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Lipid-dependent effects of halothane on gramicidin channel kinetics: A new role for lipid packing stress

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Abstract

We find that the sensitivity of gramicidin A channels to the anesthetic halothane is highly lipid dependent. Specifically, exposure of membranes made of lamellar DOPC to halothane in concentrations close to clinically relevant reduces channel life-times by an order of magnitude. At the same time, gramicidin channels in membranes of non-lamellar DOPE are little, if at all, affected by halothane. We attribute this difference in channel behavior to a difference in the stress of lipid packing into a planar lipid bilayer, wherein the higher stress of DOPE packing reduces halothane partitioning into the hydrophobic interior.

Historically, the predominant view of the anesthesia was that anesthetic gases interact with lipids in cell membranes to change their physical properties and thus modulate the function of signal transmitting structures in the nervous system. This hypothesis of “indirect” action was based upon the remarkable chemical non-reactivity of anesthetic gases (e.g., xenon) on the one hand, and the strong correlation between their solubilities in olive oil and relative potencies, known as the Meyer-Overton rule (1,2), on the other. However, changes in the physical properties of lipids induced by anesthetics appeared to be implausibly small (3). A particularly difficult problem for the survival of this hypothesis had emerged as a result of the X-ray and neutron diffraction studies by Franks and Lieb (4). These authors demonstrated that the positions of the phosphate groups in bilayers of egg lecithin with 40% cholesterol did not show any significant change upon exposure to clinical concentrations of halothane. As a consequence of these and other similar studies, the focus of more recent research has shifted to the abilities of volatile anesthetics to bind within protein cavities and thus to act on ion channels directly (3). The mechanism of this “direct” action is also elusive.

Anesthetic gases have been shown to inhibit or to potentiate a number of specific ion channels which include GABA_A and glycine receptors, two-pore-domain potassium channels, and, possibly, subtypes of sodium channels (3). Indeed, inhalational anesthetics appear to affect multiple targets, and it has been difficult to identify a single ion channel necessary and sufficient to explain their effects (5). Besides, the objects under study are complex because they contain not only a mixture of cell membrane lipids but also numerous interacting integral proteins. To account for the effects of anesthetics on quite dissimilar ion channels, Gruner and Shyamsumder (6) and Cantor (7) proposed that volatile anesthetics

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change the lateral pressure profile within the cell membrane. These pressures, hundreds of atmospheres, are able to modify channel equilibrium between open and closed states – the conjecture that was first confirmed in experiments with peptide channels (see Ref. (8) for a short review).

Leaving open the question of the particular molecular mechanism of the anesthetic action, in this Letter we describe a new possible role for the lateral pressure existing in the hydrophobic part of the lipid bilayer. Specifically, our data suggest that high lateral pressures, characteristic of the bilayers formed from non-lamellar lipids, hinder anesthetic partition into the membrane hydrophobic region thus reducing nominal activity of the drug.

We chose gramicidin A (gA), a well characterized pentadecapeptide that forms conducting dimers in lipid bilayers (9), to test the effects of halothane, a potent volatile anesthetic, in membranes of different lipid composition. We assessed the lifetime of gA channel in lipid bilayers made of non-lamellar dioleoylphosphatidyl-ethanolamine (DOPE) and lamellar dioleoylphosphatidyl-choline (DOPC) at different halothane concentrations. We also used DOPC bilayers with varying concentrations of cholesterol.

The length of the gA dimer is actually less than the hydrophobic thickness of either DOPC or DOPE bilayers. For this reason and because of hydrophobic coupling (9), channel formation involves compression and bending of lipid monolayers, so that the conductance and lifetimes of gA channels are exquisitely sensitive to the composition and stress in the bilayer.

