Dependence of Calcium Permeability of Sarcoplasmic Reticulum Vesicles on External and Internal Calcium Ion Concentrations*

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The ability of sarcoplasmic reticulum vesicles to retain calcium following ATP-supported calcium uptake in the presence of the calcium-precipitating anions oxalate and phosphate depends on $C_{a0}$ (calcium ion concentration outside the vesicles) and $C_{a}$ (calcium ion concentration within the vesicles). Calcium efflux rates at any level of $C_{a0}$ are accelerated when $C_{a}$ is increased. Higher $C_{a}$ at the time that calcium uptake reactions reach steady state is associated with a spontaneous calcium release that reflects this effect of increased $C_{a}$. Increasing $C_{a}$ at any level of $C_{a0}$ causes little or no acceleration of calcium efflux rate so that calcium permeability coefficients, estimated by dividing calcium efflux rates by $C_{a}$, the "driving force," are inversely proportional to $C_{a}$. Calcium permeability coefficients thus correlate, as a first approximation, with the ratio $C_{a}/C_{a0}$, decreasing 1000-fold as this ratio increases over a 3000-fold range ($C_{a0} = 0.1$ to $3.3 \mu M$, $C_{a} = 4$ to $750 \mu M$).

Oscillations in both the calcium content of the vesicles and $C_{a0}$ are seen as calcium uptake reactions approach steady state, suggesting that calcium permeability undergoes time-dependent variations. Sudden reduction of $C_{a0}$ to levels that markedly inhibit calcium influx via the calcium pump unmasks a calcium efflux that decreases slowly over 60 to 90 s.

The maximal calcium permeability observed in the present study would allow the calcium efflux rate from the sarcoplasmic reticulum at a $C_{a0}$ of $100 \mu M$ to be approximately $10^{-10} \text{ mol/cm}^2/\text{s}$, which is about 1 order of magnitude less than that estimated for the sarcoplasmic reticulum of activated skeletal muscle in vivo. The release of most of the stored calcium in some experiments indicates that the observed permeability changes can occur over a large portion of the surface of the sarcoplasmic reticulum.

A number of observations regarding the ability of sarcoplasmic reticulum vesicles to transport and retain calcium suggest that the calcium permeability of these membranes is not constant. Weber et al. (2) found that the level of ionized calcium outside the vesicles at which oxalate-supported calcium uptake reaches a steady state is markedly increased when larger amounts of calcium are presented to the vesicles, a finding that was suggested to mean that vesicles of increasing leakiness participated in calcium uptake as the total load of calcium was increased. These investigators also found that the rate of calcium exchange by vesicles that had reached a steady state of calcium storage in the absence of calcium-precipitating anions increased with increasing $C_{a0}$, and that the rate of $Sr^{2+}$ uptake was markedly reduced at high levels of $C_{a}$, after vesicles were preloaded with calcium (2). Further evidence that calcium permeability of sarcoplasmic reticulum vesicles may be influenced by $C_{a0}$ was obtained by Hasselbach et al. (3), who found that when $C_{a0}$ is held constant by a calcium oxalate precipitate, calcium efflux rate increases 50- to 100-fold immediately after an elevation of $C_{a}$ that also leads to renewed calcium uptake. These findings were confirmed by Makinose (4), who reported that elevation of $C_{a}$ after a small initial calcium load has been taken up by sarcoplasmic reticulum vesicles evokes the spontaneous release of a portion of the previously accumulated calcium at the same time that the added calcium is taken up.

The dependence of calcium permeability on the concentrations of $C_{a0}$ and $C_{a}$ was examined further in the present study. Evidence has been obtained that calcium permeability increases when $C_{a}$ is increased, and decreases when $C_{a0}$ is increased.

