Nature of Alcohol and Anesthetic Action on Cooperative Membrane Equilibria†

Nathan Janes,* Jack W. Hsu, Emanuel Rubin, and Theodore F. Taraschi

Department of Pathology and Cell Biology, Jefferson Medical College, 1020 Locust Street, Philadelphia, Pennsylvania 19107

Received March 12, 1992; Revised Manuscript Received July 1, 1992

ABSTRACT: A generalized, colligative thermodynamic framework is used to treat the action of solutes on cooperative membrane equilibria. Configurational entropy, the randomness imparted by solutes through the partitioning or mixing process, is implicated as the energetic driving force for the action of anesthetics on cooperative membrane equilibria. The equilibria predicted to be most sensitive to solute action—in which the dilute solute causes a perturbation equivalent to a large change in temperature—are (1) low-enthalpy processes that coincide with (2) large partitioning differences between states. The model stresses that solutes do not act at a single site, but on both states in an equilibrium, and that the perturbation is determined by the difference in entropy. Evidence for the thermodynamic framework is obtained from the partitioning behavior of the general anesthetic 1-hexanol into a model lecithin (DMPC; 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) membrane as a function of temperature and alcohol concentration. The low-enthalpy equilibrium between the gel ($L_{\beta'}$) and ripple states ($P_{\beta'}$) (pretransition) is more sensitive to 1-hexanol than the high-enthalpy equilibrium between the ripple ($P_{\beta'}$) and fluid bilayer states (L_α) (main transition). The perturbations of both equilibria are accurately described by the colligative thermodynamic framework. The results suggest that alcohols and anesthetics act through entropy to upset the natural thermal balance that maintains native membrane architecture.

The nonspecific binding of lipophilic solutes to membranes may play a key role in determining many of the acute [reviewed in Janoff and Miller (1982)] and chronic [reviewed in Taraschi and Rubin (1985)] manifestations of exposure to alcohols and anesthetics. Solutes are known to perturb membranes [reviewed in Lee (1983)]. The action of solutes on the high-enthalpy $P_{\beta'} \rightarrow L_\alpha$ phase transition of phospholipids was first treated in thermodynamic terms by Hill (1974, 1975, 1978), who employed a model using the freezing point depression to relate the shift in the equilibrium midpoint of the phase transition to the concentration of solute in the fluid bilayer. This framework provided a thermodynamic basis for those theories of anesthesia that implicated a main lipid-phase transition at the anesthetic locus [reviewed in Janoff and Miller (1982)], the Gibbs free-energy hypothesis (Hill, 1978), and impetus for the proton pump/leak hypothesis [reviewed in Bangham and Hill (1986)]. The freezing point method was widely adopted to provide indirectly-obtained fluid-phase (L_α) membrane–buffer partition coefficients [e.g., see Hill (1975), Kamaya et al. (1981), and Rowe (1982)]. Hill assumed that a solute partitioned only in the fluid state and was excluded from the gel states. Subsequently, others noted that solutes partition substantially into the gel phases [e.g., see Lee (1977), Sklar et al. (1977), Jain and Wray (1978), Pringle and Miller (1979), and Luxnat and Galla (1986)]. Most recently, Ueda and co-workers (Suezaki et al., 1990; Kaminoh et al., 1988) have investigated the effects of "gel"-state partitioning on perturbations of the main phase transition by monitoring the midpoint and width of the transition and assuming that the broadening stems solely from changes in the aqueous solute concentration.

In this work, we explore the utility of a generalized colligative thermodynamic framework based on configurational entropy in solid solution theory. In order to test this model, we investigated the effects of the anesthetic alcohol 1-hexanol on the cooperative conformational equilibria of the 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC)[1] model membrane system. The partitioning of 1-hexanol into the lecithin membrane is expressed as a function of temperature and alcohol concentration. DMPC membranes adopt three well-studied conformations, $L_{\beta'}$, $P_{\beta'}$, and L_α, whose interchange is entropically driven. The $L_{\beta'} \rightarrow P_{\beta'}$ equilibrium (gel to ripple pretransition) is a low-enthalpy event, whereas the $P_{\beta'} \rightarrow L_\alpha$ (ripple to fluid liquid-crystalline main transition) is a comparatively high-enthalpy event (Janiak et al., 1976; Chen & Sturtevant, 1981). Preliminary versions of this work have appeared previously (Janes et al., 1990, 1991).

Colligative Thermodynamics. The bilayer membrane is often considered a two-dimensional structure amenable to classical thermodynamic treatment (Hill, 1974; Bangham & Hill, 1986). In thermodynamic terms, the mixing of alcohols or anesthetics with a membrane structure increases the configurational entropy or positional randomness, and hence always results in stabilization from an energetic standpoint. The stabilization stems from an additional entropic term, configurational entropy, that is competitive with thermal entropy in altering the free energy of the membrane assembly. On a mole fraction basis, the configurational entropy, S_{cf}, of n molecules of the ith component in a mixture is deduced from probability theory as (Bent, 1965)

$$S_{cf(i)} = -(R/N_0) \ln (n_i/\sum n_i) = -R \ln N_i \qquad (1)$$

where R is the ideal gas constant, N_i is the mole fraction of the ith component on a molar basis, and N_0 is the normalization factor. A two-component system consisting of the phospholipid membrane (N_1) and dilute solute (N_2) allows for further simplification ($\ln N_1 \simeq -N_2$). The sensitivity of membrane equilibria to anesthetics is dependent on the relative magnitudes of the two entropic terms:

† This work was supported by U.S. Public Health Service Grants AA07186, AA00088, AA07215, AA07463 and the Dunglison Scholars Program.