A routine protocol of the measurements was similar to one previously described (10). First, records of channel currents were obtained with no halothane added (control condition). After that, halothane solution was added in increasing quantity to the *cis* chamber with a Gilford pipette. Following 30 seconds of mixing, 10 minutes of data were obtained at each concentration of halothane before additional halothane was added. To eliminate uncertainties in the aqueous halothane concentrations, 50 microliter aliquots of the solution in the *cis* chamber were taken at the end of each 10 minute recording and placed in sealed vials for chromatographic analysis. All experiments were performed at room temperature using 100 mV trans-membrane potential and 0.1 M KCl aqueous solution buffered at pH 7.2.

Figure 1 shows that halothane at 1.8 mM aqueous concentration produced about a 10-fold decrease in gA lifetime in DOPC bilayers, but only a small, at the border of statistical significance, reduction in gA lifetime in DOPE bilayers.

Cholesterol addition to the membrane-forming lipid solution also caused a decline in gA channel lifetime, with 18% cholesterol reducing gA lifetime by more than half. Figure 2 demonstrates the dependence on increasing cholesterol concentrations, with bilayers composed of DOPC with 55% cholesterol (see *SI, Material and Methods*) giving channel lifetimes comparable to those found in DOPE, or an order of magnitude reduction in comparison with its initial value. Interestingly, with increasing cholesterol concentration halothane produced only minor additional effect on gA channel lifetime in these mixed bilayers.

The effect of halothane on gramicidin A channels was investigated in at least two experimental studies. Using a planar lipid bilayer assay Bradley, et al. (11) reported a 40% reduction in the lifetime of n-acetyl-gA channels formed in diphytanoyl-phosphatidylcholine bilayers at 1 mM halothane. More recently, Tang, et al. (12) used ^1H nuclear magnetic resonance and photoaffinity labeling to demonstrate direct interaction between halothane and gramicidin channel. They found that the most pronounced halothane-induced change in

the resonant frequencies of indole amide protons happens in W9 residue of gA. Remarkably, the interaction required gA molecules to be in the channel conformation.

Here we find that halothane action at concentrations that are close to physiologically relevant (*SI, Methodological Issues*) is lipid dependent. Halothane produces significant effects on lifetimes of gA channels in DOPC, but not in DOPE bilayers or cholesterol-containing bilayers. To interpret this finding we assume that, independent of the particular mechanism, the magnitude of the effect is defined by the concentration of halothane in the hydrophobic region of the membrane.

Considering anesthetic partitioning to be an equilibrium process, for the chemical potentials of halothane in relative concentrations $[c_{H,DOPE}]$ and $[c_{H,DOPC}]$ in the hydrophobic regions of DOPE and DOPC bilayers we have

$$\begin{aligned}\mu_{DOPE} &= k_B T \ln [c_{H,DOPE}] + v_H \Pi_{DOPE} + const, \\ \mu_{DOPC} &= k_B T \ln [c_{H,DOPC}] + v_H \Pi_{DOPC} + const,\end{aligned}\quad [1]$$

where k_B and T have their usual meanings of the Boltzmann constant and absolute temperature, Π_{DOPE} and Π_{DOPC} are lateral pressures in DOPE and DOPC bilayers, and v_H is the volume of a halothane molecule. When halothane concentration in the membrane-bathing solutions (together with other major parameters of experiments with differing lipids) is kept constant, chemical potentials should obey $\mu_{DOPE} = \mu_{DOPC}$. Eq. [1] then reduces to

$$\frac{[c_{H,DOPE}]}{[c_{H,DOPC}]} = \exp \left\{ \frac{-v_H (\Pi_{DOPE} - \Pi_{DOPC})}{k_B T} \right\}.\quad [2]$$

To estimate the effect of DOPC to DOPE transition, we take $v_H \approx 0.15 \text{ nm}^3$ and $\Pi_{DOPE} - \Pi_{DOPC} \approx 3 \times 10^7 \text{ Pa}$ (7,13), or 300 atmospheres, and obtain about one $k_B T$ for the $v_H (\Pi_{DOPE} - \Pi_{DOPC})$ product. As a result, based on Eq. [2], we expect about 3 times lower halothane partitioning into the hydrophobic region of the membranes formed from DOPE in comparison with that for DOPC membranes.

