Calcium Transport and Monovalent Cation and Proton Fluxes in Sarcoplasmic Reticulum Vesicles

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ATP-dependent Ca\(^{2+}\) uptake by rabbit skeletal muscle sarcoplasmic reticulum vesicles has been studied in the presence and absence of artificially generated pH gradients and membrane potentials. H\(^+\) and K\(^+\) diffusion potentials were generated via the H\(^+\) and K,Na channels of sarcoplasmic reticulum by transfer of vesicles from low to high pH, or from high to low K\(^+\). Membrane potentials were measured using the voltage-sensitive fluorescent dye 3,3'-dipentyl-2,2'-oxacarbocyanine. The initial rate of Ca\(^{2+}\) uptake was found to be increased in the presence of a pH gradient and membrane potential (negative inside). In turn, the rates of decay of K\(^+\) or H\(^+\)-induced membrane potentials were accelerated during Ca\(^{2+}\) transport, suggesting that active Ca\(^{2+}\) uptake stimulated the release of K\(^+\) and H\(^+\) from the vesicles. The ratio of K\(^+\) (or H\(^+\)) release to Ca\(^{2+}\) transport was near two.

Release of K\(^+\) did not appear to be directly catalyzed by the Ca\(^{2+}\)-ATPase. Evidence against a directly coupled ATP-mediated 2 K\(^+\)-Ca\(^{2+}\) or K\(^+\)-Ca\(^{2+}\) exchange reaction was that (i) similar results were obtained when K\(^+\) was substituted by Na\(^+\) or by organic cations which could rapidly permeate through the channel of K,Na-impermeable vesicles and (ii) Ca\(^{2+}\) transport did not result in an equivalent release of "Rb" or "Na" from K,Na-impermeable vesicles.

These studies are in support of an electrogenic Ca\(^{2+}\) transport system in sarcoplasmic reticulum. The results further suggest that during Ca\(^{2+}\) transport development of a membrane potential (positive inside) is likely nullified by the countermovement of the permeant cations K\(^+\), Na\(^+\), and H\(^+\).

Isolated sarcoplasmic reticulum (SR) vesicles rapidly sequester Ca\(^{2+}\) against a concentration gradient upon energization with ATP through the action of a membrane-bound, Mg\(^{2+}\)-dependent, Ca\(^{2+}\)-stimulated ATPase (see reviews by Tada et al., 1978; Hasselbach, 1978; de Meis and Vianna, 1979, Inesi, 1979). The Ca\(^{2+}\)-ATPase is a major membrane component of skeletal muscle SR accounting for up to 90% of the total protein (Meissner, 1975). Recently, evidence has been presented that the Ca\(^{2+}\)-ATPase, when incorporated into phospholipid bilayer vesicles (Zimmniak and Racker, 1978), transfers positive charges into vesicles during Ca\(^{2+}\) transport.

The phospholipid bilayer model system offers the advantage of being relatively impermeable to ions. Study of the electrogenic nature of Ca\(^{2+}\) transport in native SR vesicles is more difficult, because these membranes are permeable to ions in a complex manner. SR vesicles are relatively impermeable to Ca\(^{2+}\), Mg\(^{2+}\), and larger ions such as gluconate-, choline-, or Tris-, and are relatively impermeable to Na\(^+\) and K\(^+\). About two-thirds of the vesicles contain a K,Na channel making them highly permeable to K\(^+\), Rb\(^+\), and Na\(^+\). The remaining one-third of the vesicles lack the K,Na channel and are therefore relatively impermeable to these cations (McKinley and Meissner, 1978). K\(^+\),Na\(^+\)-impermeable vesicles are thought to arise as a consequence of a limited number of K,Na channels in SR. Both K\(^+\),Na\(^+\)-permeable and -impermeable vesicles are highly permeable to protons and chloride suggesting that these ions can pass the sarcoplasmic reticulum membrane by a pathway separate from that of the K,Na channel (McKinley and Meissner, 1978; Meissner and Young, 1980).

In this study we have taken into consideration the particular permeability properties of native SR vesicles to investigate the effect of Ca\(^{2+}\) transport on monovalent cation and proton fluxes. The data suggest that active uptake of positively charged Ca\(^{2+}\) stimulates the release of an equivalent amount of charge including K\(^+\), Na\(^+\), or H\(^+\). With the possible exception of H\(^+\), movement of these ions does not appear to be directly catalyzed by the Ca\(^{2+}\)-ATPase. Some of these results have appeared elsewhere in a preliminary form (Meissner, 1979).

**MATERIALS AND METHODS**

Reagents—The fluorescent dye 3,3'-dipentyl-2,2'-oxacarbocyanine was the generous gift of Dr. Alan S. Waggoner (Amherst College, Amherst, Mass.). Gluconic acid (technical grade, Eastman, Rochester, N. Y.) was treated with charcoal before use to prepare the salt Tris/gluconate. Analytical grade reagents were used otherwise.

