

Editorial Views

Anesthesiology
47:1-3, 1977*Anesthetics and Pressure Reversal of Anesthesia:**Expansion and Recompression of Membrane Proteins, Lipids, and Water*

THE IMPORTANT PAPER by Kendig and Cohen in this issue¹ further strengthens the long-standing hypothesis that general and local anesthetics may have a common hydrophobic site of action in biologic membranes. They found that high atmospheric pressure restored the depression of the compound action potential produced by halothane, methoxyflurane, lidocaine, or benzocaine. Although it had been known that conduction blocks produced by inhalational anesthetics^{2,3} or ethanol⁴ could be reversed by high pressure, the present study successfully demonstrates this reversal effect for local anesthetics for the first time.

High pressure also reverses general anesthesia elicited by ethanol,⁵ inhalational agents,⁶ barbiturates,^{6,7} narcotics and neuroleptics,⁸ but such pressure antagonism in the whole animal appears to be associated with a general central nervous system stimulation,⁷ thus considerably complicating any interpretations. The choice of a simple isolated system by Kendig and Cohen facilitates an analysis of the possible mechanisms of anesthetic action and pressure reversal.

Since 1899-1935, when H. H. Meyer, Baum, Overton, and K. H. Meyer (see references in ref.8), suggested that anesthetics acted in the cell "lipoids," it has taken much effort to prove that anesthetics do indeed attach to biomembranes by hydrophobic bonding.^{8,9} As more data accumulated, it became evident that it is not so much the actual anesthetic concentration within the membrane's hydrophobic domains (lipids or proteins) that seems to be important, but rather the *volume* of hydrophobic space *disturbed* or *occupied* by these anesthetic molecules within the hydrophobic regions of the membrane.^{8,10-12} These observations have led to the

protein-unfolding theory,¹³ the membrane expansion theory,^{11,8,14} and the critical-volume hypothesis^{10,12} of anesthesia. These hypotheses agree that some micro-domain within the excitable membranes becomes expanded, but Eyring postulates this to be membrane proteins, Seeman postulates it to be membrane proteins and/or lipid, while Miller suggests it to be primarily membrane lipid. Since both proteins^{15,16} and pure lipid films^{8,17,18} are expanded by anesthetics and recompressed to varying extents by pressure, it is difficult to determine which site is more sensitive to anesthetics and to pressure reversal.

Much evidence points to the conclusion that the dominant membrane action of anesthetics is to expand membrane proteins. For example, surgical concentrations of halothane, chloroform, ether, and methoxyflurane, as well as ethanol, expand the specific volumes of erythrocyte and synaptosome membranes by 0.4-0.6 per cent,^{14,19} while pure phospholipid-cholesterol mixtures are expanded only by 0.01 per cent¹⁴ under similar conditions; the latter value is approximately the volume of the molecular space the anesthetic molecules would occupy within the lipid.^{8,20,14} Second, anesthetics are known to "fluidize" and disorder biologic^{8,21} as well as lipid membranes,^{22,23} but the anesthetic concentrations required to do this tend to be higher than those producing general anesthesia or generalized membrane expansion.^{8,24} Third, the so-called "pressure" antagonism or reversal of anesthetic-induced lipid disorder²² or anesthetic-induced leakiness in liposomes²⁵ is not true pharmacologic antagonism, since pressure by itself reduces the leakiness and disordering of lipids without shifting the actual concentration-response curve of the anesthetic; in

