Do Anesthetics Fluidize Membranes?

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The so-called membrane fluidizing effect of anesthetics as a cause of anesthesia has been questioned, mainly because the magnitude of the increase in "fluidity" is insignificant at clinically relevant anesthetic pressures. However, the term "fluidity" has an unfortunate history of being misrepresented in membrane biology. It is often expressed as the ease of movement of probe molecules incorporated into the hydrophobic region of the membrane, thereby representing the property of the microenvironment where the probe molecules reside. In surface chemistry, "membrane fluidity" means inverse viscosity. Membrane viscosity is an integral property of a total membrane (not a part of membrane), and membrane molecules must dislocate and flow against resistance. The ease of motion of probe molecules, therefore, is not fluidity, and is now expressed by the order parameter. The present study measured the effect of halothane on surface viscosity of a phospholipid monolayer spread on a water surface by an oscillating pendulum surface viscometer. The results indicate a significant decrease of about 31% in the surface viscosity by the clinical pressure of halothane; anesthetics do fluidize membranes. Two factors contribute to the surface viscosity of the lipid monolayer: the property of the membrane proper (association between phospholipid molecules) and dragging of water (association between phospholipid and water molecules). The association between phospholipid molecules is in large part related to the order parameter. The fact that anesthetics show little effect on the order parameter, whereas halothane shows a significant effect on the membrane viscosity, indicates that halothane releases surface-bound water. It is postulated that the primary effect of anesthetics on membranes is to weaken the lipid-water interaction forces. (Key words: Anesthetics, volatile: halothane. Membranes: model. Theories of anesthesia.)

Membrane fluidity has been a subject of much controversy because the term has never been rigorously defined. In physics, fluidity means inverse viscosity; therefore, it is well defined. In membrane biology, however, the term is used to express vaguely the state of membrane rigidity. Thus, because of this lack of definition, the term has been widely abused and has become a source of confusion.

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The idea of the membrane fluidizing effect of anesthetics probably originated from the work of Hubbell and McConnell,1 who used a paramagnetic spin probe, TEMPO® (2,2,6,6-tetramethylpiperidin-1-oxyl), incorporated into the hydrophobic region of an excised rabbit nerve. They reported that tetrocaine increased the electron spin signal originating from TEMPO in the lipid region, and their interpretation was that probe molecules started tumbling faster due to melting of the lipid region of the membrane. Soon, spectrophotometric methods, using fluorescence anisotropy, followed electron paramagnetic resonance to measure the tumbling motion of small fluorescent probe molecules incorporated into the hydrophobic part of the membrane.2 The membrane fluidity concept was further popularized by the fluid mosaic model about cell membranes proposed by Singer and Nicholson in 1972.3

The term membrane fluidity, however, was denounced because the property expressed by the ease of motion of probe molecules incorporated into lipid membrane core is dimensionless and is not exactly the property represented by fluidity (see, for instance, a critique by Hare et al.4). Viscosity (inverse fluidity) is known as flow resistance, and dynamic viscosity is expressed by a poise unit that has a dimension of dyn · s · cm−2 for bulk liquids. There have been attempts to express the dimensionless data obtained by probe molecules in terms of the viscosity unit (poise) by dissolving the probe molecule into solvents with known bulk viscosity and comparing the spectroscopic data (see for instance, Feinstein et al.5). The value reflects the viscosity of the microscopic region where the probe molecules reside, but it is improper to designate it as "membrane fluidity." This is because viscosity (or fluidity) is an integral property of the membrane system as a whole and deals with dislocation and distortion of an array of molecules in a solvent (in this case, water); membrane molecules must flow against friction from water molecules and among phospholipid molecules. For this reason, the property measured by probe molecules is now called "membrane orderliness," expressed in terms of "order parameter." Order parameter is a dimensionless property in which zero indicates complete disorder and unity indicates complete order.

