Pressure, anesthetics, and membrane structure: a spin-probe study

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Finch, E. D., and L. A. Kiesow. 1979. Pressure, anesthetics, and membrane structure: a spin-probe study. Undersea Biomed. Res. 6(1): 41–45.—Fatty acid spin-probe analyses at various depths in the membrane bilayer of intact erythrocytes indicate that compressional effects of pressure and fluidizing effects of lipid-soluble anesthetics are detectable only in hydrated regions of the membrane bilayer. Since current theories of anesthetic action cannot explain these observations, a proposal to do so is outlined.

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hyperbaric gases	anesthetics
biomembranes	hydration
spin probes	red blood cells

Several different hypotheses have been presented to explain the effects of general anesthetics, inert gases, and pressure on excitable membrane function. These hypotheses can be separated into two general categories: those for which the primary target of the perturbing agent is the membrane proteins and their water of hydration (Pauling 1961; Miller 1961; Eyring, Woodbury, and D'Arrigo 1973) and those in which the primary target is the membrane lipids (Meyer 1937; Miller 1974). Currently, the most widely accepted proposals for the action of general anesthetics, inert gases, and pressure are those that postulate direct changes in the physical state of membrane lipids. According to the Critical Volume Hypothesis (Miller 1974), general anesthesia and inert gas narcosis occur when the volume of the lipid portion of the membrane is expanded by about 1%; pressure-induced convulsions occur when the volume is compressed by about 1%.

The Critical Volume Hypothesis provides a qualitative explanation for the pressure reversal of anesthesia (Miller 1974). If general anesthetics and hyperbaric inert gases such as N_2 do expand membrane lipids beyond the proposed threshold for narcosis, hydrostatic pressure may indeed compress these lipids, reducing their volume below this threshold. These ideas, if correct, could have practical application in the prevention of inert gas narcosis and the so-called high pressure nervous syndrome experienced by deep-sea divers (Miller 1974; Bennett, Blenkarn, Roby, and Youngblood 1974).

Most of the evidence supporting the concept of lipid expansion and contraction is indirect and consists mainly of calculations using the solubility and molar volume of gases in oil and oil compressibility (Miller 1974). Recently, electron spin resonance (ESR) spin-probe analysis

methods have been developed that allow one to monitor structural changes in biologic membranes directly. Spin probes are stable nitroxide-free radicals that yield ESR spectra sensitive to the motional freedom of the free radical allowed by its local environment (Berliner 1976). In this study, fatty acid probes of the form:

$$CH_3\text{--}(CH_2)_m\text{-----}\overset{\displaystyle O\quad N\quad O}{C}\text{-----}(CH_2)_n\text{--}COOH$$
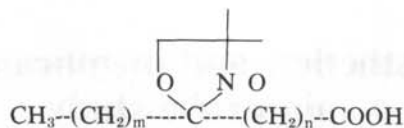
have been employed; the indices m and n, expressed as the ratio (m:n), designate the location of the nitroxide moiety along the hydrocarbon chain. When intercalated into biomembranes, these probes orient with the carboxyl group near the polar surface of the membrane.

In this report, we present the results of spin-probe analyses of human erythrocytes under pressure and anesthetic stress. Spin-probe analysis has been shown previously to be sensitive to small perturbations in red cell membrane structure (Huestis and McConnell 1974; Kury and McConnell 1975; Mason, Giavedoni, and Dalmasso 1977). The erythrocyte membrane has also been used extensively as a model for studying the mechanisms of anesthesia (Seeman 1972).

METHODS

Fresh red cells from heparinized human venous blood were washed twice and suspended in a Krebs-Ringer-phosphate buffer, pH 7.4, containing 5 mM D-glucose. Probes with (m:n) values of (12:3), (5:10), and (1:14) were employed in this study. Red cell suspensions (hematocrit ~ 80 vol %) were spin labeled according to the methods outlined by McConnell and co-workers (Huestis and McConnell 1974; Kury and McConnell 1975). ESR studies were carried out with a Varian E-4 spectrometer. To improve sensitivity, ESR derivative spectra were usually recorded using the expanded-axis technique described by Gordon and Sauerheber (1977). The sample chamber consisted of a section of high pressure nylon tubing (3.2 mm o.d., 1.6 mm i.d.) passing through the ESR sample cavity. During the pressure measurements, the nylon tubing was connected to a tank of compressed gas. For hyperbaric studies, a 7.5 cm long section of Teflon tubing (1.1 mm i.d.) was filled with the labeled red cell suspension and sealed at both ends with wax. The sample was then inserted into the nylon pressure tubing and centered in the ESR cavity; the active region of the cavity is about 2.0 cm in length. Under hyperbaric conditions, gases pass through the gas-permeable wall of the Teflon tubing and diffuse into the sample. Nitrogen gas was found to equilibrate in less than 2 h, as judged by the cessation of changes in the ESR spectra. Helium equilibrated in less than 30 min. Measurements under hydrostatic pressure were carried out with the red cell suspensions enclosed in gas-impermeable glass capillaries (1.2 mm i.d.). Nongaseous anesthetics were added to the red cell suspension before the samples were drawn into either glass or Teflon capillaries; Teflon is impermeable to halothane, chloroform, and octanol.

