

Is Membrane Expansion Relevant to Anesthesia? Mean Excess Volume

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Is Membrane Expansion Relevant to Anesthesia?

Recent disputes about the relevance of membrane expansion to the mechanism of anesthesia indicate that there is confusion about the concept of membrane expansion and stabilization. One theory suggests that the membrane is expanded when its size is increased by the size of the incorporated anesthetic molecules, whereas another theory contends that extra space must be created over the size of the incorporated anesthetic molecules in order for the membrane to be considered as expanded.

This article is intended to clarify the discrepancies between these concepts. The volume theories of anesthesia are reviewed critically. The volume change of the membrane, induced by the interaction of anesthetics, is not a simple summation of membrane volume and anesthetic volume. There are a number of factors that affect the volume when anesthetic molecules interact with the membrane in water. The theories that envision membrane expansion as the increase of volume by the size of anesthetic molecules assume that there is no interaction between membrane and anesthetic molecules (if there is interaction, there is excess volume change) and are incompatible with the pressure reversal of anesthesia.

The physical meaning of the pressure reversal of anesthesia is described, and the absolute necessity of the presence of excess volume for pressure to antagonize anesthesia is discussed. Excess volume expansion *per se* may not be the cause of anesthesia, but the mechanism by which the excess volume is created must be the key event that induces anesthesia.

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The mean excess volume hypothesis postulates that the size of the membrane is irrelevant to anesthesia. It is not the compression of the membrane size, but the reverse reaction of the multiple factors, that contributed to expansion of the system volume, is responsible for pressure antagonizing the anesthesia. Evaluation of these multiple factors may be the key to elucidation of the anesthesia mechanisms at a molecular level. (Key words: Theories of anesthesia: pressure reversal; volume expansion).

THE RELEVANCE OF MEMBRANE EXPANSION to the mechanism of anesthesia has been questioned recently.^{1,2} Franks and Lieb¹ used solution densimetry and reported that membrane volume, whether in natural or model form, stayed almost constant when interacted with anesthetic molecules. They also reported that the volume of halothane does not differ significantly in its pure liquid state, in water, and in membranes.¹ According to their criteria, the membrane is not expanded when the volume added to the membrane equals the volume of pure anesthetic molecules. On the basis of these findings, they concluded that anesthetics do not expand membranes significantly and disputed the relevance of nonspecific expansion of cell membranes to the mechanism of anesthesia. They proposed that specific receptor(s) are necessary that expand significantly when anesthetic molecules are bound.

Bull *et al.*² directly measured the erythrocyte volume and calculated the membrane area. They concluded that area expansion by anesthetics closely approximated the expansion expected by incorporation of these molecules. They postulated that the membrane expansion is irrelevant to anesthesia because tetradecanol and hexadecanol, which lack anesthetic potency, also expanded the membrane area. According to their criteria, the membrane is expanded when the anesthetics occupy the space in the membrane by the size of the anesthetic molecules.

It is obvious that the concept of anesthetic-induced membrane expansion is not consistent and is discussed by two different definitions. One theory defines that the membrane is not expanded when the size is increased by the size of incorporated anesthetics¹; an addition of extra space is required to consider a membrane to be expanded.

Another theory defines that the membrane is expanded when the size is increased by the size of incorporated anesthetics²; extra space is not required. Thus, although membrane stabilization and expansion are the terms often used to describe the nonspecific actions of anesthetics upon cell membranes, they have never been defined rigorously. The purpose of this article is to identify the difference in concepts of "membrane expansion" and to critically evaluate the theories that relate membrane expansion to the mechanism of anesthesia. We limit the present discussion to the area and volume change induced by inhalation anesthetics. Alternative theories, such as phase transition of phospholipid membranes, phase separation, cooperatively of the phase transition, membrane fluidity, protein-membrane interaction, *etc.*, are out of the present scope.

