POTASSIUM EFFLUX FROM SINGLE SKINNED SKELETAL MUSCLE FIBERS

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ABSTRACT The efflux of ^{42}K from single, skinned (sarcolemma removed) skeletal muscle fibers has been determined. Isotope washout curves are kinetically complex and can be fit as the sum of three exponentials, including a fast component ($k = 0.25\ s^{-1}$) with a pool size equivalent to 91% of the fiber volume, an intermediate component ($k = 0.08\ s^{-1}$) equivalent to 6% of the fiber volume, and a slow component ($k = 0.008\ s^{-1}$) equivalent to 0.5% of fiber volume. Only the intermediate kinetic component is significantly affected by pretreatment of fibers with detergent. Efflux curves from detergent-treated fibers could be fit as the sum of two exponentials with coefficients and rate constants comparable to those of the fast and slow component of washout of untreated controls. Similarly the washout of [^{14}C]sucrose can be described as the sum of two exponentials. We conclude that the intermediate component of ^{42}K washout results from the movement of ions from a membrane bound space within the skinned fiber. Because of its relative volume, the sarcoplasmic reticulum seems to be a reasonable choice as a structural correlate for this component. Our estimate of the potassium permeability for the sarcoplasmic reticulum (SR) based on the efflux data is 10^{-7} cm/s. This value is less than previous estimates from isolated preparations.

INTRODUCTION

The sarcoplasmic reticulum (SR) of skeletal muscle controls contractile activation by releasing calcium ion into the myoplasm, although the mechanism by which this release is initiated is unknown. One relevant area in which significant progress has been made in investigating this phenomena has been the characterization of the permeability properties of the SR to physiologically important ions (reviewed by Meissner, 1983). Studies using isolated membrane vesicles indicate that the SR is highly permeable to small, monovalent anions and cations. Specifically, permeability pathways for sodium, potassium, chloride, hydrogen ion, and organic anions and cations have been identified using tracer flux and optical techniques (Duggan and Martonosi, 1970; Kasai et al., 1979; Kometani and Kasai, 1978; Meissner, 1975; Meissner and McKinley, 1982; McKinley and Meissner, 1977, 1978; Meissner and Young, 1980). In addition, Miller and co-workers have inserted a potassium selective channel from the sarcoplasmic reticulum into lipid bilayers (Miller, 1983). Their studies indicate that the potassium channel is weakly voltage sensitive and highly conductive (Miller, 1978, 1982*a*, *b*; Miller and Rosenberg, 1979; Coronado and Miller, 1980, 1982; Coronado et al., 1980). Electron probe analysis has confirmed the existence of a potassium permeability pathway in intact fibers (Somlyo et al., 1981). The physiological role that these ionic channels might play during calcium release has been widely discussed. An obvious possibility is that they allow the rapid movement of counterions, and thus minimize the development of a potential gradient across the SR.

Our knowledge of the permeability properties of the SR comes predominantly from work with isolated channels or membrane systems. It is possible that the functioning of these channels in vivo might differ significantly from what is found following membrane extraction and isolation. The aim of the experiments described here was to measure the resting potassium permeability of SR membranes that had not been isolated or disrupted. To do this, we have used the skinned (sarcolemma removed) muscle fiber preparation because it allows direct access to the SR membrane. Our results indicate that the permeability of the sarcoplasmic reticulum measured in this preparation is somewhat less than previously reported in work using isolated membrane systems. A preliminary report of these results has appeared (Best et al., 1984).