The sample temperature was closely regulated within ± 0.1°C. This was accomplished by passing a thermostated heptane-mineral oil coolant from a circulating bath along the outer wall of the nylon pressure cell and inside a quartz Dewar jacket. The temperature of the coolant was measured with a thermistor probe near the sample. All ESR measurements reported here were carried out with the sample temperature set at 37.0 ± 0.1°C. Precise temperature regula-

tion was crucial in our experiments since pressure and anesthetic-induced changes in membrane fluidity were often small and comparable to the changes produced by small temperature variations.

Polarity-corrected order parameters (S values) were calculated from the positions of the inner and outer peaks of the ESR spectra according to the method outlined by Gaffney and McConnell (1974). An increase in S indicates increased constraints on the motion of the spin probe in the membrane. In Fig. 1, pressure- and anesthetic-induced changes in membrane fluidity are characterized as ΔS, the % change in S. The concentrations of halothane, chloroform, and octanol used (see Fig. 1) are approximately equal to those that block nerve conduction and provide 50% protection of red cells against hemolysis by osmotic shock (Seeman 1972). All hydrostatic and hyperbaric measurements were made at a pressure of 100 ATA; hyperbaric nitrogen has been reported to block nerve conduction at about 143 ATA (Seeman 1972).

RESULTS AND DISCUSSION

Hyperbaric helium (100 ATA) produced results that were essentially identical to those of hydrostatic pressure. Previous studies have shown that anesthetics and hydrostatic pressure have opposing effects on the fluidity of protein-free artificial membranes (Boggs, Yoong, and Hsia 1976; Chin, Trudell, and Cohen 1976). Similarly, our results demonstrated opposing effects in the red cell membrane and showed additionally that the absolute and relative magnitudes of pressure- and anesthetic-induced changes in spin-probe mobility depend critically on the depth of the nitroxide moiety in the membrane. For example, hyperbaric nitrogen (100 ATA) produces a net increase in S with the (12:3) probe and a net decrease in S with the (5:10) probe.

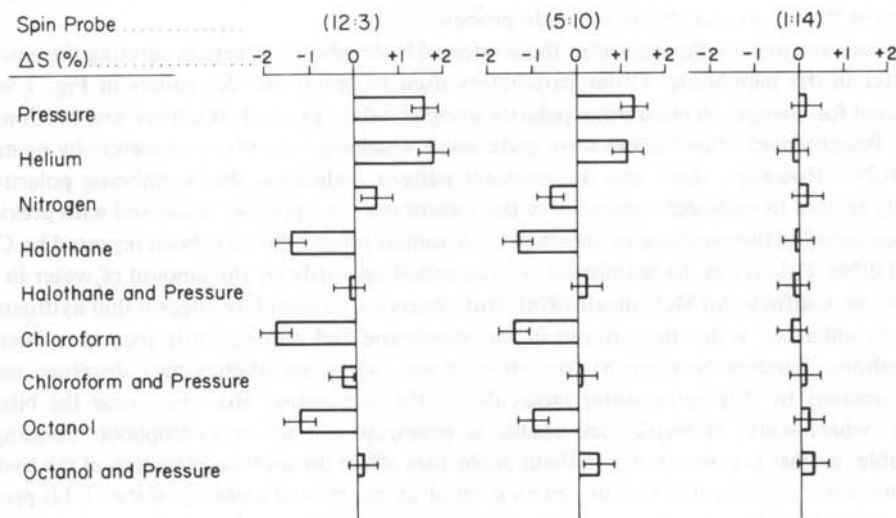Fig. 1. Bar graph showing % change (ΔS) in order parameter, S, that occurred when red cell suspensions labeled with (12:3), (5:10), and (1:14) spin probes were exposed to: pressure (100 ATA hydrostatic); helium (100 ATA hyperbaric); nitrogen (100 ATA hyperbaric); halothane (5 mM); chloroform (5 mM); octanol (0.5 mM). Graph shows average values ± SE.

