]_T\cdot\dfrac{P_A^l + P_A^g}{2}} \qquad (1)$$

where T_{to} and ΔH_o are the transition temperature and

TABLE I

Effects of lipid concentrations on the main transition temperature change

The unit is C°/M.

Alcohol C atoms (n)	Lipid concentration (mM)				
	0.2	0.5	1.0	2.0	4.0
4	–	$-3.12\cdot10^{1}$	$-3.27\cdot10^{1}$	$-3.17\cdot10^{1}$	$-2.95\cdot10^{1}$
6	$-6.89\cdot10^{2}$	–	$-6.02\cdot10^{2}$	$-6.03\cdot10^{2}$	$-6.03\cdot10^{2}$
8	$-7.72\cdot10^{2}$	–	$-5.80\cdot10^{3}$	$-5.38\cdot10^{3}$	$-2.91\cdot10^{3}$
10	–	$-2.00\cdot10^{4}$	$-1.28\cdot10^{4}$	$-7.66\cdot10^{3}$	$-3.85\cdot10^{3}$
13	–	$8.60\cdot10^{3}$	$4.32\cdot10^{3}$	$2.78\cdot10^{3}$	$1.60\cdot10^{3}$
14	–	$2.25\cdot10^{4}$	$2.03\cdot10^{4}$	$1.17\cdot10^{4}$	$5.26\cdot10^{3}$

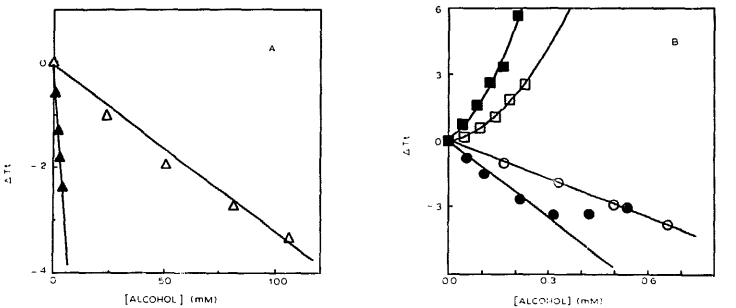

Fig. 2. Effect of *n*-alcohols on the transition temperature of 1 mM DPPC vesicle membranes. (A) △, 1-Butanol and ▲, 1-hexanol. (B) ○, 1-Octanol; ●, 1-decanol; □, 1-tridecanol, and ■, 1-tetradecanol. The ordinate is the transition temperature change, and the abscissa is the alcohol concentration.

the transition enthalpy of the lipid membranes without any additives, and R is the gas constant. The value of $\Delta H_0/RT_{t0}^2$ is 0.0437 [15]. In a reciprocal form, it becomes

$$\frac{1}{\left(\frac{dT_t}{d[A]_T}\right)_{[A]_T \to 0}} = A \cdot [L]_T + B \qquad (2)$$

$$A = -\frac{\Delta H_0}{2RT_{t0}^2} \cdot \frac{P_A^l + P_A^g}{P_A^l - P_A^g}$$

$$B = -\frac{\Delta H_0}{RT_{t0}^2} \cdot \frac{55.5}{P_A^l - P_A^g}$$

Eqn. 2 shows that the partition coefficients of anesthetics to each state (P_A^l and P_A^g) can be obtained from the slope (A) and the Y-intercept (B) of the plot between the reciprocal of $dT_t/d[A]_T$ values and the

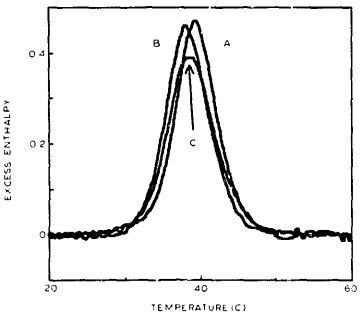

Fig. 3. Differential scanning calorimetry thermograms of the phase transition of poly(L-lysine) between α-helix and β-sheet conformations at 100 mM NaCl (pH 11.6), and effects of 1-butanol. Abscissa is the temperature and ordinate is the excess heat capacity (kJ (residue mol)$^{-1}$ deg^{-1}). The 1-butanol concentrations are: (A) 0, (B) 0.0795 M, and (C) 0.16 M.