METHODS

Fiber Preparation

A small bundle of fibers was cut from the semitendinosus muscle of *Rana temporaria*, soaked in relaxing solution for 1–2 min, and then placed under light mineral oil. A single fiber segment was isolated and its sarcolemma removed with dissecting needles. This skinned fiber was then transferred to one end of an oil-filled chamber and tied between small wires that were attached to micromanipulators with single strands of synthetic suture material. After mounting, the fiber was pulled just taut and then stretched an additional 20% of its length. The fiber diameter in oil was measured using a dissecting microscope. The mounted fiber was partially depleted of its calcium stores by immersing its entire length in a

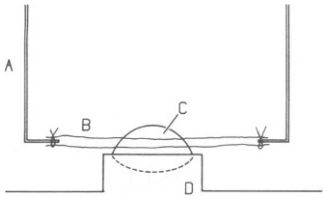

FIGURE 1 Diagram of the Plexiglas chamber used to measure isotope efflux from single skinned skeletal muscle fibers. The fiber (*B*) was tied between two wires (*A*) and placed so that its central portion passed through a bubble of washout media (*C*) that rested in a shallow depression drilled into the plastic (*D*). Each chamber contained 42 depressions into which washout media was placed after the chamber was flooded with mineral oil. Fibers were moved from bubble to bubble by sliding the chamber. The actual length of the fiber in contact with the bubble was ~90% of its total length. The ends of the fibers were never brought into contact with the loading solution.

large pool of relaxing solution to which 5 mM caffeine had been added. The fiber was then allowed to rest in relaxing solution (free of caffeine) for a few minutes. This procedure was repeated several times. If no contraction bands formed during these caffeine releases, the experiment was begun; if not, the fiber was rejected and a new one mounted. Fibers that developed contraction bands during the course of an experiment were also rejected.

The procedures used to follow the washout of isotope from the fibers were modifications of the techniques described by Stephenson (1978) and by Neville and Mathias (1979). A Plexiglas chamber was milled so that four rectangular ridges were left extending upward from the bottom. A series of shallow depressions were then drilled at equal intervals along each ridge. After the chamber was flooded with mineral oil to a level well above the height of the ridges, 50-μl bubbles of relaxing solution were pipetted into each depression. The mounted fiber was positioned so that most of its length (0.5 cm) traversed an aqueous bubble, while the ends and ties remained in the oil (Fig. 1). The fiber could be rapidly drawn through the oil and repositioned in an adjacent bubble by sliding the chamber beneath it. Fibers were loaded by placing them in isotope containing bubbles for 30 min. Control experiments (not shown) indicated that fibers did not take up additional activity after the first 20 min of exposure to the loading solution, and it will be assumed that isotopic equilibrium was reached after the standard loading time. Care was taken to make sure that the ends of the fibers and the ties never touched the loading bubble. After the loading interval the fiber was quickly washed along its entire length in nonradioactive solution to remove adherent isotope. It was then transferred from one bubble to the next at timed intervals. The Plexiglas chamber was seated in a brass block that could be rapidly cooled by circulating water. Fibers were loaded at room temperature (20°C). 5 min before the end of the loading interval, the block was chilled to 10°C and the washout of isotope was performed at this temperature. Once the washout was completed, the bubbles and fiber were collected and counted with 4 ml of Biofluor (New England Nuclear, Boston, MA) in a liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, IL). The raw counts were entered as data files on a computer (model MINC-11; Digital Equipment Corporation, Marlboro, MA). Corrections were made for background activity and, in the case of ^{42}K, for the decay of activity during the time the vials were waiting to be counted. The counts remaining in the fiber as a function of time were calculated and plotted.

Solutions

The relaxing solution used in these experiments was calculated to contain 2 mM Mg, 3.0 mM MgATP, 120 mM K + Na, 5 mM EGTA, $p^{CA} = 9.0$, pH = 7.4, $\mu = 0.15$. A computer program was used to solve the system of equilibrium reactions needed to describe this solution that contained multiple chelating species (Donaldson and Kerrick, 1975). The stability constants used in the computer program were taken from Godt and Lindley (1982) and were appropriate for 10°C. The pKa of 3-[N-morpholino] propanesulfonic acid (MOPS) was taken to be 7.28 at this temperature. The actual amounts (in millimoles per liter) of added salts used to make the relaxing solution were: 1.12 Na_2ATP, 14.77 MOPS, 5.00 Tetramethylammonium EGTA, 15.80 Tetramethylammonium hydroxide, 88.0 Potassium methane sulfonate, 5.69 $MgCl_2$.

