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EFFECTS OF SMALL NONPOLAR MOLECULES ON MEMBRANE COMPRESSIBILITY AND PERMEABILITY

A THEORETICAL STUDY OF THE EFFECTS OF ANESTHETIC GASES

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We explore from a theoretical perspective the effects of small nonpolar molecules, such as anesthetic gases, on membrane compressibility and permeability. As a model system we expand a previously proposed generalization of Nagle's model for biomembrane phase transitions. In this model anesthetic gases alter membrane compressibility, causing profound changes in membrane permeability. Anesthetics either increase or decrease membrane permeability, depending on whether the membrane lipid is originally in the solid or melted state, or in a two-phase region. These changes are reversed by high pressure, in agreement with experimental results. Anesthetic-induced changes in compressibility are predicted to inhibit fusion of phospholipid vesicles to each other and to planar bilayers, and thus might be expected to inhibit the fusion of presynaptic vesicles with the presynaptic nerve membrane. This work provides a detailed molecular theory for many of the effects of anesthetic gases on both synapse and axon, and provides a coherent framework for understanding diverse experimental results.

1. Introduction

Although general anesthetics have been in medical use for over 150 years [1], the mechanism of action, and even whether or not all general anesthetics share a common mechanism of action, is still a matter of intense debate. Although some authors maintain that anesthesia results from direct anesthetic-protein [2,3] or anesthetic-aqueous phase interactions [4,5], most believe that the site of action is in the lipid membrane. The first and most important argument in favor of this hypothesis comes from the classic observation of Meyer and Overton [6] that the potency of general anesthetics is highly correlated with their solubility in olive oil. A number of additional observations supporting this hypothesis have been made since the time of Meyer and Overton's observations. Membrane volume and fluidity changes which occur when general anesthetics are added to the membrane are also correlated with anesthetic potency [7,8]. This has given rise to the hypotheses that anesthesia occurs when the membrane volume changes by a critical amount [7] or when the membrane free energy changes by a critical amount [9]. Trudell [10] has postulated that the lateral phase separation which occurs as the membrane melting temperature is approached from above may change the membrane's functional properties by decreasing the lateral compressibility of the cell membrane, but he does not provide a detailed molecular model. Tsong et al. [11] suggest that changes in membrane permeability induced by local anesthetics are correlated with the amount of boundary lipid separating fluid-like and gel states. They further develop a cluster model of the permeability phenomena which is similar to those which have contributed greatly to the understanding of gas-liquid condensation. Hayden [12] has proposed that carrier-mediated pore formation within the membrane is sensitive to membrane thickness, and that the changes in membrane thickness which occur as a result of interaction with anesthetic molecules have an indirect effect

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on pore formation. Needless to say, the details of a lipid-mediated mechanism of anesthetic action remain controversial.

Recently, we extended Nagle's model [13–15] of the biological membrane to include interaction of small nonpolar molecules with the membrane [16]. This model seems appropriate for theoretically exploring the effects of certain general anesthetics on membrane thermodynamic properties. Within the framework of this model we have shown that changes in membrane density and phase transition temperature which are induced by small nonpolar molecules are not independent events. A Meyer-Overton-like rule arises naturally from the model. Molecules which as a result of the strength of their interaction with the membrane are highly soluble also have the greatest effects in lowering the phase transition temperature and expanding the membrane. Although this model is rigorous only for very small anesthetic gases, we believe it is reasonable to assume that larger molecules, such as moderate-length alkanes or even alcohols, exert their influence in a qualitatively similar way.

In the present paper we generalize our earlier model of small molecule-membrane interactions to allow for nonzero lateral spreading pressures. Using the generalized model we can calculate the effects of anesthetics on membrane surface area and lateral compressibility. This enables us to describe the permeability of the membrane to non-anesthetic small molecules. We show also how lateral compressibility changes may affect the incorporation of larger molecules, such as alamethicin, into the cell membrane. Finally, we discuss how the permeability and compressibility changes of biological membranes which are induced by small nonpolar molecules might affect fusion of presynaptic vesicles to the presynaptic membrane, and in so doing provide a detailed molecular model for a lipid-mediated mechanism of general anesthesia.

2. The decorated membrane model

Nagle [13–15,17] has described several two-dimensional models of the membrane lipid melting transition which account for excluded-volume effects by transforming a chain polymer model into an exactly solvable dimer model. The polymer chains are allowed to lie on a triangular lattice (fig. 1) in which one *trans* and two *gauche* rotations are possible for each bond. In the figure, the first chain is in an all-*gauche* state, while the fourth is in an all-*trans* state. Links joined in *trans* conformations are associated with a rotational energy of 0.0 kcal/mol, while *gauche* rotations contribute a configurational energy of about 0.5 kcal/mol. As long as chains are not allowed to fold back on themselves, this chain model is isomorphic with a dimer model (fig. 2) in which horizontal dimers contribute a configurational energy ϵ, and long vertical dimers contribute a configurational energy δ. We generalized one of the models [13] which did not allow for either headgroup interactions or nonzero lateral spreading pressures, to account for interactions of the small molecules with the polymer chains and with 'vacuum' in the membrane. This was done by decorating the underlying dimer model with interstitial sites, and allowing interaction of the small molecules with the interstitial site. Use of this decoration transformation allowed us to create a model in which the combinatoric problem was