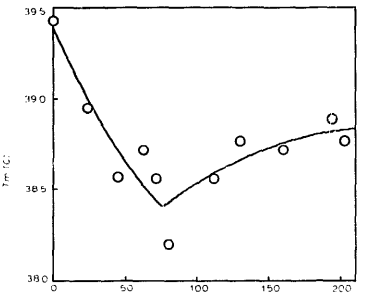

Fig. 4. Effects of 1-butanol on the α-to-β transition temperature of poly(L-lysine). Ordinate is the transition temperature, and abscissa is the 1-butanol concentration.

lipid concentrations ($[L]_T$). The partition coefficients are obtained from A and B as follows:

$$P_A^l + P_A^g = 111 \cdot A / B$$

$$P_A^l - P_A^g = 55.5 \cdot \frac{\Delta H_0}{RT_{tc}^2} \cdot \frac{1}{B} \quad (3)$$

1.1. Partition coefficients of alcohols with intermediate chain lengths

Eqn. 1 shows that the estimated values of P_A^l and P_A^g are most accurate when the value of $([L]_T \cdot [P_A^l + P_A^g]/2)$ is not very far from 55.5. Alcohols with intermediate chain lengths (1-hexanol, 1-octanol, and 1-decanol) satisfy this condition. Fig. 5 shows that the plot between the reciprocal of $dT_t/d[A]_T$ values and the lipid concentrations ($[L]_T$) is linear. From the slope and the Y-intercept, the partition coefficients (P_A^l and P_A^g), the difference ($\Delta P_A = P_A^l - P_A^g$), and the ratio ($K = P_A^g/P_A^l$) are estimated and are shown in Table II.

The partition coefficients increased by increasing the carbon-chain length. Fig. 6 is the plot of the transfer free energy ($\Delta G_{(n)}^i = -RT\ln(P_A^i)$, $i = l$ or g) of n-alcohols compared to the carbon-chain length. The free energy change of the partition of alcohols to the lipid membranes increased linearly. From the slope, the incremental transfer free energy per methylene moiety ($\Delta\Delta G_{methyl}$) is obtained by fitting the data to the following equation:

$$\Delta G_{(n)}^i = \Delta\Delta G_{methyl} \cdot n + (\text{constant}) \quad (4)$$

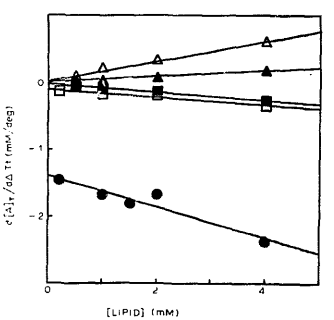

Fig. 5. Effects of the DPPC concentration on the transition temperature of vesicle membranes. Abscissa is the DPPC concentration in mM. Ordinate is the reciprocal of the $(d\Delta T_t/d[A]_T)$. Lines were drawn by the least-square linear regression. Symbols are; ●, 1-hexanol; □, 1-octanol; ■, 1-decanol; ▲, 1-tridecanol, and △, 1-tetradecanol.

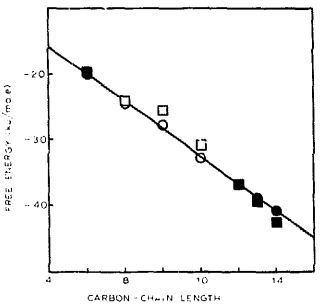

Fig. 6. Effects of the carbon-chain length of n-alcohols on the transfer free energy. Ordinate is the transfer free energy per mole of alcohols, and abscissa is the carbon-chain length of alcohols. A line is drawn for the transfer of n-alcohols to the liquid state by the least-squares regression method. Symbols are: ○, transfer to the liquid membranes, and □, to the solid membranes. Closed symbols are the estimated values from Eqn. 4.

where n is the carbon-chain length of alcohols. The value of $\Delta\Delta G_{methyl}$ was -2.08 kJ mol^{-1} methyl^{-1} for the liquid state, L_α ($r^2 = 0.986$).

