ANESTHETICS DECREASE SURFACE POTENTIAL OF PROTEIN

I. Ueda, M.D., Ph.D., T. Mashimo, M.D. and D.D. Shieh, Ph.D.

Department of Anesthesia
University of Utah Medical Center
Salt Lake City, UT 84132
and
University of Kansas Medical Center
Kansas City, KS 66103

We have proposed that anesthetics interact with hydrophobic parts of protein macromolecules and unfold their structure. In the native state, polypeptide backbone of a protein is folded in such a way to expose mainly hydrophilic ionic charges to its surface. Out of the numerous possible ways of doing this, only single structure stands out when a protein is dissolved in water. This is apparently due to the interaction between water molecules and the protein.

We have postulated that in a protein molecule in water, hydrophobic interaction is in competition with the ionic bonding, and in the native state hydrophobic interaction is present in the expense of ionic bonding. The resulting free ionic charges exert strong electrostatic force to the water dipole to form compact crystalline lattice. The crystalline water structure is an ice, but the ice formed under electrostatic force is different from the one frozen at 0°C. Ice polymorphism under pressure is well known, and Ice-III exists at 2700 bars and is 15% more dense than bulk water due to distorted hydrogen bondings. The electrostatic force of the free ionic charge exerted upon water dipole is large to form Ice-III-like structure and the attracted water molecules decrease its volume about 2.7 cm^3/mol. This reduction of volume is known as electrostriction.

The effects of anesthetics upon proteins are to relax their structures, and displace the charged sites from the surface. Then the electrostricted water molecules are released, expanding their volume concomittently. We visualize anesthesia as an interfacial phenomenon, changing the state of cluster of water molecules coopertively between electrostricted compact awake state and expanded anesthetized state. The increased hydrophobicity of the interface would interfere with the passage of hydrated ions across the membrane.

The above hypothesis predict that the surface electrical charges of proteins decrease under anesthesia. In order to test the prediction, we used a pH-indicator dye, bromothymol blue, and crystalline bovine serum albumin.

The pH at the surface of a protein is different from the bulk pH due to the surface electrostatic charge of the macromolecules interacting with the positively charged protons. The force between the surface charge and proton can be expressed quantitatively according to the Boltzmann law. The local hydrogen ion activity, a_{H+}, in the vicinity of the surface charge, ψ, is expressed by

$$a_{H+}^{s} = a_{H+}^{b}\exp(-F\psi/RT)$$

where superscripts s and b denote surface and bulk, respectively, F is Faraday's constant, R is the gas constant and T is the absolute temperature. The logarithmic form of the above equation gives

$$pH^{s} = pH^{b} + F\psi/2.3RT$$

Therefore, the difference between the bulk pH and the surface pH represents the effect of the surface potential.

In the present experiment, the bulk pH was measured by a Corning digital pH meter and a glass electrode, and the surface pH was measured by the color of the protein-bound bromothymol blue. An ultrafiltration study with an Amicon system and Diaflow PM 30 filters established that all the dye molecules were bound to the protein under the present experimental conditions (bovine serum albumin 0.04 mM and bromothymol blue 0.04 mM at 25°C). The color of the bound dye was measured precisely with a Beckman 5270 scanning spectrophotomter, after establishing the absorbance-pH curve at 615mm. (The local dielectric constant also affects the color of the dye, but the Debye-Huckel screening of the surface charge by high ionic concentrations revealed that this effect did not interfere with the present experiment.)

Addition of inhalation anesthetics to the albumin-dye solution changed the color of the bound dye in a dose-dependent manner. It was estimated that methoxyflurane and enflurane decreased the absolute value of the surface potential of bovine serum albumin about 12 mV at their clinical tensions.

The present results support our view that the conformational changes of proteins by anesthetics accompanies with the decrease of surface charge resulting in the release of electrostriction imposed upon the vicinal water molecules.