Sarcoplasmic Reticulum Vesicles—"Intermediate" density rabbit skeletal muscle sarcoplasmic reticulum vesicles used in this study have been characterized previously (Meissner, 1975). Unless otherwise indicated, vesicles (0.5 to 1.0 mg of sarcoplasmic reticulum protein/ml) were incubated for 6 to 15 h at 0°C in 480 mosM K/ gluconate or Tris/Pipes medium at pH 7. Vesicles were sedimented by centrifugation for 30 min at 35,000 rpm in a Beckman 42.1 rotor, resuspended in a small volume (15 to 20 mg of protein/ml) of the above media, incubated for another 2 to 3 h at 0°C, and then stored at −65°C for later use.

Membrane Potential Measurements—Membrane potentials were generated by gradients of permeant ions between the intravesicular cavity and the medium into which the vesicles were diluted. Membrane potentials (negative inside) were detected by the use of the fluorescent dye 3,3'-dipentyl-2,2'-oxacarbocyanine iodide (diO-C\(_{36}\)-(3)) (Sims et al., 1974) as previously described (McKinley and Meissner, 1978). Formation of positive membrane potentials and pH gradients did not affect significantly the fluorescence emission of diO-C\(_{36}\)-(3) under the conditions used in the present study (Meissner and Young, 1980).
1980). The polarity of membrane potentials are reported according to standard convention, that is, reference (ground) is extravesicular. Unless otherwise indicated, fluorescence assays were carried out at 15°C under stirring in a Fannand model 801 Fluorometer. Excitation was at 470 nm and emission was recorded at 495 nm. Slits used resulted in a half-band width of 2.5 nm. Vesicle concentrations (approximately 15 μg of protein/ml) were used which produced negligible perturbation of the fluorescence emission during dilution with incubation medium.

**Isotope Flux Measurements**—Radioisotope efflux rates from sarcoplasmic reticulum vesicles were measured as previously described (Meissner and McKinley, 1976). Briefly, vesicles were incubated for 6 to 15 h at 0°C in the presence of a radioactive compound. The vesicles were then diluted 400-fold into an unlabeled release medium with rapid mixing. Efflux of the radioactive compounds was monitored at various time intervals by placing 1.0-ml aliquots on 0.45 μm Millipore filters followed by rapid rinsing with unlabeled medium. The time required to execute filtration and rinsing was 15 to 20 s and was taken into account. The radioactivity retained on filters was counted in a liquid scintillation system.

**Ca**²⁺ Transport Measurements—Ca²⁺ uptake was initiated by the addition of SR vesicles (20 to 50 μg of protein) to 1 ml of assay medium containing 50 μM Ca²⁺ (0.01 μM/ml), 1 mM Mg²⁺, and 0.5 mM ATP. In order to stop "Ca"²⁺ uptake and "Ca"²⁺ efflux, 10 μl of 1 M LaCl₃ was added (Meissner and McKinley, 1976; Chiesi and Inesi, 1979). Only minimal "Ca"²⁺ uptake was observed when La⁺ was added to the assay media prior to the vesicles. Duplicate aliquots (0.4 ml) were placed on 0.45 μm Millipore filters and "Ca"²⁺ not taken up by the vesicles was removed by rinsing the filters with assay medium containing 10 mM La⁺ but no ATP. Radioactivity retained on filters was counted in a liquid scintillation system (Meissner and McKinley, 1976).

**RESULTS**

Effect of Ca²⁺ Uptake on K⁺- and H⁺-induced Membrane Potentials—Membrane potential measurements with the fluorescent dye diO-C₅-(3) are an effective means of distinguishing SR vesicles which contain the K,Na channel (type I) and those that do not (type II). As previously shown (McKinley and Meissner, 1978), negative membrane potentials could be generated in K⁺,Na⁺-permeable (type I) vesicles by dilution of K⁺/glucuronate-filled vesicles into Tris/glucuronate medium (Fig. 1A). The absence of a potential in K⁺,Na⁺-impermeable (type II) vesicles was due to the similarly low permeability of K⁺ and Tris⁺. On the other hand, negative potentials were exclusively formed in type II vesicles by transferring the entire SR vesicle population from K⁺/glucuronate medium to Na⁺/glucuronate medium containing the K⁺ selective ionophore valinomycin (Fig. 1B). Under these conditions, no membrane potential was formed in K⁺,Na⁺-permeable (type I) vesicles. Because of the presence of the K,Na channel, these vesicles rapidly exchanged all of their K⁺ for Na⁺ within 1 to 2 s, the experimental limit of detection. Use of valinomycin in Tris/glucuronate dilution medium enabled development of a membrane potential in all vesicles. Accordingly, the trace of Fig. 1C exhibited a deflection which was nearly the sum of the signals seen for type I (Fig. 1A) and type II (Fig. 1B) vesicles.

K⁺,Na⁺-permeable and -impermeable vesicles are permeable to H⁺ (Meissner and Young, 1980). Permeability of vesicles to both K⁺ and H⁺ resulted in rapid K⁺⁻H⁺ exchange until K⁺ and H⁺ gradients of equal size and direction were formed. The extent of K⁺⁻H⁺ exchange was minimized in the experiments of Fig. 1 with the use of the impermeable nonbuffering anion (Meissner and Young, 1980). External cation concentrations were considered to be essentially unaffected by K⁺⁻H⁺ exchange since the dilution media were an infinite bath compared to the total volume of the vesicles.