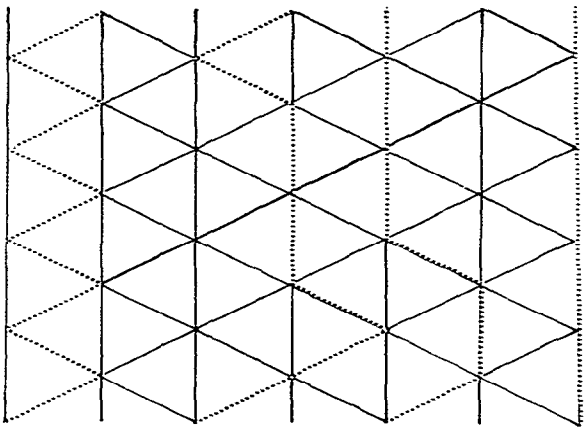

Fig. 1. Nagle's model A. Polymer chains (dotted lines) sit on an infinite triangular lattice and may under '*gauche*' rotations from the all-*trans* (completely ordered) configuration seen in the chain at the right (from ref. 16).

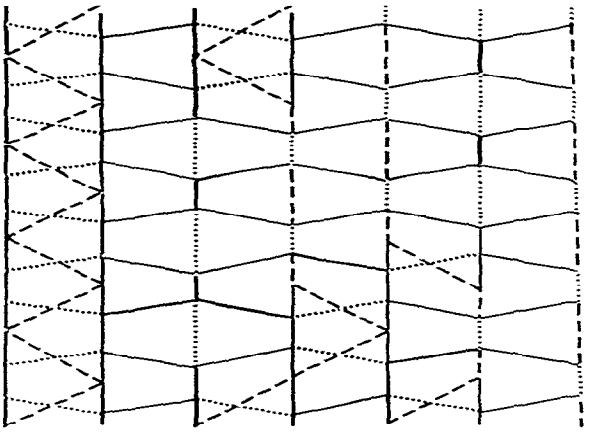

Fig. 2. Transformation of Nagle's chain model to a dimer model is done as follows: Decorate the original lattice with two new vertices placed one-third of the way from either end of each vertical link. Join each new vertex by new edges to the four closest new vertices. Place a dimer on each horizontal edge of the new lattice which crosses a horizontal chain link. Cover each vertical link of chain with a dimer placed on the corresponding short vertical edge and cover any vertex on the new lattice which has not already been covered by placing a dimer on the long vertical edge incident to that vertex. Transformation of the original triangular lattice to an appropriate rectangular lattice thus enables a one-to-one mapping of *trans* segments to short vertical dimers, *gauche* segments to horizontal dimers, and 'vacuum' to long vertical dimers [16].

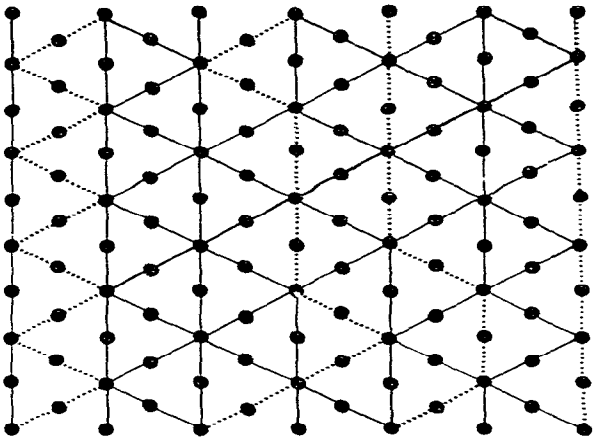

Fig. 3. Decoration of the Nagle chain model. Small molecules are permitted (in principle) to occupy interstitial sites (black dots) overlying the sides and vertices of the original triangular lattice. Transformtion of this lattice to the rectangular dimer lattice creates a decorated rectangular lattice in which there is one interstitial site for each side [16].

mathematically identical to that of the underlying dimer model, for which the solution is well known [18]. This strategy is similar to that used extensively [19] in the study of liquid solutions.

The method for decorating the original Nagle model is briefly described as follows (For a more detailed explanation, see ref. 16.): Place interstitial sites, each of which may be occupied by a small nonpolar molecule, on each primary site and halfway between each pair of primary sites. Transformation of the Nagle chain model to the dimer model yields a decorated dimer model in which every bond is covered by an interstitial site which may potentially be occupied by a small nonpolar molecule (fig. 3). This new model can be solved exactly in terms of the old. In the previous paper we calculated the effects of small nonpolar molecules on the membrane phase transition temperature and volume. In this paper our calculations extend to the membrane surface area and lateral compressibility.

Interactions of small molecules with the membrane are of two types: hard sphere repulsion when the molecule is very close to a polymer chain, and a van der Waals' attraction when it is somewhat further away. Although we previously gave the possible interactions greater generality, we assume in this paper that a small molecule cannot occupy any interstitial site which is too close to a polymer chain. Hence, we will allow anesthetic molecules to occupy only spaces which are covered by long vertical dimers, which represent vacuum in the basic Nagle model. With this assumption, the per-site partition function of the decorated dimer model on an infinite lattice is given by

$$\ln(Z)=\ln\left[Z_{\mathrm{ref}}(x,\Delta,N_x,N_y)\right] \tag{1}$$

where $y = \exp(-\delta/kT)$, $\Delta = y(1 + z\eta)$, $\eta = \exp(-\epsilon^*/kT)$, $\epsilon^* =$ energy of interaction of small molecule with vacuum, and $Z_{\mathrm{ref}} =$ partition func-

tion of the reference dimer model. Interaction energies calculated using van der Waals' interactions with only nearest neighbors for the noble gases range from approx. 0.002 (helium) to 0.2 (xenon) kcal/mol.

