Anesthetics Reduce the Magnitude of the Membrane Dipole Potential. Measurements in Lipid Vesicles Using Voltage-Sensitive Spin Probes[†]

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ABSTRACT: Lipid membranes possess a large internal dipole potential that greatly exceeds the magnitude of typical transmembrane or surface potentials. The volatile general anesthetics, halothane, isoflurane and enflurane were tested by the use of positively and negatively charged hydrophobic ion spin labels in lipid bilayer vesicles for their ability to modulate the membrane dipole potential. These anesthetics decreased the binding of negatively charged hydrophobic ion spin probes based on trinitrophenol, but increased the binding of positively charged hydrophobic ion probes based on triphenylalkylphosphoniums. They also enhanced the transit rates for both hydrophobic anions and cations; however, translocation rates were enhanced to a greater extent for the cation probes compared to the anion probes. The changes in binding constant for cations versus anions could be accurately accounted for using a simple model for the free energy profile for hydrophobic ions across membranes, and indicate that these anesthetics decrease the membrane dipole potential. From a fit of the experimental data to this model, anesthetics could promote a decrease in the dipole potential in two ways. First, anesthetics appear to modify the effective dipole moment in the membrane interface and may accomplish this by orienting their molecular dipole antiparallel to the intrinsic dipoles at the interface. Second, they modify the membrane dielectric constant, leading to a decrease in the field across the interface. At equivalent membrane concentrations, isoflurane, enflurane, and halothane produced similar changes in the dipole potential and decreased the dipole potential as much as 65 mV at a membrane mole fraction of 0.20. With conversion of partial pressures of anesthetic at 37 °C to membrane concentrations, dipole potential changes at equivalent MAC concentrations were estimated to be about 10 mV at 1 MAC. These changes in interfacial potential could modulate the conformational equilibria of membrane proteins exhibiting certain voltage-dependent conformational transitions.

Biological membranes possess a large internal potential that is independent of ionic strength and has therefore been referred to as a "dipole" potential, ϕ_d. This potential is large, and it is estimated from hydrophobic ion partitioning and transport in phosphatidylcholine vesicles and planar bilayers to be on the order of 300 mV, hydrocarbon positive (Pickar & Benz, 1978; Flewelling & Hubbell, 1986b; Franklin & Cafiso, 1993). Electrostatic measurements in monolayer systems also provide evidence for this large hydrocarbon positive potential (Simon & McIntosh, 1989; Smaby & Brockman, 1990). The origin of this potential is not completely understood; however, it appears to arise from a dipole layer within the membrane−solution interface due to the net orientation of the carbonyl oxygens and/or the water in this region (Cafiso, 1994). Because these dipole layers are symmetric about the membrane center, electrodes placed across the membrane do not measure the dipole potential.

The binding and transport of certain organic ions, referred to as hydrophobic ions, provides dramatic evidence for the presence of the dipole potential, and spin-labeled analogs of these ions provide ideal probes for this potential in lipid vesicles (Flewelling & Hubbell, 1986b; Franklin & Cafiso, 1993). Shown in Figure 1 is a schematic representation of the energy terms that contribute to the free energy profile for hydrophobic ions across membranes. The energy profiles for positive or negative hydrophobic ions such as tetraphenylborate or tetraphenylphosphonium are qualitatively represented in Figure 1B. These energy profiles are the sum of three terms: a term for the Born or electrostatic charging energy of the ion in the membrane low dielectric, ΔG_{Born}; a term for the hydrophobic interactions of the ion with the hydrocarbon interior, ΔG_{hydro}; and a term due to the electrostatic contributions of a dipole layer at each interface, ΔG_{dipole}. The resulting free energy profiles exhibit a diminished energy barrier in the bilayer center and free energy minima at the membrane−solution interface. As a consequence of the dipole potential, negatively charged hydrophobic ions transit membranes much more rapidly and bind much more strongly than do comparable cations. In fact, hydrophobic anions and cations can have translocation rate constants that differ by as much as 10^7 as a result of the dipole potential. When the binding and translocation rates for a wide range of hydrophobic ions are examined, the simple analysis depicted in Figure 1 is found to accurately account for the data (Flewelling & Hubbell, 1986b; Franklin & Cafiso, 1993).

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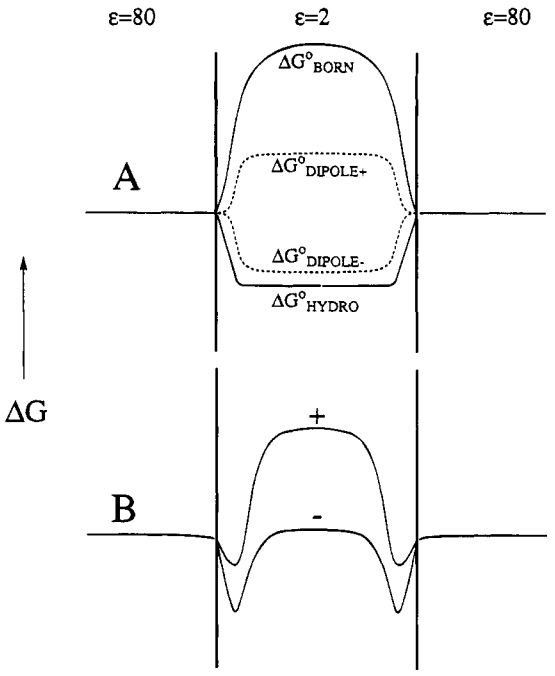


FIGURE 1: (A) Sketch of the free energy profiles for several terms that contribute to the energy of hydrophobic ions in bilayers. (B) Sum of the electrostatic charging energy, ΔG_{Born}, the hydrophobic binding energy, ΔG_{hydro}, and the dipole energy, ΔG_{dipole}, for positive and negative hydrophobic ions. The anion exhibits a deeper binding minimum in the interface and a lower energy barrier compared to the cation.