Fluorescence emission of diO-C₅-(3) reached a minimum within 1 to 2 s after the addition of the vesicles to the three polarizing media (Figs. 1, A to C). The signal returned within 10 to 20 min to that observed for nonpolarized vesicles (Fig. 1D). This gradual collapse was likely due to inward movement of Tris⁺ (Fig. 1, A and C) or Na⁺ (Fig. 1B), and the eventual dissipation of the K⁺ (and H⁺) gradients. The control nonpolarized signal was obtained by diluting Tris/glucuronate-filled vesicles into Tris/glucuronate medium (Fig. 1D). A similar trace was obtained when K⁺/glucuronate-filled vesicles were diluted into K⁺/glucuronate medium (not shown).

The ability of SR vesicles to generate and maintain membrane potentials in the presence of an ion gradient has been used to study cation movements across SR membranes which actively transport Ca²⁺. Fluorescence signals indicated that the developed membrane potentials returned faster to baseline when media contained 0.5 mM ATP, 50 μM Ca²⁺, and 1 mM Mg²⁺ (Fig. 1, A to C, i.e. when SR vesicles actively transported Ca²⁺ (see below). The effect of ATP (in the presence of Ca²⁺ and Mg²⁺) on shortening the lifetime of membrane potentials in type I (K⁺→Tris⁺) or type II (K⁺→Na⁺) vesicles was more pronounced at 21°C than at 3°C (Table I).

Addition of ATP or Ca²⁺ uptake per se were likely not responsible for the enhanced breakdown of the fluorescence signals. Russell et al. (1979) and Beeler et al. (1979) have found that the fluorescence of oxacarbocyanine dyes responds to binding of ATP and Ca²⁺ to the membranes. However, experimental conditions used by these investigators were different from the present study. We observed a normal slow rebound of fluorescence signals in type I + II vesicles when Ca²⁺ was omitted from the media or ATP was substituted by the nonhydrolyzable ATP analog adenylyl-(βγ-methylene)-diphosphonate (AMP-PCP) (Table I). In the absence of an initial potential where Tris/glucuronate-filled vesicles were diluted into Tris/glucuronate medium, fluorescence signals were not significantly changed by ATP (Fig. 1D). Addition of 10 mM Ca²⁺ to the assay media (in the absence of ATP) also showed no effect (not shown). In another control, we tested the effect of internal Ca²⁺ on diO-C₅-(3) fluorescence. Vesicles were passively loaded with Ca²⁺ by preincubation in K⁺/glucuronate medium in the presence of 1 mM or 5 mM Ca²⁺.
Valinomycin and the H'-carrier carbonyl cyanide p-trifluoromethoxyphenyl hydrazone did not cause a more rapid collapse of membrane potentials during Ca\(^{2+}\) transport (not shown). On the other hand, because of the K' permeability of the vesicles, the above experiments could not reveal whether K' release was catalyzed by an oblique exchange process directly coupled with Ca\(^{2+}\) translocation or whether active uptake of Ca\(^{2+}\) was compensated in charge by release of K' by an independent mechanism such as the K,Na channel of SR. To establish the mechanism of K' release, we studied the effect of Ca\(^{2+}\) transport on membrane potentials created by permeable ions other than K'. We expected that an oblique exchange process would be fairly specific with regard to the number and kind of ions transported during Ca\(^{2+}\) transport, while an independent mechanism would only require that the membrane is permeable in some way to the released ions.

Membrane potentials were generated in type I SR by transferring Na/ or monoethanolamine/glucosinate-filled vesicles to Tris/glucosinate medium. Both cations were previously found to pass rapidly through the K,Na channel (McKinley and Meissner, 1978). Uptake of Ca\(^{2+}\) increased the rate at which fluorescence signals returned to the base-line (Table I). These results suggest that membrane potentials formed by K', Na', monoethanolamine, or H' gradients were similarly rapidly nullified during active Ca\(^{2+}\) transport.

Negative H' diffusion potentials were established as previously described (Meissner and Young, 1980) by diluting SR vesicles from a Tris/Pipes medium at pH 6.2 into a Tris/Pipes medium at pH 7.6 (Fig. 2). In these experiments, Pipes was used as the impermeable anion to increase the internal buffering capacity of the vesicles, and thus the lifetime of the membrane potentials. As above (Fig. 1, Table II), addition of 50 μM Ca\(^{2+}\), 0.5 mM ATP, and 1 mM Mg\(^{2+}\) enhanced the rate with which the fluorescence signal returned to base-line. This result indicated that active uptake of Ca\(^{2+}\) accelerated the collapse of H'-induced membrane potentials as well. Together, the similar behavior of membrane potentials formed by K', Na', monoethanolamine, or H' gradients was in support of independent but complementary movement of K' rather than movement directly catalyzed by the Ca\(^{2+}\)-ATPase.

