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A Unitary Theory of Anesthesia Based on Lateral Phase Separations in Nerve Membranes

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This paper relates research on anesthetic effects on lipid membrane systems to mechanisms of neural function. A unitary theory of anesthesia based on anesthetic-induced changes in fluid-solid-phase separations in the lipid region of nerve membranes is presented. It is suggested that anesthetics act by fluidizing nerve membranes to a point where critical lipid regions no longer contain phase separations. As a consequence, the membranes are less able to facilitate the conformational changes in proteins that may be the basis for such membrane events as ion gating, synaptic transmitter release, and transmitter binding to receptors. It is proposed that the anesthetic-modified phase separation behavior of the membrane may alter neural function by a combination of the following effects: inhibition of conformational changes of intrinsic membrane proteins; prevention of the association of protein subunits to form polymeric ion channels; depression of transmitter release by preventing fusion of vesicles containing synaptic transmitter with the membrane of the presynaptic terminal. (Key words: Theories of anesthesia; Membrane, nerve.)

THERE CURRENTLY EXISTS no generally accepted molecular theory of action of the inhalation anesthetics. Such a theory would be of much use in our understanding of the mechanism of anesthesia and in the design of new anesthetic agents. Many attempts have been made to fill this void of knowledge. Most of the earlier theories were simply correlations of observable physical-chemical characteristics of the anesthetic drugs with anesthetic potency. For example, Meyer[1] and Overton[2] developed the correlation of anesthetic potency with solubility in olive oil, the basic principle of which still stands today.[3] In recent years, theories of anesthetic action have tended to divide themselves into two groups that differ in their explanations of the direct or indirect transmission of the anesthetic effect to membrane proteins essential to neural action. The first group includes the concept that the anesthetic molecule interacts directly with the membrane protein by changing its conformation, plugging a penetrating ion channel, or rendering the protein incapable of conformational change. These theories benefit from the simplicity of direct drug-affector interaction. Research by Woodbury *et al.*,[4] Seeman,[5] Metcalfe *et al.*,[6] and Halsey[7] provides evidence for such direct interaction.

A second group of theories is based on concern with the lipid region as the site of anesthesia. Compelling correlations of anesthetic potency with solubility in olive oil[1-3] and thermodynamic activity[8] in lipid phases have accumulated for 75 years. Mullins[9] and Miller *et al.*[10] have suggested that anesthesia obtains when a certain volume of any anesthetic molecule dissolves in a volume of nerve membrane. By using solubility theory, Miller and co-workers[11] were able to show that the site of anesthetic action has properties more like a hydrocarbon phase, such as benzene, than water or a protein.

Work by Hubbell *et al.*[12] demonstrated that anesthetic molecules serve to make the lipid regions of erythrocyte membranes and phospholipid bilayers more fluid. The anesthetic molecules were shown to penetrate the membrane, expand the surface area,[5] and increase the internal motion in the bilayer. Subsequent studies by Trudell *et al.*[13] defined the dose-response effect of anesthetic molecules on model bilayer membranes and showed that this fluidizing response occurs at concentrations in the order of magnitude of those used clinically. Further investigations demonstrated the antagonistic effects of high pressure[14-16] and anesthetics[14,17-19] on the internal fluidity, phase transition temperature, and lateral phase separations[19] in phospholipid bilayer model nerve membranes. This work has led to the suggestion that the alteration of the properties of lipid bilayers may be sufficient to explain the action of inhalation anesthetics without invoking direct anesthetic-protein interactions.[18,19] While lipid regions, as a site of anesthetic action, manifest excellent correlation with results of physiochemical measurements and membrane theory, there is no good explanation of how the anesthetic drugs dissolved in lipid regions exert their effects on membrane proteins essential to neural function. We propose the following hypothesis for a coupling mechanism.