The molecular basis for anesthesia has been the subject of a wide range of experimental studies on biological membranes and proteins (Miller, 1985; Franks & Lieb, 1994). Although a large number of membrane properties have been examined, relatively few studies examining the effect of anesthetics on the membrane dipole potential have been undertaken. Many anesthetics have modest dipole moments, and local (Kelusky & Smith, 1983; Sweet et al., 1987; Auger et al., 1988) as well as general anesthetics (Trudell & Hubbell, 1976) are present to some extent at the membrane–solution interface. As a result, the membrane dipole potential is an important membrane property that is likely to be modulated by at least some anesthetics. Previous work on the conductance rates of lipophilic ions in bilayers (Reyes & Latorre, 1979) and on lipid monolayers (Simon & Bennett, 1980) indicates that chloroform and alcohols depress the dipole potential, but studies on the effects of other general anesthetics have not been determined.

A connection between the dipole potential and the gating of channels or receptors is not difficult to imagine. Transmembrane electrical potentials modulate the activity of ion channels, and a depolarization of a few tens of millivolts is known to gate ion channels such as the sodium channel of the nerve axon. Although the molecular mechanisms that underlie this process are not yet understood, they must involve the movement of charges or dipoles within the membrane interior so that the free energies of open and closed channel conformations are modulated by the transmembrane electric field. Voltage-dependent conformational events may also be modulated by changes in the dipole potential. The dipole potential is likely to fall across a relatively short distance, resulting in an extremely large electric field within the membrane interface. Conformational changes in ion channels or receptors that involve the movement of charges or dipoles across the membrane interface could be strongly influenced by this potential.

In this work, we utilize the spin-labeled hydrophobic ions **I**–**III** shown in Chart 1 to examine the effects of halothane, enflurane, and isoflurane on the membrane dipole potential. The procedure that we use is similar to one we used previously to examine the effects of phloretin and 6-ketocholestanol on the magnitude of the membrane dipole potential in vesicles (Franklin & Cafiso, 1993). The data provides strong evidence that these anesthetics diminish the magnitude of the dipole potential and produce changes that could be significant at clinical anesthetic concentrations.

EXPERIMENTAL PROCEDURES

Materials

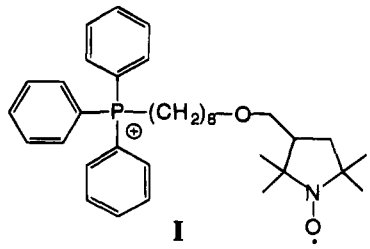
Egg yolk phosphatidylcholine (egg PC)[1] was isolated from fresh hen eggs following the procedure of Singleton et al. (Singleton et al., 1965) and was stored in a chloroform solution at −20 °C until use. The spin-labeled phosphonium **I** was synthesized as previously described except that dioctyl bromide was coupled to a five-membered proxyl nitroxide rather than the six-membered TEMPO nitroxide (Flewelling & Hubbell, 1986a). The synthesis and characterization of spin-labels **II** and **III** are described in detail elsewhere (Franklin et al., 1993). Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) was obtained from Halocarbon Laboratories (Hackensack, NJ), enflurane (2-chloro-1,1,2-trifluorethyl difluoromethyl ether) was from Ohio Medical Products (Madison, WI), and isoflurane (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether) was from Anaquest (Madison, WI).

Methods

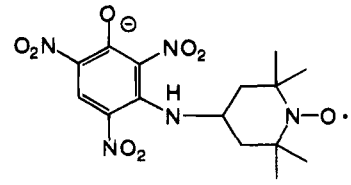
Preparation of Lipid Vesicles. Aliquots of egg PC in chloroform were dried under a stream of nitrogen and vacuum desiccated for a minimum of 15 h at room temperature. The lipids at a concentration of 80–90 mM were then dispersed in a buffer containing 25 mM MOPS (4-morpholinepropanesulfonic acid) and 100 mM NaCl, pH = 7.0, and freeze–thawed five times. The lipid dispersion was then extruded through polycarbonate membranes of 0.05-μm pore size 10 times under high-pressure nitrogen gas. The vesicle suspension was then centrifuged at approximately 12000*g* for 10 min to remove any multilamellar material. The lipid suspension was then diluted in buffer to final concentrations that were used in the binding measurements. The phospholipid concentrations in the final vesicle suspensions were determined using a Fiske–Subbarrow phosphate assay similar to that described previously (Bartlett, 1959). Anesthetics and the spin probes **I**–**III** were added directly to the vesicle suspensions, vortexed, and then transferred to an EPR flat cell, which was then sealed. Following EPR spectroscopy, aliquots of the samples from the EPR cells were withdrawn and injected into vials with Teflon septums containing a chloroform/methanol 65/35 mixture. Samples of anesthetics were analyzed using a Hewlett Packard 5890

[1] Abbreviations: PC, phosphatidylcholine; halothane, 2-bromo-2-chloro-1,1,1-trifluoroethane; enflurane, 2-chloro-1,1,2-trifluorethyl difluoromethyl ether; isoflurane, 1-chloro-2,2,2-trifluoroethyl difluoromethyl ether; TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl; MOPS, 4-morpholinepropane sulfonic acid; EPR, electron paramagnetic resonance; MAC, minimum alveolar concentration.