The cartoon in Figure 3, left illustrates the distribution of lateral pressure in a lipid bilayer. The integral of Π over the total thickness of the membrane is zero for a relaxed bilayer because the interfacial tension – peaks pointing to the left – is compensated by the pressure of head-head and tail-tail repulsion – peaks pointing to the right. Figure 3, right shows exchange of halothane molecules between the bulk aqueous solution and a site on the gramicidin molecule in the vicinity of such a pressure peak. The tighter lipid packing of DOPE changes the equilibrium of halothane partitioning in favor of the bulk and, according to our interpretation, reduces halothane effect on the channel (Figure 1). The residuals of the hydrocarbons used for bilayer formation (*SI, Material and Methods*) do not seem to distort packing stress significantly (14).

Interestingly, addition of cholesterol appears to act similarly as far as the environment for the gA molecule is concerned. At cholesterol concentrations similar to those used in X-ray experiments (4) the effect of halothane is virtually lost. It is known that both compression and bending moduli for such membranes increase markedly with cholesterol concentration (15), and there is a slight increase in membrane thickness (16). In agreement with Lundbaek et al. (17), we find that the lifetime of gA channel in DOPC bilayers is inversely related to cholesterol concentration (Figure 2). We also show that cholesterol decreases channel

sensitivity to halothane, which may suggest that the lateral pressures for DOPC membranes with cholesterol concentrations above 40% (see *SI*) are similar to those for DOPE. This conclusion is in qualitative accord with the finding by Coorssen and Rand (18) that presence of cholesterol helps transition to non-bilayer hexagonal structures. Direct measurements of anesthetic solubility in lipid bilayers (19) also support our hypothesis. At room temperatures cholesterol addition reduced halothane partitioning by a factor up to 2. Importantly, this reduction reflects the overall solubility, not the partitioning into the regions of high lateral pressures (Figure 3) discussed in the present study, which may be even more sensitive to the presence of cholesterol.

Concerning mammalian cells, the situation is even more complex as the distribution of lipids within cell membranes is still poorly studied, though it is recognized that membranes maintain an elaborate lateral organization of lipids and proteins (20,21,22). Also, at this stage we can not discriminate between indirect action (6,7) and the possibility that volatile anesthetics may specifically bind to the gA channel (12). Our tentative conclusion is that the regulation of the channel by halothane does not happen through the halothane-induced change in lateral pressure (see *SI, Methodological Issues*). Rather, a different mechanism related to a direct halothane-peptide interaction is involved. Nonetheless, whatever the mechanism, our findings suggest a new role for the stress of lipid packing: *Regulation of anesthetic partitioning into the membrane hydrophobic region*. This previously overlooked factor may contribute to the reported deviations from the Meyer-Overton rule (4,5,23).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) Gramicidin channel lifetimes in DOPC (circles) and DOPE (squares) bilayers at increasing concentration of halothane in the membrane-bathing solution. Clinically relevant concentrations are in the 0.25 – 0.50 mM range. (B) Current traces of gA channels in DOPC (a), in DOPC +1.5 mM halothane (b), and in DOPE (c) bilayers. A crude estimate shows that 1 mM of halothane in aqueous solution leads to 1:10 halothane/lipid ratio in DOPC bilayers (*SI, Methodological Issues*).

Figure 2. (A) Effect of cholesterol addition to DOPC bilayers on the channel lifetime in the absence (filled circles) and presence (open circles) of 1.5 mM halothane. Cholesterol content is given as the concentration of cholesterol in the lipid mixture used for bilayer formation (*see SI for details*). (B) Current traces of gA channels in DOPC bilayers with 6% cholesterol (a), 66% cholesterol (b), and 66% cholesterol + 1.5 mM halothane (c).

Figure 3. Stress of packing of the non-lamellar DOPE into a planar bilayer structure drives halothane molecules (black diamonds) out of the hydrophobic region of the membrane.