Diamond and Katz [23] reported that the values of $\Delta\Delta G_{methyl}$ of alcohols (between methanol and 1-butanol) to DMPC (dimyristoylphosphatidylcholine) membranes were -1.88 (C1-C2), -2.72 (C2-C3) and -2.18 kJ mol^{-1} methyl^{-1} (C3-C4). Franks and Lieb [24] reported that the $\Delta\Delta G_{methyl}$ of the cholesterol containing vesicle was -3.4 kJ mol^{-1} methyl^{-1}. Ahmed et al. [25] measured the transfer free energies of phenothiazine derivatives at the temperature below and above the transition of DMPC membranes, and reported that $\Delta\Delta G_{methyl}$ values were -1.62 at 30°C and -1.06 kJ mol^{-1} methyl^{-1} at 15°C. Considering the difference in the membrane phospholipids they used, the scatter of these values may be in the acceptable range.

The present analysis is limited to the initial part of the biphasic response of the main-transition temperature. A detailed discussion about the biphasic effect of long-chain alcohols can be found in our recent articles [8,26].

1.2. Partition coefficient of short-chain alcohols

For alcohols of shorter chain length than 1-butanol, the partition coefficient to DPPC is expected to be small. When the partition coefficients are small, $([L]_T \cdot [P_A^l + P_A^g]/2)$ becomes much smaller than 55.5, and Eqn. 1 is approximated as

$$\left(\frac{dT_t}{d[A]_T}\right)_{[A]_T \to 0} = -\frac{RT_{tc}^2}{\Delta H_0} \cdot \frac{P_A^l - P_A^g}{55.5} \quad (5)$$

TABLE II

The partition coefficients of n-alcohols to the liquid and solid states of DPPC

P_A^l was estimated from Eqn. 4, and P_A^s from ΔP_A (for butanol) or K (for tridecanol and tetradecanol). The numerical values are expressed by the ratio of mole fractions. To convert to the molarity scale, divide by 42.4 [11].

Alcohol C atoms (n)	P_A^l	P_A^s	ΔP_A	K
4	2060	1980	80	0.963
6	11200	9380	1810	0.838
8	39700	17200	22500	0.433
10	268000	125000	143000	0.466
12	1180000	1180080	0	1.000
13	2600000	3410000	−810000	1.310
14	5760000	11600000	−5840000	2.012

Here, the alcohol effect on the transition temperature becomes independent of the lipid concentration (see Table I, 1-butanol). Eqn. 5 indicates that only the difference in the partition coefficients can be estimated from the transition temperature depression, and the values for P_A^l and P_A^s cannot be obtained from the plotting. Hence, to estimate these values, we assume that $\Delta\Delta G_{methyl}$ between 1-butanol and 1-hexanol is similar to that between 1-hexanol and 1-decanol. Then, the partition coefficient of 1-butanol to the liquid state (L_α) of DPPC membranes was estimated to be 2060 according to Eqn. 4. From this value and ΔP_A, the values of P_A^s and K were calculated to be 1980 and 0.963, respectively (Table II).

1.3. Partition coefficient of long-chain alcohols

It has often been contended that long-chain alcohols do not interact with membranes [3,27]. This non-interaction is presumed to cause the loss of anesthetic potency of larger molecules. However, the overall partition coefficients of long-chain alcohols to the phospholipid model membrane continues to increase beyond the cutoff length [24].

To calculate the partition coefficients of long-chain alcohols to the liquid and solid states, we first estimated the partition coefficient to the liquid state from Eqn. 4. Because the partition coefficients of these alcohols are very large, Eqn. 5 is approximated as

$$\left(\frac{dT_t}{d[A]_T}\right)_{[A]_T \to 0} = -\frac{RT_{to}^2}{\Delta H_o} \cdot \frac{1-K}{[L]_T} \qquad (6)$$

Similar to the short-chain alcohols, only the ratio ($K = P_A^s/P_A^l$) of the partition coefficients between liquid and solid membranes can be obtained from the transition temperature change (Table II). The values of K for the long-chain alcohols are larger than 1. This indicates that the affinity of these alcohols to the solid membranes is higher than to the liquid membranes.

From Eqn. 4, the partition coefficients of 1-tridecanol and 1-tetradecanol to the liquid state (L_α) of DPPC membranes were estimated to be 2600000 and 5760000, respectively. From P_A^l and K, the values of P_A^s and ΔP_A are calculated and shown in Table II. Because of the higher affinity to the solid membrane ($P_A^l < P_A^s$, Table II), the long-chain alcohols increase the transition temperature of DPPC membranes.