Membrane Stabilization and Expansion

In 1957, Shanes^{3,4} first identified drugs that suppress the action potential of excitable cells without affecting the resting membrane potential as membrane stabilizers. The term "membrane stabilization" was proposed to differentiate drugs from those that hyperpolarize the excitable membranes. He assumed that the membrane stabilizers nonspecifically expand the membrane area and collapse the sodium pore by increased lateral pressure. Later, Shanes and Gershfeld⁵ demonstrated two-dimensional expansion by membrane stabilizers in lipid monolayers spread at the air/water interface. In Shanes' membrane stabilization concept, expansion was discussed by area; membrane volume was not considered.

Seeman⁶ extended the idea of stabilization from the bioelectric event of excitable membranes to mechanical protection of nonexcitable erythrocyte membranes against hypotonic hemolysis. He and his collaborators demonstrated (see review of Seeman⁷) that the so-called membrane stabilizers suppressed hypotonic hemolysis of human erythrocytes and shifted the erythrocyte fragility curve to the left. They also demonstrated that the antihemolytic effect is accompanied by expansion of the erythrocyte membrane area and estimated that the extent of expansion is about 0.4% by the surgical concentrations of general anesthetics and about 2% by the nerve blocking concentration of local anesthetics. The difference in the magnitude of expansion between general and local anesthetics is caused by the difference in their concentrations: general anesthetics block nerve conduction when the concentration is increased.

Seeman⁷ was the first to introduce the idea of membrane volume in the membrane stabilization concept. By assuming that expansion is uniform in all directions, he⁷ calculated that the volume expansion was about one order of magnitude larger than the van der Waals volume of incorporated anesthetic molecules. This estimation was

criticized by Trudell⁸ on the ground that the membrane thickness decreases when the membrane area is increased. Trudell⁸ re-examined the data reported by Seeman⁷ and calculated that the volume increased by anesthetics was about equal to the van der Waals volume of incorporated anesthetic molecules and that the advocated excess volume increase did not exist.

Volume Occupied by Anesthetic Molecules

Unrelated to the above membrane stabilization-expansion concept, Mullins⁹ proposed in 1954 that the volume of anesthetic molecules in the membranes has prime importance for the mechanism of anesthesia. By examining the Overton-Meyer hypothesis, which correlates anesthetic potency to olive oil solubility, he found better correlation between anesthetic potency and the volume of anesthetic molecules dissolved in olive oil. According to the Overton-Meyer hypothesis, anesthesia occurs when the number of anesthetic molecules reaches a certain value in the cell membrane. According to Mullins' concept, anesthesia occurs when the volume of anesthetic molecules reaches a critical value in the cell membrane. Extra volume is not considered. The volume fraction of the incorporated anesthetic molecules in the membrane determines the depth of anesthesia.

Pressure Reversal of Anesthesia

The pressure reversal of anesthesia was first discovered by Johnson, Eyring, and co-workers¹⁰⁻¹² in 1942 with luminous bacteria. They found that a variety of anesthetics suppressed the light intensity of luminous bacteria and that hydrostatic pressure in the range of 100-150 atm restored the light intensity.

In 1951, Johnson and Flagler¹³ further demonstrated the pressure-reversal of anesthesia in tadpoles. These amphibian larvae, anesthetized with ethanol or urethane and staying at the bottom of the container, instantly started swimming again when a hydrostatic pressure of 100-150 atm was applied. Later, the pressure reversal of anesthesia was confirmed in tadpoles,¹⁴ newts,^{15,16} and mice¹⁵ anesthetized with modern gaseous anesthetics.

According to thermodynamics, a change in the equilibrium constant (K), such as the depth of anesthesia, by pressure (P) expresses the volume change (ΔV). By using R for the gas constant and T for the absolute temperature,

$$(\partial \ln K / \partial P)_T = -\Delta V / RT \quad (1)$$

Here, ΔV is the excess volume difference in the total system and is not the volume occupied by the anesthetic molecules. The following example clarifies its property. When 50 ml ethanol is mixed with 50 ml water, the total volume does not quite reach 100 ml. The decrement is the excess volume, and in this case it carries a negative sign. In contrast, when 50 ml ethanol is mixed with 50

ml of an apolar solvent, such as decane, the total volume exceeds 100 ml. The increment is the excess volume, and in this case, it carries a positive sign. The excess volume is the difference in the system volume before and after the interaction, for instance, between anesthetics and membranes. It does not matter whether anesthetics or membranes or both change the volume.