**TABLE I**

| Potential-creating media | Vesicle type | Temperature (°C) | Fluorescence signal (t₀/₂) |
|--------------------------|-------------|------------------|---------------------------|
|                          |             |                  | -ATP | +ATP |
| K → Tris                 | I           | 3                | 75   | 22  |
|                          | I           | 13               | 25   | 6   |
|                          | II          | 16               | >2   |     |
| K → Na + Val             | II          | 3                | 86   | 34  |
| Na → Tris                | I           | 13               | 24   | 8   |
| EIOH-NH₂ → Tris          | I           | 13               | 30   | 10  |

**TABLE II**

| Potential-creating media | Additions to dilution medium | Fluorescence signal |
|--------------------------|-----------------------------|---------------------|
|                          | % decrease | Fluorescence signal | t₀/₂ |
| A. K → Tris + Val        | None       | 49                 | 45   |
|                         | Mg         | 49                 | 40   |
|                         | Mg, Ca     | 49                 | 45   |
|                         | Mg, EGTA, ATP | 51                 | 50   |
|                         | Mg, Ca, ATP | 47                 | 7    |
|                         | Mg, Ca, AMP-PCP | 48                 | 40   |
| B. pH 6.1 → pH 7.6       | None       | 19                 | 15   |
|                         | Mg, Ca     | 18                 | 16   |
|                         | Mg, EGTA, ATP | 20                 | 15   |
|                         | Mg, Ca, ATP | 18                 | 3    |
|                         | Mg, Ca, AMP-PCP | 19                 | 14   |

and slow transient fluorescence signals similar to those seen in Fig. 1 were observed in the presence and absence of ATP, respectively. Further, light-scattering effects could not account for the observed rapid changes in fluorescence intensity. No or only minimal changes in light intensity at 495 nm (λₑ = 470 nm) were recorded during Ca\(^{2+}\) uptake when the dye was omitted.

Our explanation for the Ca\(^{2+}\) uptake-induced enhancement of potential breakdown is an increase in the rate of K' (and H') release during Ca\(^{2+}\) transport. It appeared likely that intrinsic K' and H' permeability was sufficient to mediate increased cation efflux since addition of increasing amounts of

**Fig. 2.** Effect of Ca\(^{2+}\) transport on time course of fluorescence signals elicited by H'-induced membrane potentials. SR vesicles containing 480 mM Tris/Pipes at pH 6.2 were diluted 100-fold at 15°C into an isosmolar solution of Tris/Pipes at pH 7.6. The dilution medium contained 1.5 μM diO-C₃-(3), 1 mM Mg\(^{2+}\), 50 μM Ca\(^{2+}\), no or 0.5 mM ATP.
Rates of Ca\textsuperscript{2+} uptake were observed in spite of the fact that the Ca\textsuperscript{2+} uptake capacity of vesicles was twice as great at pH 6.2 (Fig. 3). Establishment of a negative membrane potential (theoretical Nernst potential \( \Delta V = -75 \text{ mV} \)) by transferring vesicles from pH 6.2 to 7.6, nearly doubled the initial rate of Ca\textsuperscript{2+} uptake. On the other hand, initial Ca\textsuperscript{2+} uptake was slightly reduced when a positive membrane potential was formed by diluting vesicles from pH 7.6 to 6.2. Thus, the Ca\textsuperscript{2+} transport system of SR was affected by a H\textsuperscript{+}-induced diffusion potential, a pH gradient, or both.

Membrane potentials were also generated in all SR vesicles using K\textsuperscript{+} gradients in the presence of the K\textsuperscript{-}-ionophore, valinomycin. In standardization experiments in the absence of a potential and gradient Ca\textsuperscript{2+} uptake was faster in 480 mOsm K/glucionate medium than in Tris/glucionate medium containing K/glucionate at a concentration of 48 mOsm (K \( \rightarrow \) K, Tris \( \rightarrow \) Tris in Fig. 4). The increased rate suggested an activating effect of K\textsuperscript{-}, in agreement with earlier reports (Shigekawa and Pearl, 1976; Dugan and Jacob, 1977; Ribeiro and Vianna, 1978). When vesicles were diluted from high (480 mOsm K/glucionate, pH 7) to low K\textsuperscript{-} concentration (48 mOsm K/glucionate + 432 mOsm Tris/glucionate, pH 7), a negative potential (theoretical Nernst potential \( \Delta V = -55 \text{ mV} \)) and a 10-fold H\textsuperscript{+} gradient (pH\textsubscript{i} = 6) were formed. Under these conditions, a maximal initial rate of Ca\textsuperscript{2+} uptake was observed that was 1.5-fold greater than the rate in K/glucionate medium (K \( \rightarrow \) Tris\textsuperscript{-} and K\textsuperscript{-} \( \rightarrow \) K\textsuperscript{+} in Fig. 4). Therefore, Ca\textsuperscript{2+} uptake was stimulated, despite a decreased K\textsuperscript{-} concentration in the dilution medium. Establishment of 10-fold K\textsuperscript{+} and H\textsuperscript{+} gradients of opposite direction (\( \Delta V = +55 \text{ mV} \), pH\textsubscript{i} = 8) had a retarding effect on initial Ca\textsuperscript{2+} uptake (Tris\textsuperscript{-} \( \rightarrow \) K\textsuperscript{+}). Ca\textsuperscript{2+} uptake was therefore slowed down, despite an increased K\textsuperscript{-} concentration in the dilution medium (Tris\textsuperscript{-} \( \rightarrow \) K\textsuperscript{+} and Tris\textsuperscript{-} \( \rightarrow \) Tris\textsuperscript{-} in Fig. 4). At later times, membrane potentials collapsed (cf. Fig. 1) and similar amounts of Ca\textsuperscript{2+} were accumulated by vesicles transferred to K\textsuperscript{+} or Tris\textsuperscript{-} media. These results showed that in the presence of a membrane potential, Ca\textsuperscript{2+} uptake was accelerated and slowed down as predicted by the electrogenic model. Simultaneous formation of a pH gradient prevented, however, ruling out an alternative explanation, that stimulation and retardation in Ca\textsuperscript{2+} uptake was attributable to a pH gradient rather than a membrane potential.