2. Anesthetic potency and transition temperature change

The anesthetic potency is generally described by the bulk concentration of anesthetic in water (mM) or in the gas phase (partial pressure). However, these values vary according to the anesthetic distribution within the gas phase, the aqueous phase, and the target site. The potency expressed by the bulk concentration represents

TABLE III

The potency of n-alcohols for anesthesia and transition temperature

The EC_{50} values are from Alifimoff et al. [2]. The unit of concentration C is molarity (M).

Alcohol C atoms (n)	Anesthesia (tadpole)			Transition temperature		
	EC_{50}	C_{anesth}^{membr}	α_{anesth}	C_{trans}^{water}	C_{trans}^{membr}	α_{trans}
4	$1.08 \cdot 10^{-2}$	$5.33 \cdot 10^{-1}$	0.136	$3.19 \cdot 10^{-2}$	$1.55 \cdot 10^{0}$	0.062
5	$2.90 \cdot 10^{-3}$	$3.22 \cdot 10^{-1}$	0.230	–	–	–
6	$5.70 \cdot 10^{-4}$	$1.35 \cdot 10^{-1}$	0.535	$1.33 \cdot 10^{-3}$	$3.16 \cdot 10^{-1}$	0.305
7	$2.30 \cdot 10^{-4}$	$1.21 \cdot 10^{-1}$	0.593	–	–	–
8	$5.70 \cdot 10^{-5}$	$6.62 \cdot 10^{-2}$	1.081	$1.08 \cdot 10^{-4}$	$1.25 \cdot 10^{-1}$	0.770
9	$3.70 \cdot 10^{-5}$	$9.50 \cdot 10^{-2}$	0.753	–	–	–
10	$1.26 \cdot 10^{-5}$	$7.15 \cdot 10^{-2}$	1.000	$1.70 \cdot 10^{-5}$	$9.63 \cdot 10^{-2}$	1.000
11	$8.10 \cdot 10^{-6}$	$1.02 \cdot 10^{-1}$	0.703	–	–	–
12	$4.70 \cdot 10^{-6}$	$1.30 \cdot 10^{-1}$	0.548	0.00	0.00	0.000
13	no anesthesia			$-3.00 \cdot 10^{-6}$	$-1.85 \cdot 10^{-1}$	−0.522
14	no anesthesia			$-4.16 \cdot 10^{-7}$	$-9.88 \cdot 10^{-3}$	−1.704

an apparent potency. The intrinsic potency must be expressed by the concentration at the target site.

2.1. Apparent potency

We [16] showed that the difference in the affinity of local anesthetics correlates well with their apparent anesthetic potencies. When the lipid concentration is very small, the potency of alcohol in water (C_{trans}^{water}) to decrease the transition temperature by 1 C° is

$$C_{trans}^{water} = \frac{55.5 \cdot \Delta H_0}{RT_{to}^2} \cdot \frac{1}{P_A^l - P_A^s} \quad (7)$$

The reciprocal of this concentration is the apparent potency to change the transition temperature. C_{trans}^{water} was calculated and shown in Table III.

Alifimoff et al. [2] reported the EC_{50} of *n*-alcohols in the inhibition of the righting reflex of tadpoles. Fig. 7 shows the excellent linear relationship between $\ln(C_{trans}^{water})$ and $\ln(EC_{50})$ with a slope of 1.11 ($r^2 = 0.999$). The difference in the partition coefficients between the liquid and solid states is proportional to C_{trans}^{water} (Eqn. 7). The apparent anesthetic potency of *n*-alcohols correlates well with the difference in the partition coefficient between the liquid and solid states.

2.2. Intrinsic potency

The intrinsic potencies of alcohols to induce anesthesia in tadpoles [2] and to decrease the transition temperature of lipid membranes are compared by estimating their concentrations in the liquid state of the membranes. We assume that the liquid state is equivalent to the resting state. The membrane concentrations of alcohols required for anesthesia (C_{anesth}^{membr}) are estimated by multiplying the EC_{50} by the partition coefficients (P_A^l) of DPPC membranes as shown in Table III. The reciprocal of C_{anesth}^{membr} represents the intrinsic anesthetic potency.