In order for a system to become susceptible to pressure, the volume of the total system of the anesthetized state must be larger than the unanesthetized state. The size of cell membranes is irrelevant to the pressure reversal. In other words, pressure reversal of anesthesia does not occur if anesthetic molecules are transferred from the aqueous phase into the cell membrane and expand the membrane volume by the volume they occupied in the aqueous phase without changing the total volume.

Free Volume Hypothesis

Based on pressure reversal of anesthesia, Stern and Frisch¹⁷ proposed the free volume hypothesis for the mechanisms of anesthesia in 1973. They postulated that there is a range of free volume between the unanesthetized and anesthetized states. Physiologic response is assumed to be a sole function of free volume, v_f , and they proposed that anesthesia ensues when v_f reaches a threshold value. They defined free volume as follows:

$$v_f = v_s + \alpha(T - T_s) - \beta(P - P_s) + \phi \bar{v}_A \quad (2)$$

where v_s is the volume of the site of anesthetic action at the standard state, T is the absolute temperature, P is the hydrostatic pressure, α is the thermal expansibility, β is the compressibility, ϕ is the anesthetic concentration expressed by the volume fraction, \bar{v}_A is the partial molal volume of anesthetics, and subscript s signifies the standard state. This equation expresses that anesthetic potency depends upon temperature, pressure, and volume fraction of anesthetic molecules.

Critical Volume Hypothesis

In the same year, Miller *et al.*¹⁶ used high pressure to differentiate the anesthetic action between the number hypothesis (Overton-Meyer) and the volume hypothesis (Mullins), and supported Mullins' concept. They proposed the critical volume hypothesis, which states that anesthesia occurs when the volume of the site of action reaches a critical value resulting from volume occupation by anesthetics. The free volume hypothesis and the critical volume hypothesis appear to be similar, but they differ in the basic concept; the former is deduced from thermodynamic analysis of the pressure reversal of anesthesia, while the latter is an extension of Mullins' concept, although the experiment was performed under high pressure.

According to the critical volume hypothesis, pressure reverses anesthesia by compressing the volume of the site of action. Compressibility of the membrane is the key factor for the pressure reversal; excess volume increase was not considered. They expressed the volume change in the site of action by anesthetics as

$$E = \frac{\bar{v}_A \cdot P_A}{H_A \cdot V_m} - \beta P_A \quad (3)$$

where E is the fractional volume expansion, \bar{v}_A is the partial molal volume of anesthetics at the site of action, P_A is the partial pressure of volatile anesthetics in the gas phase, β is the compressibility of the site of action, H_A is Henry's solubility constant for the anesthetic, and V_m is the volume of the site of action. Henry's constant is equivalent to the reciprocal of the mole fraction solubility designated by x in the original notation.¹⁶ Capital letter V is used for the system volume and lower case v is used for the molal volume. This equation states that anesthesia depends upon the third and fourth term of equation (2) of the free volume hypothesis.

Their strategy in demonstrating the validity of the critical volume hypothesis over the Overton-Meyer hypothesis was to measure the pressure effect on anesthesia and analyze the data according to the equations that express the volume and number theories. The structure of the fitting equation is shown in the Appendix. Linearity of the line drawn through the data points, plotted according to the two fitting equations, was used for the comparison.

In the fitting equation, however, the volume of the site of action (V_m) was assumed to be constant when the anesthetics are bound. This assumption is inconsistent with the theory that postulates volume expansion of the site of anesthetic action for the anesthesia mechanisms. Because V_m and v_i in the fitting equation are not constant but variable, dependent upon anesthetic concentrations as well as pressure, the equation does not stipulate linearity for the plot. It also ignores contributions from other factors to the volume (see next section). It is not surprising that Halsey *et al.*¹⁸ and Smith *et al.*¹⁹ reported nonlinear plots for the critical volume hypothesis.