Trudell et al. have shown that inhalation anesthetics decreased the orderliness of model phospholipid membranes, and high pressure in the range of 100–150 atm reversed the anesthetic effect.6,7 Despite these results, Trudell concluded that membrane orderliness is irrelevant to anesthesia mechanisms because the change in order parameter is insignificant at clinically relevant anes-
thetic pressures. He proposed that there must be some mechanisms that amplify the small change in the order parameter and suggested that depression of the phase-transition temperature of lipid membranes may be the amplifying mechanism. Phospholipid membranes exist in at least two states: solid-gel and liquid-crystalline. Transition between the two states is analogous to transition between ice (solid) and water (liquid). At clinical concentrations, anesthetics decrease the transition temperature several degrees.

In this article we report anesthetic effects on surface viscosity, using a phospholipid (dipalmitoylphosphatidylcholine) monolayer spread on the air–water interface. When lipids are spread on the water surface, they form a membrane of monomolecular thickness, orienting hydrocarbon tails to the air and hydrophilic groups to the water. Although monolayers only represent one-half of the bilayer membrane, two-dimensional membrane fluidity can be measured accurately by surface viscometry.

Methods

Synthetic dipalmitoylphosphatidylcholine (1,2-dipalmitoyl-sn-glycerophosphorylcholine) was obtained from Sigma (St. Louis, Missouri). Purity was checked by thin-layer chromatography and confirmed to show a single spot. Water was purified by distillation, followed by passage through two-stage ion-exchanger columns, an activated charcoal column, and an ultrafilter in a Millipore® water purification system (Bedford, Massachusetts). Halothane was a gift from Halocarbon Laboratories (Hackensack, New Jersey).

Dipalmitoylphosphatidylcholine was dissolved in 90% ligroin and 10% ethanol (v/v) and spread on the water surface with microsyringe. The solvent is evaporated in a few minutes at room temperature, evidenced by a constant surface tension. Halothane was vaporized in the copper kettle of an anesthesia machine, using nitrogen as a carrier gas, and diluted with nitrogen gas. The anesthetic concentration in the gas was estimated from the temperature of the vaporizer and the flow of the nitrogen and confirmed by gas chromatography. The ambient pressure was measured by a mercury barometer, and the anesthetic concentration was converted to partial pressure.

In the present study, an oscillating pendulum surface viscometer was used to measure anesthetic effects on viscosity (reciprocal of surface fluidity) of a phospholipid monolayer membrane, spread on an air–water interface. This method measures damping of rotatory swing of a ring or a disc, touching the surface of a monolayer. Because perfect placement of the ring or disc onto the interfacial region is technically difficult, the obtained values are often called apparent surface viscosity. But, this is not a drawback because the purpose of the present study is to estimate the ratio of the viscosity change by anesthetics rather than to obtain absolute surface viscosity values. For detailed discussions of surface viscometry methods, the readers are referred to suitable textbooks (Davies and Rideal) and reviews (Kanner and Glass).

An oscillating pendulum-type surface viscometer, similar to that described by Kimizuka, was fabricated using a Teflon® disc that was milled so that the disc touches the water surface at the rim (fig. 1). The diameter and the weight of the disc was 34.3 mm and 24.143 g, respectively. The Teflon® disc is suspended by a nichrome wire of 50 μm in diameter and about 60 cm in length from a rotating pivot on a stand. The pendulum is set in motion by a twist of the pivot and oscillated with gradually decreasing amplitude, sliding on the water surface. Oscillations are measured by harnessing a small reflecting mirror to the wire and illuminating it from a focusing light source. The angular amplitude of the reflected light beam is read on a circular metal strip surrounding the mirror.

A linear plot between logarithmic amplitude and the number of swings indicates Newtonian viscosity. The surface viscosity, , is calculated from the following equation.

\[
\eta_s = [(\Delta\lambda)/(2\pi T)] \cdot [1/R_1^2 - 1/R_2^2]
\]
where \( I \) is the moment of inertia of the disc, \( R_1 \) and \( R_2 \) are radii of the disc and the container, respectively, \( T \) is the period of oscillation, and \( \Delta A \) is the difference in natural logarithmic decrements of the successive angular amplitudes of the disc oscillation on the water surface and water covered by the monolayer. In the present case of the cylindrical disc, the moment of inertia is one-half of the mass (weight) of the disc multiplied by the square of the disc radius: 

\[
I = (M \times r^2)/2
\]

where \( M \) and \( r \) are the mass and radius of the Teflon® disc, respectively.

