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The Lateral Pressure Profile in Membranes: A Physical Mechanism of General Anesthesia

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ABSTRACT: A mechanism of general anesthesia is suggested and investigated using lattice statistical thermodynamics. Bilayer membranes are characterized by large lateral stresses that vary with depth within the membrane. Incorporation of amphiphilic and other interfacially active solutes into the bilayer is predicted to increase the lateral pressure selectively near the aqueous interfaces, compensated by decreased lateral pressure toward the center of the bilayer. General anesthesia likely involves inhibition of the opening of the ion channel in a postsynaptic ligand-gated membrane protein. If channel opening increases the cross-sectional area of the protein more near the aqueous interface than in the middle of the bilayer, then the anesthetic-induced increase in lateral pressure near the interface will shift the protein conformational equilibrium to favor the closed state, since channel opening will require greater work against this higher pressure. This hypothesis provides a truly *mechanistic* and *thermodynamic* understanding of anesthesia, not just *correlations* of potency with structural or thermodynamic properties. Calculations yield qualitative agreement with anesthetic potency at clinical anesthetic membrane concentrations and predict the alkanol cutoff and anomalously low potencies of strongly hydrophobic molecules with little or no attraction for the aqueous interface, such as perfluorocarbons.

Although there is general agreement that the site of action of general anesthetics involves postsynaptic ligand-gated ion channels, a *mechanistic* understanding of general anesthesia does not yet exist (Miller, 1985; Forman & Miller, 1989; Franks & Lieb, 1994; Forman et al., 1995). On the one hand, the well-studied correlation between anesthetic potency and membrane concentration would seem to imply an *indirect* mode of action of general anesthetics on membrane proteins, effected through some perturbation of the lipid bilayer. Explanations of this type have been offered, involving phase separation or changes in bilayer thickness, order parameters, or curvature elasticity, and have been extensively reviewed (Miller, 1985; Janoff & Miller, 1982; Koblin, 1994). These approaches generally suffer from three weaknesses. Membrane perturbations are relatively small at clinical anesthetic levels, often duplicated with a small variation of a different property (e.g., a temperature increase of 1 °C) that does not induce anesthesia (Franks & Lieb, 1982, 1994). Also, exceptions such as the cutoff in potency for long *n*-alkanols and the anomalously low potency of perfluorinated hydrocarbons and the lighter inert gases remain unexplained (Franks & Lieb, 1985; Miller et al., 1989; Koblin, 1994). [The additional requirement of some degree of aqueous interfacial activity in addition to membrane solubility (Yoshino et al., 1994; Pohorille et al., 1996; Pohorille & Wilson, 1996) eliminates some of these anomalies, consistent with results presented here.] Most importantly, with a few exceptions such as the work of Trudell (1977) discussed below, they usually do not provide a *causal* (mechanistic) relationship between anesthetic potency and the perturbed structural or thermodynamic property. In a seminal paper, Gruner and Shyamsunder (1991) have considered an effect closely related to that presented here, although they do not suggest a *mechanism*, as described in greater detail below.

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Other evidence suggests a mechanism in which general anesthetics inhibit (or possibly potentiate) an ion channel protein by binding *directly* to it (Franks & Lieb, 1984, 1987, 1994). The correlation between anesthetic potency and inhibition of the water-soluble protein luciferase favors such a mechanism, particularly because it exhibits a cutoff in inhibition for long *n*-alkanols. A direct binding mechanism is also supported by the observation of a mild difference in potency among stereoisomers of some anesthetics, although this result does not rule out a bilayer-mediated mechanism, given the existence of chiral molecules in the membrane. Recent kinetic studies on the inhibition of the nicotinic acetylcholine receptor of the neuromuscular junction in *Torpedo* are consistent with the action of anesthetics on a well-defined site distinct from the agonist site (Wood et al., 1995; Forman et al., 1995). However, they do not rule out a bilayer-mediated mechanism that shifts the equilibrium populations of the closed and open protein states.

