SODIUM AND POTASSIUM ION PERMEABILITY OF SARCOPLASMIC RETICULUM VESICLES

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1. Introduction

In muscle, Ca^{2+} is released into and absorbed from the sarcoplasm through the action of an intracellular membranous system, the sarcoplasmic reticulum. Purified sarcoplasmic reticulum vesicles retain a permeability barrier for Ca^{2+} [1], sucrose and larger ions, such as Tris, choline or gluconate [2]. We have now used tracer flux techniques and the fluorescent dye 3,3′-dipentyl-2,2′-oxacarbocyanine which indicates membrane potentials [3] to further examine Na^+ and K^+ permeability in rabbit skeletal-muscle sarcoplasmic reticulum vesicles. The data reported here suggest the presence of two types of sarcoplasmic reticulum vesicles – one type is highly permeable to Na^+ and K^+, and a second type is relatively impermeable to Na^+ and K^+. In vivo sarcoplasmic reticulum may therefore contain channels for small cations such as Na^+ or K^+.

2. Materials and methods

Gluconic acid (technical Grade; Eastman, Rochester, NY) was treated with charcoal before use. Other reagents used were of analytical grade. Radioisotopes were obtained from New England Nuclear (Boston, Mass.). The fluorescent dye 3,3′-dipentyl-2,2′-oxacarbocyanine [diO-C_5-(3)] was the generous gift of Dr Alan S. Waggoner (Amherst College, Amherst, Mass.).

Sarcoplasmic reticulum vesicles used in this study have been characterized previously [4]. Vesicles were prepared by zonal centrifugation of rabbit skeletal muscle homogenates and were subsequently extracted in 0.6 M KCl, 15% sucrose for 1 h at 0°C.

Isotope flux measurements were performed essentially as previously described [2].

Membrane potential changes were created by changing the ionic environment of the vesicles as outlined in the legend of fig.2 and were measured using the fluorescent dye diO-C_5-(3) [3]. Fluorescent assays were carried out at 4°C under stirring in a Perkin-Elmer MPF-3L Fluorometer. Excitation was at 470 nm and emission was recorded at 495 nm. Both slits were set at 0.52 mm to give a half-band width of 4 nm. The amount of added vesicles (about 17 μg protein/ml) was adjusted so that no change in fluorescence emission occurred when vesicles were diluted into a medium identical to the incubation medium.

3. Results

3.1. *[^{3}H]Choline, ^{22}Na and ^{86}Rb ion spaces and exchange rates*

Isotope flux measurements for [^{3}H]choline, ^{22}Na and ^{86}Rb ions were carried out under conditions of equilibrium between the outside and inside of purified sarcoplasmic reticulum vesicles. Vesicles were incubated in the presence of radioactive compounds and then diluted into an unlabeled medium of identical

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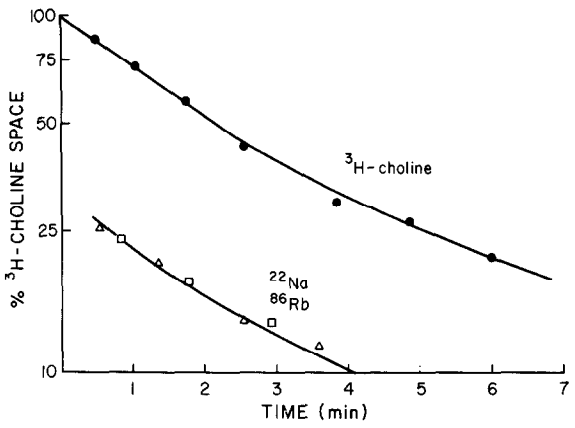

Fig.1. Measurement of [^{3}H]choline, ^{22}Na and ^{86}Rb ion-exchange rates and isotope spaces. Sarcoplasmic reticulum vesicles (18 mg protein/ml) were incubated for 24 h at 0°C in the following medium with [^{3}H]choline and either ^{22}Na or ^{86}Rb added: 200 mM choline Cl, 20 μM $CaCl_2$, 1 mM $MgCl_2$, 1 mM NaCl, 1 mM RbCl and 5 mM Pipes–Tris, pH 7.0. Vesicles were then diluted 200 times into an unlabelled medium of identical composition at 4°C. Filters were rapidly rinsed and the radioactivity remaining in the vesicles was determined [2]. Data are expressed as percent apparent [^{3}H]choline space extrapolated to zero time versus time. Rb^+ has been used rather than K^+ since there is no convenient radioisotope for K^+. Rb^+ and K^+ were found to behave similarly in the membrane polarization studies.