**Ca\textsuperscript{2+} Transport and K\textsuperscript{+}, H\textsuperscript{+} Efflux Rates from K\textsuperscript{-}- and H\textsuperscript{-}-permeable Vesicles**—It has been observed in Figs. 1 and 2 that Ca\textsuperscript{2+} uptake enhances the rate of potential collapse. Since, as noted above, the addition of valinomycin or the proton carrier carbonyl cyanide p-trifluoromethoxyphenyl hydrazone did not promote faster potential breakdown, it appeared that intra- and extravesicular K\textsuperscript{+} and H\textsuperscript{+} were at electrochemical equilibrium at all times during Ca\textsuperscript{2+} transport. The likely explanation for Ca\textsuperscript{2+} uptake-induced enhancement of potential breakdown was therefore the induction of faster

![Ca\textsuperscript{2+} Uptake by SR Vesicles](image1)

**Fig. 3. Ca\textsuperscript{2+} uptake by SR vesicles in the presence and absence of H\textsuperscript{+}-induced membrane potentials.** Vesicle and dilution media contained 480 mOsm Tris/Pipes at the indicated pH. Dilution media contained in addition 1 mM Mg\textsuperscript{2+}, 50 \( \mu \)M "Ca\textsuperscript{2+}, and 0.5 mM ATP. Ca\textsuperscript{2+} transport was initiated at 15°C by 100-fold dilution of vesicles and stopped at the indicated time by the addition of 10 mM La\textsuperscript{3+} (cf. "Materials and Methods").

![Dependence of Fluorescence Emission of diO-C5-(3) on Size of Ion Gradients in the Absence of Ca\textsuperscript{2+} Transport](image2)

**Fig. 5.** Dependence of fluorescence emission of diO-C5-(3) on size of ion gradients in the absence of Ca\textsuperscript{2+} transport. Size of K\textsuperscript{+} gradients was varied by replacing in the vesicle medium part of the K\textsubscript{i}/glucionate with Tris/glucionate so that the sum of the two salts remained at 480 mOsm. Composition of dilution media was kept constant so that final Tris/glucionate and K/glucionate concentrations were 432 and 48 mOsm, respectively. Type II vesicles were rendered permeable to K\textsuperscript{+} by the addition of 0.5 \( \mu \)M valinomycin to the dilution media. Similarly, size of H\textsuperscript{+} gradients was varied with the use of vesicles that were present in Tris/Pipes media of different pH (pH 6.2 to pH 7.6) and were diluted into Tris/Pipes media of constant final pH (pH 7.6). Fluorescence decreases extrapolated back to the time of vesicle addition are indicated.
Ca\(^{2+}\) Transport in SR

Table III

| Potential-creating media | Time (s) | Ca\(^{2+}\) uptake \(\text{nmol/mg protein}\) | \(-\text{ATP}\) | \(+\text{ATP}\) | \(\Delta K^{+}/\text{Ca}^{2+}\) | \(\Delta H^{+}/\text{Ca}^{2+}\) |
|-------------------------|---------|--------------------------------|-------------|-------------|----------------|----------------|
| K\(\rightarrow\) Tris + Val | 0       | 0                             | 29 ± 1      | 29 ± 2      |                  |                  |
|                         | 3       | 89 ± 9                        | 27.5 ± 1    | 21.5 ± 1    | 1.5 ± 0.7       |                  |
|                         | 5       | 122 ± 15                      | 26 ± 1      | 15 ± 1.5    | 1.9 ± 0.6       |                  |
|                         | 10      | 148 ± 15                      | 22 ± 1      | 8 ± 1.5     | 1.7 ± 0.5       |                  |
| pH 6.2 \(\rightarrow\) pH 7.6 | 0       | 0                             | 19 ± 1      | 19 ± 2      |                  |                  |
|                         | 3       | 44 ± 4                        | 16 ± 1      | 8.5 ± 0.5   | 2.05 ± 0.6      |                  |
|                         | 5       | 52 ± 6                        | 14.5 ± 1    | 6 ± 0.5     | 2.4 ± 1.0       |                  |
|                         | 10      | 59 ± 4                        | 11 ± 1      | 4.5 ± 0.5   | 2.5 ± 1.0       |                  |