Chart 1



I



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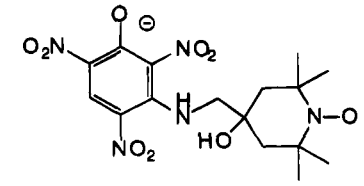

III

series II gas chromatograph using a 30-m HP-1 capillary column with a 0.53 mm i.d. and a 2.65-μm film thickness using a procedure similar to one described previously (Kusmierz et al., 1989).

The PC bilayer/buffer partition coefficients for these anesthetics were determined at 23 °C for the vesicles and buffers used here by dialysis. Approximately 1.5 mL of extruded egg PC vesicles containing anesthetic was dialyzed against a solution of 25 mM MOPS and 100 mM NaCl, pH = 7.0, using a 12−14K MW cutoff membrane. After 24 h of equilibration in a Teflon-sealed container, the anesthetic levels in the vesicle suspension and in the buffer were assayed as described above. Unless otherwise noted, these partition coefficients are given in units of (moles of anesthetic per kilogram of membrane)/(moles per liter in buffer) and were used to determine the membrane anesthetic concentrations in the experiments described below.

EPR Spectroscopy. EPR spectra were recorded on a Bruker ESP 300 spectrometer using 100-μL quartz flat cells and a standard X-band microwave cavity, and all experiments were carried out at an ambient temperature of 23 °C. An incident microwave power of 20 mW was used along with a peak-to-peak modulation amplitude of 2 G. The binding of hydrophobic ions **I**−**III** was determined as previously described from the amplitudes of the $m_I = -1$ resonance of the nitroxide spectrum (Franklin & Cafiso, 1993). The amplitude of the $m_I = -1$ resonance amplitude is proportional to the number of spins in the aqueous phase, and the ratio of membrane-bound to aqueous spins, λ, can be given by

$$\lambda = \frac{(A_f^o - A)}{[A - (b/a)A_f^o]} \tag{1}$$

where the baseline to peak amplitude of the $m_I = -1$ resonance is given by A, A_f^o is the amplitude of this resonance in the absence of vesicles, and the ratio b/a reflects the contributions that the membrane and aqueous spins make to the $m_I = -1$ resonance amplitude (Cafiso & Hubbell, 1981). The value of b/a was 0.04 for probe **I** and 0 for probes **II** and **III**.

One important parameter that will be obtained from the phase partitioning of hydrophobic ions **I**−**III** is the partition coefficient, β, which is defined as the ratio of the surface density of bound probe to the aqueous probe concentration. An expression that relates β to the phase partitioning λ is given in eq 2 and is described in detail elsewhere (Flewelling & Hubbell, 1986a).

$$\frac{1}{C_L} = \beta A_L \frac{1}{\lambda} + V_L \tag{2}$$

In eq 2, C_L is the lipid concentration, A_L is the area per lipid, and V_L is the volume per mole of lipid. The partition coefficient β has units of length, and dividing β by the thickness of the domain where the hydrophobic ion binds yields the binding constant, K, for the ion. For the probes used here we use a thickness of 4 Å for the binding region and values for A_L and V_L of 66 Å^2 and 1255 Å^3.

Transmembrane ion currents for hydrophobic ions were determined as described previously (Cafiso & Hubbell, 1982; Franklin & Cafiso, 1993). This was accomplished by rapidly mixing spin probe **III** or **I** with lipid vesicles into the EPR cavity using a precision mixing ram (Model 715, Update Instrument, Inc., Madison, WI). A time-dependent change in the $m_I = -1$ EPR resonance amplitude, A, accompanies this mixing and is a direct result of the transmembrane migration of the hydrophobic ion. The time dependence in the amplitude of A can be related to the ion current across the vesicle by

$$i = \frac{bzF}{S_o}\left[\frac{KV_{mo}/V_o}{1 - \sigma_o/\sigma_i}\right]\left[\frac{dA}{dt}\right] \tag{3}$$

where K is the binding constant for the probe to the membrane interface, V_o and V_i are the external and internal vesicle aqueous volumes, V_{mo} and V_{mi} are the external and internal membrane volumes occupied by the probe, S_o is the external vesicle surface area, $\sigma_o = (1 + KV_{mo}/V_o)$, and $\sigma_i = (1 + KV_{mi}/V_i)$ (Cafiso & Hubbell, 1982). When the initial current for the inward flow of hydrophobic ion, i_o, is determined using eq 3, the initial rate of change in the signal intensity $(dA/dt)_{t=0}$ can be related to the forward rate constant, k_f, by

$$k_f = \frac{i_o S_o}{FK_o V_{mo}[I_o]} \tag{4}$$

where $[I_o]$ is the external aqueous concentration of probe. A detailed discussion of the use and derivation of these expressions can be found elsewhere (Cafiso & Hubbell, 1982).