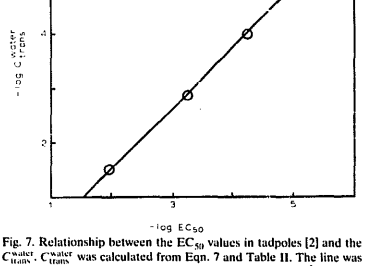

Fig. 7. Relationship between the EC_{50} values in tadpoles [2] and the C_{trans}^{water}. C_{trans}^{water} was calculated from Eqn. 7 and Table II. The line was drawn by the least-squares method. The slope was 1.11 ($r^2 = 0.999$). The difference in the partition coefficients ($\Delta P_A = P_A^l - P_A^s$) is proportional to C_{trans}^{water} (Eqn. 7).

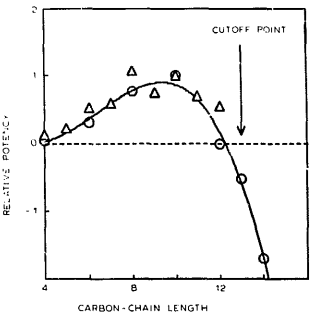

Fig. 8. Effects of the carbon-chain length on the relative potencies of *n*-alcohols in reference to 1-decanol. Ordinates are the relative potencies for anesthesia (△), and temperature change (○). The zero and negative values are for the transition temperature only. Negative values (below the horizontal broken line) indicate elevation of the transition temperature and no anesthetic potency.

The alcohol concentration in the liquid DPPC membranes required to depress the transition temperature 1 C° (C_{trans}^{membr}) is

$$C_{trans}^{membr} = \frac{55.5 \cdot \Delta H_0}{RT_{to}^2} \cdot \frac{1}{1 - K} \quad (8)$$

The reciprocal of C_{trans}^{membr} in Table III is the intrinsic potency of alcohols to change the transition temperature of model membranes. To compare the intrinsic anesthetic potencies with the potencies of the depression of the transition temperature, the relative potencies (α_{anest} and α_{trans}) were calculated in reference to 1-decanol, because this alcohol showed the maximal effects. Fig. 8 is the relation between the relative potencies and the carbon-chain length. When the carbon-chain length increases, both relative potencies increase up to 1-octanol, then decrease after 1-decanol. The anesthetic potency disappeared with alcohols longer than 1-dodecanol. Simultaneously, the potency to change the transition temperature was negated, and the transition temperature was elevated by these alcohols. The correlation between these two potencies was excellent (Fig. 8).

3. Mechanism of anesthesia and cutoff phenomena

3.1. Two-state model of nerve excitation and anesthetic action

Nerve excitation generates heat [28,29]. The heat-flow profile can be superimposed on the action potential on a millisecond scale. It suggests that at least two thermodynamically identifiable states exist for nerve excitation [28,29]. We envision that nerve excitation involves transition of the integral excitation machinery consisting of the sodium channel protein and the lipid matrix between the two states. After all, pore structures cannot be formed unless channel proteins are incorporated into the membrane architecture. Presumably, both proteins and lipids change their conformations at excitation.

Because excitation (depolarization) is exothermic and recovery to the resting state (polarization) is endothermic, high temperature supports the resting potential level and low temperature supports the action potential level. This is not a hypothetical model. In a squid giant axon, conditioned to sustain metastable action potential [30], Spyropoulos demonstrated that application of a heat pulse during action potential suppressed the depolarization [30]. Inoue et al. [31] unambiguously demonstrated that high temperature supports the resting potential and low temperature supports the action potential in a squid giant axon. By scanning the temperature between 2°C and 20°C, they demonstrated that cooling induced an abrupt depolarization at 5°C. The membrane stayed at the depolarized state when heated, until about 16°C where the membrane abruptly repolarized. This sequence could be repeated with hysteresis. The result suggests that nerve excitation is a transition between two states: polarized and depolarized. The transition resembles the main phase transition of lipid membranes between liquid and solid, or the transition of proteins between β-sheet and α-helix conformations. Both liquid-to-solid transition in lipids and β-to-α transition in proteins are exothermic and the reverse is endothermic.