Results of lattice statistical thermodynamic calculations are presented that strongly support a novel bilayer-mediated mechanism of action of general anesthetics. This mechanism does not suffer from the shortcomings typical of bilayer-mediated mechanisms proposed previously, as discussed above. Rather, it provides a truly *mechanistic* and *thermodynamic* understanding of general anesthesia that correlates well with anesthetic potency at clinical anesthetic concentrations, including the alkanol cutoff and perfluorocarbon anomalies, and is largely consistent with observed structural effects such as membrane order parameter and thickness changes.

THE LATERAL PRESSURE PROFILE

Fluid interfacial regions, such as found in self-assembled monolayers and bilayers, are of molecular thickness. The concentration of the large interfacial free energy over this microscopically narrow region leads to enormous local transverse stresses (lateral pressures) corresponding to bulk pressures of many hundreds of atmospheres (Gaines, 1966). The local lateral pressure depends strongly on location within the interfacial region, i.e., the lateral pressure profile is nonuniform (Ben-Shaul, 1995; Safran, 1994). As described below, the incorporation of anesthetics in bilayers is calculated to perturb the lateral pressure profile in a highly nonuniform manner. Although the perturbation of the local pressures at clinical anesthetic concentrations is typically *relatively* small, it is large in absolute magnitude since the pressures themselves are enormous. In particular, *for interfacially active solutes* (i.e., at least part of which is attracted to the aqueous interface) except long *n*-alkanols, a large stress increase is predicted to occur near the aqueous interface, with a compensating decrease near the middle of the bilayer.

By what mechanism might these changes in the lateral pressures induce anesthesia? It is possible that general anesthesia involves inhibition of the agonist-induced opening of the ion channel in a postsynaptic receptor (Franks & Lieb, 1994; Miller, 1985; Forman & Miller, 1989). In general, the opening of the channel is expected to be accompanied by a *nonuniform* change in the cross-sectional area occupied by the protein in the membrane. If, for example, the protein were to expand most near an aqueous interface and expand less (or shrink) near the middle of the bilayer, then the protein would experience a significantly increased local pressure where its area increases the most. In that case, the thermodynamic equilibrium between the closed (resting) and open states of the ion channel protein would shift toward the resting state. The hypothesis is simple: a shift in lateral pressure from the center of the bilayer toward the aqueous interfaces results in inhibition, since greater mechanical work is then needed to open the ion channel. Anesthetic potency is determined by the membrane concentration of anesthetic required to shift the protein conformational equilibrium substantially toward the closed state.

As mentioned above, the idea that anesthetic-induced variations in the lateral pressure profile might somehow be coupled to altered protein function is not new (Gruner & Shyamsunder, 1991; Seddon & Templer, 1995). In a particularly elegant presentation, Gruner and Shyamsunder discussed the possibility of a mechanism in which anesthetics induce changes in the *spontaneous curvature* of the monolayer leaflets. As they noted, this contribution (and others, such as the elastic moduli) to the elastic curvature stress of the bilayer is a function of the lateral pressure profile. However, they did not suggest a *mechanism* by which the coupling (of resulting changes in the stress profile to altered protein function) might occur.

THERMODYNAMIC ANALYSIS

For simplicity, the protein (with or without bound agonists) is assumed to exist in only two conformational states, cl (closed) and op (open). In each state, the protein cross-sectional area may vary with depth within the membrane. Let $A_{cl}(z)$ and $A_{op}(z)$ represent these area functions, z indicating the position along the axis perpendicular to the bilayer plane. The conformational shift from cl to op is accompanied by a depth-dependent change in the cross-sectional area: $\Delta A(z) = A_{op}(z) - A_{cl}(z)$. Defining $\delta\pi(z)$ as the depth-dependent lateral pressure acting over a thin slice of the bilayer of thickness δz, then $p(z) = \delta\pi(z)/\delta z$ represents the lateral pressure density at depth z. The interfacial tension resulting from contact between water and hydrocarbon segments leads to a large negative pressure at the interface, while the strong entropically driven repulsions among the chain tails result in large positive lateral pressures in the bilayer near the interface, decreasing toward the middle of the bilayer (Ben-Shaul, 1995). Since the fluid membrane is *self-assembled* (unlike spread monolayer films), the *total* lateral pressure in the membrane is zero (or nearly so); i.e., $\pi = \int \delta\pi(z) = \int p(z)\ \delta z \approx 0$. In other words, the bilayer will expand or contract laterally in order to minimize the membrane free energy.