Estimating the Magnitude of Dipole Potential Changes. The magnitude the membrane dipole potential was estimated by comparing the free energy profiles calculated using a total potential model for hydrophobic ions in membranes with the measured binding data for hydrophobic ions **I**−**III**. A more detailed description of this model and its validity can be found elsewhere (Flewelling & Hubbell, 1986b; Franklin & Cafiso, 1993). Briefly, this electrostatic model places point dipoles due to the membrane and the anesthetic on separate square lattices at fixed distances from the bilayer center. An energy profile for the ion in the membrane is determined by summing the dipole, Born charging, and hydrophobic energies for these probe ions. This model assumes that anesthetics modify the dipole potential by inserting their molecular

dipole into the interface, although in principle alterations in the structure of the interface that change the intrinsic dipole moment of the membrane would produce identical changes. The binding energies produced by this model can be determined and compared to the experimental data by taking the energy difference between the bulk solution and the binding mimina where the ion resides, ΔG_B°. The binding constant in this case is simply $K = \exp(-\Delta G_B^\circ/RT)$. The energy difference between the minima in the interface and the center of the bilayer yields $\Delta G^{\circ\ddagger}$, where the rate constant is given by $k = f \exp(\Delta G^{\circ\ddagger}/RT)$ and f is the universal frequency factor of 6×10^{12} s^{-1} at room temperature. To model the hydrophobic ion behavior observed here, the positions of the intrinsic dipole layer, the ion sizes, and the hydrophobic interaction energies were identical to those found previously for spin probes **I** and **III** (Franklin & Cafiso, 1993). In our modeling we also allowed the dielectric constant of the membrane interior to vary with the addition of anesthetic according to $\epsilon = 2.0 + \gamma\chi$, where γ is a quantity that describes the incremental change in the dielectric constant produced by the anesthetic and χ is the mole fraction of anesthetic. Because the fits were not highly dependent on the position of the dipole layer induced by the anesthetic, we assigned this layer a distance of 19 Å from the bilayer center, coplanar with the intrinsic dipole layer. Thus, fits to the data involved varying two parameters, the size of the effective dipole moment contributed by the anesthetics, and the incremental change produced in the membrane dielectric by the addition of anesthetic.

RESULTS

Isoflurane Increases the Membrane Binding of Hydrophobic Cations and Decreases the Binding of Hydrophobic Anions. The membrane binding of hydrophobic ion spin labels to lipid bilayer vesicles in the presence and absence of isoflurane was measured directly from their EPR spectra as described above. In Figure 2, panels A and B, $1/\lambda$ (the free to bound probe ratio) is plotted as a function of the reciprocal of the lipid concentration for probes **I** and **III** in the presence and absence of isoflurane. As expected, the data are well-fit by a linear dependence of $1/\lambda$ on the reciprocal of the lipid concentration according to eq 2, and the slopes of these lines are related to the inverse of the partition coefficient for the probe. The addition of isoflurane to the bilayer increases the partition coefficient, β, for probe **I** from 5.2×10^{-5} to 8.9×10^{-5} cm (Figure 2A). In contrast, the binding of the negative trinitrophenol probe **III** decreases upon the addition of isoflurane, and the binding curves shown in Figure 2B represent a decrease in β from 23×10^{-5} to 10×10^{-5} cm. A similar decrease is also observed for the other negatively charged trinitrophenol probe, **II**. These changes occur with total isoflurane concentrations of about 2.5 mM, which corresponds to a membrane concentration of 17 mol % using the buffer/bilayer partition coefficient of 94 determined here. The differential changes that are observed in the partitioning of hydrophobic ion probes **I** and **III** to the membrane interface argue strongly that these changes are electrostatic in origin. Probe **II**, as well as several analogs of **I** with varied chain length, exhibits changes in binding with anesthetic that are determined solely by its valence, also arguing that these changes are electrostatic in origin. The increased cation binding and decreased

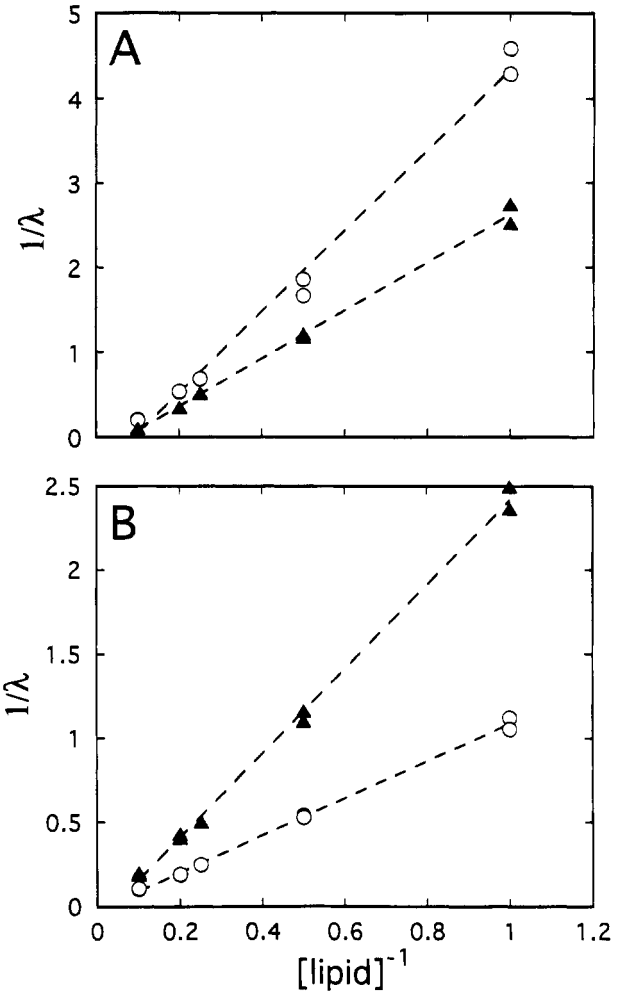

FIGURE 2: Free to bound probe ratio ($1/\lambda$) versus the reciprocal of the egg PC concentration ($1/C$) (mM^{-1}) in the absence (○) and presence (▲) of isoflurane for (A) the positive phosphonium ion (probe **I**) and (B) the anion (probe **III**). The partition coefficient for the cation probe increases, and the partitioning for the negative probe decreases, in the presence of anesthetic. The PC/buffer partition coefficient for isoflurane was found to be 94 ± 15, and the concentrations were adjusted in each sample to produce an anesthetic mole fraction in the membrane of 0.17. The total isoflurane concentration used in each case was near 2.5 mM, and greater than 70% of the anesthetic was in aqueous phase for all but the highest lipid concentration shown.

anion binding seen in Figure 2 are consistent with a decrease in the membrane dipole potential upon the addition of isoflurane.