As an example for proteins, the α-to-β transition thermograms of a polypeptide are shown in Fig. 3. Poly(L-lysine) was chosen because this homopolymer peptide can be formed into well-defined α-helix. The endothermic thermograms were obtained by differential scanning calorimetry. It shows that 1-butanol decreased the transition temperature. In this analogy, the liquid state of lipid model membranes and the β-sheet conformation of proteins correspond to the resting state, whereas the solid state and the α-helix conformation correspond to the excited state [16,32].

The existence of two states in functional proteins is not limited to the excitation machinery in cell membranes. Most membrane enzymes show a clear break in the Arrhenius plots [33,34]. Two activation enthalpies – with higher values in the low temperature range – again suggest that these enzymes exist in at least two different states. The two states may be ascribed to the conformation of the enzyme protein, the membrane lipids where the enzyme is embedded, or a combination of both.

Anesthesia occurs when a compound has a higher affinity to the resting state than to the excited state, and the anesthetic binding stabilizes the resting state. The higher affinity of anesthetics to membranes in the high temperature range (resting state) may be a reason for the close correlation between the anesthetic potency and the depression of the transition temperature in lipid membranes. In the following, the effect of anesthetics on lipid membranes is used as a functional model to analyze the thermotropic phase-transition of the excitation machinery.

3.2. Critical temperature hypothesis of anesthesia

We use local anesthetic data to show the discrepancy between the membrane binding of anesthetics and their effects. Although the effects of local and general anesthetics are not identical, the temperature dependency of their effects is comparable. The nerve blocking potency of local anesthetics increases at low temperature despite the decrease in anesthetic binding (negative temperature dependence) [35–37]. The interaction of local anesthetics with lipid membranes is endothermic, and the enthalpy change for the interaction with the solid state is larger than with the liquid state [32]. The van't Hoff plot of local anesthetic partition to model phospholipid membranes shows a break [32]. When the temperature decreases, the affinity to the solid state decreases more than to the liquid state. The difference in the partition coefficients between the two states increases with the decrease in the temperature. The cause of the increase in the nerve blocking potency at low temperature is the increased difference between the affinities of the resting and excited states [32]. The total number of anesthetics bound to the action site has no meaning; the difference in the anesthetic affinities to the high entropy structure and the low entropy structure determines the anesthetic effects.

The difference in the affinities decreases at high temperature, and becomes zero. Above this temperature, the affinity to the solid state becomes greater than to the liquid state. Under this condition, the nerve blocking potency disappears. This temperature is the 'critical temperature of anesthesia' [32].

3.3. Mechanism of cutoff phenomena

The critical temperature of anesthesia differs with each drug. The present results showed that 1-dodecanol (12 carbon atoms) did not change the transition temperature, while 1-tridecanol elevated it. These results

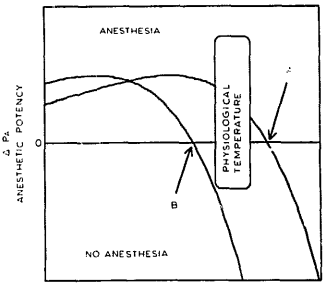

Fig. 9. Schematic representation of the temperature dependence of the anesthetic potency expressed by the difference in the partition coefficients of *n*-alcohols between the liquid and solid membranes. Ordinate is ΔP_A and abscissa is temperature. ΔP_A corresponds to the anesthetic potency (see text). A: T_{eq} of the short- and intermediate-chain alcohols. B: T_{eq} of the long-chain alcohols without anesthetic potency.

indicate that the critical temperatures of 1-dodecanol and 1-tridecanol (13 carbon atoms) in the DPPC model membrane system are just above and below the transition temperature of DPPC (42°C), respectively. Our previous results [7] also show that the transition temperature elevates when the carbon-chain length of *n*-alcohols increases above 1-dodecanol. Thus, the critical temperatures of the short-chain alcohols are expected to be higher than those of the long-chain alcohols.

Fig. 9 explains the relationship between the physiological temperature and the critical temperature. The critical temperatures of the short-chain and intermediate-chain alcohols (A) are higher than the physiological temperature, hence anesthesia ensues. The critical temperature of the long-chain alcohols (B) is lower than the physiological temperature. These alcohols interact preferentially with the excited state. They fail to induce anesthesia.

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