Loading solutions were made by addition of isotope to the basic relaxing solution described above. High specific activity ^{42}K (2–5 mCi/mg K) was purchased from New England Nuclear as an aqueous solution containing a total of 5 mCi of radioactivity. This was dried and redissolved in 1 ml of relaxing solution that had no added K salt. The difference between the amount of K added as isotope plus carrier, and that required by the solution recipe was calculated. This difference was made up by adding cold KCl solution (~20 μl of a 2-M stock). [^{14}C]Sucrose solutions were prepared by adding 1 ml of relaxing solution to 250 μCi of isotope. The total amount of sucrose present depended on the specific activity of the [^{14}C]sucrose stock but was never >2 mM.

All solutions contained 40 μM quercitin to block the Ca-ATPase. The drug was added as an ethanolic solution with the final concentration of ethanol never exceeding 2% (vol/vol). The pH of all solutions was checked and adjusted if necessary (at 10°C) after addition of isotope and drugs. The K and Na concentrations were checked with a flame photometer.

Data Analysis

The efflux curves were plotted as log counts remaining vs. time. They were resolved into their individual components using a least squares fitting routine. In all cases the curves could be described as being the sums of either two or three exponentials. The coefficient of each exponential (pool size) was converted to a fractional fiber volume. To do this, the number of counts in each pool was converted to an equivalent volume by dividing by the specific activity of the loading bubble. This equivalent volume was then divided by 0.80 times the volume of the fiber segment exposed to the aqueous bubbles. The actual length of the fiber in contact with the bubble was ~90% of its total length. If radioisotope entered the portion of the fiber in oil and was included in the washout, our estimates of equivalent fiber volume would be incorrect by ~10%. The factor 0.80 corrects for the portion of the fiber volume taken up by the myofibrillar proteins (Mobley and Eisenberg, 1975).

RESULTS

^{42}K Efflux from Untreated Fibers

The washout of ^{42}K from skinned fibers is kinetically complex (Fig. 2). When plotted semilogarithmically, the

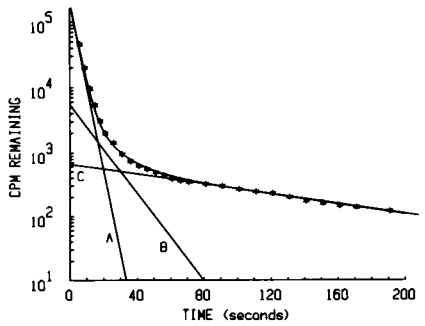

FIGURE 2 Efflux curve showing the time course of ^{42}K washout from a skinned fiber. The fitted curve was calculated from the equation $Y = 206{,}000\ \exp(-0.3t) + 5{,}500\ \exp(-0.08t) + 661\ \exp(-0.009t)$. The straight lines are the stripped exponentials, and correspond to the fast (*A*), intermediate (*B*), and slow (*C*) components of efflux. Fiber JH43.

efflux curves of untreated fibers can be described as a sum of three exponentials. Initially, ^{42}K efflux is very rapid, with isotope leaving the fiber with a time constant of 2–3 s. The pool size of this fast component of efflux expressed as a percent of the fiber volume (see Methods) averaged 91%. A second component of washout is resolved at longer times, has a time constant of ~10–15 s, and represents a pool 5.7% of the total fiber volume. Finally, there is a very slow component of efflux with a time constant of ~80 s that represents <1% of the fiber volume. The averaged parameters from 11 fibers are shown in Table I. For the rest of this discussion, we shall refer to these components of efflux as the fast, intermediate, and slow components.

