TITLE: CYTOCHEMICAL LOCALIZATION OF CA-ATPASE IN HUMAN SKELETAL MUSCLE

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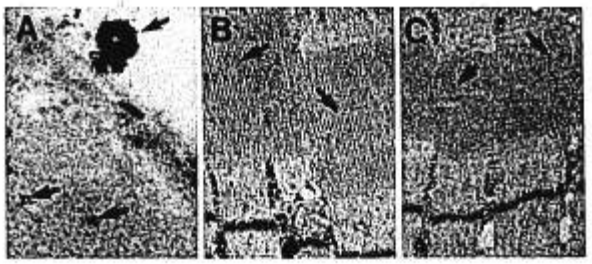
Impaired regulation of myoplasmic calcium concentrations in skeletal muscle appears to be an important mechanism in the development of malignant hyperthermia (MH). Calcium adenosine triphosphatase (Ca-ATPase) is a membrane-bound, energy dependent calcium extrusion pump that is involved in the regulation of intracellular calcium (Ca) levels. Biochemical studies have produced conflicting results regarding Ca transport by the sarcoplasmic reticulum (SR) of skeletal muscle from MH-susceptible (MHS) individuals. The objective of this study was to determine if Ca-ATPase activity could be localized cytochemically in muscle from individuals undergoing diagnostic testing for MH.

All studies were conducted on tissue that remained after diagnostic testing (N=11). Small muscle bundles were dissected from each sample, immobilized and fixed by immersion in cold buffered 2.5% paraformaldehyde. After buffer rinsing, a one-step method for demonstrating Ca-ATPase activity was employed (1). Muscle was incubated in medium containing 250 mM glycine, 3mM ATP, 10 mM Ca chloride, 4 mM lead citrate and 8mM levamisole. Muscle from normal patients served as controls, and cytochemical controls included omission of either ATP or Ca. Samples were post-fixed in osmium, and routinely processed for transmission electron microscopy.

An electron dense precipitate, dependent on the presence of ATP and Ca, indicated the presence of Ca-ATPase activity. Accumulations of precipitate were observed along the sarcolemma as filamentous, ring-shaped structures (Fig. A). Precipitate localized within the myofibril was less dense and more evenly distributed than that along the sarcolemma (Figs B/C). Differences in staining intensity and distribution in muscle from control and MHS individuals were not apparent when qualitatively compared.

Results of this study demonstrate the presence of Ca-ATPase activity at the ultrastructural level in human skeletal muscle from control and MHS patients. However, conclusions regarding SR function or the amount of CaATPase activity cannot be made.



CaATPase activity along the sarcolemma (A) in MHS muscle (60,000X); within the myofibril from control (B; 20,000X) and MHS patients (C; 19,000X).

1. Acta Histochem Cytochem 14:705-726, 1981.

A679

TITLE: ANESTHETICS FLUIDIZE MEMBRANE CORE AND SURFACE DIFFERENTLY

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Membrane fluidity has not been well defined, and "order parameter" is often preferred to express the membrane property. Nevertheless, order parameter represents the conformation of the lipid tails, and gives an erroneous impression that the property of the lipid core represents the property of the total membrane structure. Because membranes are highly structured, the properties of the core and surface are not necessarily identical. We report here that anesthetics increase the "fluidity" of the membrane core but decrease the "fluidity" of the interfacial region.

Infrared spectroscopy measures the state of vibrations of connections between atoms in a molecule. It can estimate presence of functional groups, three dimensional structure, intra- and inter-molecular interactions, etc., from the vibration of submolecular groupings. The vibrational characteristics of hydrophobic lipid tails and hydrophilic head-groups of dipalmitoylphosphatidylcholine molecules were assessed in unilamellar vesicle membranes. A Perkin-Elmer Fouriet-transform infrared (FTIR) spectroscope model 1750 interfaced with a 7300 computer and an attenuated total reflection cuvette was used. The behavior of the lipid tails was assessed by the vibrations of C-H stretching, and the behavior of the head group was assessed by the $P=O^-$ stretching, $(CH_3)_3\text{-}N^+$ stretching, and C=O stretching of the glycerol skeleton.

The main phase transition of phospholipid membranes between the solid-gel and liquid-crystalline states was characterized by a sudden shift of the C-H stretching to the higher frequency region. In the presence of volatile anesthetics (halothane, enflurane, and chloroform), the main transition temperature was depressed (the frequency-jump occurred at lower temperatures). In contrast, the $P=O^-$ stretching mode shifted to the lower frequency by the addition of anesthetics.

The shift of a peak to high frequency means that the bond started vibrating faster. Conversely, the low frequency shift means that the bond started vibrating slower. If one defines that the membrane fluidity is expressed by the movability of molecular structure, the anesthetics decreased the fluidity of the interface and increased the fluidity of the membrane core. A similar result was reported by Hitzemann[1] who used fluorescence of surface-bound and core-bound probe molecules and concluded that ethanol decreased the fluidity of the surface of the DPPC vesicle membrane.

The present result shows that the water molecules bound to the phosphate moiety of the membrane are replaced by the anesthetic molecules. The binding of the larger mass of anesthetic molecules would make the $P=O^-$ stretching to vibrate slower, and explains why alcohols decreased the fluidity of the membrane surface when measured by the fluorescence anisotropy[1]. The study further showed that these anesthetics partially released water molecules from the C=O moiety that is situated closer to the surface.

The notion that anesthetics fluidize cell membranes is misleading. The response of cell membrane structure to anesthetics is much more complicated than previously considered.

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Reference
1. Biochim Biophys Acta 983: 205-211, 1989