The monolayer trough had a 99.5 mm diameter and was placed in a water bath. The temperature, monitored by a thermistor inserted into the trough with 0.01° C resolution (United Systems, Dayton, Ohio), was controlled by circulating water from a constant-temperature water bath. The trough was contained in a Plexiglas® casing, and anesthetics were introduced into the gas phase.

Surface tension of the monolayer was measured according to Langmuir's hanging plate method with a platinum plate and a high-sensitivity force transducer (Shinko Company, Kanagawa, Japan). The transducer output was connected to a surface-tensoimeter (Acorn, Tokyo, Japan) and recorded on a strip chart recorder. The platinum plate surface was roughened by scrubbing with a scouring powder to ensure complete wetting and washed under running water for at least 15 min, rinsed with purified water, then heated to white brightness by a Bunsen burner. The monolayer surface pressure (\( \pi \)) is defined as a difference between the surface tensions measured in the presence (\( \gamma \)) and absence (\( \gamma_0 \)) of the surface monolayer.

\[
\pi = \gamma_0 - \gamma
\]

All experiments were performed at 25.0 ± 1° C. The equilibrium penetration of halothane into the membrane system was ascertained by the constancy of the surface tension, which usually was reached within 20 min. At least four surface monolayers were examined with three measurements for each monolayer. The obtained data were stored in an Apple® IIe microcomputer interfaced with a PDP® 11/23 minicomputer. The nonlinear least-squares polynomial curve fitting, data averaging, and estimation of data scatter were performed with a statistics program. The data were highly reproducible, and the standard deviations were within 2%. For this reason, standard deviation for the data and the error bars for the figure were omitted.

**Results**

Surface viscosity is a function of the surface concentration of phospholipid molecules. Therefore, viscosity was measured at four different compressional states of the membrane. When expressed by the surface area available to one phospholipid molecule, they were 80, 65, 60, and 55 Å². The surface pressures at these phospholipid densities were 7.5, 11.6, 12.7, and 14.5 dyn·cm⁻¹, respectively. In the absence of anesthetics, the apparent surface viscosity values were: 0.14, 0.41, 0.68, and 1.24 surface millipoise units (×10⁻⁴ dyn·s·cm⁻¹), respectively, at 25° C. Addition of anesthetics in the gas phase decreased viscosity, dose dependently. At a halothane partial pressure of 1 · 10⁻⁴ atm, the values were 0.12, 0.35, 0.47, and 0.87 × 10⁻⁴ dyn·s·cm⁻¹, respectively. The data are summarized in table 1. A decrease of about 31% in apparent viscosity was observed with the membrane at high

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Membrane density</th>
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<tbody>
<tr>
<td>Lecithin surface density*</td>
<td>80</td>
</tr>
<tr>
<td>Surface pressure†</td>
<td>7.5</td>
</tr>
<tr>
<td>Viscosity‡</td>
<td>12.7</td>
</tr>
<tr>
<td>Control</td>
<td>0.14</td>
</tr>
<tr>
<td>In the presence of</td>
<td></td>
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<tr>
<td>1.0 · 10⁻⁴ atm halothane</td>
<td>0.12</td>
</tr>
</tbody>
</table>

* Expressed by Å² per each lecithin molecule.
† Expressed as dyn·cm⁻¹.
‡ Expressed as ×10⁻⁴ dyn·s·cm⁻¹.