A major goal of this study was to identify structural correlates for the various components of the washout data. In particular we were interested in determining which, if any, of the pools represented membrane-bound space within the skinned fiber. Our approach was to treat the fibers with agents that should have specifically affected ^{42}K movement across membranous structures and to determine which, if any, of the components of ^{42}K washout were affected. Since we wanted to compare the efflux kinetics of fibers before and after such treatment, we first determined whether the kinetics of washout of ^{42}K were similar for repeated washouts from the same fiber. The results for a double washout experiment are shown in Fig. 3. The fiber was loaded with ^{42}K and the washout followed as described previously. At the end of the first washout period the fiber was placed in relaxing solution for an additional 15 min to insure that all isotope had been removed. The fiber was then reloaded and the ^{42}K efflux followed a second time. As seen in Fig. 3, the kinetics of the second washout are similar to those of the first. Results from multiple washout experiments are summarized in Table II with the rate constants and pool sizes expressed as ratios of those found for the first washout compared with those from the second. None of the parameters shows significant changes between the first and second washout.

Effects of Detergent on the Kinetics of ^{42}K Efflux

Treatment of skinned fibers with detergents is known to destroy membranous structures without significantly affecting the contractile proteins (Kitazawa et al., 1982). The effect of a 30-min soak in 0.5% Triton X-100 on the kinetics of ^{42}K efflux from a skinned fiber are shown in Fig.

TABLE I
PARAMETERS FROM FITS TO ^{42}K EFFLUX CURVE (MEAN ± SD; $n = 11$)

Component	Compartment size	Rate constant
	%	s^{-1}
Slow	0.5 ± 0.5	0.008 ± 0.001
Intermediate	5.7 ± 0.2	0.081 ± 0.022
Fast	90.5 ± 0.1	0.249 ± 0.059

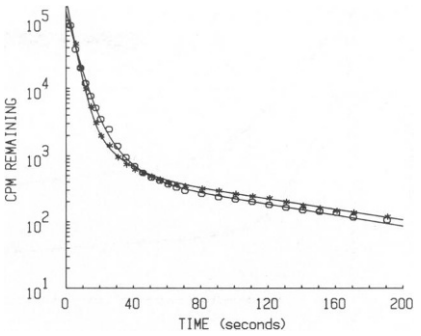

FIGURE 3 Double ^{42}K washout. At the ends of the first washout period (*), the fiber was reloaded and the efflux followed a second time (O). Fitted curves are $Y = 166{,}000 \exp(-0.3t) + 9{,}500 \exp(-0.08t) + 601 \exp(-0.009t)$ (*) and $Y = 125{,}000 \exp(-0.23t) + 12{,}000 \exp(-0.08t) + 575 \exp(-0.009t)$ (O). Fiber JH42.

4. After detergent treatment the efflux is adequately fit as a sum of two exponentials rather than three exponentials, as found in the control washout. From the averaged data shown in Table III, it can be seen that after exposure to detergent the slow component of efflux has a rate constant and a relative pool size that are not different from the slowest component of the untreated fiber (compare Tables I and III). The rate constant of the fast component is not significantly different from that of the fastest component of the control washout, while the relative pool size is somewhat smaller. From these results it seems that only the intermediate component of efflux identified in untreated fibers is greatly affected by the action of detergents. This indicates that the intermediate component may represent the movement of ^{42}K from a membrane-bound space within the skinned fiber.

The effect of detergent on ^{42}K washout was studied in eight fibers. Of these, six could be fit with two exponentials. The averaged parameters from these fits are included in Table III. The remaining two fibers gave washouts after detergent treatment that were fit as three exponentials. The rate constants and coefficients for the fast and slow components were similar to those shown in Table III. The intermediate component for these two fibers had a rate constant of 0.09 s^{-1} and a pool size <1%. Thus, for two of the eight fibers, detergent treatment reduced the size of the intermediate component but did not eliminate it.