The per-site free energy of the dimer model is given by

$$F = -kT\ln(Z_{ref}) \tag{2}$$

and the entropy by

$$S = kV\ln(Z) - 2kV\rho_x\ln(x) - 2kV\rho_y\ln(y) + 2kV\rho_z\epsilon^* \tag{3}$$

where ρ_z is the site occupation density of the small molecule. To calculate the free energy of the chain model one realizes that it differs from that of the underlying dimer model only in energy, not entropy. In the case of the first model (lateral spreading pressure constrained to zero) the free energy of the dimer model differs from that of the chain model by a term $2V\rho_y\delta$, thus accounting for the fact that the energetic contributions of vacuum dimers to the dimer model do not directly contribute to the energy of the membrane. When the lateral spreading pressure of the membrane is not constrained to zero, the models also differ by a term $2V\rho_x\beta$, where $\beta = [\epsilon + kT\ln(x)]$. In addition, chains are held together by a van der Waals' interaction of the form aV^{-b}, where $a = 1.84$ kcal/mol and $b = 3/2$. This gives us for the Helmholtz free energy

$$F = -kT\ln(Z_{ref}) + 2kTV\rho_x\ln(x) + 2kTV\rho_y\ln(y) + 2\epsilon\rho_x V + aV^{-b} \tag{4}$$

and for the Gibbs free energy

$$G = -kT\ln(Z_{ref}) + 2kTV\rho_x\ln(x) + 2kTV\rho_y\ln(y) + 2\epsilon\rho_x V + aV^{-b} + PV + \Pi A. \tag{5}$$

The volume and area of the chain model are given in terms of the densities of x and y dimers as [14]

$$A = (1 - 2\rho_y - \rho_x)^{-1}$$
$$V = (1 - 2\rho_y)^{-1} \tag{6}$$

where the dimer densities are given by

$$\rho_x = 0.50 - (0.5/\pi)\cos^{-1}\left[1 - (1+\Delta)^2/(2x^2 + 2\Delta)\right]$$
$$\rho_y = 0.25 - 0.5\,\rho_x \mp (0.25/\pi)\cos^{-1}\left[1 - (1-\Delta)^2/2x^2\right] \tag{7}$$

where the upper sign is used if $\Delta < 1$, and $x = \exp(-\epsilon/kT)$, $y = \exp(-\delta/kT)$, $\Delta = y(1 + z\eta)$, $\ln(Z) = \ln[Z_{ref}(x, \Delta, N_x, N_y)]$; and k is Boltzmann's constant, T the temperature (K) and z the small molecule activity. The partition function Z is given by

$$\begin{aligned} &\ln(Z) = 0 && \text{for } \Delta \leq 1 - 2x \\ &\ln(Z) = 0.5\ln(\Delta) && \text{for } \Delta \geq 1 + 2x \\ &\ln(Z) = 2\int \rho_x \mathrm{d}\ln(x) + 2\int \rho_y \mathrm{d}\ln(\Delta) && \text{for } 1 - 2x \leq \Delta \leq 1 + 2x \end{aligned} \tag{8}$$

The site-occupation density of the small nonpolar molecule occupying the interstitial sites is given by

$$\rho_z = z\rho_y\eta/(1 + z\eta) \tag{9}$$

From the definitions

$$P = -(\partial F/\partial V)_{T,P} = -2\epsilon\rho_x - abV^{-b} + \{T[kV\ln(Z) - 2kV\rho_x\ln(x) - 2kV\rho\ln(y) - k\ln(y) + 2k\ln(x)] + 2\epsilon\}/V \tag{10}$$

$$\Pi = -(\partial F/\partial A)_{T,V} = -2V[\epsilon + kT\ln(x)]/A \tag{11}$$

we derive the lateral compressibility

$$\kappa = A^{-1}(\partial A/\partial\Pi)_T. \tag{12}$$

which may be computed numerically. The Π vs. A curves for the model are readily calculated using the procedure outlined by Nagle [14] for the nondecorated model. Thus, the earlier model is easily generalized to the case of nonzero lateral spreading pressure. This is important for considering interactions of molecules with membranes of fixed area, as well as for understanding the electrostatic effects of small molecules such as calcium on the membrane [20].

3. Effects of small nonpolar molecules on the membrane phase diagram

Figs. 4 and 5 illustrate the effect of adding small molecules to the membrane. The phase diagram is shifted to higher lateral spreading pressures, and the isotherms are spread out. The phase diagram retains the same basic shape, however. In the case illustrated, the small nonpolar molecule shifts the membrane critical point from $\Pi \cong 2.6$

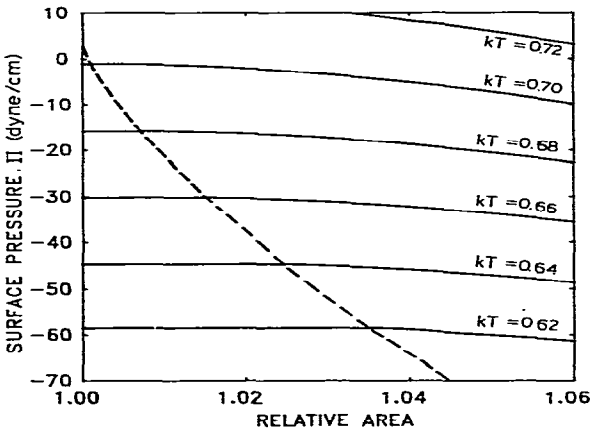

Fig. 4. Π vs. A curves for the membrane model with no anesthetic present. To the left of the dashed line both fluid and solid phases exist. To the right, only a single phase is found. The critical point is at $\Pi \cong 2.6$ dyne/cm, $kT \cong 0.705$ kcal/mol.