How does the fraction of protein in the open state depend on variations in the pressure profile? Define $p_0(z)$ as the pressure profile in the absence of anesthetic and $[r]_0$ as the corresponding equilibrium concentrations of the protein in each of the two conformational states (r = cl or op). Addition of an anesthetic alters the lateral pressure profile by an amount $\Delta p(z)$, shifting the equilibrium concentrations of each of the protein conformations from $[r]_0$ to [r]. To good approximation, the chemical potential of the protein in state r can then be written as (Cantor, 1997)

$$\mu_r = \mu_r^* + RT \ln [r] + N_{Av} \int A_r(z)\ \Delta p(z)\ \delta z \qquad (1)$$

where N_{Av} is Avogadro's number, μ_r^* represents the standard

chemical potential of the protein in conformation r, i.e., at unit activity and pressure profile $p_0(z)$, and $A_r(z)$ is assumed to be unaffected by $\Delta p(z)$. In the absence of anesthetic, the chemical potential is thus $\mu_r^\circ = \mu_r^* + RT \ln [r]_0$. At equilibrium, the chemical potentials of the two protein states must be equal, both with anesthetic present ($\mu_{cl} = \mu_{op}$) and without ($\mu_{cl}^\circ = \mu_{op}^\circ$). Subtracting the second equality from the first, μ_r^* is eliminated, and the fraction of protein in the open configuration is easily shown to be $F = (1 + y_0 e^\alpha)^{-1}$, where $y_0 = [cl]_0/[op]_0$, and $\alpha = (k_BT)^{-1} \int \Delta A(z)\, \Delta p(z)\, \delta z$ is proportional to the concentration of anesthetic in the membrane. Presumably, in the absence of agonist $[cl]_0 \gg [op]_0$, so $F \ll 1$ for all anesthetic concentrations. However, after the agonist binds, $[cl]_0 \ll [op]_0$, so $F \approx 1$ in the absence of anesthetic ($\alpha = 0$) but drops precipitously once α exceeds $-\ln(y_0)$. If $F_{max} = (1 + y_0)^{-1}$ is the value of F in the absence of anesthetic, then

$$f = F/F_{max} = (1 + y_0)/(1 + y_0 e^\alpha) \qquad (2)$$

represents the fractional inhibition, i.e., the concentration of the open conformation with anesthetic present relative to that in its absence. Note that if the shift in the protein cross-sectional area were *independent* of bilayer depth, i.e., if ΔA were independent of z, then the equilibrium would be unaffected by anesthetic, since for that case $\alpha = (k_BT)^{-1} \Delta A \int \Delta p(z)\, \delta z = 0$. In general, however, the lateral expansion or contraction of the protein varies with z, so $\alpha \neq 0$.

As mentioned above, the theory of Trudell (Trudell, 1977; Janoff & Miller, 1982) does provide a mechanical and thermodynamic hypothesis of anesthesic action. It presumes coexistence of gel and liquid crystalline phases in the absence of anesthetic and that the addition of anesthetic melts the gel state. *By analogy with spread monolayer films*, it is supposed that the pure liquid crystalline phase has decreased lateral compressibility compared to the two-phase system, thus preventing the protein from opening. However, the analogy is inappropriate for bilayer dispersions, which are *self-assembled*, i.e., they exist at zero (or nearly zero) total lateral pressure: the bilayer is free to expand or contract laterally to minimize the free energy. As discussed above, if the expansion of the protein were uniform (ΔA independent of z), anesthetic-induced melting of the putative gel phase would have no effect on the protein conformational equilibrium.