Lateral phase separations[20] occur in phospholipid bilayer membranes when highly ordered gel-phase phospholipids and disordered fluid-phase phospholipids coexist. This equilibrium condition confers many special properties on the membrane, including high lateral compressibility. How lateral phase separations may facilitate conformational changes in proteins is illustrated in figure 1.

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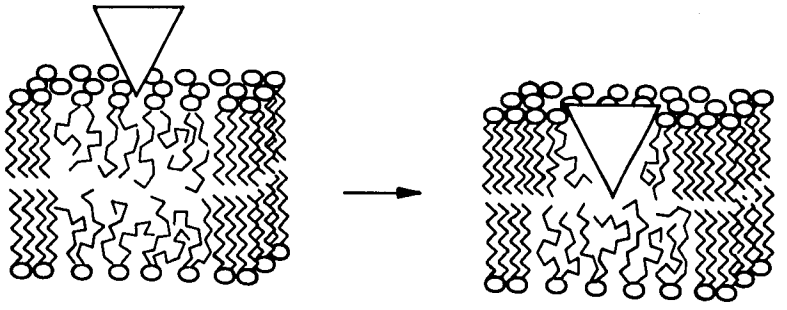

FIG. 1.*A* (*left*), representation of a phospholipid bilayer in which the circles depict the hydrophilic phosphate head groups and the zigzag lines depict fatty-acid chains. The solid wedge represents the element of volume required when a membrane-solvated protein expands or is inserted into the plane of the membrane. On the left and right edges of the bilayer segment there are regions in which the fatty-acid chains are highly ordered. In the center of the bilayer there are regions in which the fatty-acid chains are depicted as being disordered.

B (*right*), the insertion of the wedge represents the expansion or insertion of a membrane-solvated protein. It is accomplished by the packing of some of the high-volume disordered lipids into a low-volume ordered array.

Figure 1*A* represents a cross section of a phospholipid bilayer component of the boundary membrane of a nerve cell. The circles indicate the highly-charged phospholipid head groups and the zigzag lines represent the two fatty-acid chains. On the left and right edges of the bilayer segment are regions in which the fatty-acid chains are depicted as fitting tightly together, requiring a relatively small volume per chain and manifesting little vibrational motion. The regions are said to be highly ordered or solid-phase. In the center of figure 1*A*, the fatty-acid chains are depicted as kinked or bent. These fatty-acid chains require a relatively large volume per chain and manifest vibrational motion. They are said to be disordered or fluid-phase. Those boundaries between solid and fluid phases in a phospholipid bilayer are termed lateral phase separations.

In figure 1*A*, a solid wedge is shown poised above the bilayer surface. The volume of the wedge may be considered to be the insertion, rotation, or expansion of a membrane-solvated protein. In figure 1*B*, the wedge has been pushed down into the bilayer. The bilayer is able to accommodate insertion of the wedge by transforming some of the high-volume disordered fatty-acid chains in its central section into regions of lower-volume more highly ordered chains. This transformation of high-volume disordered chains to lower-volume ordered chains[20] allows the bilayer to accommodate the wedge insertion without disturbing the overall matrix of the phospholipid bilayer membrane. The ability of a membrane containing a lateral phase separation to accommodate volume changes is termed high lateral compressibility.

Proteins have been shown to undergo large volume changes during their function.[21] These volume changes may result from changes in three-dimensional structure of conformation. Clearly, if the function of a protein involves passing from the exterior of a cell into the interior of the membrane, the volume change in the membrane would be significant. These considerations suggest that the existence of lateral phase separations may be essential for some cellular processes. The importance of lateral phase separations to the function of membrane-solvated proteins has been demonstrated in bacterial systems in the laboratories of Linden and associates[22] and McElhaney.[23] In order for anesthetic modification of phase separations to be important in mammalian systems, it is necessary that regions of lipid capable of undergoing phase transitions occur near proteins. Griffith *et al.* used electron paramagnetic resonance techniques to demonstrate a boundary layer of lipids that surround membrane proteins.[24] Similarly, Stier and Sackmann have described a phase transition-dependent halo of lipid surrounding the cytochrome P-450 reductase hydroxylating system.[25] These and other[26] studies demonstrate that there are regions of phospholipid bilayer that could contain lateral phase separations surrounding important membrane proteins, and that these separations are important to cellular function.