rates of K\(^{+}\) and H\(^{+}\) efflux during Ca\(^{2+}\) transport. We used the dye technique to estimate K\(^{+}\) and H\(^{+}\) efflux rates from vesicles during and in the absence of Ca\(^{2+}\) transport. Calibration experiments of Fig. 5 indicated that the magnitude of the dye signal was dependent on the size of the K\(^{+}\) or H\(^{+}\) gradient, so that the amount of K\(^{+}\) or H\(^{+}\) released within 3, 5, or 10 s after dilution could be estimated from the re-bound portion of fluorescence signals (cf. Figs. 1 and 2). The decrease in intravesicular K\(^{+}\) or H\(^{+}\) concentration was calculated assuming that the composition of the large extravesicular medium remained unchanged after the initial dilution step. Buffer capacity of Tris/Pipes medium used in Fig. 5 was determined at 15°C by measuring the \([\text{H}^{+}]\) as pH prior and after the addition of small aliquots of 1 N HCl. Internal vesicle spaces were estimated by measurement of apparent \([\text{H}^{+}]\)sucrose and \([\text{H}^{+}]\)choline\) spaces (McKinley and Meissner, 1978), so that the total amounts of K\(^{+}\) or H\(^{+}\) released by the vesicles could be calculated. These were found to be 2.5 \(\mu\text{g/mg protein}\) for vesicles present in K\(^{+}\)/glucuronate medium and 2.1 \(\mu\text{g/mg protein}\) for vesicles present in Tris/Pipes medium at pH 6.2. \(\Delta K^{+}/\text{Ca}^{2+}\) and \(\Delta H^{+}/\text{Ca}^{2+}\) are the difference in the amount of K\(^{+}\) and H\(^{+}\) that were released by the vesicles 3, 5, and 10 s after vesicle dilution in the presence and absence of Ca\(^{2+}\) transport. These amounts and the amounts of Ca\(^{2+}\) taken up by the vesicles (Ca\(^{2+}_{\text{int}}\)) are given on a molar basis. Data are the average of four experiments ±S.E.

Fig. 6 shows the uptake of \(\text{Rb}^{+}\), \(\text{Na}^{+}\), and \([\text{H}^{+}]\)choline ion spaces and efflux rates in the presence and absence of Ca\(^{2+}\) transport. SR vesicles present in 10 mM \(\text{RbCl}\) (or \(\text{NaCl}\)), 190 mM \([\text{H}^{+}]\)choline Cl, 5 mM Pipes, 10 mM Tris, pH 7, were diluted 400-fold into an unlabeled medium of the same composition at 15°C, except that the dilution medium contained 1 mM Mg\(^{2+}\) and 50 \(\mu\text{M Ca}^{2+}\), and 0.5 mM ATP where indicated. \(\text{Rb}^{+}\), \(\text{Na}^{+}\), and \([\text{H}^{+}]\)choline\) efflux from the vesicles was determined by Millipore filtration as described under "Materials and Methods." \(\text{Rb}^{+}\) has been used rather than K\(^{+}\) since there is no convenient radioisotope of K\(^{+}\).
Addition of ATP resulted in the uptake of about 55 nmol of Ca\(^{2+}\)/mg of protein within 30 s (Table IV). It appeared unlikely that minimal stimulation of \(^{68}\)Rb\(^{+}\) or \(^{22}\)Na\(^{+}\) efflux by ATP in K\(^{+}\)-impermeable vesicles was due to low Ca\(^{2+}\) uptake. Fluorescence measurements suggested that K\(^{+}\)-impermeable and impermeable vesicles can accumulate Ca\(^{2+}\) with a similar rate (Fig. 1). Further, the amount of Ca\(^{2+}\) accumulated within 30 s did not seem to be greatly affected by the impermeability of type II vesicles to Rb\(^{+}\), since similar amounts of Ca\(^{2+}\) were taken up at this time by vesicles in the presence or absence of valinomycin (not shown). It was therefore concluded that about one-third of the Ca\(^{2+}\) (18 nmol/mg of protein) was taken up by type I vesicles. Accordingly, Rb\(^{+}\) and Na\(^{+}\) efflux from K\(^{+}\),Na\(^{-}\)-impermeable vesicles was increased by less than 0.1 Rb\(^{+}\) or Na\(^{+}\)/Ca\(^{2+}\) taken up (Table IV). Moreover, as indicated by the enhanced \([\text{H}^{+}]\text{choline}\) efflux rates during Ca\(^{2+}\) transport, these small increases in Rb\(^{+}\) and Na\(^{+}\) fluxes were likely due to nonspecific membrane permeability increases during Ca\(^{2+}\) uptake. In agreement with such an interpretation, the following three observations. First, increase in Rb\(^{+}\) and Na\(^{+}\) concentrations to 200 mm did not affect appreciably isotope efflux rates in the presence or absence of Ca\(^{2+}\) transport. At 200 mm, amounts of Rb\(^{+}\) or Na\(^{+}\) were released which approached those seen for \([\text{H}^{+}]\text{choline}\) at a concentration of 190 mm (Table IV). Second, replacement of the permeable chloride anion (McKinley and Meissner, 1978) by the impermeable gluconate anion resulted in a greater stimulation of Rb\(^{+}\) and Na\(^{+}\) release from actively transporting vesicles (Table IV). Third, addition of 5 mm oxalate which increased Ca\(^{2+}\) uptake 2- to 3-fold at 15\(^{\circ}\)C, had little effect on isotope release rates (not shown).