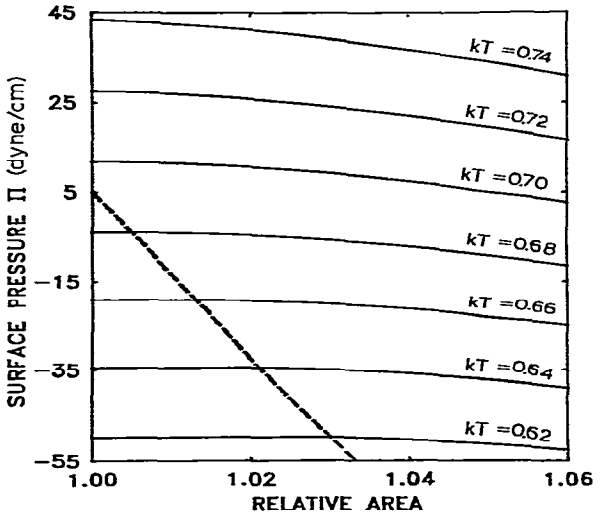

Fig. 5. Π vs. A curves for the membrane model in the presence of small molecules with activity $z = 0.5$, interaction energy $\epsilon^* = 0.5$ kcal/mol. The critical point is shifted to $\Pi \cong 5.0$ dyne/cm, $kT \cong 0.690$ kcal/mol. In addition, the vertical axis is expanded somewhat with respect to the separation of the isotherms.

dyne/cm, $kT = 0.705$ kcal/mol, to $\Pi \cong 5.0$ dyne/cm, $kT = 0.690$ kcal/mol. These changes are reversed by palcing the system under sufficiently high pressures. For a given interaction energy with the membrane, the changes in the phase diagram are monotonic with increasing activities of small molecules. For molecules with different interaction energies, the change in the activity required to produce any given membrane change is readily predicted from eq. 9 (at a given temperature T). It is apparent that addition of small molecules to the system can move the membrane from a two-phase to a one-phase region when the surface area is held constant. The importance of this finding will be discussed later in the paper.

While we have not investigated in detail the behavior of the model's critical exponents, a cursory examination suggests that there is little change from that of the undecorated model when the small molecule activity z is small. This aspect of the model is under continuing investigation.

4. Effects of small nonpolar molecules on the surface area

In fig. 6 we illustrate the change of surface area seen on addition of small nonpolar molecules to a membrane with lateral spreading pressure constrained to zero. The small molecules expand the membrane, especially at the phase transition temperature. The importance of this effect diminishes significantly within a few degrees above the phase transition temperature. The effect on membrane volume is much smaller than the effect on surface area, resulting in a decrease in membrane thickness which is almost inversely related to the increase in membrane surface area (adding small molecules with $\epsilon^* = 0.5$ kcal/mol, $z = 0.2$ at $T = 355$ K results in a 0.172% increase in volume and a 2.4% increase in surface area). Both membrane expansion and decrease in thickness have been shown experimentally to occur on addition of anesthetic molecules to the membrane. These findings support the contention of Trudell [21] that the increase in membrane volume is not a 3/2 power of surface expansion, as previously supposed [22]. The relative magnitudes of the volume and surface

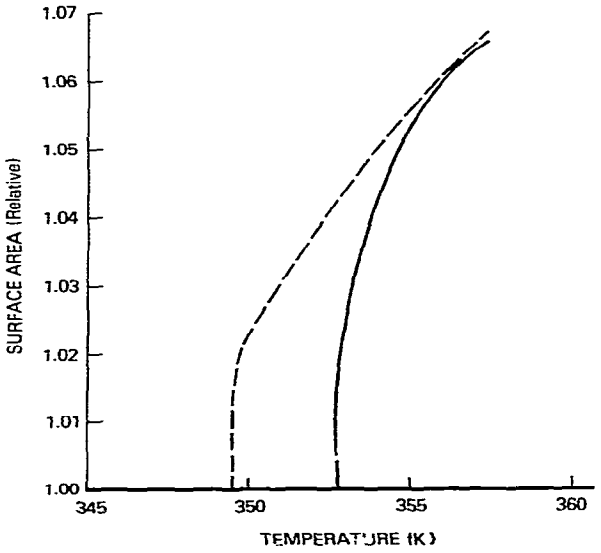

Fig. 6. Relative surface area vs. temperature for the membrane model with (- - - - - -) and without (————) small molecules present with activity $z = 0.2$, interaction energy $\epsilon^* = 0.5$ kcal/mol, and pressure $P = 1.5$ atm.