[1] Abbreviations: DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine.

0006-2960/92/0431-9467$03.00/0

$$\Delta G = \Delta H - T(\Delta S_t + \Delta S_{cf}) \quad (2)$$

The free energy, enthalpy, and thermal entropy are G, H, and S_t, respectively. The change in enthalpy remains constant over the range of dilute solute concentrations relevant to this study (Mountcastle et al., 1978; Sturtevant, 1982); therefore, the enthalpy change at the midpoint is

$$\Delta H = T_0 S_t \quad (3)$$

T_0 is the unperturbed midpoint temperature. Combining eq 2 and 3, and noting that T_0 is approximately equal to the perturbed midpoint for temperatures (Kelvin) and solute concentrations of biological relevance, yields

$$\Delta T/T_0 = \Delta S_{cf}/\Delta S_t \quad (4)$$

ΔT is the perturbation of the equilibrium midpoint induced by the solute.

Since this mode of anesthetic action occurs through an entropic mechanism, its effects are manifested in terms of equivalent temperature (or pressure) units of perturbation. If the solute partitions differently among the various competing conformational assemblies that comprise the membrane, then the relative free energies (ΔG) and equilibria among the assemblies vary accordingly. If, on the other hand, the drug partitions equally into all membrane assemblies, then all assemblies are equally stabilized, the equilibria between states remain unaltered, and there is no apparent net perturbation. Therefore, the unequal distribution of anesthetic among different membrane states entropically drives competing equilibria among these states, stabilizing some more than, and at the expense of, others. The net effect of altering the total entropy change implies that the midpoint temperature must be altered in order to maintain the original energetic balance. In this manner, anesthetic action upsets the natural thermal balance of the membrane.

Thus, the ratio of configurational and thermal entropies determines the magnitude of the perturbation in absolute fractional temperture units. If the thermal entropy is very large, the equilibrium is resistant to anesthetic action. If the configurational entropy is a substantial fraction of the thermal entropy, the equilibrium will be exquisitely sensitive to anesthetic action. This is equivalent to the statement that the equilibria most sensitive to anesthetics, for a given differential partitioning, are low-enthalpy events, since the change in enthalpy at the equilibrium midpoint is proportional to the change in thermal entropy.

The colligative thermodynamic analysis embodied in eq 4 therefore implicates two factors which determine anesthetic potency in membranes. Membrane processes most sensitive to solute action are (1) low-enthalpy equilibria wherein (2) the intramembrane solute concentrations differ between the initial and final states. The model predicts that the absolute degree of partitioning is not a crucial determinant and is relevant only to the extent that it correlates with the partitioning difference between states.

Analysis of Partitioning. In order to interpret the partitioning data quantitatively, certain relationships between the lipid assembly and the partition coefficients must be derived. The formalism developed is analogous to that used in differential scanning calorimetry. The degree of anesthetic partitioning into a membrane system is sensitive to and characteristic of the state of lipid assembly. Near the equilibrium midpoint between two states with characteristic partition coefficients, the partition coefficient will exhibit a discontinuity. The temperature dependence of the partition coefficient through the change in states can be modeled using the van't Hoff relation (Lewis & Randall, 1961):

$$\frac{d \ln K_{eq}}{dT} = \frac{\Delta H_{vH}}{RT^2} \quad (5)$$

The van't Hoff enthalpy, ΔH_{vH} is an idealized enthalpy for a completely cooperative equilibrium, and is related to the true calorimetric enthalpy by the degree of cooperativity.

The equilibrium constant, K_{eq}, is related to the partitioning of the solute, K_p, by assuming that the change in partitioning at the transition temperature is proportional to the extent of the reaction. A similar assumption is often used in calorimetry, in which the change in specific heat is assumed to be proportional to the extent of the phase change (Mabrey & Sturtevant, 1978). The temperature dependence of the partitioning exhibits the functional form:

$$K_p = \frac{K_p^{\alpha} + K_p^{\beta} \exp[C(T - T_m)]}{1 + \exp[C(T - T_m)]} \quad (6)$$

$$C = \frac{\Delta H_{vH}}{RTT_m} \simeq \frac{\Delta H_{vH}}{RT_m^2} \quad (7)$$

The partition coefficients for the membrane states α and β are K_p^{α} and K_p^{β}, respectively. These partition coefficients are not necessarily constant and may be altered to include a temperature dependence. The total partition coefficient is K_p. The midpoint temperature is T_m. A fit of the experimental data to this function yields partition coefficients for each phase, the midpoint temperature, and the van't Hoff enthalpy (ΔH_{vH}).