In summary, isotope efflux and fluorescence experiments suggested that Rb\(^{+}\) and Na\(^{+}\) efflux from K\(^{+}\), Na\(^{-}\)-impermeable and impermeable vesicles was differently affected by the active uptake of Ca\(^{2+}\). An initial Rb\(^{+}\) or Na\(^{+}\) release to Ca\(^{2+}\) transport ratio of less than 0.1 was found in K\(^{+}\), Na\(^{-}\)-impermeable (type II) vesicles. In contrast, a transport ratio near 2 was estimated for type I vesicles as well as type II vesicles, if the latter were made permeable to K\(^{+}\) with valinomycin.

### DISCUSSION

Membrane potential and ion flux experiments reported here suggest that ATP-mediated Ca\(^{2+}\) uptake by sarcoplasmic reticulum may be compensated in charge by the counter movement of the permeable cations K\(^{+}\), Na\(^{+}\), and/or H\(^{+}\). Compensating movement of these biologically relevant cations during Ca\(^{2+}\) transport would seem to prevent the development of a membrane potential, positive inside, which could impede further Ca\(^{2+}\) uptake. Transport of K\(^{+}\) and Na\(^{+}\) appeared to be mediated by the K,Na channel of SR rather than by an obligate exchange process directly coupled with Ca\(^{2+}\) translocation.

In isolated SR vesicles, either intrinsically K\(^{-}\)-permeable (type I) or rendered permeable to K\(^{+}\) by valinomycin (type II), a sufficient amount of K\(^{+}\) was released outward to neutralize the charge transported by Ca\(^{2+}\) into the vesicles. Na\(^{+}\) or monoethanolamine\(^{-}\) could be substituted for K\(^{+}\), supporting the notion of a complementary movement of ions not directly catalyzed by the Ca\(^{2+}\)-ATPase. Furthermore, during Ca\(^{2+}\) transport, Rb\(^{+}\) or Na\(^{+}\) efflux from K\(^{+}\), Na\(^{-}\)-impermeable vesicles was only minimally stimulated when these contained the permeable ion Cl\(^{-}\). Thus, it was unlikely that the observed ATP-mediated 2 K\(^{+}\)-Ca\(^{2+}\) exchange reaction occurred via the Ca\(^{2+}\)-ATPase.

The lack of SR vesicles impermeable to H\(^{+}\) prevented a direct test of a putative 2 H\(^{+}\)-Ca\(^{2+}\) coupled transport system. Indirect studies revealed an increase in the initial rate of Ca\(^{2+}\) uptake when a proton gradient and a negative membrane potential were induced by H\(^{+}\) or by dilution of vesicles from high to low K\(^{+}\) media. Since decreased K\(^{+}\) lowered Ca\(^{2+}\) uptake in the absence of a membrane potential, it was conceivable that enhanced Ca\(^{2+}\) uptake may have been due to the negative potential rather than to the ions present in the media. The ability of vesicles to increase Ca\(^{2+}\) uptake in response to a membrane potential was compatible with the presence of a fully electrogenic Ca\(^{2+}\) transport system or an electrogenic H\(^{+}\)-Ca\(^{2+}\)-ATPase. Because of the simultaneous formation of pH gradients the present experiments did not rule out, however, a directly coupled electroneutral 2 H\(^{+}\)-Ca\(^{2+}\) exchange reaction.

The existence of an electrogenic Ca\(^{2+}\) transport system in sarcoplasmic reticulum was suggested in earlier studies with native SR vesicles (Dupont, 1979; Akerman and Wolff, 1979) or a purified Ca\(^{2+}\)-ATPase incorporated in phospholipid bilayer vesicles (Zimniak and Racker, 1978). The rate of Ca\(^{2+}\) uptake was found to be affected by a membrane potential. Use of the dyes 8-anilino-1-naphthalenesulfonic acid (Zimniak and Racker, 1978) or diS-C\(_2\)(5) (Dupont, 1979) suggested the formation of a membrane potential, positive inside, of 50 to 60 mV during Ca\(^{2+}\) transport. Because of the ineffectiveness of the dye diO-C\(_2\)(5)-3 as an indicator for positive membrane potentials under our experimental conditions (Meissner and Young, 1980) we did not measure positive potentials in the present investigation. A possible involvement of H\(^{+}\) or the existence of an obligatory K\(^{+}\)-Ca\(^{2+}\) exchange reaction was not ruled out in the earlier studies. In this connection it may also be noted that interpretation of the earlier experiments with native vesicles (Dupont, 1979; Akerman and Wolff, 1979) was complicated by the failure to consider the presence of vesicles that were impermeable to H\(^{+}\) and differed in their K\(^{+}\),Na\(^{-}\) permeability. Permeability properties of the phospholipid bilayer vesicles used by Zimniak and Racker (1978) were not directly studied. Our studies do not support the suggestion of Madeira (1978, 1979) that a H\(^{-}\)-impermeant SR membrane