CALCULATIONS AND RESULTS

Well-tested lattice statistical thermodynamic methods were used to predict the effect of anesthetics on the lateral pressure profile. First, existing lattice methodology was employed (Cantor, 1993, 1996) that was developed to describe lipid *monolayers* in selective (good) solvents, i.e., at the oil/water interface. The advantage of this approach is that it provides an accurate description of chain conformational contributions to the entropy, including bond orientational correlations, using a simple cubic lattice model for the chains. The molecular interfacial area can be varied continuously, so that free energy minimization is easily constrained to zero total lateral pressure, appropriate to self-assembled systems. Using this approach, the effect of varying the strength of attraction of small anesthetics to the aqueous interface could be explored. The major disadvantage of this approach is that a monolayer is *not* half a bilayer; the mixing of chain tails from opposing sides of a bilayer is quite different from the mixing of chain tails with monomeric solvent in a monolayer. So, in a second set of calculations the theory was modified to model bilayers, requiring considerable simplification as described below. Here the anesthetic, like the lipid, was presumed to be strongly amphiphilic, i.e., comprised of a flexible hydrophobic chain bonded to a compact hydrophilic head-group constrained to remain in contact with the aqueous interface. The effect of varying its chain length was explored, to investigate the cutoff in potency for *n*-alkanol anesthetics.

(1) Monolayers. In the lattice approach (Cantor, 1996), the monolayer is divided into thin layers of finite thickness, labeled $i = 1, 2, ...$, by proximity to the aqueous interface, each layer characterized by a lateral pressure $\delta\pi_i$. Upon addition of anesthetic, the change in pressure in layer i is given by $\Delta(\delta\pi_i)$, so in this discretized model, $\alpha = (k_BT)^{-1} \sum_i [\Delta A_i\, \Delta(\delta\pi_i)]$. In a first step, existing theory for monolayers was easily modified to consider a fixed total concentration of anesthetic, in which the anesthetic solute, like the hydrophobic solvent, occupies a single cubic lattice site. The effect of varying the interfacial activity of the anesthetic (i.e., its attraction for the aqueous interface) on the pressure profile was examined for this special case. Zero mixing energy between anesthetic and lipid segments or oil was assumed, except at the interface where an energetic preference for the anesthetic was incorporated. As in previous work, the interfacial tension ($\gamma \approx 50$ dyn cm^{-1}) is assumed constant and localized at the interface. The concentration of anesthetic within each layer of sites was allowed to vary, subject to a predetermined constraint on the total solute concentration within the monolayer. Minimization of the free energy with respect to the chain probability distribution and the lateral area per lipid, subject to the constraint on the total anesthetic concentration, resulted in expressions for the lipid chain segment and bond distributions, the anesthetic concentration profile, surface area per lipid, monolayer thickness, and the lateral pressure profile $\delta\pi_i$. Results were obtained for a range of values of n_{lipid}, the lipid chain length, and E_{bend}, the internal chain bending energy (a measure of chain internal stiffness). In each case, calculations were first performed without added anesthetic and then for a mixture of 98 mol % double-chained lipid and 2 mol % solute (corresponding to ~30 mM anesthetic, a typical clinical bilayer concentration), yielding predictions of changes in the lateral pressure profile, $\Delta(\delta\pi_i)$, examples of which are presented in Figure 1.

For a strongly interfacially active anesthetic, i.e., with large energetic preference to reside in the layer adjacent to the aqueous interface, the anesthetic increases the competition for sites among the lipid chains in that layer. As a result, the lateral pressure in the first layer increases significantly, with compensating decrease in pressure spread over the remaining layers. However, in the other extreme of no attraction of the anesthetic for the interface, i.e., for a solute equivalent in hydrophobicity to the methylene groups which comprise most of the bilayer, the change in pressure profile of the monolayer is predicted to vary gradually with distance from the aqueous interface, the details depending on chain length and internal stiffness. If (as hypothesized) the anesthetic mechanism requires the lateral pressure on the ion channel protein to increase selectively near the aqueous