composition. Efflux of the radioactive compounds from the vesicles was monitored at various times at 4°C by Millipore filtration. Figure 1 shows that the apparent isotope spaces for Na^+ and Rb^+ were each only about 1/3 that of choline$^+$. No significant change in apparent isotope spaces occurred when Na^+ or Rb^+ concentrations were increased 10-fold in the incubation and dilution media. Assuming that [^{3}H]choline space (3 μl/mg protein) is a measure of internal vesicle space, these data are consistent with the interpretation that about 2/3 of $^{22}Na^+$ and $^{86}Rb^+$ was lost from the vesicles within 20–30 s after dilution into the exchange medium. This would suggest a population of vesicles which was highly permeable to Na^+ and Rb^+. In contrast, the remaining $^{22}Na^+$ and $^{86}Rb^+$ passed the membrane with a rate similar to that of [^{3}H]choline suggesting a second population of vesicles which was relatively impermeable to Na^+ and Rb^+.

$^{86}Rb^+$ was fully exchanged within 20 s when the dilution medium contained the ionophore valinomycin (10^{-8} M). In the presence of the ionophore X537A (10 μg/ml), both $^{86}Rb^+$ and $^{22}Na^+$ were rapidly released. [^{3}H]Choline exchange rates were unaffected by the two ionophores.

3.2. *Membrane potential changes in sarcoplasmic reticulum vesicles*

Membrane potential measurements with the fluorescent dye diO-C_5-(3) were used to obtain additional evidence for the presence of sarcoplasmic reticulum vesicles which differ in their Na^+ and K^+ permeabilities. Sims et al. [3] previously showed that the fluorescence emission of diO-C_5-(3) decreases when membranes become negatively charged inside. Membrane potential measurements involved transfer of vesicles filled with 200 mM Kgluconate to iso-osmolar media containing either K-, Na- or Tris gluconate. Gluconate$^-$, a relatively slow penetrating anion, was used to minimize osmotic effects [2]. K^+ (2 mM) was added to the dilution media to control the magnitude of the developed potentials (about 110 mV negative inside). Figure 2 shows that K gluconate filled vesicles elicited no change in fluorescence when diluted into K or Na gluconate containing media, indicating that no membrane potentials were created. However, when vesicles were diluted into Tris gluconate medium, in the presence or absence of valinomycin, or Na gluconate medium, in the presence of valinomycin, there were rapid decreases in fluorescence. Within 1 h, fluorescence signals returned to their original values. This gradual reduction in membrane potential was likely due to slow inward movement of Tris$^+$.

4. Discussion

Ion flux and membrane polarization experiments reported here suggest that purified rabbit skeletal-muscle sarcoplasmic reticulum vesicles are composed of two types which differ in their permeability to small cations. Vesicles designated Type I are relatively impermeable to larger cations like choline$^+$ or Tris$^+$, but are at least 10-times more permeable to small cations such as Na^+, Rb^+ or K^+. Type II vesicles differ from Type I in that Na^+ and Rb^+, or K^+ permeation rates are as slow as those of choline$^+$ or Tris$^+$. Na,K-