### Table IV

| Composition of media | Ca\(^{2+}\) uptake | Isotope | Apparent isotope spaces | \(\Delta M^{\text{out}}/M^{\text{in}}\) | \(\Delta M^{\text{out}}/M^{\text{in}}\) | \(\Delta M^{\text{out}}/M^{\text{in}}\) | \(\Delta M^{\text{out}}/M^{\text{in}}\) |
|----------------------|-----------------|--------|------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| RbCl or NaCl, 190 CholCl | 55 | \(^{68}\)Rb, \(^{22}\)Na | 0.6 | 0.05 | 0.06 | 0.05 | 0.06 |
| Na Glu, 190 | 60 | \(^{22}\)Na | 2.0 | 1.8 | 2.0 | 1.8 | 2.0 |
| Chol Glu | 10 | \(^{22}\)Na | 0.75 | 0.45 | 0.15 | 0.45 | 0.15 |
| 200 RbCl or NaCl | 75 | \(^{68}\)Rb, \(^{22}\)Na | 0.7 | 0.65 | 0.4 | 0.65 | 0.4 |
| 200 Na Glu | 80 | \(^{22}\)Na | 0.7 | 0.6 | 0.75 | 0.6 | 0.75 |
forms a H⁺ gradient during Ca²⁺ uptake and that a transmembrane proton gradient may provide the motive force.

The high permeation rates of H⁺, K⁺, and Na⁺ allow for their rapid redistribution during Ca²⁺ transport. Carvalho and Leo (1967) demonstrated that the active uptake of Ca²⁺ by SR initiates the release of an equivalent amount of K⁺, H⁺, and/or Mg²⁺ from vesicles within 15 to 30 min (the time required to sediment the vesicles). It seems unlikely that Mg²⁺ is released in an obligatory manner during Ca²⁺ uptake as discussed by Kanazawa et al. (1971), Froehlich and Taylor (1976), or Beeler et al. (1979). Use of the fluorescence probe chlorotetracycline (Nagaaki and Kasai, 1980) and membrane potential measurements with Mg/glucuronate-filled vesicles have indicated that Mg²⁺, like Ca²⁺ or Mn²⁺ (Meissner and McKinley, 1976), is a relatively impermeant cation. An obligatory Ca²⁺ for Mg²⁺ exchange would therefore appear to be incompatible with a K⁺ or H⁺ release to Ca²⁺ transport ratio of about two. Also, Ueno and Sekine (1978) found that Ca²⁺ uptake could far exceed the internal Mg²⁺ concentration of the vesicles.

We envision an electrogenic Ca²⁺ transport system of SR as part of a membrane which is permeable to various biologically relevant ions, as summarized in Model A presented in Fig. 7. The cation permeability of sarcoplasmic reticulum in vivo is likely reflected by vesicles permeable to H⁺, K⁺, and Na⁺ rather than by H⁺- or K⁺-Na⁺-impermeable vesicles. Studies with sonicated (McKinley and Meissner, 1978) and reconstituted (Young et al., 1981) vesicles have led us to suggest that SR contains a limited number of randomly distributed K,Na channels, approximately 50 µm². Because of this low number of channels, fragmentation of the reticulum structure during homogenization yields two vesicle fractions, one of which lacks the K,Na channel. That no H⁺- and Cl⁻-impermeable vesicles (Meissner and McKinley, 1976; Meissner and Young, 1980) are observed would suggest a higher density of H⁺ or Cl⁻-channels. As indicated by the present study, both H⁺ and K,Na channels are capable of promoting sufficiently rapid countercurrent movement of H⁺ and K⁺ to maintain these ions at electrochemical equilibrium during Ca²⁺ transport. In this regard it is of interest that a voltage-gated, K⁺-conducting channel has been found to display single channel conductance fluctuations between an "open" and "closed" state when SR vesicles were fused with planar bilayers (Miller, 1978). Whether the translocation of 2 Ca²⁺ at the expense of 1 ATP (Hasselbach and Makinose, 1963; Weber et al., 1966) by an electrogenic Ca²⁺-ATPase occurs independently of other ions (Model A, Fig. 7) or is coupled to the outward movement of n H⁺ (n = 1 to 3) or inward movement of phosphate anions (formed during ATP hydrolysis) (Model B, Fig. 7), could not be resolved in the present study since SR is permeable to H⁺ and phosphate anions. Ca²⁺ transport appears to be not directly coupled to Cl⁻ since Ca²⁺ uptake was observed in essentially chloride-free media.

We propose that a physiological function of H⁺ and K,Na channels is to act as a complementary part of the Ca²⁺ transport system by allowing rapid H⁺ and K⁺ movement to counter Ca²⁺ fluxes during muscle relaxation. Ca²⁺ uptake could generate a momentary positive membrane potential causing the efflux of H⁺ and/or K⁺, the exact number of which would be determined by the proton-buffering capacity and permeant ion concentrations. A consequence of a monovalent cation and proton permeant membrane, together with the similar ionic composition of SR and myofibrillar spaces (Somlyo et al., 1977), is that SR may not undergo significant potential changes during muscle relaxation, even though it appears to contain an electrogenic Ca²⁺ transport system.

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