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The abbreviations used are: $C_{a0}$, calcium ion concentration inside the microsomal vesicles; $C_{a}$, calcium ion concentration in the medium outside the microsomal vesicles, calculated as described previously (5); EGTA, ethylene glycol bis(β-aminoethyl ether)N,N,N',N'-tetraacetic acid. As used in this article, "calcium uptake" and "calcium release" refer to the rate of net gain or loss of calcium by the vesicles, "calcium influx" and "calcium efflux" refer to rates of unidirectional calcium fluxes into and out of the vesicles, respectively.
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MATERIALS AND METHODS

Preparation of microsomes and measurements of calcium uptake and release were as described in Ref. 5. All experiments were carried out in 0.12 M KCl, 40 mm histidine buffer at pH 6.8, and 5 mm MgATP at 25°C. Calcium influx was measured by addition of tracer amounts of high specific activity, carrier-free ⁴⁰CaCl₂ (1 mCi/μmol) to a reaction mixture in which ⁴⁰CaCl₂ was used for the initial calcium uptake reaction. The added tracer calcium increased total CaCl₂ concentration in the medium by <0.02 nmol/liter, which could be ignored. A duplicate reaction was started with ⁴⁰CaCl₂ instead of ⁴⁰CaCl₂ to permit assessment of net calcium uptake and release prior to and after addition of the tracer calcium, as well as to allow calculation of the total calcium concentration outside the vesicles at the time of tracer addition. All measurements were initiated at the time that calcium uptake reached steady state, as determined in a series of pilot reactions.

The rate of uptake of the tracer calcium was measured with serial samples taken at 15-s intervals and analyzed by the Millipore filtration method (5). Calcium influx rates were calculated from the rate of tracer uptake, adjusted to reflect the average calcium concentration outside the vesicles during each 15-s interval, and expressed as nanomoles of influx per mg of protein. The apparent rate of total calcium influx measured by the tracer experiment was high during the first 15-s interval, due possibly to exchange with traces of calcium in the glassware. Over the subsequent 60 to 90 s, however, the rate of disappearance of the tracer became linear with time. Calcium influx rates were thus calculated from curves obtained from tracer uptake during four to six consecutive 15-s intervals.

RESULTS

Effects of Ca⁺ on Spontaneous Calcium Release after Anion-supported Calcium Uptake—The level of Ca⁺ at the time that calcium uptake in the presence of calcium-precipitating anions approached steady state influenced the ability of the sarcoplasmic reticulum to retain calcium. When reactions were started with a high ratio between total calcium and protein, spontaneous calcium release began earlier and was much greater in extent than in reactions started with a low total calcium:protein ratio (Fig. 1). The more rapid spontaneous release of calcium can be seen in Fig. 1 to result from the higher level of Ca⁺ at the time that calcium uptake reached a steady state, and not to be dependent on the absolute calcium or protein concentration at the start of the reaction.

As shown in the preceding paper (5), an increase in calcium permeability will lead to greater calcium release when Ca⁺ is high because more calcium must be added to the external medium from stores in the vesicles to restore the equilibrium between calcium influx and the increased calcium efflux rate than when Ca⁺ is low (Fig. 15 in Ref. 5). This relationship cannot, however, explain fully the findings in Fig. 1 because calcium release began after t = 10 min in the presence of high Ca⁺, in contrast to the continued uptake of calcium without spontaneous release until t = 30 min when Ca⁺ was low. This finding suggests that high Ca⁺ has an additional effect to increase calcium permeability. A similar spontaneous calcium release at high Ca⁺ has also been reported in cardiac sarcoplasmic reticulum vesicles (6), and spontaneous calcium release by cardiac preparations is remarkably reduced at low concentrations of Ca⁺. ² High Ca⁺ also was noted to promote spontaneous calcium release in studies of calcium storage in the absence of calcium-precipitating anions (7).