The sophisticated data fitting was unnecessary to denounce the validity of the Overton-Meyer hypothesis under high pressure. This is because there is a constraint that the solubility of a gas (Henry's constant) is only dependent on temperature in ideal cases. Hence, the number of anesthetic molecules in membranes in equilibrium with the gas phase is affected little by high pressure. The non-ideality of anesthetic gases may deviate the results to a certain degree, but the number of anesthetic molecules in the membrane must be nearly constant at the pressure range between ambient and moderately high 200 atm. If the number of anesthetic molecules in the membrane

determines the depth of anesthesia, pressure cannot reverse anesthesia.

By using Henry's law, the fitting equation for the critical volume hypothesis can be transformed into the following form:

$$x_A^B = \text{constant} \cdot P_{He} + x_A^A \quad (4)$$

where x is the mole fraction concentration of anesthetics in the membrane. The meaning of this equation is simply that anesthetic concentration must be increased in proportion to the applied pressure to maintain an adequate level of anesthesia; volume is not included.

Multiplicity of the Factors Involving the System Volume

The volume V is thoroughly written by the following thermodynamic expression.

$$V = V(T, P, n_A, n_W, \dots, n_i, \dots) \quad (5)$$

Here, T, P, n signify absolute temperature, pressure, and number of moles, respectively. Subscripts A and W indicate anesthetic and water, respectively, and i is used to describe other components.

The volume change dV is expressed by taking the total differential of the above expression.

$$\begin{aligned} dV &= \left(\frac{\partial V}{\partial T} \right)_{P, n_j} dT + \left(\frac{\partial V}{\partial P} \right)_{T, n_j} dP + \left(\frac{\partial V}{\partial n_A} \right) dn_A \\ &\quad + \left(\frac{\partial V}{\partial n_W} \right) dn_W + \dots + \left(\frac{\partial V}{\partial n_i} \right) dn_i \dots \\ &= \left(\frac{\partial V}{\partial T} \right)_{P, n_j} dT + \left(\frac{\partial V}{\partial P} \right)_{T, n_j} dP + \bar{v}_A dn_A \\ &\quad + \bar{v}_W dn_W + \dots + \bar{v}_i dn_i + \dots \end{aligned} \quad (6)$$

Dividing both sides by $\sum n_j$ ($j = A, W, \dots, i, \dots$) and also by the mean molal volume of the unanesthetized state (v_0), one obtains

$$\begin{aligned} \frac{dv}{v_0} &= \alpha dT - \beta dP + \frac{\bar{v}_A}{v_0} dx_A \\ &\quad + \frac{\bar{v}_W}{v_0} dx_W + \dots + \frac{\bar{v}_i}{v_0} dx_i + \dots \end{aligned} \quad (7)$$

where α is the thermal expansibility and β is the compressibility. This equation states that for analysis of the volume change by anesthetics, the effects of temperature, pressure, anesthetics, water, and other component molecules must be considered.

When this equation is integrated under the condition that α, β , and \bar{v}_A are constant, ignoring the change of x_W and x_i by anesthetic binding, one obtains the identical equation described in the free volume hypothesis and the critical volume hypothesis. We contend that these parameters cannot be ignored and should be evaluated to elucidate the molecular mechanism of anesthesia.

Mean Excess Volume

General anesthesia appears to always be reversed by high pressure. Under the principle that the contraposition theorem is always true, it can be stated that any condition that is not antagonized by high pressure is not anesthesia. Restated, without excess volume expansion, anesthesia does not occur.

This logic appears to be difficult for many readers to follow. It is helpful to recall an early geometry class. When a proposition (contraponend) "all A is B " is true, then the contrapositive "all not- B is not- A " is inevitably true. Contraponend and contrapositive are equivalent statements. If one accepts one statement, he cannot refuse the other, whereas the converse proposition "all B is A " and the inverse proposition "all not- A is not- B " may or may not be true. In the present argument, the contraponend is "all anesthesia (induced by inhalation anesthetics) is reversed by pressure" and the contrapositive is "all not reversed by pressure is not anesthesia." These are one and the same statement. If the contraponend is true, contrapositive does not need experimental proof to support the statement. Therefore, the statement "without excess volume expansion, anesthesia does not occur" stands regardless of a myriad of alternative hypotheses that explain the anesthesia mechanism. All alternative hypotheses simply must be compatible with the mean excess volume expansion.