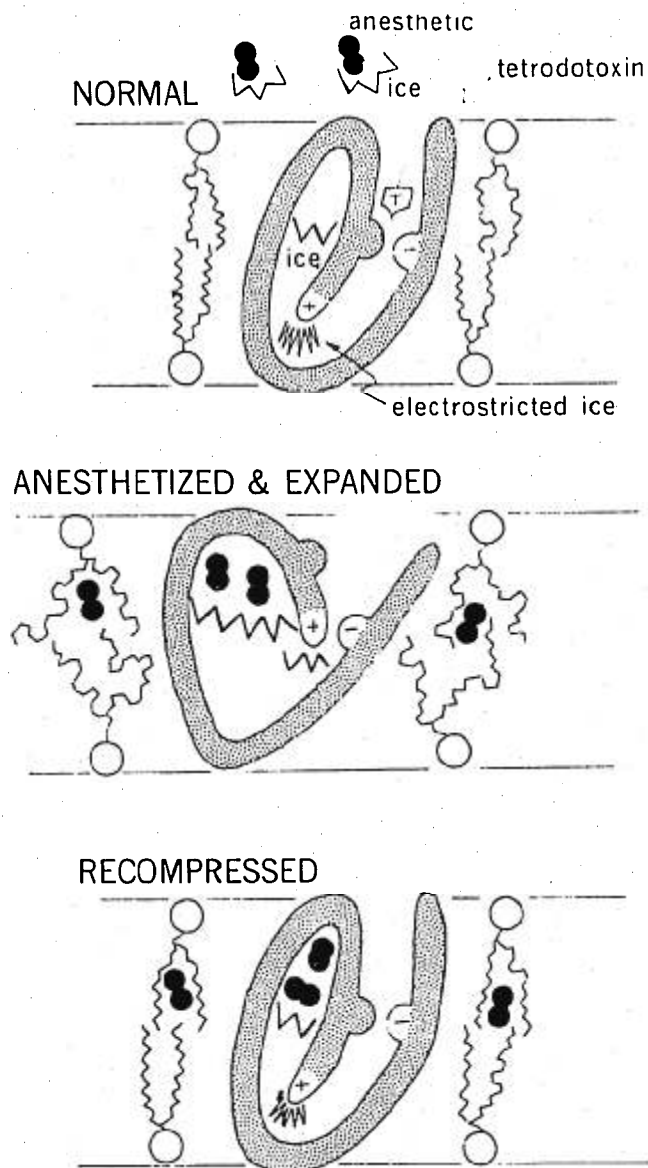


FIG. 1. A model for the events described by Kendig and Cohen.¹

Top: The normal excitable membrane of the nerve cell contains coiled proteins and semi-fluid lipid molecules. The anesthetic molecules are known to be associated with "organized" or "ice-like" water; such organized water (W-shaped symbols) also exists in association with hydrophobic regions of proteins. Tetrodotoxin (T) indicates the site of the sodium conductance channel, which becomes blocked in anesthesia. The charged sites (+) of proteins are associated with very dense ice (electrostricted water).

Center: Anesthetized state. The nerve cell membrane now contains the anesthetic molecules, which have attached to proteins and lipids by hydrophobic bonding. The anesthetic within the protein not only directly expands the protein, but also brings more ice, which expands the membrane even further. Electrostricted ice will contribute to expansion if the charge site (+) is neutralized. The sodium conductance channel is now distorted and the cell's excitability is blocked. Expansion and fluidization of membrane lipids also occurs, but the magnitude of the lipid expansion is much less than that of the membrane proteins.

Bottom: Pressure reversal of anesthesia. The membrane excit-

other words, the concentration-response curves at different pressures are all parallel,^{22,25} whereas they should converge for true antagonism.

The overall expansion of the excitable membrane by the anesthetic, as well as the amount of recompression by high pressure, is made up of different contributions from membrane proteins, lipids and water. Eyring *et al.*¹³ and Ueda *et al.*¹⁰ have emphasized the importance of protein-associated water in anesthetic-induced expansion.

Hence, the results of Kendig and Cohen require some kind of pressure-sensitive site common to general and local anesthetics (but not tetrodotoxin). Such a site is schematized in figure 1. The top of the figure illustrates the normal membrane with the anesthetic starting to enter the membrane. The anesthetic molecules are drawn with a partial ice-cover (indicated by the W-shaped markings), since hydrophobic molecules have long been known to induce ice formation (see references in ref. 8). The hydrophobic region of the membrane protein also contains some such hydrophobically-associated ice. The ionized regions of the protein, however, are associated with electrostricted water (very dense ice). In the center of the figure, the anesthetic molecules have formed a hydrophobic bond with the hydrophobic region of the membrane protein. The protein has expanded as a consequence of several factors: 1) the hydrophobic region of the protein has enlarged slightly not only due to the presence of anesthetic, but also because there will be more hydrophobically-associated ice-water; 2) the electrostricted water will be released if ion-pairs are formed, further expanding the protein; 3) lipid expansion and fluidization also occur, leading to secondary changes in protein conformation.

Finally, the recompression events at high pressure are depicted at the bottom of the figure. The anesthetic remains within the hydrophobic domains,²⁶ but the absolute reformation of the excitable protein (the sodium-conductance channel protein) is presumably primarily restored by the pressure-induced melting of the hydrophobic ice-caps, and the restoration of electrostricted water; lipid re-ordering must also contribute to normalizing the membrane protein excitability. These qualitative contributions must be precisely measured in future efforts to resolve the details of the anesthetic

able protein is now recompressed to almost its original state, despite the continued presence of the anesthetic molecules. The recompression is made up of several components, including the melting of hydrophobic "ice caps," the direct refolding of the protein, the restoration of some electrostricted water, and similar events (but smaller in magnitude) in the lipid regions of the membrane.

Note that the action of tetrodotoxin is to block the conductance channel directly without eliciting membrane expansion; thus, pressure reversal of tetrodotoxin block does not occur.

process. In addition to the nuclear magnetic resonance and electron paramagnetic resonance spectrometers, an increasingly valuable tool will turn out to be the high-precision density meter.¹⁴

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