Spontaneous Changes in Calcium Uptake "Capacity"—Analysis of the behavior of sarcoplasmic reticulum vesicles in the presence of high Ca⁺, consistently showed instability in calcium uptake capacity as calcium uptake approached an initial steady state. These changes, which can be seen in many of the control reactions at high Ca⁺ in Ref. 5 (e.g. Figs. 9A and 10C), were associated with large changes in Ca⁺ when measurements were made as calcium uptake in 50 mm phosphate approached a steady state. As seen in Fig. 2, where mean values for five similar reactions carried out concurrently are plotted, Ca⁺ first reached a minimum, then increased and finally decreased again as calcium uptake approached steady state. The differences between Ca⁺ at t = 22 min from those at t = 18, 19, and 26 min (Fig. 2) were highly significant (p < 0.01) and arise from oscillations in the calcium content of the vesicles.

Effects of Ca⁺ and Ca⁻ on Calcium Permeability—The effects of Ca⁺ on calcium permeability were studied in paired experiments in which initiation of the calcium uptake reaction with ⁴⁰Ca allowed calcium uptake or release rates to be measured, while simultaneous measurements of tracer uptake after addition of small amounts of ⁴⁰CaCl₂ to identical reactions initiated with ⁴⁰Ca allowed calcium influx rates to be calculated (see "Materials and Methods"). Calcium efflux rates could then be calculated from those of calcium influx, from which calcium uptake rate during this time was subtracted or calcium release rates added. Results in a typical experiment are shown in Fig. 3, in which calcium influx is plotted at three levels of Ca⁺. Calcium influx rates decreased as Ca⁺ was lower at the time, 15 min after the start of the reaction, when these measurements were initiated.

Calcium efflux rates, calculated from measurements of calcium content and of calcium influx, are shown in Fig. 4 as a function of Ca⁺ at two levels of Ca⁻. The latter were maintained at 20 or 4 μM by the presence of 1.0 or 5.0 mm oxalate as the calcium-precipitating anion. Calcium efflux rate at any level of Ca⁺ was not increased significantly at the higher Ca⁻ (i.e. at the lower oxalate concentration) even though the higher Ca⁻
concentration within the vesicles might be expected to accelerate calcium efflux.

Calcium permeability coefficients ($K_e$) were calculated from data such as those shown in Fig. 4 with the assumption that calcium efflux rate is a direct linear function of $Ca_o$, according to the equation:

$$ \text{Calcium efflux rate} = K_e \times Ca_o $$  (1)

Levels of $Ca_o$ were calculated from the solubility products of calcium oxalate and calcium phosphate as described in Ref. 5. As shown in Fig. 5, the calcium permeability coefficient increased with increasing $Ca_o$ and decreased with increasing $Ca_i$.

The effects of both $Ca_o$ and $Ca_i$ on the calcium permeability coefficient reflect a close relationship between this coefficient and the ratio $Ca_o/Ca_i$. The data from Fig. 5, obtained in a series of experiments in which $Ca_o$ was varied by the use of two oxalate concentrations, fell on a single line when the calcium permeability coefficients were plotted as a function of the ratio $Ca_o/Ca_i$ (Fig. 6). When data from eight series of experiments carried out with varying oxalate or phosphate concentrations were plotted in this manner, the calcium permeability coefficient was found to correlate with the ratio $Ca_o/Ca_i$ over a more than 3,000-fold range (Fig. 7).

Effects of Sudden Reduction of $Ca_o$ on Calcium Permeability - Sudden reduction of $Ca_o$ following $^{40}Ca$ uptake, by greatly slowing calcium influx via the calcium pump, unmasked a rapid calcium efflux that caused the rapid release of a small amount of calcium. The extent of the initial calcium release during the first 15-s interval increased only slightly when the final level of $Ca_o$ was lowered in the range between 1.0 to 0.05 $\mu$M by addition of varying amounts of EGTA (Fig. 8). After 60 to 90 s, calcium release slowed (Fig. 8A) or, following addition of smaller amounts of EGTA, was followed by reuptake of the previously released calcium (Fig. 8, B and C). These findings indicate that calcium permeability slowly decreases following addition of EGTA, which by chelating $Ca^2+$ outside the vesicles, increased the ratio $Ca_o/Ca_i$. When only small amounts of EGTA were added, the lower level of $Ca_o$ appeared to reduce calcium permeability to a greater extent than it inhibited the