Figure 3A shows changes in the binding of **I** and **III** which are measured as a function of the aqueous concentration of isoflurane. In the absence of isoflurane, the partition coefficient of **III** to egg PC liposomes is 5 times that of **I**. However, above a concentration of 3 mM, the binding of **I** actually exceeds that of probe **III**. Shown in Figure 3B are the ratios of the binding constants for probes **I** and **III** in the presence and absence of anesthetic plotted as a function of the membrane concentration of anesthetic. The solid lines in Figure 3B are discussed below and represent fits of the experimental data using a model for hydrophobic ion energies across membranes.

Enflurane and Halothane Also Promote Differential Changes in the Binding of Hydrophobic Anions and Cations. Enflurane and halothane were also examined to determine whether they alter the binding of probes **I**–**III**. As shown

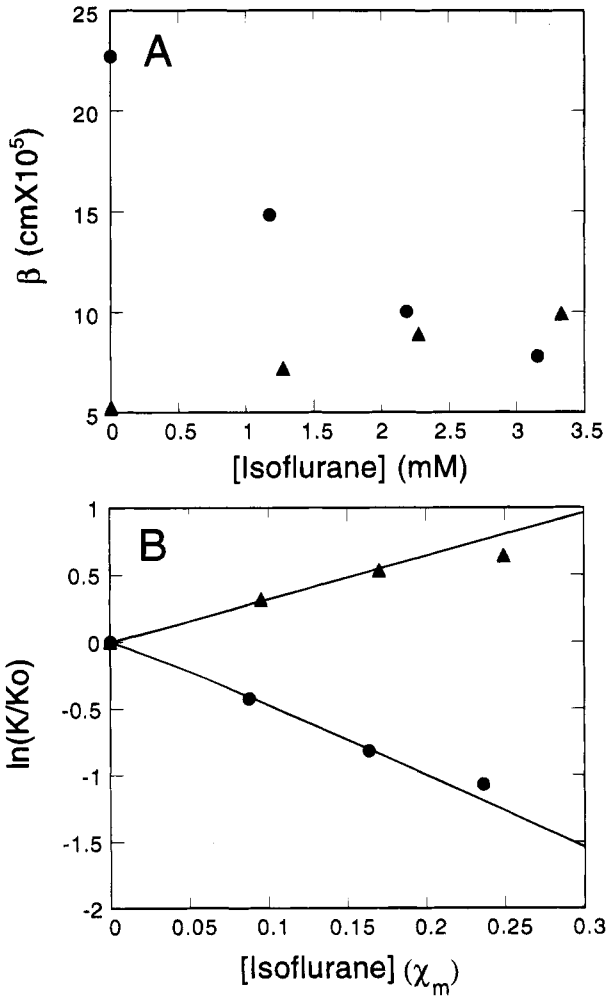

FIGURE 3: (A) Partition coefficient for the hydrophobic cation probe **I** (▲) and the anion probe **III** (●) as a function of the aqueous isoflurane concentration. The cation probes show an increase in binding with anesthetic, while the anion probes show a decrease. In (B) the ratio of the binding constants in the presence and absence of anesthetic (K/K_o) have been plotted on a log scale as a function of the mole fraction of anesthetic in the membrane. The solid lines in (B) represent the best fit of the experimental data to the electrostatic model described in the text. The parameters used in this fit are given in Table 1.