TABLE II
COMPARISON OF PARAMETERS FROM FITS TO DOUBLE ^{42}K EFFLUX CURVES

Component	Compartment size	Rate constant
Slow	1.17 ± 0.20	0.95 ± 0.19
Intermediate	0.87 ± 0.17	1.14 ± 0.21
Fast	1.10 ± 0.26	1.12 ± 0.41

Values are the ratios of the parameters from the first washout compared with those from the second. None of the values is significantly different from 1.0 (t-test, $\alpha = 0.01$) Values given are mean ± SD; $n = 3$.

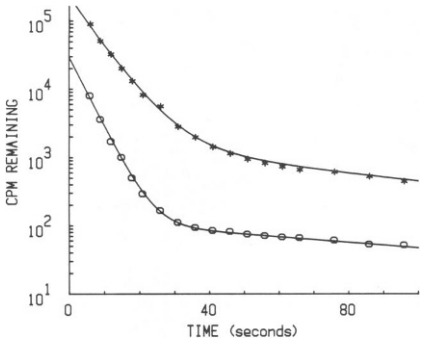

FIGURE 4 Effect of detergent treatment on ^{42}K efflux. After the first washout was determined (*), the fiber was exposed to 0.5% Triton X-100 for 30 min, reloaded with isotope, and the second curve measured (O). The fitted functions are $Y = 211{,}183 \exp(-0.17t) + 12{,}340 \exp(-0.07t) + 1{,}655 \exp(-0.013t)$ (*) and $Y = 29{,}693 \exp(-0.25t) + 120 \exp(-0.009t)$ (O). Fiber J045.

Washout of [^{14}C]sucrose

If the tentative conclusion reached above concerning the membrane dependence of the intermediate component of tracer efflux is true, we would predict that this component of efflux would be reduced or absent if a membrane impermeant tracer were used. Since sucrose is relatively impermeable to the SR membrane of skinned fibers, as shown by its osmotic effect on SR volume (Asayama et al., 1983), we repeated the efflux experiments using [^{14}C]sucrose. The results are shown in Fig. 5 and Table IV. The washout kinetics of [^{14}C]sucrose from untreated fibers are adequately fit as the sum of two exponentials. The slow component of sucrose washout has a rate constant of 0.013 s^{-1} and a pool size of 2.1% of the fiber volume. These values are significantly different from those obtained for the slow component of ^{42}K washout of untreated fibers. The fast component of [^{14}C]sucrose efflux has a rate constant of 0.15 s^{-1}, which is significantly slower than the fastest efflux rate for ^{42}K in either untreated or detergent-treated fibers, but still considerably faster than that for the intermediate pool. The size of the fast [^{14}C]sucrose pool is 80% of total fiber volume. This is not statistically different from the size of the fast ^{42}K pool. We have also studied the effect of detergent treatment on the washout of [^{14}C]sucrose. In three fibers there was no significant effect of

TABLE III
PARAMETERS FROM FITS TO ^{42}K EFFLUX CURVES FROM DETERGENT-TREATED FIBERS (MEAN ± SD; $n = 6$)

Component	Compartment size	Rate constant
	%	s^{-1}
Slow	0.2 ± 0.1	0.006 ± .003
Fast	73.0 ± 0.2*	0.258 ± .103

*Denotes significant difference from comparable parameter in Table I (two sample t-test, $\alpha = 0.01$).

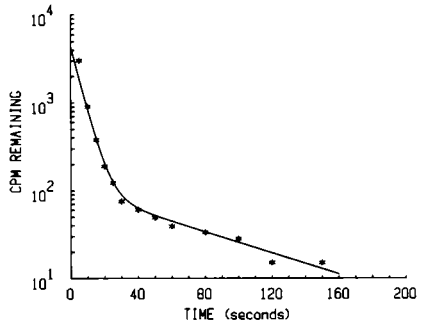

FIGURE 5 [^{14}C]Sucrose washout. The fitted curves is $Y = 4330 \exp(-0.175t) + 102 \exp(-0.014t)$. Fiber CK31.

detergent on the washout kinetics (data not shown). If the slow and fast components of sucrose efflux describe the movement of isotope from the same pools as the slowest and fastest component of ^{42}K efflux, then these results suggest again that the intermediate pool seen in the ^{42}K experiment is associated with a membrane-bound space. Since sucrose is prevented from entering this space because of its low permeability, the sucrose efflux curve does not contain the intermediate component seen with ^{42}K.