![Fig. 3](http://www.jbc.org/) Calcium influx rates, measured after tracer $^{40}CaCl_2$ addition to a reaction 15 min after its initiation with $^{40}CaCl_2$ as described under "Materials and Methods." Protein concentration was 10 $\mu$g/ml and 1.0 mM oxalate was the calcium-precipitating anion. Final $Ca_o$ concentrations at the time of tracer addition were 2.4 $\mu$M (O), 0.88 $\mu$M (■), and 0.10 $\mu$M (□) following addition of $CaCl_2$ at the start of the reactions to final concentrations of 37, 26, and 18 $\mu$M, respectively.

![Fig. 2](http://www.jbc.org/) Oscillations in $Ca_o$ as calcium uptake approaches steady state at high $Ca_o$. Data from five independent reactions in which calcium uptake by 51 $\mu$g of protein/ml was measured at a total calcium concentration of 100 $\mu$M in the presence of 50 mM phosphate as described under "Materials and Methods" are shown (O——O). The horizontal bars represent ±S.D. Also plotted for reference is the initial calcium uptake reaction (■——■) taken from a pilot run.

![Fig. 4](http://www.jbc.org/) Dependence of calcium efflux rate on the level of $Ca_o$. Calcium uptake reactions were carried out with 10 $\mu$g of protein/ml and 18 to 80 $\mu$M $CaCl_2$ as described under "Materials and Methods." Calcium efflux rates were measured, beginning at $t = 10$ min in experiments with 5.0 mM oxalate (O), and at $t = 12$ min when oxalate was 1.0 mM (□).
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5. Dependence of the calcium permeability coefficient on Ca. The calcium permeability coefficient, calculated as the ratio calcium efflux rate/C%, was measured at 20 and 4 μM Ca, maintained by 1.0 mM (○) and 5 mM (●) oxalate, respectively (data from Fig. 4).

6. Relationship between calcium permeability coefficient and the ratio Ca/Ca. Data and symbols shown in Figs. 4 and 5.

calcium pump, thereby allowing for a slow renewal of calcium uptake (Fig. 8C'). Addition of larger amounts of EGTA reduced Ca, to levels where calcium uptake became minimal (Fig. 8A). Under these conditions, marked slowing of the rate of calcium release indicated that calcium permeability gradually decreased at the lower Ca.

Effects of X537A on Calcium Permeability—Although it is well established that the ionophore X537A is able to induce a cation-selective leak in lipid membranes, and that this ionophore can increase calcium fluxes across the membranes of the sarcoplasmic reticulum (see Ref. 5), the effects of low concentrations of X537A on calcium release after oxalate- and phosphate-supported calcium uptake were quite complex. In addition to its ability to induce a small, rapid calcium release, the ionophore greatly slowed the rate of spontaneous calcium release seen in the presence of high Ca, (Fig. 9). Calcium efflux rate calculated as described under "Materials and Methods," fell from approximately 310 to 160 nmol/mg of protein/min 30 s after the initial X537A-induced calcium release shown in Fig. 9. Because Ca, was maintained constant by the calcium oxalate precipitate within the vesicles, these findings indicate that after causing an initial calcium release, the ionophore reduced the high calcium permeability seen at high Ca,.

Reduction of the rate of spontaneous calcium efflux at high levels of Ca, after addition of X537A could be shown to result from a paradoxical effect of the ionophore to decrease the rate of calcium efflux. Following its effect to promote a rapid calcium release, 10 μM X537A markedly reduced the rates of spontaneous calcium release and influx at high Ca, (Fig. 9). Calcium efflux rate calculated as described under "Materials and Methods," fell from approximately 310 to 160 nmol/mg of protein/min 30 s after the initial X537A-induced calcium release shown in Fig. 9. Because Ca, was maintained constant by the calcium oxalate precipitate within the vesicles, these findings indicate that after causing an initial calcium release, the ionophore reduced the high calcium permeability seen at high Ca,.