The analytical framework presented is not specific to the partitioning analysis. It is broadly applicable to any technique in which the observable is characteristic of each state, and the change is proportional to the extent of the reaction.

MATERIALS AND METHODS

Partition coefficients were obtained using a dual-radiolabel centrifugal technique modified from Katz and Diamond (1974a). The technique is suitable for extremely dilute solute concentrations, well below the levels involved in general anesthesia. The trace hexanol membrane concentrations in mole fraction (mf) units vary from 0.006% to 0.3%, depending on the lipid state, compared to an upper limit of about 5% mf (0.07 M) required for general anesthesia (Dluzewski et al., 1983; Meyer 1937). The technique is a ratio method; consequently, it is insensitive to uncertainties in the specific activity of the sample or to the adsorption of radiolabels to the walls of sample tubes, or evaporation.

Membrane Preparation. The purity (>99%) of phospholipids (Avanti Polar Lipids, Alabaster, AL) was confirmed by thin-layer chromatography. The lipid solutions (in chloroform) were dried in a round-bottom flask by rotary evaporation. The lipid film was then placed under high vacuum overnight (<5 mtorr) to remove any residual chloroform. Radiolabels were prepared as stock aqueous solutions of [^{14}C]hexanol (activity 1.4 Ci/mol; American Radiolabeled Chemicals, St. Louis, MO) and [^{3}H]water (activity 5 Ci/L; Amersham, Arlington Heights, IL) and added to the buffer prior to lipid hydration. One hundred milligrams of the dried lipid film was dispersed in 10 mL of buffer (0.1 M KCl, 0.01 M Tris-HCl, pH 7.0, radiolabels, and unlabeled hexanol when appropriate). The total hexanol concentrations in the buffer before lipid hydration were 0.0714, 1.475, 2.950, and 4.425 mM, corresponding sequentially to panels A–D in Figure 1. The typical activity of the buffer was 1.5 mCi/L ^{3}H and 0.1 mCi/L ^{14}C. Multilamellar vesicles were formed by vigorous

vortexing at a temperature above the main transition. The pH remained unchanged after hydration of the lipid. The phospholipid dispersions were allowed to equilibrate at the experimental temperature for a minimum of 3 h in the L_α state, and overnight otherwise. The data are presented as the mean ± the standard deviation from a minimum of four determinations.

Centrifugal Separation. The equilibrated liposome suspension was divided in half, pipetted into each of two sealed 10-mL Oak Ridge polycarbonate centrifuge tubes (Nalge Co., Rochester, NY), and pelleted for 60 min at 35 000 rpm (100000*g*) in a Beckman 75-TI rotor and L5-65B ultracentrifuge (Palo Alto, CA). Both the centrifuge and rotor had previously been equilibrated at the experimental temperature. The rotor temperature was calibrated on a water blank run under the same conditions. Phosphate assays (Bartlett, 1959) were performed on the supernatant to ensure complete centrifugal-phase separation. Supernatant phosphorus was less than 1% of the total. From each centrifuge tube, an aliquot of pellet and two aliquots of supernatant were quickly transferred to a tared glass 20-mL scintillation vial. Sample weights generally ranged from 50 to 100 mg. All weights were determined to ±0.1 mg. The sample temperatures were maintained outside of the centrifuge by utilizing the considerable heat capacity of the rotor. The uncertainty in temperature throughout the procedure was typically ±1 °C, although the thermal stability of the centrifuge was greatest below ambient temperature.

Radiolabel Counting. The radiolabels were counted in a Packard Tri-Carb Model 1900CA liquid scintillation analyzer (Packard Instrument Co., Downers Grove, IL) equipped with a barium-133 external γ-ray source, using a dual-window analysis (0–9.1 keV and 9.1–156 keV). Typical counting times were 5–10 min per sample. To each glass scintillation vial containing the sample were added a cocktail of 9 mL of Biosafe II scintillation fluid (Research Products International Corp., Mount Prospect, IL) and 1 mL of water. The cocktail water minimized variations in quench caused by variations in sample volume. The resulting cocktail remained well above the unstable region (>20% water). Quench standards for both ^{14}C and ^{3}H were prepared using water (250-μL increments) as a quenching agent. The quench standards were routinely run with the samples. The external γ source serve to calibrate the quench. After preparation, the sample vials were stored overnight in the dark to allow the mixture to equilibrate to a stable quench and to minimize chemiluminescence.

Calculation of Partition Coefficients. The determination of the partition coefficients follows the derivation and notation of Katz and Diamond (1974a):

$$K_p = [(C_{bp}/C_{bo}) - (C_{tp}/C_{to})]/(1 - C_{tp}/C_{to}) \quad (8)$$

where C represents radioactive disintegrations per minute per unit mass, the subscripts b and t represent carbon and tritium, respectively, and the subscripts p and o denote the pellet and supernatant, respectively. The units are molal units, expressed as (mole of hexanol in membrane/kg of membrane)/(mol of hexanol in water/kg of water), or equivalently (g of hexanol/g of membrane)/(g of hexanol/g of buffer). The reported molal K_ps are expressed in terms of total membrane mass, inclusive of intramembrane solute. Molal K_ps can also be expressed in terms of lipid mass alone, exclusive of intramembrane solute. The difference between the two expressions is less than the experimental uncertainty at all alcohol concentrations employed here. For all thermodynamic treatments, the intramembrane solute concentrations are converted to mole fraction units, $N_2/(N_1 + N_2)$.