These considerations may be developed into a unitary theory of anesthesia based on the demonstration that at a given temperature clinical concentrations of anesthetics will alter or eliminate lateral phase separations in a phospholipid bilayer.[17–19] The assumption that these bilayer properties are important to neural function is implicit in the following discussion.

Discussion

Three components of neural function that may be susceptible to an alteration in membrane phase separation behavior are considered. The first proposed mechanism of action is based on the hypothesis that a transmembrane protein forms a selective ion channel for sodium or potassium ions. A small

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conformational change in this channel would serve to stop ionic conductance and thus act as a gate. A similar conformational change in a protein may also be involved in recognition of transmitter by its receptor at the synapse and subsequent initiation of the permeability changes that form the basis for many postsynaptic potentials.

A possible mechanism by which an anesthetic may inhibit these essential conformational changes is illustrated in figure 2. Figure 2*A* depicts a phospholipid bilayer containing a membrane-solvated protein that contains a small pore. While the drawing depicts a hypothetical sodium channel, it is important to emphasize that the same considerations may apply to any protein in a nerve membrane that must move or change its shape in order to function, whether to open an ion pore or to recognize a synaptic transmitter. The phospholipid bilayer in figure 2*A* is seen to contain regions of high-volume disordered fatty-acid chains, as well as lower-volume ordered chains. In figure 2*B*, the transmembrane protein has undergone a conformational change in order to expand the sodium channel to initiate a sodium flux and the initial phases of an action potential. In doing so, some of the neighboring high-volume fluid-phase fatty-acid chains have been transformed into the lower-volume highly ordered solid phase, as described in figure 1. This allows the protein to expand without encountering high lateral pressures or disordering the entire matrix of the membrane. However, the application of inhalation anesthetic molecules to the membrane