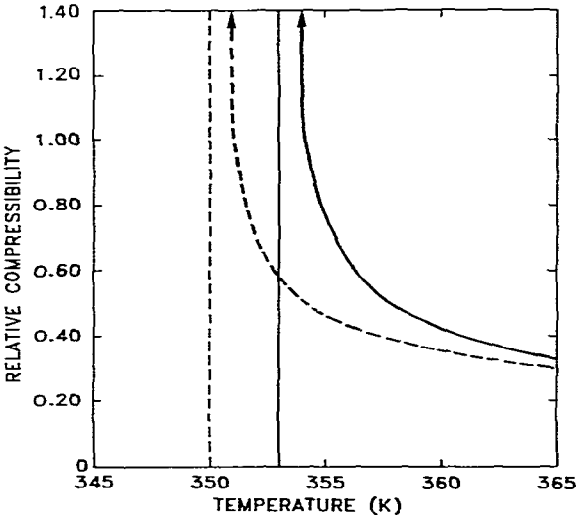

Fig. 7. Lateral compressibility relative to that at 355 K vs. temperature for the membrane with (- - - - - -) and without (——) small molecules at activity $z = 0.5$, interaction energy $\epsilon^* = 0.5$ kcal/mol. and pressure $P = 0.0$ atm.

area increases are similar to those observed experimentally: a 1.4% volume expansion of a dipalmitoylphosphatidylcholine bilayer was measured under conditions where the surface area increased by 21% [23]. The anesthetic-induced membrane expansion can account for increased lateral diffusion rates in the membrane through the use of the free volume diffusion model of Galla et al. [24].

5. Effects on the lateral compressibility and membrane permeability when the lateral spreading pressure is held constant

In fig. 7 we see a plot of the membrane lateral compressibility versus temperature with and without small molecules present. The lateral compressibility has a peak at the phase transition temperature, and falls off instantly at lower temperatures and slowly at higher temperatures. The decrease with increasing temperature has been experimentally verified for the red blood cell; the peak was not observed, however, perhaps as a result of cholesterol in the membrane or the relatively high experimental temperatures [25]. The effect of the anesthetic is to shift the lateral compressibility curve to lower temperatures and to broaden the peak slightly, similar to its effect on the heat capacity curve [16]. Small amounts have little effect on the maximum peak height, but larger concentrations can have appreciable effects. Nagle and Scott [26] have related the noncarrier-mediated small molecule permeability g of the membrane to the lateral compressibility, arriving at the expression

$$g = C_0 + C_2\kappa \tag{13}$$

Since the permeability is directly related to lateral compressibility, which is in turn a function of anesthetic concentration, we may see that an increase in the concentration of anesthetic might be expected to rapidly increase membrane permeability at temperatures below the original phase transition temperature, and to more gradually decrease

it at temperatures above the phase transition temperature. Qualitatively similar results would be expected using the permeability theory of Doniach [27]. Thus, depending on the temperature, either an increase or a decrease in permeability to such species as Cl^-, Na^+ and H_2O might be found. Increasing the anesthetic activity causes a monotonic decrease in the compressibility (and hence the permeability (fig. 8). Although general anesthetics have been found to increase the permeability of membranes to the above molecules [9,25] and there is a decrease in uptake of catecholamines by chromaffin granules in the presence of anesthetics [28], no experiments have determined the detailed temperature dependence of this phenomenon, and none has provided information on the experimental temperature relative to the phase transition temperature of the model system studied. Such results are available for local anesthetics, however, and show a temperature-dependent effect similar to that predicted above [11]. The change in phase transition temperature, the predicted ordering of the permeability changes, and the widening of the permeability curves which we predict for general anesthetics are all seen with local anesthetics. While these results are encouraging, they do not provide a sufficient experimental test of these theoretical results. Conversely, experiments relating anesthetic concentrations to membrane or vesicle permeability without considering detailed temperature effects are not very valuable. The effect of anesthetic on the isothermal compressibility curve is strongly dependent on the strength of interaction with the membrane. The result is that, for a given anesthetic activity, the isothermal compressibility (and thus the membrane permeability) is inversely correlated with the solubility of anesthetic in the membrane (fig. 9). This relationship between membrane function and lipid solubility is analogous to the Meyer-Overton rule.

The idea that lateral compressibility may be related to ionic permeability may extend to carrier-mediated ion transport as well. Consider, for example, the effect of the lipid on the barrel-stave model of pore formation [29,30]. In this model, linear subunits which may form trimer and tetramer ion-permeable channels are associated with the membrane. In the resting state the majority of subunits are found on the surface, with only a few units embedded in the membrane. When a potential is applied to the membrane, units insert into the membrane and associate to form the trimeric

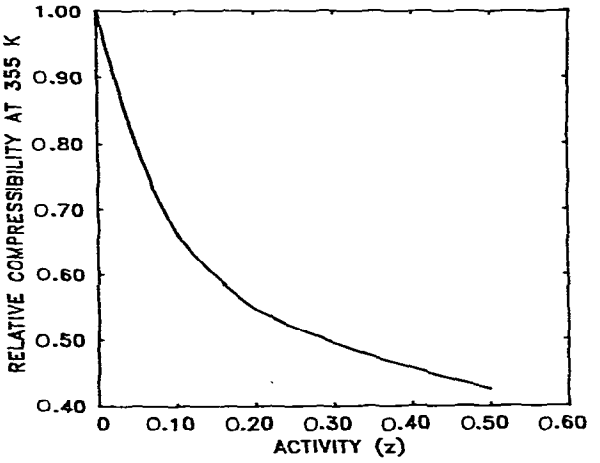

Fig. 8. Lateral compressibility relative to that of the membrane without anesthetic at 355 K, $\Pi = 0.0$ dyne/cm, pressure $P = 0.0$ atm, vs. the activity of a small molecule with interaction energy $\epsilon^* = 0.5$ kcal/mol in the membrane.