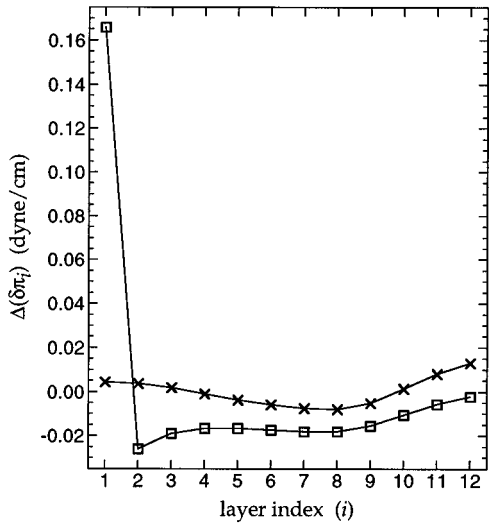

FIGURE 1: Predicted changes in the lateral pressure profile $\Delta(\delta\pi_i)$ for a lipid monolayer ($n_{lipid} = 12$, $E_{bend} \approx 1.2k_BT$) upon addition of 2 mol % anesthetic, assumed to occupy a single lattice site. Layer index (i) is numbered from the aqueous interface. Results presented for two extremes of anesthetic interfacial activity: no energetic preference for the interface (×); strong energetic preference for the interface (□).

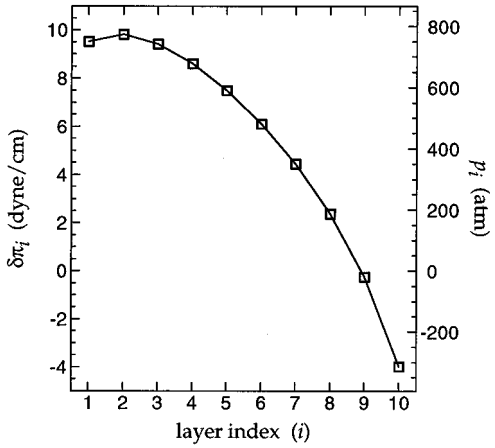

FIGURE 2: Predicted lateral pressure profile in a bilayer for $n_{lipid} = 16$ and $E_{bend} \approx 1.6k_BT$. The first layer is adjacent to the aqueous interface; the tenth layer is near the bilayer center. The left ordinate gives the lateral pressure $\delta\pi_i$ in each layer (i); the right ordinate gives the lateral pressure density $p_i = \delta\pi_i/\delta z$.