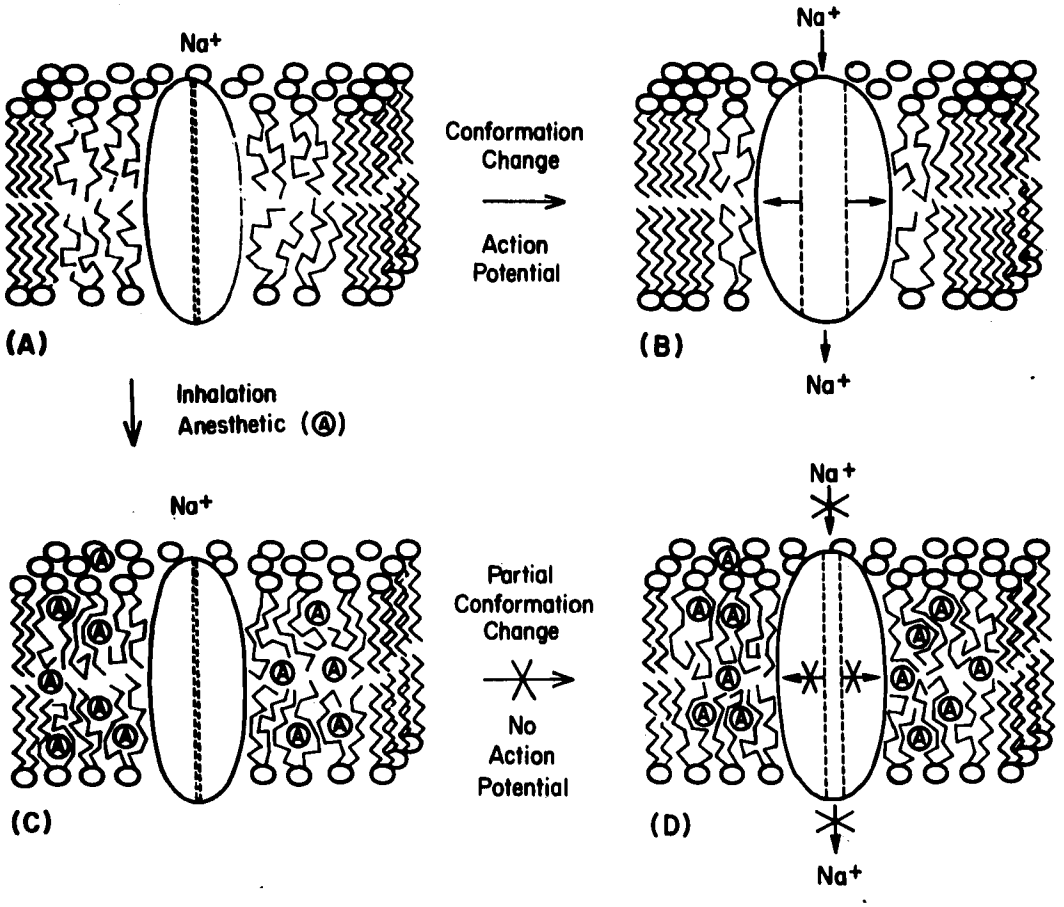

FIG. 2.*A*, a phospholipid bilayer containing a membrane-solvated globular protein that has a sodium channel in the closed configuration.

B, the globular protein has expanded in conformation to allow a sodium-ion flux. The expansion is accomplished by converting some high-volume fluid-phase lipids into the low-volume solid phase.

C, anesthetic molecules have fluidized the entire bilayer and destroyed the regions of solid phase.

D, conversion of the high-volume fluid-phase lipids into the low-volume solid phase is a high-energy process. Therefore, the protein is unable to expand or change conformation, and the excitation process does not occur.

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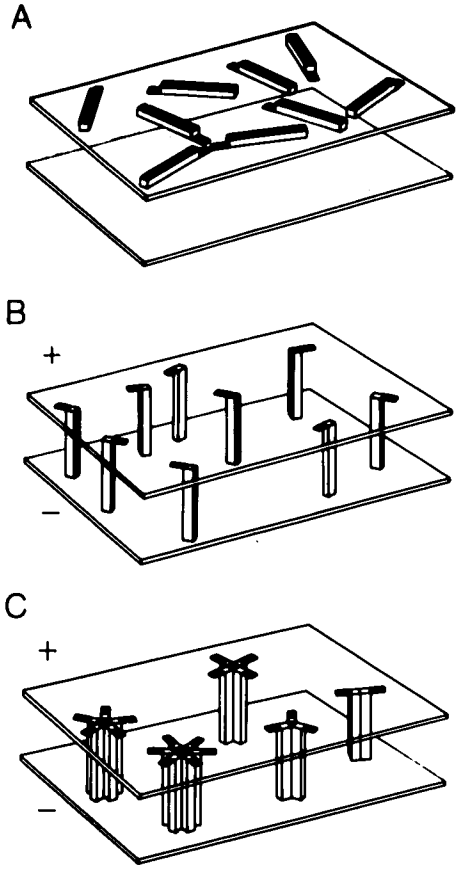

FIG. 3. Model of the excitation process in a nerve membrane.

A, at rest, protein subunits lie flat on the membrane surface represented by the upper plane.

B, an applied field has pulled the molecules into the membrane toward the *trans* surface.

C, lateral diffusion within the membrane leads to aggregation of the monomers into oligomers. Trimers, tetramers, pentamers, and hexamers form a central opening acting as a channel for the flow of ions.