An additional, but small, uncertainty in the molal K_p stems from nonsolvent or membrane-bound water, not accessible to hexanol. A correction proposed in the absence of alcohol (Katz & Diamond, 1974b) may be approximated by adding to the K_p reported here 0.12 molal unit below, and 0.33 molal unit above, the main transition. Such considerations are negligible with respect to the experimental error in the partitioning changes that are employed in the thermodynamic treatment.

Analysis of Partitioning Curves. The partitioning of hexanol is presented as a function of temperature for each alcohol concentration. These curves were analyzed by fitting them to eq 6 in the following manner.

(1) The partition coefficients of hexanol into any given state did not exhibit a pronounced temperature dependence, so eq 6 was used without modification (K_p^α and K_p^β were assumed constant).

(2) The temperature titration at trace hexanol levels (Figure 1A) was analyzed as follows. Each cooperative equilibrium was initially treated independently. A least-squares four-parameter fit of eq 6 to the data was performed. The entire curve was analyzed using the appropriate modification of eq 6 for two equilibria. Overlap between the two curves was corrected by fitting the combined equilibria to a three-parameter least-squares fit (both ΔH_{vH}s and the ripple K_p), while other parameters remained fixed. Overlap between the equilibria was always small. Standard errors are reported for each fitted parameter.

(3) The temperature titrations at higher hexanol concentrations (Figure 1B–D) were treated similarly except that the K_ps for the gel and ripple phases were considered independent of hexanol concentration, and values derived from the trace hexanol titration were used. The L_α K_p demonstrated a clear concentration dependence and was fit for each titration. The $L_{\beta'}$ K_p was assumed constant since there was no obvious concentration dependence, and ice formation precluded accurate K_p determination at higher alcohol concentrations. The $P_{\beta'}$ K_p did not exhibit a significant or consistent concentration dependence; therefore, its value was fixed from the trace hexanol concentration.

(4) The molal K_p values obtained from the simulations were subsequently converted into intramembrane mole fraction (mf) units for further thermodynamic treatment. Previously, a single intramembrane mf alcohol concentration was reported for each membrane state directly from molal K_p (Janes et al., 1990, 1991). Here, intramembrane concentrations are reported at the equilibrium midpoint to account for modest changes in aqueous alcohol concentrations during the equilibrium. Thus, two intramembrane concentrations for the ripple state are reported, corresponding to each equilibrium midpoint.

RESULTS

DMPC undergoes two entropically driven equilibria. The gel ($L_{\beta'}$) to ripple ($P_{\beta'}$) equilibrium (pretransition) is a low-enthalpy (ΔH = 3.5 kJ mol^{-1}) process that occurs at 14.4 °C in the absence of an anesthetic (Chen & Sturtevant, 1981). The ripple ($P_{\beta'}$) to liquid-crystalline (L_α) equilibrium (main transition) is a comparatively high-enthalpy process (ΔH = 21.05 kJ mol^{-1}) that occurs at 23.6 °C in the absence of an anesthetic (Chen & Sturtevant, 1981). The pretransition is well-known to be much more sensitive to "impurities" than the main transition [e.g., see Eliasz et al. (1976)], and the