The fact that anesthesia, induced by a variety of drugs, is antagonized by the same magnitude of pressure indicates that the size of the excess volume increase is uniform among all anesthetics at the same depth of anesthesia; anesthesia occurs when the mean excess volume of the total system exceeds a limiting value.

It is the mean excess volume of the total system that is relevant to the pressure reversal of anesthesia and not the membrane volume. In this respect, the mean excess volume concept differs from the critical volume hypothesis in that the volume occupied by anesthetics in the membrane is not important in the excess volume concept. The excess volume may be created at any place in the total system.

The idea that the membrane expands by the volume of incorporated anesthetic molecules ignores the interaction between the anesthetic molecules and membrane molecules. Interaction between two molecules is always accompanied by an excess volume change (positive or negative) except in an imaginary ideal solution. If there is no interaction, the energy level of the anesthetized state remains unchanged from the unanesthetized state and pressure reversal cannot occur.

We are not implying that the excess volume expansion is the cause of anesthesia. It may well be the result of anesthesia or a side effect. Nevertheless, the mechanism by which the excess volume is created must be involved in achieving the anesthetized state.

The mechanisms of creation of excess volume involve multiple factors, as described in the previous section. Evaluation of each factor contributing to the system volume should be the key to elucidation of anesthesia mechanisms at a molecular level.

Among these factors, Eyring *et al.*²⁰ postulated that the major contributor may be the structural change in interfacial water clusters. The polymorphism of water structure under pressure is well known, and the water cluster at the macromolecule/water interface is condensed strongly (see, for instance, Kauzmann,²¹ Drost-Hansen,²² and Millero²³). The most efficient way to create excess volume is to release the water molecules condensed at the interface into the bulk water phase. Evidence for such release of interfacial water molecules by anesthetics is accumulating,²⁴⁻²⁷ and the possible effect of the destruction of interfacial water structure on anesthesia mechanisms has been reviewed.²⁸

In this context, pressure reverses anesthesia, not by compression of the size of the membrane, but by the reverse reaction of multiple factors that contributed to the positive mean excess volume change (possibly by restructuring the interfacial water).

Partial Molal Volume

Although the critical volume hypothesis does not discuss excess volume increase, membranes expand with excess volume when anesthetic molecules are transferred from the aqueous phase to the membrane. This is because the partial molal volume of anesthetics in water is smaller than that in the hydrophobic domain. When anesthetic molecules are surrounded by water molecules, which are polar and have stronger intermolecular forces than apolar molecules, the distance between the next neighbor molecules are shorter than when residing in an apolar domain. Hence, the free volume of anesthetics in water is smaller than that in membranes or in its pure liquid state, and the transfer of anesthetic molecules from water to membrane is accompanied by an excess volume increase. In this context, system volume represents the strength of the intermolecular forces, and the volume of each molecule has little meaning.

Franks and Lieb,¹ in contrast, reported that the partial molal volume of halothane was not different in water and in the membrane and equals the molal volume of the pure liquid state. The results violate the above general rule. There are ample reports that the volume of hydrophobic molecules, such as halothane, decreases when transferred into water (see, for instance, Friedman and Scheraga²⁹). Presumably, the precision of their study was inadequate to detect the significant differences among these values. Contradictory data, showing the excess volume increase of anesthetic molecules when incorporated into the hydrophobic domain, are reported for phospholipid membranes by Kita *et al.*³⁰ and Kaneshina *et al.*²⁴

The method and result of the study of Franks and Lieb¹ do not warrant their conclusion that the nonspecific membrane expansion is irrelevant to anesthesia.

Is Membrane Expansion Relevant to Anesthesia?