No pressure- or anesthetic-induced change in red cell membrane fluidity was detected with the (1:14) probe (see Fig. 1). The nitroxide moiety on this probe sits deep in the membrane near the bilayer center. The mobility of the nitroxide moiety is most constrained near the bilayer surface ((12:3) probe) and most unconstrained near the bilayer center ((1:14) probe). The (1:14) probe resides in a region of the membrane in which the fluidity most closely resembles that of oils that have been used as physical models in the development of membrane lipid expansion-contraction theories of anesthesia, i.e., motion is nearly isotropic and fluidity is high (McConnell and McFarland 1972). The absence of detectable changes using the (1:14) probe indicates that theories of anesthesia based on the expansion and contraction of membrane lipids are, at best, an oversimplified view of anesthetic-membrane-pressure interactions. Lipid expansion and contraction theories in their current form cannot explain the gradient of anesthetic-pressure-induced changes within biologic membranes, such as those reported here. We are therefore outlining a new proposal for the action of pressure and anesthetics on membrane structure.

It is well known that the dominant attractive force that holds the phospholipid matrix of biological membranes together is the hydrophobic force; the evidence has been reviewed by Tanford in his book *The Hydrophobic Effect: Formation of Micelles and Biological Membranes* (1973). Hydrophobic effects stem from the strong attractive forces between water molecules. To minimize the disruption of these attractive forces, amphiphilic molecules such as phospholipids are arranged in membrane bilayers so that contact of the nonpolar portion of the molecule with surrounding water is reduced. It has generally been thought (Tanford 1973), because of the absence of water molecules in these regions, that hydrophobic forces have little effect on the internal structure of biological membranes. However, recent ESR (Griffith and Dehlinger 1974) and neutron diffraction studies (Zaccai, Blasie, and Shoenborn 1975) indicate that water molecules penetrate into biological membranes to depths near the nitroxide moiety of the (12:3) and (5:10) spin probes but not the (1:14) probe. Consequently, the dynamic structure of hydrocarbon chains should be affected by hydrophobic effects in regions of the membrane where water molecules penetrate, which in turn should have an effect on the motion of the (12:3) and (5:10) nitroxide probes.

Pressure and anesthetics may alter these internal hydrophobic effects by altering the amount of water in the membrane. Order parameters used to obtain the ΔS values in Fig. 1 were corrected for changes in membrane polarity using standard methods (Gaffney and McConnell 1974). Polarity correction factors were quite small, changing the order parameters by no more than 0.2%. However, there was a consistent pattern, indicating that membrane polarity is directly related to motional constraint in the membrane, i.e., polarity increased with pressure and decreased in the presence of anesthetics. A similar observation has been reported by Chin et al. (1976). Polarity in the membrane is determined primarily by the amount of water in the membrane (Gaffney and McConnell 1974). Our observations therefore suggest that hydrostatic pressure enhances water penetration in the membrane and consequently increases internal hydrophobic bonding between hydrocarbon chains, while anesthetics may decrease membrane polarity by displacing water molecules in the membrane. However, near the bilayer center, where water molecules are unable to penetrate and where hydrophobic bonding is negligible, neither pressure nor anesthetic molecules affect the molecular motion of the hydrocarbon chains, as indicated by our observation of an unaffected mobility of the (1:14) probe. This proposal for the role of water in determining pressure and anesthetic effects on membrane structure differs from other hypotheses (Pauling 1961; Miller 1961; Eyring et al. 1973), since it involves changes in membrane water penetration rather than water structure per se.

Finch, E. D., and L. A. Kiesow. 1979. La pression, les anesthesiques, et la structure des membranes: étude par résonance paramagnetique électronique. Undersea Biomed. Res. 6(1):41–45.— Des analyses d'acides gras par une méthode de résonance paramagnetique électronique à diverses profondeurs de la couche double de la membrane de globules rouges intactes montrent que les effets mécaniques de la pression et les effets des anesthésiques solubles dans les lipides ne se laissent dépister que dans les régions hydratées de la membrane. Les hypothèses courantes de l'activité des anesthésiques ne rendant pas compte de ce phénomène, les auteurs en proposent une nouvelle.

gaz hyperbariques   globules rouges
membranes biologiques   anesthésiques
résonance paramagnetique électronique   hydratation

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