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bilayer results in the disordered bilayer in figure 2*C*. This anesthetic-containing bilayer is in a more highly disordered state because of the influence of the anesthetic molecules.[14,17–19] The protein in figure 2*C* is not transformed to an active form as in figure 2*B*, because the disordered fatty-acid chains near the protein now require a large input of energy in order to be transformed into the lower-volume solid phase.[19] The protein cannot expand without encountering high lateral pressures. Therefore, the conformational change does not occur in this example (the pore does not fully open and an action potential is not observed). When a number of such proteins become unable to function in a nerve membrane, the membrane could be said to be in an anesthetized state. It will regain function only when the anesthetic molecules are removed.

A second and distinctly different neural protein function that may depend on lateral phase separations is based on the theory of membrane excitability proposed by Baumann and Mueller, diagrammed in figure 3.[27] Their theory includes protein subunits lying on the surface of a nerve membrane (fig. 3*A*) which, under the electrostatic influence of a conducted action potential, are pulled into the bilayer (fig. 3*B*) and aggregate to form a polymeric protein bundle with an ion pore in the center (fig. 3*C*). If one considers the protein subunits to be straight rods, the ion pore may be visualized as the long narrow space formed between four parallel rods held tightly together. It is proposed that the insertion of the protein subunits and their subsequent migration in the plane of the membrane to form a polymeric bundle would require much less energy if the membrane contained lateral phase separations. Thus, if the Baumann–Mueller theory is correct, the inability of the membrane to accommodate volume changes when anesthetics are present, illustrated in figure 2, would produce anesthesia.

The model of membrane excitation proposed by Blumenthal describes similar polymeric protein ion channels; however, these channels would not dissociate into surface monomers in their nonconducting state. Instead, the ionic conductance would be controlled by a pattern of wave propagation over the membrane surface.[28] This pattern is likely to be a function of membrane lateral compressibility, which is in turn dependent on lateral phase separations in the plane of the membranes, as described above.[20] In this case, it is proposed that anesthetics would affect the propagation of neural excitation by changing the membrane wave pattern that controls communication to the individual ion channels.

A third nerve membrane function that may be dependent on lateral phase separations involves synaptic transmitter release. It is likely that this nerve membrane function does not involve proteins, but rather the fusion with the presynaptic membrane of small bilayer vesicles filled with synaptic transmitter (fig. 4). The vesicle fusion illustrated in figure 4*B* results in exocytosis of the transmitter substance (T) into the synaptic cleft (fig. 4*C* and *D*).[29,30] Since membrane fusion has been shown to be greatly facilitated by lateral phase separations,[31,32] it is proposed that the alteration or elimination of these lateral phase separations by an anesthetic may reduce the amount of transmitter released in response to an action potential (fig. 4*E*).

It can be seen that there are several mechanisms by which anesthetics may affect neural function by altering lateral phase separations in membranes. Moreover, it is possible that the neural proteins in various parts of the anatomy are surrounded by