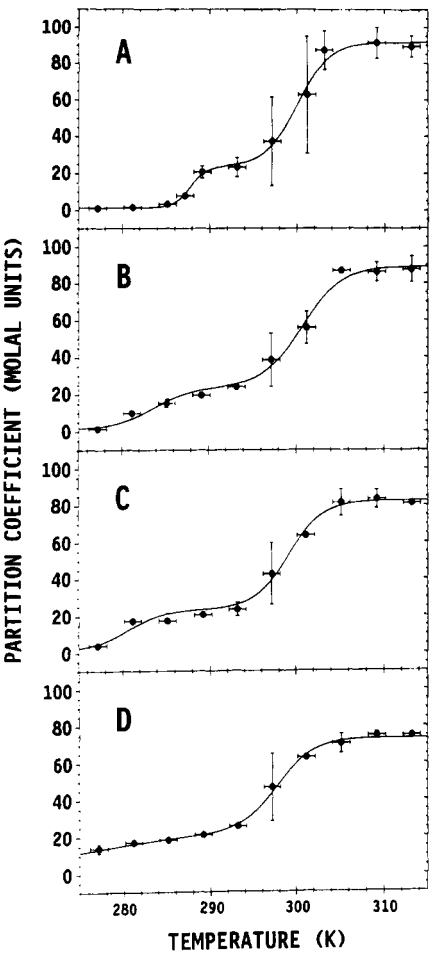

FIGURE 1: Molal partition coefficient of 1-hexanol into multilamellar dimyristoyllecithin membranes is shown as a function of temperature and alcohol concentration. Panel A represents the partitioning of trace hexanol concentrations into the essentially unperturbed bilayer. Panels B–D represent sequential increases in the alcohol concentration. The fit corresponds to the theoretical multiparameter least-squares analysis described in the text. The percent mole fraction intramembrane 1-hexanol concentrations [moles of hexanol/(moles of lecithin & hexanol)] at the $L_{\beta'} \rightarrow P_{\beta'}$ equilibrium midpoint are as follows: (panel A) $L_{\beta'}$ = 0.006%, $P_{\beta'}$ = 0.10%; (panel B) 0.12%, 2.06%; (panel C) 0.24%, 4.02%; (panel D) 0.35%, 5.91%. The mole fraction 1-hexanol concentrations at the $P_{\beta'} \rightarrow L_\alpha$ equilibrium midpoint are as follows: (panel A) $P_{\beta'}$ = 0.072%, L_α = 0.28%; (panel B) 1.48%, 5.4%; (panel C) 2.98%, 9.75%; (panel D) 4.54%, 12.9%. For comparative purposes, general anesthetic intramembrane concentrations are considered less than 5 mf %.

comparison provides a quantitative test for the predictions of the thermodynamic model. The partitioning behavior into the essentially unperturbed membrane, at trace hexanol concentrations, is shown along with the best theoretical fit in Figure 1A. Figure 1B–D illustrates the effects of progressive increases in the alcohol concentration.

Magnitude of Partitioning. Each of the three membrane states exhibits a substantially different receptivity to hexanol partitioning. The receptivity of $L_{\beta'}$ for the alcohol marginally exceeds the receptivity of the buffer (K_p = 1.3). A dramatic increase in partitioning occurs with the appearance of the more fluid $P_{\beta'}$ state (K_p = 23.5). A larger partitioning increase in absolute terms, but more modest in relative terms, accompanies the appearance of the L_α state (K_p = 91.7, Figure 1A). No significant temperature dependence of partitioning into a given state is observed, consistent with a small entropy of transfer. A slight negative concentration dependence is also observed for L_α (K_p = 73.3, Figure 1D). This differential receptivity to partitioning leads to different concentrations of

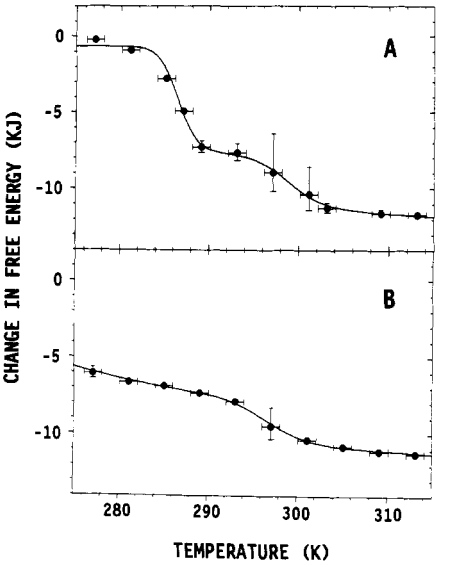

FIGURE 2: Free energy of hexanol partitioning is shown as a function of temperature and alcohol concentration. Panels A and B correspond to the extremes in alcohol concentration shown in Figure 1, panels A and D, respectively.

intramembrane alcohol, and different degrees of free energy stabilization (lowering) among the different lipid states. Differential partitioning is the inherent requirement for solute action via an entropic mechanism.

Many reports of lecithin–solute binding are indirect and assume negligible partitioning into the ripple state (vide infra); however, hexanol partitions substantially into the ripple state. The receptivity of the states to partitioning parallels changes in phospholipid surface density (De Young & Dill, 1988). Viewed from the standpoint of the free energy of the partitioning process ($\Delta G = -RT \ln K_p$; Katz & Diamond, 1974c), the changes at $L_{\beta'} \rightarrow P_{\beta'}$ dwarf the corresponding changes at $P_{\beta'} \rightarrow L_\alpha$ as shown in Figure 2 for the extremes of alcohol concentration presented in Figure 1. The partitioning change associated with $P_{\beta'} \rightarrow L_\alpha$ is 2.2–3.1-fold larger than $L_{\beta'} \rightarrow P_{\beta'}$. By contrast, the ratio of the analogous calorimetric endotherms, 6.1, is substantially greater (Chen & Sturtevant, 1981).

Equilibrium Midpoint. The data allow an assessment of the thermodynamic criteria advanced above which predict that (1) alcohols will perturb membrane equilibria if the partitioning between the product and reactant states differs and (2) the magnitude of the perturbation is dependent on the ratio of the change in partitioning to the change in enthalpy. In Figure 3, the observed perturbation of the equilibrium midpoint temperature is contrasted with the perturbation predicted by the thermodynamic model. The close correspondence of the observed and predicted behavior supports the thermodynamic principles presented above. We targeted low-enthalpy equilibria as likely candidates for sensitivity to the action of anesthetics. As predicted, the midpoint temperature of the low-enthalpy equilibrium exhibited a marked response to alcohol. At intramembrane concentrations of pharmacological relevance, less than 5 mf %, a dramatic shift in the midpoint, up to –9 °C, is observed. By contrast, the high-enthalpy equilibrium midpoint temperature is relatively insensitive to the alcohol at these pharmacological levels. No significant change is observed, while a shift of about –1 °C is predicted. The insensitivity exhibited by the $P_{\beta'} \rightarrow L_\alpha$ equilibrium is in agreement with the observations of others in related lecithin/anesthetic systems (Hill, 1974).