Decamethonium Does Not Affect the Kinetics of ^{42}K Efflux

Coronado and Miller (1980) have described a K channel from the SR that is blocked by the drug decamethonium. The effect of 120 μM decamethonium on ^{42}K efflux from skinned fibers was studied using a double washout protocol. The initial efflux of ^{42}K was followed as described

TABLE IV
PARAMETERS FROM FITS TO [^{14}C]-SUCROSE EFFLUX CURVES (MEAN ± SD; $n = 3$)

Component	Compartment size	Rate constant
	%	s^{-1}
Slow	2.1 ± 0.1*	0.013 ± 0.001*
Fast	80.0 ± 0.1	0.152 ± 0.023*

*Denotes significant difference from comparable parameter in Table I (two sample t-test, $\alpha = 0.01$).

TABLE V
COMPARISON OF PARAMETERS FROM FITS TO DOUBLE ^{42}K EFFLUX CURVES: EFFECT OF DECAMETHONIUM

Component	Compartment size	Rate constant
Slow	1.33 ± 0.31	1.15 ± 0.27
Intermediate	1.14 ± 0.37	1.20 ± 0.26
Fast	1.3 ± 0.28	0.95 ± 0.19

Values are the ratios of the parameters for the control washout compared with those from the washout in the presence of 120 μM decamethonium. None of the values is significantly different from 1.0 (t-test, $\alpha = 0.01$). Values are means ± SD; $n = 3$.

above. After the first washout was completed, the fiber was reloaded with ^{42}K for 25 min. It was then exposed for 5 min to a loading solution of the same specific activity to which 120 μM decamethonium had been added. The washout of ^{42}K was followed in the normal manner, except that drug was present in all the washout bubbles. The summarized results from double washouts of three fibers are shown in Table V. The rate constants and pool sizes determined during experiments with the drug are expressed as fractions of the values of the paired control. There were no significant differences found between the efflux in the presence of decamethonium and the paired control washouts.

DISCUSSION

The major aim of this study was to estimate the resting permeability of the SR of skinned fibers to potassium ion. We chose the skinned fiber preparation because it allows direct access to the SR without significant disruption of the membrane. Due to the complex nature of the ^{42}K washout curves, it was necessary to identify structural correlates of the various kinetic components of the efflux.

Our data strongly suggest that only the intermediate component of ^{42}K washout represents movement of ions from a membrane-bound space. First, this component of the efflux was not present in fibers treated with detergent. Kitazawa et al. (1982) have shown with electron micrographs that exposure of skinned fibers to Triton X-100 under conditions nearly identical to those used in this study removes virtually all membranous organelles. However, the structure of the contractile proteins as well as their function is preserved after detergent treatment (Kitazawa et al., 1982; Orentlicher et al., 1974). Second, [^{14}C]sucrose washes out of skinned fibers with a time course that can be adequately described as a sum of only two exponentials. Only the intermediate component of ^{42}K washout is not represented in the [^{14}C]sucrose data. Sucrose is relatively impermeant to biological membranes, and should be excluded from membrane-bound space within the skinned fiber.

Skinned fibers contain several membrane-bound organelles (including SR, mitochondria, and possibly sealed off transverse tubules) that could be involved in determining the kinetics of ^{42}K efflux. Perhaps the most direct way of identifying the structure involved is to look at the relative fiber volume it represents. The average pool size of the intermediate component was 5.7% of total fiber volume. This value is in good agreement with estimates of the volume of the terminal cisterns of the SR (Peachey, 1965; Mobley and Eisenberg, 1975). The volumes of the other possible membrane-bound spaces within a skinned fiber (mitochondria: 1.0%, transverse tubules: 0.3% [Mobley and Eisenberg, 1975]) are too small to account for this pool even if added together. This does not mean that potassium ion is prevented from moving across mitochondrial and transverse tubule membranes during our efflux experiments. It does suggest, however, that the SR is the major contributor to the intermediate pool.