**FIG. 2.** Halothane-induced, dose-dependent depression of apparent surface viscosity of dipalmitoylphosphatidylcholine monolayer at surface density of 60 Å² per lecithin molecule and at 25° C. The surface viscosity is expressed by the surface millipoise unit (×10⁻⁴ dyn·s·cm⁻¹). Halothane concentration is expressed by the partial pressure (×10⁻⁴ atm). The filled circle signifies the control value in the absence of halothane. Error bars are omitted because the standard deviation was less than 2%.
phospholipid concentration (condensed state) and about 15% at low phospholipid concentration (expanded state).

The surface area of the expanded monolayer (low phospholipid concentration) is composed of a mixture of phospholipid and water molecules, and the lipid tails are in random orientation, presumably laying flat on the water surface.\(^{13}\) When the monolayer is compressed into a condensed state, the surface water molecules are squeezed out into the bulk aqueous phase, and the lipid tails become oriented perpendicular to the monolayer surface. Compared with expanded monolayer, condensed monolayers probably represent the properties of lipid bilayers better, because lipid tails in bilayer membranes are oriented perpendicular to the membrane surface. At the most compressed state, the surface area occupied by a phosphatidylycholine molecule is in the range of 45–60 Å\(^2\) and is independent of the length of the lipid tail (because tails are in a perpendicular orientation relative to the surface). With planar lipid bilayers, the surface area occupied by a phospholipid molecule at close-packed state was reported\(^{16}\) to be 58 Å\(^2\). Therefore, the dose-dependent effect of halothane was studied with the phospholipid monolayer at a 60 Å\(^2\)-compressional state (fig. 2). At a halothane partial pressure of 0.5·10\(^{-2}\), 1.0·10\(^{-2}\), and 2.0·10\(^{-2}\) atm, the values were 0.54, 0.47, and 0.40 \(\times 10^{-3}\) dyn \(\cdot\) s \(\cdot\) cm\(^{-1}\), respectively. The dose–response curve was typical sigmoid.

**Discussion**

The present results clearly demonstrate that halothane significantly decreases surface viscosity (increases monolayer membrane fluidity), and contradicts the data obtained with probe molecules. The discrepancy is caused by the difference in the properties that these studies measured. With probe molecules, the finding is mainly limited to the property of the microscopic area where the probe molecules reside: membranes stay stationary, and the probe molecules rotate and wobble in the hydrophobic domain. In the viscosity study, the property of the membrane system as a whole, including water–membrane interaction, is represented; the arrays of phospholipid molecules dislocate and slip on the water surface, dragging water molecules that adhere to the membrane surface.

In this context, it is interesting to note that inhalation anesthetics (methoxyflurane, chloroform, halothane, enflurane, and fluoroxxene) significantly decreased the membrane rigidity (expressed by compressional modulus) when the membrane molecules were dislocated and slipped on the water surface. The surface compressional modulus is the reciprocal of surface compressibility and has a dimension of dyn \(\cdot\) cm\(^{-1}\). It is measured by compressing the area of monolayer membranes on the water surface by a Teflon\(^{\circ}\) bar in a dynamic surface-tension balance. In this case, the monolayer array of the phospholipid molecules is dislocated according to the compression. Although compressional modulus is not exactly fluidity, it gives information on the property of the membrane as a whole. Inhalation anesthetics (methoxyflurane, chloroform, halothane, enflurane, and fluoroxxene) decreased compressional moduli of the dipalmitoylphosphatidylcholine monolayer by as much as 40% at clinical pressures.\(^{11}\) The value is in reasonable agreement with the present study.

The fact that lipid membranes cannot be formed without water suggests that the water–membrane interaction is an important part in the membrane property and function. Apparently, halothane weakens the lipid–water association force and releases the dragging water molecules. The interfacial action of anesthetics that weakens the association force between water and macromolecules has been demonstrated in membranes, micelles, and proteins.\(^{17–27}\) The present surface-visibility study supports our view that the primary action site of polar inhalation anesthetics is the membrane–water interface, releasing bound water molecules. This means that the membrane surface becomes less hydrophilic (more hydrophobic) under anesthesia. Presumably, the increased hydrophobicity of the membrane surface is intimately related to the state of anesthesia.

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**References**