The basic idea of the membrane expansion-stabilization concept is that the expansion interferes with the flux of the current-carrying ions. Conclusive evidence that the dimension of membrane is irrelevant to anesthesia was provided by the study of Bull *et al.*² They showed by direct measurement that long chain alkanols, devoid of anesthetic potency, increased the size of the erythrocyte membranes.

Another piece of evidence that anesthesia may not involve change in membrane size is our finding³¹ on anesthetic inhibition of the cell-free light-emitting enzyme (luciferase) extracted from firefly tails. The firefly luciferin-luciferase system does not emit light in the absence of ATP. Addition of ATP induces a burst of light, and the light intensity is proportional to the amount of added ATP. Despite the fact that the soluble enzyme protein does not contain a phospholipid membrane structure, inhalation anesthetics inhibited the ATP-induced light intensity. Together with our demonstration of excess volume expansion by inhalation anesthetics in the aqueous solution of lipid-free (lipid content less than 0.005%) crystalline bovine serum albumin²⁶ and a synthetic polypeptide,²⁵ it is suggested that the action of anesthetics is nonspecific and increases excess volume of all macromolecular systems in water, regardless of lipid membranes or proteins. The membrane size may not be relevant to anesthesia, but expansion of the mean excess volume is essential for the anesthetic effect. The possible involvement of interfacial water structure for the excess volume expansion has been discussed.

APPENDIX

The fractional volume expansion (E) of the membrane at anesthetic partial pressure P_A and at helium pressure P_{He} described by Miller *et al.*¹⁶ is rewritten as follows:

$$E = \left(\frac{\bar{v}_A/H_A}{V_m} - \beta \right) P_A + \left(\frac{\bar{v}_{He}/H_{He}}{V_m} - \beta \right) P_{He} \quad (A-1)$$

Here, H is Henry's solubility constant, subscripts A and He signify anesthetic and helium, respectively, and total pressure is $P_A + P_{He}$.

The condition for pressure reversal is that the expansion by anesthetics is antagonized by compression by pressure and the membrane volume does not change, or $dE = 0$. According to Miller *et al.*¹⁶ \bar{v}_i and V_m are assumed to be constant. Then,

$$dE = \left(\frac{\bar{v}_A/H_A}{V_m} - \beta \right) dP_A + \left(\frac{\bar{v}_{He}/H_{He}}{V_m} - \beta \right) dP_{He} = 0 \quad (A-2)$$

Rearranged

$$\frac{dP_A}{dP_{He}} = - \frac{\bar{v}_{He}/H_{He} - \beta V_m}{\bar{v}_A/H_A - \beta V_m} \quad (A-3)$$

By integrating this equation, one obtains the fitting equation for the critical volume hypothesis that Miller *et al.*¹⁶ derived as follows.

Let P_A^A designate the ED_{50} value of an anesthetic partial pressure at one atmospheric pressure. When the anesthetic concentration is increased from P_A^A to P_A^B according to the pressure increase, E is increased from E^A to E^B . Consider the condition where P_A^B becomes ED_{50} by pressurizing the system by helium to P_{He} , then

$$\frac{P_A^B}{P_A^A} = \left\{ \frac{\beta}{E^A} - \frac{\bar{v}_{He}}{E^A \cdot H_{He} \cdot V_m} \right\} \cdot P_{He} + 1 \quad (A-4)$$

where H_{He} is Henry's constant for helium.

They obtained linear plots between P_A^B/P_A^A versus P_{He} for N_2O , N_2 , CF_4 , and SF_6 and concluded that the molecular mechanism of the pressure reversal of anesthesia is explained by the critical-volume hypothesis.

They expressed the Overton-Meyer hypothesis at high pressure by the following equation.

$$\frac{1}{\bar{v}_A} \ln \frac{P_A^B}{P_A^A} = \frac{1}{2RT} (P - P_A) \quad (A-5)$$

where P is the total pressure. The plots between $(1/\bar{v}_A) \ln(P_A^B/P_A^A)$ versus $(P - P_A)$ produced inferior linearity compared with the previous plots. From this result, they concluded that the Overton-Meyer hypothesis is not compatible with the pressure reversal of anesthesia.

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