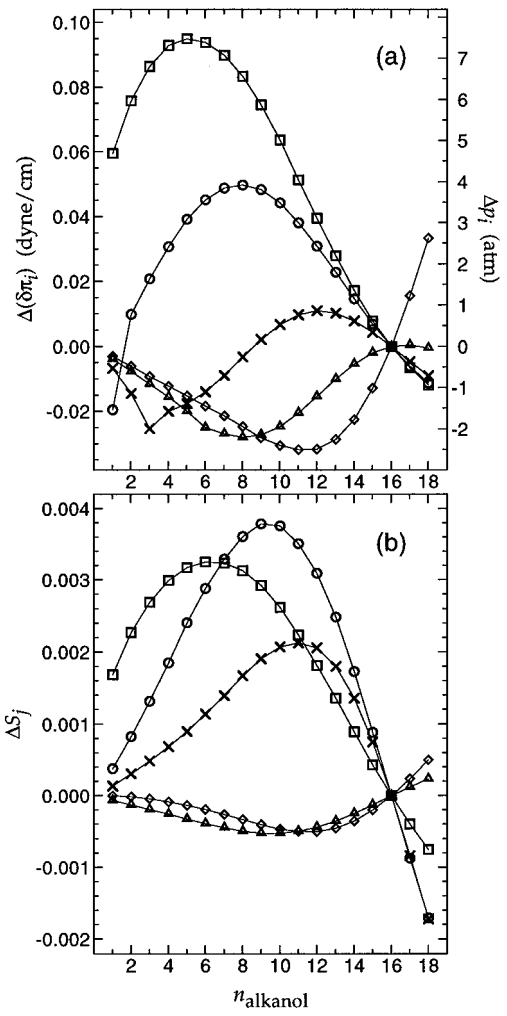

FIGURE 3: Predicted changes in a bilayer upon addition of 2 mol % alkanol (corresponding to ~30 mM, a typical clinical anesthetic concentration) as a function of $n_{alkanol}$, the alkanol chain length. Results are presented for $n_{lipid} = 16$ and $E_{bend} \approx 1.6k_BT$ over the range $1 \leq n_{alkanol} \leq 18$. (a) Lateral pressure. The left ordinate gives the change in lateral pressure $\Delta(\delta\pi_i)$; the right ordinate gives the corresponding change in lateral pressure density Δp_i. Values are plotted for representative layers: (□) $i = 1$; (○) $i = 2$; (×) $i = 4$; (△) $i = 7$; and (◇) $i = 10$. (b) Changes in the order parameter of the lipid backbone ΔS_j . Values are plotted for representative bond positions j, numbered by distance along the chain from the head group: (□) $j = 1$; (○) $j = 5$; (×) $j = 8$; (△) $j = 11$; and (◇) $j = 14$.

interface (with compensating decreased pressure in the interior), then anomalously low anesthetic potency is predicted for solute molecules which are *too* hydrophobic, i.e., with little or no preference for the surface of the predominantly methylenic environment of the bilayer interior. This requirement of at least some affinity for the aqueous interface is then consistent with the anomalously low potency of some nonpolar molecules, as has previously been suggested (Pohorille & Wilson, 1996; Yoshino et al., 1994). For example, calculations of excess chemical potentials of perfluorinated alkanes (Pohorille et al., 1996; Pohorille & Wilson, 1996) indicate that they are sufficiently hydrophobic not to be attracted to the aqueous interface, and it is thus not surprising that only CF_4 is (mildly) anesthetic. Although the anesthetic Xe is nonpolar, it is more polarizable by interfacial water than the CH_2 groups it would replace at the interface and is thus interfacially active, while Ne, He, and H_2 are not, consistent with their anomalously low anesthetic potency. Alkanes can also be considered from this perspective. If CH_3 (but not CH_2) groups have some interfacial activity in a bilayer in contact with water, short alkanes (with a sufficiently high CH_3/CH_2 ratio by volume) might be expected to have anesthetic potency, but not the longer alkanes, as observed experimentally. Consistently, the anesthetic cyclopropane, while nonpolar, is calculated to be interfacially active (M. Wilson, private communication).

(2) Bilayers. To extend the theoretical description of chain statistics to bilayers, it was necessary to simplify the analysis considerably. The bilayer was treated as two "compact" monolayers, a reasonable approximation given the limited interdigitation in membranes (Slater & Huang, 1992). The free energy was minimized subject to a set of constraint equations with associated Lagrange multipliers to ensure constant density, i.e., complete filling of the lattice sites in each layer. A simplified version of the packing entropy that ignores bond correlations was used, from which a pressure profile with a simply identified layer dependence was easily

obtained, similar in many respects to techniques (Ben-Shaul, 1995; Marqusee & Dill, 1986) used to investigate a wide range of amphiphilic aggregates.

For the bilayer calculations, the anesthetic (like the lipid) was modeled as a flexible-chain amphiphile, i.e., with one end constrained to reside in the layer adjacent to the aqueous interface. This is appropriate for *n*-alkanols, which are strongly interfacially active both in films spread at the oil/water interface (Motomura, 1980) and in self-assembled aggregates (Auvray, 1994). This approach was used to perform calculations for $n_{\text{lipid}} \leq 18$, for a wide range of anesthetic chain lengths. The chain stiffness energy was varied from $E_{\text{bend}} = 0$ (flexible) to $E_{\text{bend}} \approx 2k_BT$ (quite stiff); similar qualitative trends in the results were found over most of this range. In general, addition of anesthetic is predicted to increase the molecular interfacial area slightly more than the membrane volume, i.e., slightly decreasing the membrane thickness, in agreement with experiment (Janoff & Miller, 1982; Franks & Lieb, 1982) as is the predicted lipid molecular area of ~63 Å^2. In Figures 2 and 3, results are presented for $n_{\text{lipid}} = 16$, corresponding to the length of the acyl chains in typical membrane lipids.