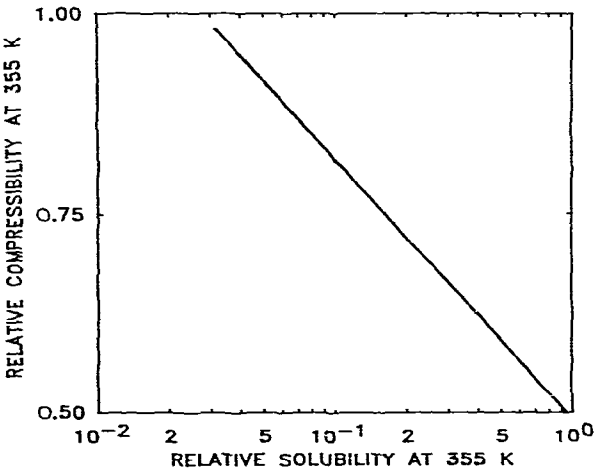

Fig. 9. Lateral compressibility relative to that of the anesthetic-free membrane vs. the solubility of small molecules at activity $z = 0.1$ at 355 K, $\Pi = 0.0$ dyne/cm.

and tetrameric conducting channels in the membrane. The energy required to embed a unit in the membrane is given by

$$E = E_L + E_A + E_R \tag{14}$$

where E_L is the free energy of lipid-barrel-stave interaction, E_A the free energy required to expand the membrane, enough to incorporate a unit, and E_R the energy required to rotate a molecule from horizontal to vertical. If we assume the energy of lipid-stave interaction to be independent of the fluidity of the membrane, then the fraction of units embedded in the membrane is given by

$$N/N_0 = \exp(-E/kT) \tag{15}$$

where N_0 is the number of available units. The free energy cost of expanding the membrane (and hence E_A) is expected to change as

$$E_A = 0.5\Delta A^2/(A\kappa) \tag{16}$$

and the steady-state Na^+ conductivity, which is proportional to the number of trimers in the membrane, is thus given by

$$g = KN_0^3 \exp[-3(E_L + E_R)/kT] \exp[-1.5(\Delta A)^2/A\kappa kT] \tag{17}$$

Increasing the temperature once above the phase transition temperature has the effect of excluding pore-forming molecules from the membrane and giving rise to a higher fraction of closed pores. Such an effect has been experimentally observed for the alamethicin system [31]. Increasing the concentration of anesthetic may either increase or decrease the permeability, depending on the temperature relative to the membrane phase transition temperature, as a result of these lateral compressibility changes. A biphasic response of the membrane is not expected. Similar effects would be expected for two-conformation molecular channels (such as gramicidin) buried within the membrane. The membrane permeability should be proportional to the number of open channels. This in turn will depend, at constant temperature and pressure, on the energy cost of compresssing the membrane (eq. 15) giving

$$g \propto \exp(-0.5\Delta A^2/A\kappa). \tag{18}$$

The number of open channels thus decreases with increasing anesthetic concentration. The compressibility effect again mimics that of increasing temperature, the channel-closing effects of which have also been demonstrated, albeit at a temperature somewhat below the phase transition temperature for the pure-lipid system [32]. The discrepancy in threshold temperature may simply reflect the composition dependence of phase transition temperature in lipid-protein systems, while the fact that conductance does not cease entirely probably results from the fact that (unlike the Nagle model) the compressibility does not drop to zero below the phase transition temperature. Such an effect could possibly explain the effect of anesthetics on the postsynaptic membrane as well as on the axon. This effect was first hypothesized by Trudell [10], who did not, however, provide a detailed molecular model. In addition, by decreasing the activation energy required for channel closure, the compressibility effect could in principle explain the reversible dose-dependent speeding up of Na^+ channel inactivation which is caused by anesthetics [33]. A related membrane-mediated effect has been proposed by Hayden [12], who relates an increase in bilayer thickness to channel closure. such a model, though reasonable for hydrocarbon molecules which might be expected to partition themselves between the layers of the bilayer biological membrane [34], does not seem reasonable for small anesthetic gases, or even alcohols, which would be expected to thin the membrane as predicted by this model, rather than thicken it.

6. Effects on the lateral compressibility and membrane permeability when the surface area is constant

The compressibility changes calculated earlier are valid only for a membrane constrained to a fixed lateral spreading pressure. It seems likely in many biological systems that the surface area, rather than the surface pressure, will be invariant. This is because nerves, as well as most noncirculating cells, have abundant inelastic cytoskeletal elements which will tend to preserve membrane size and shape, and are surrounded by an abundant

collagen matrix. In this case the membrane behaves in a qualitatively different manner. In figs. 4 and 5 we have seen the Π vs. A curves plotted for the membrane in the presence and absence of anesthetic molecules. Note that the presence of anesthetic may cause the membrane to change from a state in which two phases are present to a state in which a single phase is present. If the membrane is originally in a single-phase region, the compressibility will decrease with any increase in anesthetic concentration and the permeability and other changes can be expected to behave much as described in the previous section. In the two-phase region this is incorrect, however. We expect the solid phase to be impermeable to small molecules; therefore

$$g = C_0 + C_2\kappa' X \tag{19}$$

where X is the mole fraction of the liquid phase, given by

$$X = \left[(A_{\text{mem}} - 1)/(A_{\text{liq}} - 1)\right] \tag{20}$$

where A_{mem} is the relative area of the membrane, A_{liq} the area of a molecule in the liquid region relative to that in the solid phase, and κ' the compressibility of the liquid phase. As the amount of small molecule in the membrane increases, and as long as the membrane remains within the two-phase region, both the compressibility and the mole fraction of the liquid phase increase, with a concomitant increase in the permeability of the membrane (fig. 10). Once the membrane is in a one-phase region, however, the permeability is expected to decrease, as above. In other words, a biphasic response to increasing concentrations of anesthetic in the membrane is predicted. This biphasic change in the compressibility will be reflected in other membrane functions which depend directly on the compressibility, as discussed above and below.