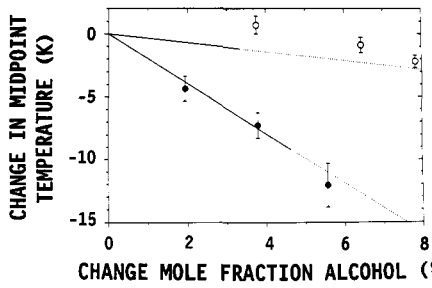

FIGURE 3: Dependence of the equilibrium midpoint temperature of DMPC on the presence of 1-hexanol. The 1-hexanol intramembrane concentration difference between the initial and final states at the equilibrium midpoint is shown. Data are presented for the low-enthalpy $L_{\beta'} \rightarrow P_{\beta'}$ (pretransition; filled circles) and the high-enthalpy $P_{\beta'} \rightarrow L_\alpha$ (main transition; open circles) equilibria. The colligative thermodynamic predictions (eq 10) are represented by the lines. The solid portions of the lines designate the absolute intramembrane concentrations of pharmacological relevance (<5 mf %).

DISCUSSION

Colligative thermodynamics implicates configurational entropy as the energetic driving force for the action of alcohols and anesthetics on cooperative membrane equilibria. The thermodynamic framework addresses the fundamental question of why some membrane equilibria are sensitive to anesethetics whereas others are insensitive. Anesthetics act through entropy, as does temperature. In both cases, the inherent requirement for solute action is that the entropy of the product and reactant states differs. For temperature, the thermal entropy must differ. For anesthetics, the configurational entropy must differ; consequently, the partitioning must differ. Both stimuli alter the relative free energies of the states, driving the interchange when the free energy of the product is lowered below that of the reactant. Thus, a requirement for the action of dilute anesthetics or the action of modest temperature changes is that the free energies of the states are not too different. This is the case in the vicinity of the equilibrium midpoint temperature, where the free energies are nearly equal. We define sensitivity to anesthetics relative to the sensitivity to temperature. Equilibria sensitive to anesthetics are those in which low levels of solute induce a perturbation equivalent to a substantial change in temperature. The free energy change of such equilibria is insensitive to thermal action, meaning that the thermal entropy change is small. Thus, sensitivity depends on the ratio of the thermal and configurational entropies as shown in eq 4. Sensitive equilibria have (1) large configurational entropy changes and (2) small thermal entropy changes. Neither entropy, however, is typically measured directly. Configurational entropy is deduced from partitioning. Thermal entropy is deduced from the enthalpy change of the equilibrium. Therefore, cooperative membrane equilibria that are most sensitive to the action of anesthetics exhibit (1) a large change in partitioning and (2) a small change in enthalpy.

This framework predicts that there is no single site of solute action. Solutes alter the free energies of both states in an equilibrium, and the difference determines the perturbation. Therefore, if solute potency correlates with partitioning into a bulk solvent, such as octanol, the "site" of action does not necessarily resemble that solvent.

Absolute and Differential Partitioning: Hill's Approach. To date, thermodynamic treatments of solute action on membranes have focused on the main phase transition. Hill (1974, 1975, 1978) first applied colligative thermodynamics to solute/membrane interactions using a freezing point depression model (assuming that partitioning occurs solely into the L_α state). The depression of the main phase transition induced by anesthetics was employed to deduce partitioning indirectly. These indirect partition coefficients are partitioning *differences* between the $P_{\beta'}$ and L_α states. These K_p values will always underestimate the actual values. For the hexanol/DMPC system in Figure 1A, the indirect approach would yield the L_α K_p (91.7 molal units) less the $P_{\beta'}$ K_p (23.5 molal units) or 74% (68.2 molal units) of the actual value for L_α. A more potent (local) anesthetic, chlorpromazine, exhibits proportionately similar molar partitioning behavior in DPPC [$K_p(L_{\beta'}) \approx 100$; $K_p(P_{\beta'}) \approx 1000$; $K_p(L_\alpha) \approx 3500$], such that the $P_{\beta'} \rightarrow L_\alpha$ partitioning difference is 71% of the total (Luxnat & Galla, 1986). Similar results are reported for benzyl alcohol in DPPC [$K_p(P_{\beta'}) \approx 5$; $K_p(L_\alpha) \approx 13$; $\Delta K_p \approx 60\%$ (Colley & Metcalfe, 1972)], halothane in DPPC [$K_p(P_{\beta'}) \approx 30$; $K_p(L_\alpha) \approx 100$; $\Delta K_p \approx 70\%$ (Simon et al., 1979a)], benzene in DMPC [$K_p(P_{\beta'}) \approx 27$; $K_p(L_\alpha) \approx 115$; $\Delta K_p \approx 76\%$ (De Young & Dill, 1988)], and hexane in DMPC [$K_p(P_{\beta'}) \approx 270$; $K_p(L_\alpha) \approx 1070$; $\Delta K_p \approx 75\%$ (Simon et al., 1979b)]. A comparison of the partitioning of 11 alcohols into egg lecithin (L_α model) and "gel"-state DPPC at 24 °C yielded qualitatively similar results for the two states (Jain & Wray, 1978)—the "gel" DPPC is presumably in the ripple state on the basis of the alcohol/lipid ratios reported for most cases. The largest discrepancy was observed for the long-chain alcohols [1-nonanol: K_p("gel") = 1020; $K_p(L_\alpha)$ = 1400 molal units; $\Delta K_p \approx 27\%$]. Long-chain alcohols are known to raise the $P_{\beta'} \rightarrow L_\alpha$ midpoint, an effect consistent with greater solute partitioning in $P_{\beta'}$ than in L_α [e.g., see Eliasz et al. (1976) and Pringle and Miller (1979)]. A lesser discrepancy from this trend is reported for the local anesthetic tetracaine in DMPC, albeit over a 12 °C span [$K_p(P_{\beta'}$, 18 °C) $\approx$ 1840; $K_p(L_\alpha$, 30 °C) $\approx$ 3200; $\Delta K_p \approx 42\%$ (Kaminoh et al., 1988)].