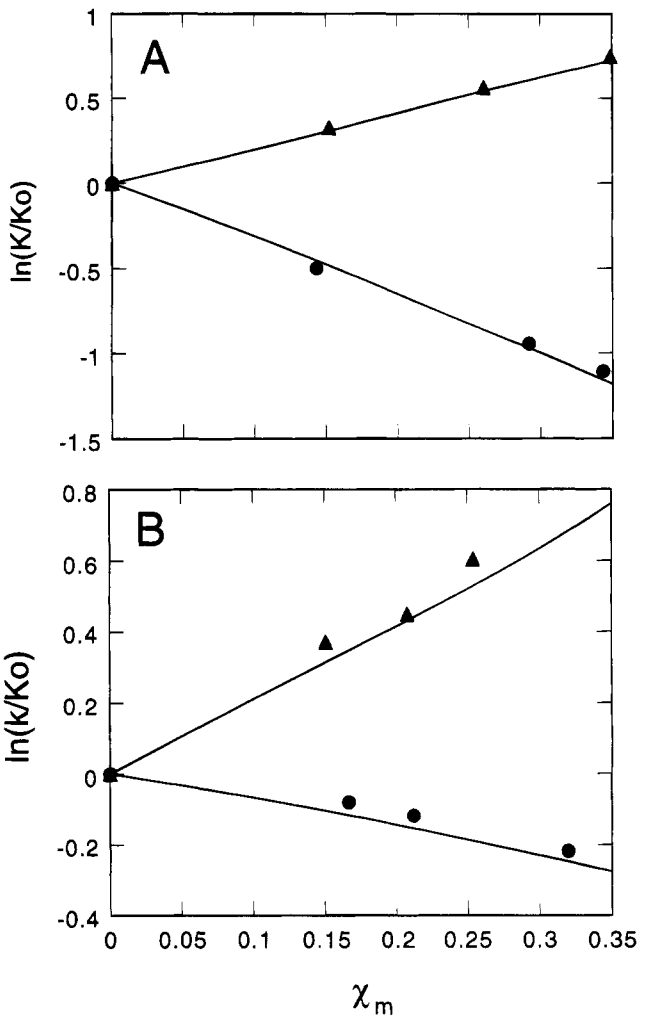

FIGURE 4: Ratio of the binding constants for the hydrophobic cation probe **I** (s) and the hydrophobic anion probe **III** (l) in the presence and absence of anesthetic (K/K_o) plotted on a log scale as a function of the mole fraction of (A) enflurane or (B) halothane in the membrane. The membrane concentrations of anesthetic were calculated using the PC/bilayer partition coefficients measured in these membranes (see Experimental Procedures). For enflurane we determined a PC bilayer/buffer partition coefficient of 64 ± 10, and for halothane we determined a partition coefficient of 93 ± 15. In each case the anesthetic was found to increase the binding of the cation but decrease the binding of the anion. The solid lines in each plot represent the best fit of the experimental data to the electrostatic model described in the text. The parameters used to fit this data are given in Table 1.

in Figure 4, panels A and B, both halothane and enflurane increase the membrane binding of the positively charged phosphonium probe **I**, but decrease the binding of the negatively charged nitrophenol **III**. This is qualitatively similar to the data shown in Figure 3, and these changes in binding are also expected if enflurane and halothane reduce the magnitude of the dipole potential. Again, the solid lines in these figures represent fits to the data using an electrostatic model and will be described below. On a molar basis, halothane had less of an effect than did either isoflurane or enflurane on the binding of these hydrophobic ions. Although the membrane concentrations of anesthetics used here are quite high, the effects of anesthetics on the dipole potential are linear and can be extrapolated to clinical anesthetic levels (see below).

Translocation Rates for Anions and Cations Are Both Increased in the Presence of Halothane, Enflurane, and Isoflurane. As discussed in the introduction, the membrane dipole potential should be revealed in both the binding and the translocation rates for hydrophobic ions. A decrease in the dipole potential should also produce differential changes in the translocation rates for these ions. Shown in Figure 5 are changes in the translocation rates that are measured in lipid vesicles in the presence of halothane for probes **I** and **III**. The translocation rates for the cation and anion probe both increase, although the cation rate is increased to a greater extent than is the anion rate. Similar changes are observed for probes **I** and **III** upon the addition of enflurane or isoflurane. The increase in the rate constants for both ions seen in Figure 5 is not entirely unexpected. Anesthetics are likely to increase the membrane dielectric constant, and this should reduce the magnitude of the electrostatic charging energy, ΔG_{Born}, for either ion. The larger enhancement in cation permeability relative to that for the anion is consistent with a reduction in the dipole potential. Similar changes in the transmembrane currents of hydrophobic ions were observed previously for chloroform in planar bilayers (Reyes & Latorre, 1979).

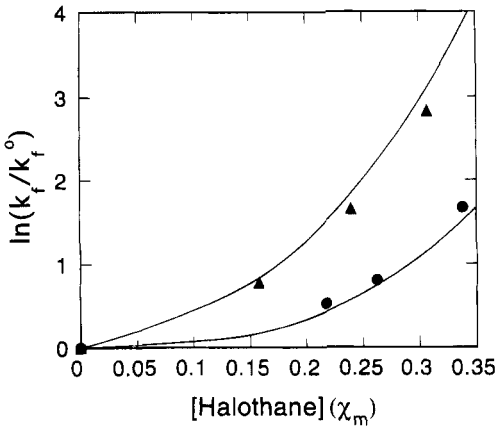

FIGURE 5: Plot of the ratio of the forward rate constants for the transmembrane migration of the hydrophobic cation **I** (▲) and the hydrophobic anion **III** (●) in the presence and absence of halothane (k_f/k_f^o) as a function of the membrane mole fraction of halothane. The solid line represents the fit that can be obtained with the data using the same change in dipole moment that was used to fit the binding data (see Figure 4B) and a value of 0.15 for γ.

Magnitude of the Dipole Potential Estimated from Hydrophobic Ion Binding. The increase in cation binding and the decrease in anion binding that are seen with these anesthetics provide a strong indication that the changes are electrical in origin. A number of changes in the lipid interface are likely to occur with anesthetic addition, but changes that alter lipid dynamics or packing are unlikely to produce differential changes in the binding of oppositely charged ions. Assuming that the binding changes are electrical in origin, it is possible to make an estimate of the magnitude of the change in the dipole potential produced by enflurane, halothane, and isoflurane from the data in Figures 3 and 4. This procedure is described briefly above and in detail elsewhere (Flewelling & Hubbell, 1986b; Franklin & Cafiso, 1993). Because the translocation rates appear to be strongly affected by changes in the membrane dielectric constant, we used the binding constant data to evaluate changes in the dipole potential and allowed the value of the dielectric constant to increase with the concentration of added anesthetic.

The fits to the binding data using this model are shown in Figures 3B and 4, and a fit to the translocation rate data in the presence of halothane is shown in Figure 5. These were the best fits that could be obtained by varying two parameters: the magnitude of the "effective dipole moment" placed at the interface per anesthetic molecule bound to the membrane and the dielectric constant increment produced by the anesthetic. As discussed below, this modeling does not necessarily distinguish the actual molecular source of the dipole potential change. Given the simplicity of the model used, the fits to the data are excellent, and the parameters used are summarized in Table 1. The binding data for isoflurane and enflurane could be well-fit without any change in the dielectric constant using effective dipole moments of 1.0 and 0.65 D, respectively, per anesthetic molecule added. The halothane binding data required increases in the membrane dielectric constant to adequately fit the data, but required a much smaller effective dipole moment (about 0.12 D) than either isoflurane or enflurane. The translocation rate data for halothane shown in Figure 5 could also be fit using the same dipole moment provided that a much smaller change in the membrane dielectric constant was used, in this case $\gamma = 0.15$.