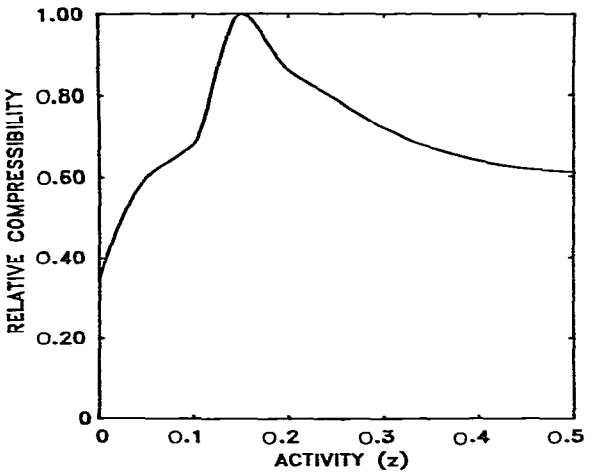

Fig. 10. Lateral compressibility relative to that of the anesthetic-free mebrane vs. the activity of small molecules with interaction energy $\epsilon^* = 0.50$ kcal/mol when the membrane is constrained to a fixed area $A = 1.013$.

7. Effects on the presynaptic nerve terminal

Remler [35] has proposed a model for the presynaptic release of neurotransmitter in which the process may be separated into two components—reaching of the synapse wall by the vesicle and fusion of the vesicle to this wall. Concentration of vesicles near the synapse, and thus the probability of reaching the synapse wall, is proposed to be determined by the presence of a local Na^+ leak, reducing the membrane voltage by 10%. In the present theory, such a leak would be reduced by the presence of anesthetic molecules, the local concentration of vesicles would decrease, and the probability of collision of a vesicle with the membrane would similarly decrease. Remler proposes that the compound probability of release of a vesicle from the presynaptic nerve terminal is given by

$$P = MI/[1-(1-M)(1-I)] \tag{21}$$

where I is the probability of reaching the synapse wall and M the probability of release (to be discussed further below). Under resting conditions I is small and rate limiting. In this case, the frequency of spontaneous miniature end-plate potentials is expected to be reduced by anesthetics because a smaller number of presynaptic vesicles reach the presynaptic membrane due to the anesthetic-induced decrease in Na^+ leakage. We are not aware

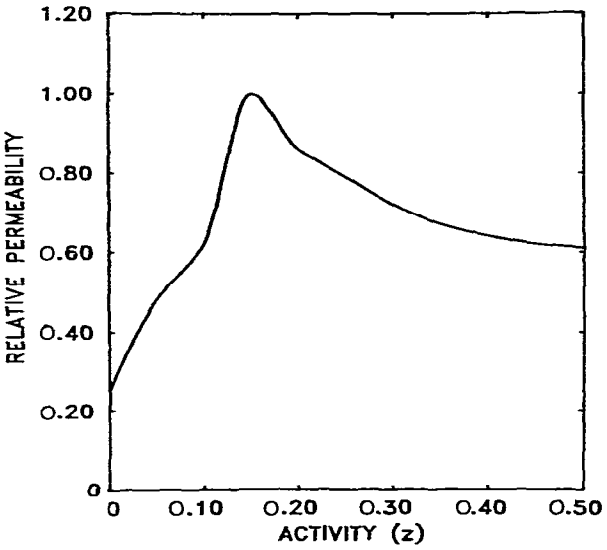

Fig. 11. Relative permeability of the membrane at 355 K vs. the activity of small molecules with interaction energy $\epsilon^* = 0.5$ kcal/mol when the membrane is constrained to a fixed area $A = 1.013$. The initial area and membrane parameters were chosen here to accentuate the possible permeability increase. A much less pronounced increase is seen for many possible combinations of parameters and membrane area.

of any evidence tending either to confirm or refute this suggestion.

Remler speculates that when the action potential is propagated to the synapse, the fusion of vesicles with the membrane begins to dominate. There is experimental evidence [36] to show that fusion of vesicles to each other or to planar membranes is greatly enhanced as the membrane phase transition is approached, an effect which may be related to bilayer compressibility or to cluster formation (lateral phase separation). It may be speculated that the role of the Ca^{2+} surge which accompanies neurotransmitter release at the presynaptic nerve terminal may be to promote such a fusion by raising the membrane phase transition temperature to a level nearer the operational temperature of the organism. The effect of Ca^{2+} is thus similar to that of increased pressure. Our model is readily generalized to include a speculation by Papahadjopoulos et al [36] regarding the role of membrane compressibility in vesicular fusion. Consider the schematic diagram of vesicular fusion shown in fig. 11. We propose that fusion of vesicles to the presynaptic membrane goes via a transition state (b) in which both the vesicle and the presynaptic membrane must form a hole of critical area A. Though not quite identical to other models which have been proposed, this hypothesis is consistent with experimental observations of point defects in bilayer fusion [32]. The energy required to form each such defect is given by

$$E = 1/2(\Delta A)^2/A\kappa \tag{22}$$

giving a contribution to the activation energy for such a process proportinal to $\Delta A^2/A\kappa$. The probability of fusion M of a phospholipid vesicle when encountering the membrane will now have the form

$$M = K' \exp\left[-(\Delta A)^2/A\kappa_{ves}kT\right] \exp\left[-(\Delta A)^2/A\kappa_{mem}kT\right]. \tag{23}$$