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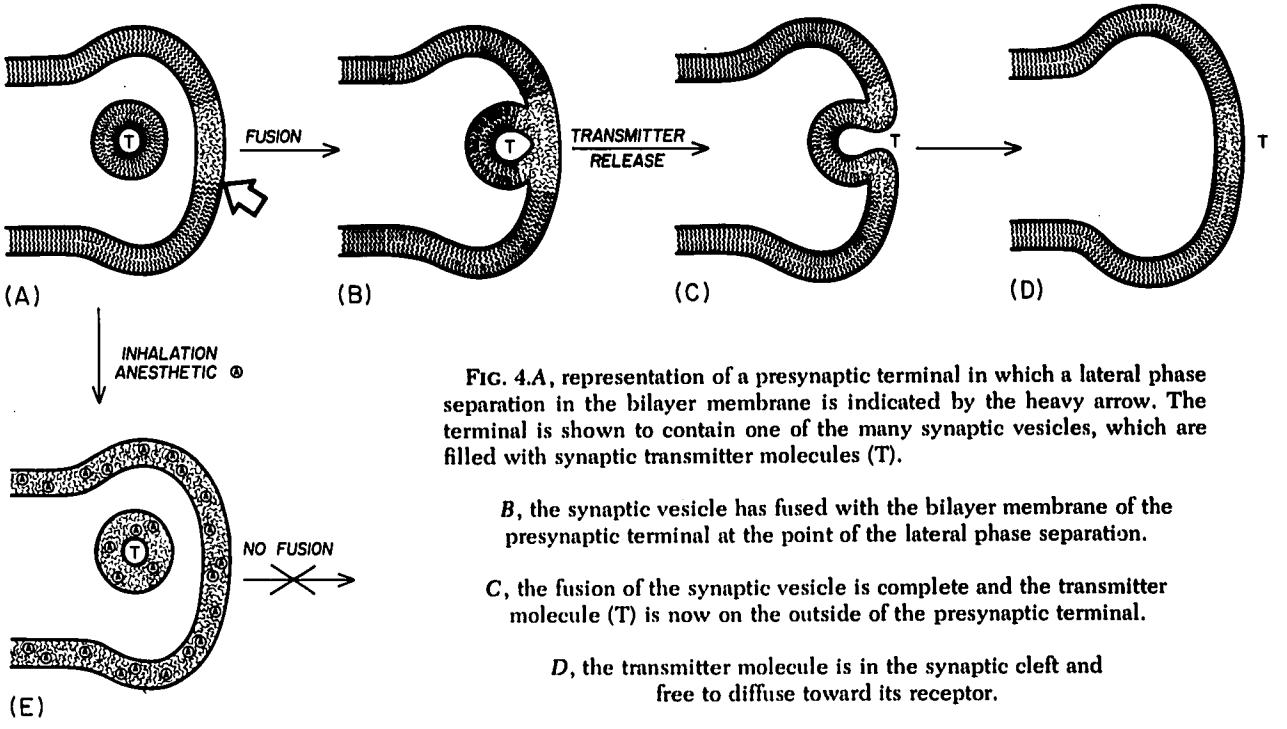

FIG. 4.*A*, representation of a presynaptic terminal in which a lateral phase separation in the bilayer membrane is indicated by the heavy arrow. The terminal is shown to contain one of the many synaptic vesicles, which are filled with synaptic transmitter molecules (T).

B, the synaptic vesicle has fused with the bilayer membrane of the presynaptic terminal at the point of the lateral phase separation.

C, the fusion of the synaptic vesicle is complete and the transmitter molecule (T) is now on the outside of the presynaptic terminal.

D, the transmitter molecule is in the synaptic cleft and free to diffuse toward its receptor.

E, the bilayer membrane of the presynaptic terminal is fluidized by the presence of anesthetic molecules (A). Lateral phase separations no longer exist. The studies in references 31 and 32 suggest that subsequent fusion will be inhibited by the lack of lateral phase separations.

boundary layers of differing phospholipid compositions. The effect of an equipotent concentration of an anesthetic on membrane lateral phase separations is dependent on both phospholipid composition[19] and the structure of the anesthetic.[17,18] Thus, a minimum alveolar concentration of an anesthetic will produce an effect on the function of a nerve cell that is dictated by the particular phospholipid composition of the cell and the molecular structure of the anesthetic. This suggests that a given anesthetic that is an effective central nervous system depressant may also exert a strong effect on nerves associated with cardiac function, whereas a second equally potent anesthetic may instead exert a profound effect on nerves associated with respiration. In this way, many of the side effects of anesthetics may be explained. The same concept of differential effects, applied to the interaction of excitatory and inhibitory synapses, may explain why some structural isomers of clinically used anesthetics are convulsants.

It is clear that no single theory will explain the variety of effects of the many different anesthetic agents. It is proposed that the differential alteration of lateral phase separations may offer a unitary framework in which to consider anesthetic action at the molecular level.

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