ELECTRICAL PROPERTIES OF CARDIAC SARCOPLASMIC RETICULUM MEMBRANE VESICLES

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Abstract

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The massive Ca^{2+} movements which occur across the sarcoplasmic reticulum during accumulation and release would be likely to generate a transmembrane potential. Previous studies of sarcoplasmic reticulum in intact tissues have supported the speculation that electrical fluctuations occur across this organelle during Ca^{2+} regulation. While conventional microelectrodes have been employed to study the electrical properties of the cardiac sarcolemma, the physical characteristics of the sarcoplasmic reticulum have rendered their use impractical on this membrane. It has recently become possible to monitor the electrical difference across cells, vesicles, and organelles by recording the absorbance and/or fluorescence of the membranes stained with a transmembrane voltage sensitive dye. The purpose of this research was to characterize the optical responses of the positively charged transmembrane voltage sensitive cyanine dye diS-C_3-(5) in order to elucidate the basic electrophysiological properties of cardiac sarcoplasmic reticulum membrane vesicles during Ca^{2+} handling.

Ca^{2+} fluxes across the vesicles were monitored spectrophotometrically with arsenazo III and by millipore filtration with $^{45}Ca^{2+}$. The absorbance at 670 nm of diS-C_3-(5) increased during ATP-dependent Ca^{2+} binding by the vesicles apparently as a function of both the intravesicular $[Ca^{2+}]$ and transmembrane potential. Dye calibration in the absence of Ca^{2+} revealed that when K^+ diffusion potentials were imposed, the absorbance of diS-C_3-(5) increased with inside positive transmembrane potential. Dye calibration in the absence of a transmembrane potential showed that added Ca^{2+} (mM) decreased absorbance when it was added to the extravesicular medium, or when it was preloaded within the vesicles. Therefore, the increase in dye absorbance produced during Ca^{2+} accumulation by sarcoplasmic reticulum vesicles could be represented by the difference between the electrical signal

which would increase the absorbance, and a concomitant enhancement of intravesicular Ca^{2+}-dye interactions which would decrease the absorbance of diS-C_3-(5).

These data suggest that Ca^{2+} accumulation by cardiac sarcoplasmic reticulum membrane vesicles generates an inside positive transmembrane potential. Two possible mechanisms exist for this electrical development: an electrogenic Ca^{2+}-ATPase; and the passive inward movement of K^+ down its chemical gradient after being extruded from the vesicles by the Ca^{2+}-ATPase.

The effect that selected cation ionophores had on the transmembrane potential following active Ca^{2+} accumulation by the vesicles was used to postulate a depolarization-repolarization cycle across the sarcoplasmic reticulum during the cardiac cycle. In accordance with the theory that alamethicin is a channel forming ionophore, a rapid Ca^{2+} efflux and a decrease in the absorbance of diS-C_3-(5) was observed upon introducing alamethicin to the vesicles following Ca^{2+} accumulation. This apparent alamethicin-induced depolarization was subsequently demonstrated to be due to a nonspecific alamethicin-dye interaction. The absorbance of diS-C_3-(5) returned to the baseline and resisted optical changes in the presence of alamethicin. After the transmembrane potential was generated by Ca^{2+} accumulation, the addition of the K^+ specific ionophore valinomycin appeared to produce a hyperpolarization. This finding indicates the existence of a K^+ concentration asymmetry across the membrane. This chemical gradient could have been generated by K^+ being extruded as the counterion to Ca^{2+} during Ca^{2+}-ATPase activity. A23187 dissipated the actively generated Ca^{2+} gradient and partially depolarized the membrane. A component of the A23187 mediated electrical signal appeared to be due to an electroneutral Ca^{2+},H^+-exchange. These data suggest that an electrical cycle could occur across the sarcoplasmic reticulum. The release of Ca^{2+} would depolarize the membrane, followed by repolarization which could occur by an induced increase in K^+ conductance.

Finally, replacing the impermeable anion methanesulfonate with the more permeable anion chloride was shown to depolarize the sarco-

plasmic reticulum membrane vesicles. However, this anion exchange did not induce Ca^{2+} efflux from the vesicles. This observation does not support the hypothesis of a depolarization induced Ca^{2+} release from the sarcoplasmic reticulum. The fact that an A23187 mediated Ca^{2+} efflux depolarized the membrane, and that anion exchange depolarized but did not induce Ca^{2+} release from the vesicles, suggests that depolarization of the sarcoplasmic reticulum was a consequence, not a cause, of Ca^{2+} efflux.

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