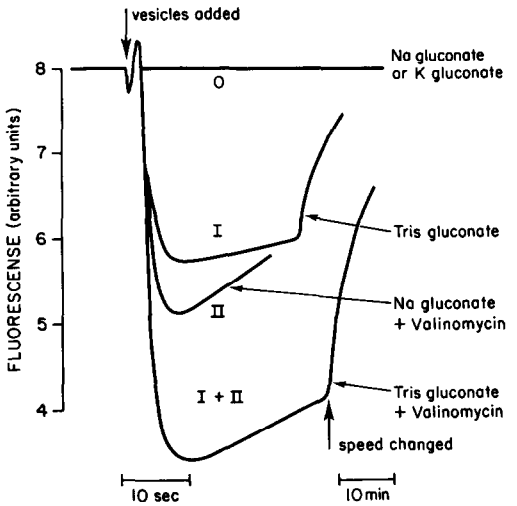

Fig.2. Changing ionic environments of sarcoplasmic reticulum vesicles and fluorescence emission of diO-C_5-(3). Vesicles (25 mg protein/ml) were incubated for at least 24 h at 0°C in a medium containing 200 mM K gluconate, 1 mM Mg gluconate, 20 μM Ca gluconate, and 10 mM Pipes–Tris, pH 7.0. Fluorescent assays were carried out by diluting vesicles about 1500 times under stirring into 3 ml media containing 1 mM Mg gluconate, 20 μM Ca gluconate, 10 mM Pipes–Tris, pH 7.0, 1.67 10^{-6} M diO-C_5-(3) (5 μl 1 mM acetone solution), and either 200 mM K gluconate or 198 mM Na gluconate plus 2 mM K gluconate (Curve 0), 198 mM Tris gluconate and 2 mM K gluconate (Curve I), 198 mM Na gluconate, 2 mM K gluconate and 10^{-6} M valinomycin (Curve II), or 198 mM Tris gluconate, 2 mM K gluconate and 10^{-6} M valinomycin (Curve I + II). Curves are labeled according to the types of vesicles that are predicted to give a dye response on transfer to the indicated dilution medium.

permeable, Type I, vesicles make up about 70% of the ion space of all vesicles, while Na,K-impermeable, Type II, vesicles are about 30%.

Fluorescence experiments suggest the presence of vesicles (Type I) which are more permeable to K^+ than to $Tris^+$ and which therefore form a membrane potential negative inside on transfer from K gluconate to Tris gluconate. The developed potential in turn causes a decrease in the fluorescence emission of the diO-C_5-(3). Increases in fluorescence signals seen on addition of valinomycin point to the presence of a second population of vesicles (Type II) which have normally a K^+ permeability similar to Na^+ and $Tris^+$ but become permeable to K^+ in the presence of the ionophore. These vesicles are then able to develop a membrane potential so that the signal seen in the presence of valinomycin on transfer to Tris gluconate represents Type I and II vesicles, while only Type II vesicles elicit a fluorescence signal on transfer to Na gluconate. The latter is true since Type I vesicles are predicted to have rapidly exchanged all their K^+ for Na^+ (fig.1), so that no potential can be developed. It is of interest that the decreases in fluorescence assigned to Type I and II vesicles are nearly additive and correlate with the ion spaces of the two types of vesicles as determined from tracer flux measurements.

In previous Millipore filtration experiments carried out at 23°C [2], the presence of Type II vesicles was not recognized. At 4°C, ion permeation rates are diminished allowing a more reliable measurement of ion spaces. Kasai and Miyamoto [5] recently presented evidence for the presence of sarcoplasmic reticulum vesicles which form a permeability barrier for Na^+ and K^+. Their Millipore filtration experiments indicate that Na and K ion spaces correspond to about 60% of the sucrose space. No interpretation for these different solute spaces was given.

An intriguing question is the significance of vesicles that differ in permeability to Na^+ and K^+. Type I, Na,K-permeable, vesicles are not thought to be simply leaky vesicles, since both types of vesicles have similar permeability to choline, Tris and sucrose [2]. Rather in vivo sarcoplasmic reticulum may contain a limited number of channels for small cations such as Na^+ and K^+. Consequently, during homogenization some vesicles are obtained which contain Na,K channels while other vesicles have none. Assuming a random distribution of channels and an average vesicle surface area of 0.02 μm^2, a density of about 50 channels/μm^2 is calculated. A possible physiological function of Na,K channels would be to allow rapid K^+ and Na^+ movement across the sarcoplasmic reticulum membrane, thereby minimizing charge and osmotic effects during Ca release and uptake.

Acknowledgements

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References

[1] MacLennan, D. H. and Holland, P. C. (1975) Ann. Rev. Biophys. Bioeng. 4, 377–404.
[2] Meissner, G. and McKinley, D. (1976) J. Membr. Biol. 30, 79–98.
[3] Sims, P. J., Waggoner, A. S., Wang, C. and Hoffman, J. F. (1974) Biochemistry 13, 3315–3330.
[4] Meissner, G. (1974) Meth. Enzymol. 31, 238–246.
[5] Kasai, M. and Miyamoto, H. (1976) J. Biochem. (Tokyo) 79, 1067–1076.