Note that the peak rate of fusion is expected to correspond to the permeability maximum, an effect which has been confirmed experimentally [37]. Thus, the effect of anesthetic-induced compressi-

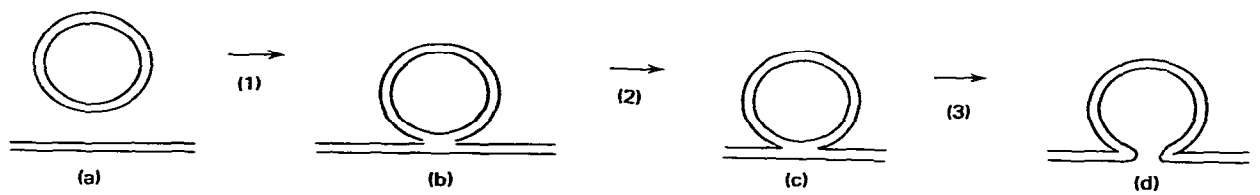

Fig. 12. Proposed steps for fusion of phospholipid bilayers. Step 1, the formation of small defects (b) in the opposing bilayers, is assumed to be rate limiting and to lead to the formation of a partially fused complex (c) in which the progression to a completely fused single bilayer (d) is favored.

bility changes is to reduce the number of vesicles released when the nerve is stimulated. Such an effect has been observed experimentally for both the barbiturates and halothane [38]. While these compressibility effects may explain the biphasic nature of the anesthetic response seen both clinically and in isolated nerve preparations, they do not readily explain the profound changes in the spike pattern of the isolated nerve which seems to be dependent not only on the presence of anesthetics, but also on the nature of the anesthetic agent [39].

8. Discussion

We have provided a detailed molecular theory of the mechanism of general anesthetic effects, by generalizing our previous theoretical analysis of the effects of general anesthetics on biological membranes to include the effect on surface area, membrane thickness and lateral compressibility (and thus to both direct and carrier-mediated ion transport, and vesicular fusion). The lipid state of the membrane is shown to influence the membrane permeability via changes in the lateral compressibility, as shown by Nagle and Scott [26]. Perturbations of the lipid state caused by anesthetic molecules may thus have an effect on both mediated and nonmediated ion transport. The generalized model of the anesthetic-membrane interaction thus developed predicts the experimentally observed anesthetic-induced membrane permeability increase at temperatures below the normal phase transition temperature of the membrane and predicts reversal of this change by pressure. At temperatures above the membrane's normal phase transition temperature, an anesthetic-induced permeability decrease is predicted. This effect is also antagonized by pressure. This pressure effect, which occurs as a result of excluding anesthetic molecules from the membrane, is well known clinically and experimentally. Both carrier-mediated and noncarrier-mediated permeability changes induced by small nonpolar molecules might be expected to result in many of the physiologic changes seen in general anesthesia. A similar explanation may be responsible for the inhibition of red cell hemolysis by general anesthetics [40].

The model also predicts increases in compressibility, and hence permeability and fusion rate, when the area of the membrane is held fixed. In reality, neither the assumption of fixed membrane surface pressure nor surface area is likely to be correct, but the biologically correct assumption, that certain external ion concentrations are held fixed, is expected to give behavior somwhere between the two extremes which we have considered.

A model for the fusion of presynaptic vesicles to the presynaptic membrane predicts a similar dependence of the probability of fusion on the lateral compressibility. As a result, increasing the concentration of anesthetic at temperatures above the phase transition temperature of the membrane decreases the rate of fusion. Thus, the suggestion that the anesthetic may then exert its effects via lateral compressibility changes is in keeping with experimental observations on the mechanism of action.

Although analysis of the van der Waals' interactions results in a correct ordering of the relative potencies of the noble gases as anesthetics, it predicts that helium should serve as an extremely weak anesthetic, in that it causes the same permeability changes in this membrane as caused by the other noble gases. The pressure effect does not overcome the membrane-expanding effects, which seem to occur for any reasonable energy of interaction with the membrane. This result is strongly in contradiction to experiment [41], but the reason for the discrepancy is unclear. One likely explanation is the two-dimensional nature of the model, which accentuates the effects of excluded-volume-type interactions relative to three dimensions, in addition to the fact that the model ignores the effects of membrane expansion on lipid-small molecule van der Waals' interactions.

The model we have chosen, which is exactly solvable, is particularly appropriate for studying the effects of anesthetics on membrane permeability and fusion, since both phenomena appear to be related to the occurrence of critical fluctuations in the membrane. While physically more realistic models for biomembrane phase transitions have been proposed, the mean-field approximations required to solve them are expected to give qualita-

tively inferior results when discussing membrane properties near the critical point [17]. It is the fact that the present work relates anesthetic effects to the occurrence and size of critical fluctuations in the membrane, as well as the detailed physical modeling, which distinguishes it from all previously proposed theories of anesthesia.

While the mechanism we have used for accounting for the interaction of anesthetics with the membrane, the decoration transformation, is particularly interesting when applied to Nagle's models, similar manipulations may easily be carried out for other models [42]. In addition, preliminary results obtained using an approach similar to the scaled particle theory of solutions [43], suggest that the results which we have presented are qualitatively the same for molecules inserting in the membrane at the lipid/water interface. Study of the qualitative differences and similarities in the interactions modeled through the use of these and other [34,44,45] models should greatly enhance our understanding of the mechanisms of action of anesthetic drugs.

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