Table 1: Dipole Potential Changes Produced by Volatile Anesthetics[a]

anesthetic	effective dipole moment (D)	γ	$\Delta\phi_d$ @ 20 mol % (mV)	$\Delta\phi_d$ @ 1 MAC (mV)
enflurane	0.65	0	−44.5	−10.5
isoflurane	1.0	0	−67.2	−10.5
halothane	0.12	1.8	−51.2	−6.7

[a] Dipole potential changes were determined by generating free energy curves for the hydrophobic ions I and III that produce good fits to the binding data. Two adjustable parameters were used to simultaneously model cation and anion binding data: the effective dipole moment of the anesthetic in the interface and γ, which describes the change in dielectric constant, ϵ, produced by the anesthetic (where $\epsilon = 2.0 + \gamma\chi$, and χ is the anesthetic mole fraction). Equivalent membrane concentrations of anesthetic at 1 MAC were estimated from the published MAC pressures and PC bilayer/gas partition coefficients at 37 °C (Firestone et al., 1986) (for this estimate we assumed that enflurane had the same partition coefficient as isoflurane).

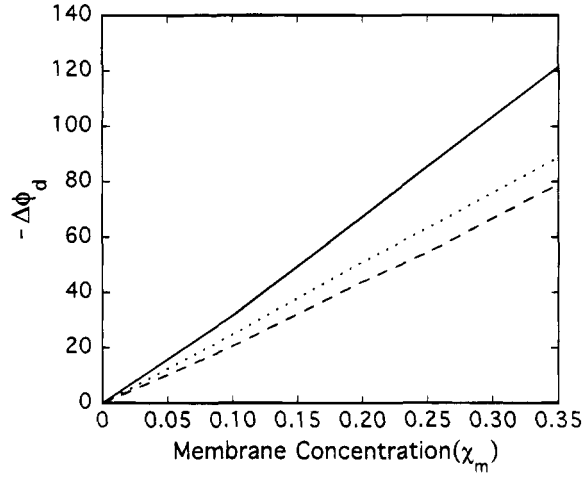

FIGURE 6: Dipole potential changes produced by halothane (•••), enflurane (− −), and isoflurane (—) as a function of the membrane mole fraction of anesthetic. These changes in dipole potential were calculated using the electrostatic model described in the text using the parameters shown in Table 1. The dipole potential changes produced by these anesthetics are approximately linear with the membrane concentration of anesthetic, and values for $\Delta\phi_d$ extrapolated to an anesthetic membrane concentration of 20 mol % and 1 MAC are given in Table 1.

The dipole potential change, $\Delta\phi_d$, as a function of the membrane concentration of halothane, enflurane, and isoflurane is shown in Figure 6. These values of $\Delta\phi_d$ are calculated using the electrostatic model and the parameters shown in Table 1 that were obtained by fits to the binding data (Figures 3 and 4). The model predicts a roughly linear dependence of $\Delta\phi_d$ on anesthetic concentration, and the dipole potential change for each anesthetic at a membrane concentration of 20 mol % is shown in Table 1. While isoflurane shows the greatest effect per membrane-bound anesthetic, the effect of each anesthetic on the dipole potential is similar. Table 1 also shows the changes in the dipole potential expected at a membrane concentration corresponding to anesthetic pressures at 1 MAC. These estimates are approximate, as there are a number of assumptions inherent in this calculation. Membrane concentrations of anesthetic at 1 MAC were determined from the pressures and PC bilayer/gas partition coefficients at 37 °C listed previously (enflurane was assumed to have the same partition coefficient

as isoflurane) (Firestone et al., 1986).

Upon inspection of Table 1 it is interesting to note that both halothane and isoflurane produce almost the same shift in the dipole potential even though the effective point dipoles that they contribute differ by a factor of 8. While the addition of a dipole layer to the interface that opposes the intrinsic dipole moment will alter the magnitude of the membrane dipole potential, changes in the dielectric constant of the membrane interior also produce significant changes in the dipole potential by increasing the polarizability of the membrane interface and the membrane interior. From the fits to the data, this appeared to be the case for halothane, where large shifts in the membrane dielectric but a smaller change in the interfacial dipole moment were required to account for the data. As discussed below, differences between halothane and the substituted methyl ethyl ethers might be the result of differences in the dynamics of these anesthetics in the membrane interface.

DISCUSSION

The spin-labeled hydrophobic ion binding data presented here provide strong evidence that the volatile general anesthetics halothane, enflurane, and isoflurane reduce the magnitude of the membrane dipole potential. These electrical changes have not been reported previously for these anesthetics, but are consistent with data for chloroform in planar bilayers, where the translocation rates of hydrophobic ions could be accounted for by a reduction in the dipole potential (Reyes & Latorre, 1979). The anesthetics examined here all produced significant changes in the membrane dipole potential at moderate membrane mole fractions. When extrapolated to clinically relevant anesthetic levels, the changes in $\Delta\phi_d$ are small, but comparable to the transmembrane voltage changes necessary to excite the nerve axon. Several assumptions were made in order to provide an estimate for $\Delta\phi_d$ at 1 MAC, and the actual potential changes produced by these anesthetics in biological membranes may be greater or less than those indicated in Table 1. If the partition coefficients of these anesthetics to biologically relevant membranes are different from those used here, quite different estimates for $\Delta\phi_d$ might be obtained. In addition, the calculation of $\Delta\phi_d$ in Table 1 assumes a homogeneous distribution of anesthetic in the plane of the membrane. Localization of these anesthetics near membrane protein−lipid interfaces might produce much larger local shifts in the value of $\Delta\phi_d$ than those estimated here.