The limited data indicate that partitioning into the various membrane assemblies is roughly proportional and, therefore, correlates with oil partitioning (Janoff & Miller, 1982). We can expect, therefore, that the partitioning difference between two membrane states generally correlates with oil partitioning. The analysis presented here supports the usefulness of indirect partition coefficients obtained using Hill's approach in comparing *relative* partitioning for many, but not all, alcohol and anesthetic agents.

Generality of the Colligative Treatment. The thermodynamic treatment implies that the membrane can be approximated as a bulk isotropic solvent. The solute essentially "replaces" a lipid in the membrane in a completely random manner, while maintaining similar intermolecular binding forces. The energetic driving force arises solely from positional randomness, as derived from probability theory (not disordering from the standpoint of membrane fluidity). The framework accurately predicts that other low-enthalpy equilibria should be sensitive to solute (Taraschi et al., 1991). The regularity of differences in partitioning between the $P_{\beta'}$ state and L_α state for a variety of diverse agents further supports "well-behaved" solute action. The greatest divergence is observed for the long-chain alcohols that approach the "freezing point elevation" approximation.

This study addresses the energetic features of membrane equilibria that promote susceptibility to alcohol and anesthetics. The model DMPC equilibria were chosen for their well-defined energetic features, not for any putative physiological role. We see little compelling evidence that either equilibrium occurs in eukaryotic cell membranes near physiological temperatures [McElhaney, 1982; Seelig, 1978; Maraviglia et al., 1982; Gordon & Mobley, 1985; Palade,

1985; but see, e.g., Jain and White (1977) and Wolf et al. (1990)]. We propose that the origins of sensitivity to alcohol and anesthetic action derive from thermodynamic features that distinguish the $L_{\beta'} \rightarrow P_{\beta'}$ equilibrium (or the lamellar to hexagonal, $L_\alpha \rightarrow H_{II}$, equilibrium; Janes et al., 1991; Taraschi et al., 1991) from the $P_{\beta'} \rightarrow L_\alpha$ equilibrium and that other sensitive equilibria possess those thermodynamic features. We envision *no* role for the ripple state, per se, in biological membranes.

Implications for Biological Membranes. Thermodynamics elucidate the energetics of equilibria sensitive to alcohols and anesthetics but remain rather abstract mechanistically. We envision the (neuronal) membrane as a structurally diverse, heterogeneous, two-dimensional fluid—a dynamic mosaic of microdomains, energetically balanced for sensitivity to chemical stimulus. Alcohol and anesthetic action, assessed from the vantage of the *average* free energy of the membrane, is entropically equivalent to a mosaic of local thermal heating and cooling, which alters the energetic balance and architecture. We postulate that anesthetics act by disrupting the domain structure of lipids in biological membranes, effectively altering the native thermal balance of the membrane. All processes that require a defined lipid architecture are liable to disturbance. Proteins that require defined domains for function are also potential targets. The differential affinity of membrane components for different lipid domains may induce a compartmentation among proteins and their effectors. Processes that rely on lipid heterogeneity to maintain lateral segregation among membrane components are potential targets. The thermodynamic formulation postulates that the membrane equilibria most sensitive to anesthetic action are those that involve a low enthalpy change and a large partitioning difference.

REFERENCES

Bangham, A. D., & Hill, M. W. (1986) *Chem. Phys. Lipids 40*, 189–205.

Bartlett, G. R. (1959) *J. Biol. Chem. 234*, 466–468.

Bent, H. A. (1965) *The Second Law*, pp 179ff, 229ff, Oxford University Press, New York.