The predicted lateral pressure profile is presented in Figure 2. The left ordinate gives the lateral pressure $\delta\pi_i$ in each layer i; the right ordinate gives the corresponding lateral pressure density $p_i = \delta\pi_i/\delta z$, where the layer thickness is given by $\delta z = 1.27$ Å, the vertical distance along the director of an all-trans alkane. The pressure is highest nearest the aqueous interface where the chains are the most conformationally constrained. The curve resembles the predicted orientational order profile (not shown), consistent with typical experimental results except for the low values near the middle of the bilayer (layers 9 and 10), an artifact of the forced separation of the two monolayers in the theory.

In Figure 3a are plotted the predicted changes in the lateral pressures in representative layers as a function of n_{alkanol}, the length of the (*n*-alkanol) anesthetic. Only close to the aqueous interface does the lateral pressure increase over a wide range of n_{alkanol}. Changes in the pressure densities near the aqueous interface are well in excess of 1 atm, even at this clinically relevant anesthetic concentration. The *intrinsic* alkanol potency (inversely proportional to *membrane* concentration necessary for channel inhibition) is thus predicted to pass through a *maximum* at intermediate n_{alkanol}, dropping to zero as n_{alkanol} approaches n_{lipid}. [For equal chain length, lipid and alkanol become identical at this level of approximation, so zero anesthetic effect is predicted, as has already been suggested (Miller et al., 1987, 1989).] The cutoff of potency with increasing n_{alkanol} is clearly predicted. For $n_{\text{alkanol}} > n_{\text{lipid}}$, a *negative* pressure change is predicted near the interface; i.e., such alkanols would tend to *reverse* anesthesia. These predictions (e.g., location of the maximum of the curve) should certainly not be taken as quantitatively precise. However, the general trends and the prediction of the alkanol cutoff are significant. That local lateral pressures are enormous means that pressure *changes* are still large in magnitude (although *relatively* small), serving to *amplify* the effect of solubilization of anesthetic. Arguments that the effects on the bilayer of anesthetics at clinical concentration are small (Franks & Lieb, 1982, 1987, 1994) may be valid for structural properties and even some thermodynamic properties, but not for the lateral pressure profile.

Correlations between potency and the magnitude of the decrease in the lipid order parameter in the membrane interior have been found for alkanols of varying length (Miller et al., 1987). Predicted changes in the order parameter profile ΔS_j as a function of bond position j are graphed in Figure 3b. Measurements (Miller et al., 1989; Raines et al., 1993) have often used a probe labeled at the 12th carbon of stearic acid, roughly equivalent to the 10th or 11th bond in these calculations. At this position a decrease in order parameter is predicted, with maximal effect for alkanols of (roughly) 8−10 segments, the effect going to zero at $n_{\text{alkanol}} = n_{\text{lipid}} = 16$ and becoming positive for $n_{\text{alkanol}} > 16$. While these predictions are qualitatively consistent with the observed trends (Miller et al., 1987, 1989; Firestone et al., 1994), they are smaller by nearly a factor of 10. Smaller decreases in order parameter have been observed (Firestone et al., 1994) using a probe labeled at the fifth carbon, in complete disagreement with the large *increase* predicted here. The reason for this discrepancy is not clear. It may result from the lack of intermolecular bond correlations in the simplified description of the chain conformational entropy.