The membrane dipole potential can be probed by measuring either hydrophobic ion binding or translocation rates; however, the binding data provided the clearest evidence for these electrical changes. For all the anesthetics examined, the translocation rates of both the cation and anion probes were enhanced upon the addition of anesthetic. This likely occurs because membrane crossing rates are very sensitive to the magnitude of the electrostatic ion charging energy, ΔG_{Born}, which is highly dependent upon changes in the membrane dielectric constant that accompany anesthetic addition. Indeed a similar behavior was also observed for hydrophobic ion currents in planar bilayers in the presence of chloroform (Reyes & Latorre, 1979). Compared to the translocation rates, the binding measurements are not very sensitive to changes in the membrane dielectric constant and clearly show differential changes between hydrophobic cations and anions with the addition of these anesthetics.

The model used here to fit the binding and translocation rate data was developed previously to describe the binding and translocation rate differences between tetraphenylborate and tetraphenylphosphonium (Flewelling & Hubbell, 1986b), and it was recently used to quantitate the effects of phloretin and ketocholestanol on the behavior of spin-labeled hydrophobic ions (Franklin & Cafiso, 1993). While it is a simple approximation to a real bilayer, the model does a remarkably good job of accounting for the binding and transport rates of these organic ions. The data obtained here also fit well to this model, although several interesting and unexpected features were observed. The binding data and translocation rate data for halothane could both be fit using the same effective dipole moment added per molecule of anesthetic. However, the fit of the translocation rate data required a much smaller shift in the membrane dielectric constant than did the fit of the binding data. This is not unreasonable, as this model assumes a uniform hydrocarbon dielectric constant which would require a uniform distribution of anesthetic. The higher dielectric change required to account for the binding data (compared to the translocation rate data) could result from a nonuniform distribution of anesthetic. Another intriguing feature in Table 1 is the difference between the parameters needed to fit the halothane data and those for enflurane and isoflurane. From these fits, halothane appears to make little contribution to the intrinsic dipole moment, at the same time producing a large change in the apparent dielectric constant of the interface. In contrast, enflurane and isoflurane make much larger contributions to the dipole moment, but do not significantly alter the interfacial dielectric constant. Differences in anisotropy and rates of motion of these anesthetics in the membrane interface might account for these differences. If the motion of halothane in the interface were relatively isotropic, its molecular dipole moment would be sufficiently averaged so that it produced a minimal net dipole moment. At the same time, it could orient in response to a strong electric field and contribute to the dielectric constant in the interface. If the motion of isoflurane and enflurane were highly anisotropic, their permanent dipoles would not be motionally averaged, which might account for their larger dipole contributions. However, if they are at least partially oriented, they would be less likely to respond to local electric fields and might not contribute significantly to the dielectric constant at the interface.

As indicated above, the model used here places dipole moments at the membrane interface with the addition of anesthetic, and the electrical changes that the model simulates could arise in several ways at the molecular level. For example, the anesthetic may act, as the model implies, by contributing a portion of its molecular dipole along the bilayer normal within the membrane, and the magnitudes of the molecular dipole moments of halothane and several reasonable configurations of enflurane and isoflurane are large enough to account for the dipole potential changes seen in Table 1. For example, several low-energy configurations of enflurane have dipole moments between 1.7 and 3.0 D, while for isoflurane these dipole moments were between 2.0 and 2.3 D. Thus, the observed changes in dipole potential could originate from the molecular dipoles of these anesthetics. Alternatively, it is possible that these anesthetics modulate the organization of water or the structure of the membrane interface. Without further structural information, our modeling cannot distinguish between these possibilities,

as they are electrically equivalent.

How could the membrane dipole potential modulate the activity of membrane proteins and promote anesthesia? In principle, any structural change in a membrane protein that involves the movement of charge or dipole moments through the membrane interface should be sensitive to the membrane dipole potential. Such structural transitions in membrane proteins remain largely uncharacterized, although for the membrane protein rhodopsin such an electrically active conformational transition has been observed. During the MI−MII transition in visual rhodopsin the protein polarizes the membrane interface, an event that appears to be coupled to the movement of a proton across the membrane interface (Cafiso & Hubbell, 1980). This charge uptake is likely to be coupled with other structural changes that have recently been observed for this membrane protein (Farahbakhsh et al., 1993). The similarity of this protein to other G-protein-coupled receptors suggests that similar structural transitions may trigger transmembrane signaling events in these proteins. Such a transition, where charge must transit the membrane−solution interface, is expected to show sensitivity to the membrane dipole potential.

In summary, the anesthetics halothane, isoflurane, and enflurane reduce the magnitude of the membrane dipole and produce small voltage changes at clinically relevant anesthetic levels that might modulate the activity of membrane proteins. Currently, it is difficult to determine whether the membrane dipole potential is likely to mediate the effects of anesthetics. To address this possibility, we are examining the effects of a wider range of anesthetics and nonanesthetic molecules on the value of the dipole potential in membrane vesicles. We are also directly examining the effects of dipole potentials on membrane proteins, to determine whether this interfacial potential has the capacity to modulate certain electrically active conformational equilibria in membrane proteins.

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