Chen, S. C., & Sturtevant, J. M. (1981) *Biochemistry 20*, 713–718.

Colley, C. M., & Metcalfe, J. C. (1972) *FEBS Lett. 24*, 241–246.

De Young, L. R., & Dill, K. A. (1988) *Biochemistry 27*, 5281–5289.

Dluzewski, A. R., Halsey, M. J., & Simmonds, A. C. (1983) *Mol. Aspects Med. 6*, 459–573.

Eliasz, A. W., Chapman, D., & Ewing, D. F. (1976) *Biochim. Biophys. Acta 448*, 220–230.

Gordon, L. M., & Mobley, P. W. (1985) in *Membrane Fluidity in Biology* (Aloia, R. C., & Boggs, J. M., Eds.) Vol. 4, pp 1–49, Academic Press, Orlando, FL.

Hill, M. W. (1974) *Biochim. Biophys. Acta 356*, 117–124.

Hill, M. W. (1975) *Biochem. Soc. Trans. 3*, 149–152.

Hill, M. W. (1978) *Ann. N.Y. Acad. Sci. 308*, 101–110.

Jain, M. K., & White, H. B. (1977) *Adv. Lipid Res. 15*, 1–60.

Jain, M. K., & Wray, L. V. (1978) *Biochem. Pharmacol. 27*, 1294–1295.

Janes, N., Hsu, J. W., Rubin, E., & Taraschi, T. F. (1990) *Molecular and Cellular Mechanisms of Alcohol and Anesthetics*; presented at a meeting of The New York Academy of Sciences, Calgary, Alberta, Canada, June 1990; Abstract M-23.

Janes, N., Hsu, J. W., Rubin, E., & Taraschi, T. F. (1991) *Biophys. J. 59*, 125a.

Janiak, M. J., Small, D. M., & Shipley, G. G. (1976) *Biochemistry 15*, 4575–4580.

Janoff, A. S., & Miller, K. W. (1982) in *Biological Membranes* (Chapman, D., Ed.) Vol. 4, pp 417–476, Academic Press, New York.

Kamaya, H., Kaneshina, S., & Ueda, I. (1981) *Biochim. Biophys. Acta 646*, 135–142.

Kaminoh, Y., Inoue, T., Ma, S.-M., Ueda, I., & Lin, S. H. (1988) *Biochim. Biophys. Acta 946*, 337–344.

Katz, Y., & Diamond, J. M. (1974a) *J. Membr. Biol. 17*, 69–86.

Katz, Y., & Diamond, J. M. (1974b) *J. Membr. Biol. 17*, 87–100.

Katz, Y., & Diamond, J. M. (1974c) *J. Membr. Biol. 17*, 101–120.

Lee, A. G. (1977) *Biochemistry 16*, 835–841.

Lee, A. G. (1983) in *Membrane Fluidity in Biology* (Aloia, R. C., Ed.) Vol. 2, pp 43–88, Academic Press, New York.

Lewis, G. N., & Randall, M. (1961) *Thermodynamics* (revised by Pitzer, K. S., & Brewer, L., Eds.) McGraw-Hill, New York.

Luxnat, M., & Galla, H.-J. (1986) *Biochim. Biophys. Acta 856*, 274–282.

Mabrey, S., & Sturtevant, J. M. (1978) *Methods Membr. Biol. 9*, 237–274.

Maraviglia, B., Davis, J. H., Bloom, M., Westerman, J., & Wirtz, K. W. A. (1982) *Biochim. Biophys. Acta 686*, 137–140.

McElhaney, R. N. (1982) *Chem. Phys. Lipids 30*, 229–259.

Meyer, K. H. (1937) *Trans. Faraday Soc. 33*, 1062–1068.

Palade, G. E. (1985) in *The Cell in Contact* (Edelman, G. M., & Thiery, J.-P., Eds.) pp 9–24, John Wiley & Sons, New York.

Pringle, M. J., & Miller, K. W. (1979) *Biochemistry 18*, 3314–3320.

Rowe, E. S. (1982) *Mol. Pharmacol. 22*, 133–139.

Seelig, J. (1978) *Biochim. Biophys. Acta 515*, 105–140.

Simon, S. A., McIntosh, T. J., Bennett, P. B., & Shrivastav, B. B. (1979a) *Mol. Pharmacol. 16*, 163–170.

Simon, S. A., Stone, W. L., & Bennett, P. B. (1979b) *Biochim. Biophys. Acta 550*, 38–47.

Sklar, L. A., Hudson, B. S., & Simoni, R. D. (1977) *Biochemistry 16*, 819–828.

Suezaki, Y., Tatara, T., Kaminoh, Y., Kamaya, H., & Ueda, I. (1990) *Biochim. Biophys. Acta 1029*, 143–148.

Taraschi, T. F., & Rubin, E. (1985) *Lab. Invest. 52*, 120–131.

Taraschi, T. F., Lee, Y.-C., Janes, N., & Rubin, E. (1991) *Ann. N.Y. Acad. Sci. 625*, 698–706.

Wolf, D. E., Maynard, V. M., McKinnon, C. A., & Melchior, D. L. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 6893–6896.