The effect of adding cholesterol to the bilayer can be estimated. At the level of approximation of this mean-field theory (without bond correlations), if cholesterol is modeled as a rigid rod, then it has no influence on $p_0(z)$, the pressure profile in the absence of anesthetic. However, since $\Delta p(z)$ depends on the ratio of anesthetic to flexible lipid, addition of cholesterol will increase the effect of a given membrane concentration of anesthetic by a multiplicative factor $(1 - x_{\text{chol}})^{-1}$. Thus, cholesterol magnifies the anesthetic effect in that it lowers the required membrane concentration of anesthetic. (However, since the partition coefficient between membrane and aqueous phases decreases significantly with added cholesterol, the net effect may be a *decrease* in anesthetic potency, at fixed anesthetic chemical potential.) The effect of cholesterol on order parameter profiles is more subtle. In recent work (Cantor, 1996), it was demonstrated that inclusion of bond correlations is critical to understand the packing entropy of chain molecules of different stiffness, leading to predictions of cholesterol−lipid phase separations. An understanding of the effect of anesthetics on order parameters will likely require a more sophisticated description of chain conformational statistics.

Are the predicted pressure changes at clinical anesthetic concentrations large enough to keep the ion channel closed? For the acetylcholine receptor with bound neurotransmitter, perhaps 85% of the protein is in the open state in the absence of anesthetic (Matsubara et al., 1992), i.e., $y_0 = [\text{cl}]_0/[\text{op}]_0 \approx 0.2$. Using this value in eq 2, the fractional inhibition is $f = 0.5$ at $\alpha = 2$, $f = 0.1$ at $\alpha = 4$, and $f = 0.0003$ at $\alpha = 10$. Using an order-of-magnitude estimate of $\Delta A(z)$ of the protein, α can be predicted. [Note that $\Delta A(z)$ refers to the *entire* protein, not just the ion channel. In general, the depth dependence of the area change of the ion channel will not be related in any simple way to $\Delta A(z)$.] At clinical concentrations of an anesthetic such as a medium-length alcohol, the total pressure increase is predicted to be of order 0.3 erg cm^{-2} (summing over the layers adjacent to the two aqueous interfaces and considering the effect of added cholesterol) with compensating negative values distributed over the bilayer interior. A typical protein radius in the closed state might be 35 Å, as for the nicotinic acetylcholine receptor (Unwin, 1993) near the aqueous interface (but

smaller in the membrane interior). Little is known about $\Delta A(z)$; by arbitrarily supposing a change in radius that is 15% greater near the aqueous interfaces than at the center of the bilayer (e.g., increasing to 40 Å near the aqueous interfaces but unchanged in the center), α is estimated to be of order 1. Considering the approximations and simplifications of the lattice model from which the $\Delta\pi_i$ are predicted, the experimental uncertainty in y_0, and particularly the lack of information about $\Delta A(z)$ for ion-channel proteins, the agreement is encouraging.

DISCUSSION

Even at membrane anesthetic concentrations of only a few mole percent, it seems possible that resulting changes in the lateral pressure profile can alter conformational equilibria sufficiently in ion channel proteins to induce general anesthesia. This sensitivity derives in part from the concentration of very large transverse stresses within a narrow interfacial region. Also, whereas the chemical potential of each protein conformation depends approximately *logarithmically* on its own concentration, it depends *linearly* (through Δp) on the concentration of the anesthetic, as evident from eq 1. Thus, a variation in anesthetic concentration requires a much larger shift in the relative concentrations of the protein conformational states, in order to maintain equality of their chemical potentials.

If, as has been presumed, anesthetics act by *inhibiting* the conformational change of an intrinsic membrane protein, then the predictions are consistent if the conformational change is accompanied by a shift in the cross-sectional area greater near the aqueous interface than in the middle of the bilayer, i.e., $\Delta A(\text{interface}) > \Delta A(\text{center})$. However, if anesthetics act rather by *potentiating* some protein conformational change, then the theory is still consistent if the protein is found to have the *opposite* ΔA distribution, i.e., with $\Delta A(\text{interface}) < \Delta A(\text{center})$.

Clearly, there are many other processes involving conformational changes in intrinsic membrane proteins whose sensitivity to variations in membrane composition may also result from induced changes in the lateral pressure profile. It is tempting to speculate that adaptation of membranes to altered composition is driven by the need to establish the particular lateral pressure profile required to maintain the conformational population distribution of intrinsic membrane proteins.

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