Coronado and Miller (1980) have isolated a potassium channel from the SR and shown that it is sensitive to block by the drug decamethonium. The data reported in this study suggest that decamethonium did not affect the rate of ^{42}K efflux from the SR of skinned fibers. However, Coronado and Miller applied drug to what they define as the "inside" of the SR membrane. It is therefore possible that in our preparation the drug could not reach its blocking site. A second possible explanation for our failure to find block of potassium efflux by decamethonium is that the drug's effect on isolated potassium channels is enhanced by voltage. If the resting membrane potential of the SR is near zero millivolts, which seems likely (Kitazawa et al., 1984), the decrease in channel conductance may have been too small to be resolved by the methods used in this study.

The identity of structures that give rise to the other two pools is less certain. It seems reasonable to suggest that the fast pool represents isotope trapped in the myofilament space that can diffuse directly into the collecting bubbles. The fact that the average pool size for the fast component of ^{42}K efflux is 91% of the total fiber volume, a value that is appropriate for the nonorganellar volume of a muscle cell, supports this notion (Mobley and Eisenberg, 1975). The pool size for the fast component of [^{14}C]sucrose was also a large fraction of the cell volume. In addition, the average rate constant for efflux of [^{14}C]sucrose is ~60% of that for ^{42}K, a trend that might be expected from differences in the diffusion coefficients of potassium and sucrose in aqueous media.

If the fast component of efflux is due purely to diffusion, then its kinetics should not follow an exponential time course, but rather have the form of a diffusion curve (Harris and Burns, 1949). Unfortunately, we found it impossible to obtain samples more rapidly than every 3–5 s, and only a few data points were obtained at very early times during a washout. The number of points collected at early times was insufficient to allow us to determine whether the kinetics were, in fact, nonexponential, and we did not feel the data justified more complex modeling. If the fast component of efflux does represent a diffusional process, then our failure to treat it as such would bias our estimates of the parameters describing the intermediate component. However, any potential error introduced in this way should not have affected the conclusions we have drawn from our data. For instance, arguments based qualitatively on the presence or absence of a particular component of efflux under various concentrations would be unaffected. The potential error in our value for the size of the intermediate pool can be estimated by considering the relative pool size and efflux kinetics as suggested by Hodgkin (see appendix in Solomon, 1960). For our data, the amount of this error would have been <50%. Our conclusion concerning the identity of the intermediate pool

based on its apparent volume would not be invalidated by a correction of this magnitude. Finally, the relative sizes of the fast and intermediate pools (they differ by a factor of 15) should have minimized any error introduced into our estimate of the rate constant of the intermediate pool by cycling of isotope back into the SR before it left the myofilament space.

We can only speculate on the structure that gives rise to the very slow component of isotope washout. One possibility is that the slow component is due to isotope that has become associated with protein binding sites. Alternately, the slow component might represent a small amount of contaminant isotope that is associated with the portion of the fiber that is in oil during the load period.

If we assume that the intermediate component of the ^{42}K efflux is entirely determined by the movement of isotope across the SR membrane, then we can use the rate constant of that component to estimate the potassium permeability (Sten-Knudsen, 1978). For a system in which ions are moving from a small membrane bound space into an infinite bath, it can be shown that: $P = K \cdot V/A$, where P is permeability, V is volume, A is surface area, and K is the rate constant of efflux determined as the slope of the line relating isotope remaining in the space and time. From our average value of the rate constant of the intermediate compartment ($K = 0.08\ s^{-1}$) and assuming a surface-to-volume ratio for the SR of 20 μm^{-1} (Mobley and Eisenberg, 1975), we can calculate the permeability of the SR to be 1.7×10^{-7} cm s^{-1}.

