

Transverse tubules from frog skeletal muscle. Purification and properties of vesicles sealed with the inside-out orientation

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Transverse tubule vesicles were isolated from frog skeletal muscle by a procedure initially described by Roseblatt (J. Biol. Chem. 256, 8140-8148 (1981)) and later modified by Hidalgo (J. Biol. Chem. 258, 13937-13945 (1983)). A large fraction of the isolated vesicles (80-90%) were sealed, as indicated by the detergent induced increase in (Na⁺ + K⁺)-ATPase activity and ATP-dependent ouabain binding. To determine the orientation of the sealed vesicles binding of digoxin, a lipid soluble derivative of ouabain, was measured. The same values of ATP-dependent digoxin binding were found with or without detergents, indicating that all the vesicles that are sealed have the ATP site accessible, and hence are sealed with the cytoplasmic side-out (inside-out orientation). The transverse tubule preparation isolated from frog muscle is highly purified, as indicated by its cholesterol content and its (Na⁺ + K⁺)-ATPase activity; negligible contamination with sarcoplasmic reticulum was observed, as in