The permeability of the SR membrane to potassium ions has been studied extensively in isolated membrane vesicles (Meissner, 1983). Most of this work has been done using mammalian muscle. The relative permeability of the potassium ion channel has been deduced from the response of the vesicles in a variety of experimental situations. For instance, changes in light scattering have been used to monitor osmotic effects of various ions on vesicle volume (Kasai et al., 1979; Kometani and Kasai, 1978). These studies indicate that SR vesicles are highly permeable to Na and K. The creation of diffusion potentials by ion substitution has been monitored with voltage sensitive dyes. Again, the results indicate an SR membrane permeable to potassium ions (McKinley and Meissner, 1978). Meissner and McKinley (McKinley and Meissner, 1978; Meissner and McKinley, 1976) have identified an Na/K channel in vesicles from rat muscle using isotope-flux techniques. They suggest that the channel is uniformly distributed along the SR at a density of 50 pores/μm^2. They estimate that the SR potassium permeability is $>10^{-6}$ cm s^{-1}, which is equivalent to a zero voltage conductance of $\sim 10^{-3}$ S/cm^2 (Meissner, 1983) and a factor of 10 greater than our own. Miller and co-workers have inserted isolated vesicles into planar bilayers and studied the observed conductance pathways under voltage clamp conditions (Miller, 1983). They have identified a potassium channel that is moderately voltage dependent. Channels isolated from frog skeletal muscle have two open states—one having a conductance 30% of the other (Labarca and Miller, 1981). The specific conductance of the SR membrane calculated from their data is given as 6×10^{-2} S/cm^2 (Miller, 1983), and indicates a membrane even more permeable than that described by Meissner and co-workers. Garcia and Miller (1984) have studied the cation permeability of isolated SR vesicles using a stopped-flow technique that permits rapid measurement of ion fluxes. They conclude that the potassium channel studied by Miller and his colleagues in planar bilayers is present in large quantities in SR vesicles. The calculated potassium conductance of the vesicles was of the order of 0.1 S/cm^2.

The value for the SR potassium conductance estimated from our results in skinned fibers is thus significantly less (about a factor of 10) than that determined by Meissner, and very much smaller (at least a factor of 100) than that reported by Miller's group. The only comparable estimates from intact cells were made by Bezanilla and Horowicz (1975) and Vergara et al., (1978) from models used to describe optical response of cells stained with voltage sensitive dyes. Their estimates of the resting conductance of the SR of 2.8×10^{-5} and 7.2×10^{-5} S/cm^2, respectively, are compatible with the results from this study in that they suggest isolated SR preparations are more permeable than intact SR membranes to potassium ion. Addition of the potassium ionophore valinomycin to intact muscle increases the amount of potassium accumulated by the sarcoplasmic reticulum during a tetanus (Kitazawa et al., 1984). This result is consistent with the idea that the drug is capable of increasing the normal potassium permeability of the SR, and supports the notion that the permeability is not extraordinarily large in the native state. Recently, Baylor et al. (1984) used kinetic modeling to compare the estimated time course of SR calcium release to other optical signals that might reflect changes in SR membrane potential. They conclude that none of the signals is early enough to support the notion that a change in SR potential initiates calcium release. Further, they suggest that the only way the optical signals could reflect an SR potential change that results from calcium efflux is if the SR membrane has a higher resistance than that reported in isolated preparations. From our results, we would conclude that such an assumption may be justified. The reason for the discrepancy between the measured potassium permeability of isolated SR channels and vesicles, and estimates or measurements made in intact muscle cells and skinned fibers, is not clear. Possibly, the techniques used to isolate SR membranes change the permeability properties of the potassium channels. Alternatively, as suggested by Garcia and Miller (1984), physiological control mechanisms may suppress the conductance of the channel in the native state.

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