DISCUSSION

The present findings indicate that as calcium uptake in the presence of a constant level of Ca, approaches steady state, spontaneous calcium release begins sooner, and is greater in magnitude when the levels of Ca, are high (1 to 5 μM) than when Ca, is low (0.1 to 0.5 μM) (Fig. 1). This spontaneous calcium release cannot be attributed simply to deterioration of the membranes as it can be halted when Ca, is suddenly lowered by addition of EGTA (Fig. 8B and C). Furthermore, the findings of significant oscillations in calcium uptake "capacity" (5) and in the level of Ca, at the time that the reaction approaches a steady state (Fig. 2) are difficult to reconcile with the view that this spontaneous calcium release is due to a

3 J. Dunnett and W. Nayler, personal communication.
Fig. 8. Effects of EGTA addition on calcium release. Calcium uptake was carried out with 7 μg of protein/ml in 2.5 mM Tris oxalate as described under "Materials and Methods." After 8 min, EGTA was added to final concentrations of 1.4 mM (A), 0.7 mM (B), and 75 μM (C). Subsequent calcium release (○) and the calcium retained by an aliquot of the control reaction to which no EGTA was added (●) were followed. The numbers in parentheses on the graphs are the calculated Ca, levels.

nonspecific deterioration of the membranes. Instead, it appears that these spontaneous changes in calcium uptake "capacity" reflect the operation in these vesicles of a mechanism that controls the membrane permeability to calcium.

The findings described above and in the preceding paper (5) indicate that the level of C, has two important effects on calcium release from sarcoplasmic reticulum vesicles containing an insoluble calcium precipitate. The first arises from the relationship between the Ca, concentration gradient, C, / C, and C, in which higher levels of C, increase the amount of calcium released following a given increase in calcium permeability (5). This effect, which is shown diagrammatically in Fig. 10 of Ref. 5, reflects the ability of increased C, to amplify the net calcium release caused by a given increase in membrane permeability to calcium. Amplification of calcium release by high C, is due to the constraints of an experimental design in which most of the calcium in the reaction mixture comes to be stored within the vesicles and so is unavailable to the calcium pump sites, which are stimulated only by Ca, at the outside of the vesicles. A second effect of C, which is described in the present report, can be attributed to an increase in calcium permeability that accompanies an increase in C, at any given level of C, (Fig. 5). These latter findings are in accord with previously reported data (2-4) which indicate that elevation of C, increases the rate of calcium exchange.

Evidence that calcium permeability decreases when C, is increased is found in Fig. 15 of Ref. 5, which illustrates the finding that both the rate and extent of spontaneous calcium release can decrease when oxalate concentration is reduced, or when phosphate instead of oxalate is used as the calcium-precipitating anion. These findings appear paradoxical as the rate of net calcium release from vesicles of constant leakiness might be expected to increase when C, is increased from 4 to 150 μM, when 50 mM phosphate is substituted for 5 mM oxalate (Fig. 15 of Ref. 5). Thus, the phenomena illustrated in this figure may be manifestations of the same mechanism observed by Weber et al. (2) whereby an increase in C, slows Sr, influx. The present findings are thus in accord with those reported earlier, and indicate that calcium permeability decreases when C, is increased, an effect that is independent of changes in C, (Fig. 5). Additional evidence that calcium permeability decreases following an increase in C, is seen in Ref. 5, where the effects of X537A on vesicles before and after the initiation of oxalate-supported calcium uptake are contrasted. The apparent "protective" effect of prior calcium uptake (Fig. 4 of Ref. 5) may arise from the ability of the increased C, to reduce calcium permeability, and thus to slow the rate of calcium efflux following addition of X537A to levels below those of the calcium influx which can be achieved by the calcium pump.

The relative independence of calcium efflux rate and C, (Fig. 4) might reflect the existence of a calcium efflux channel that is controlled by a carrier with a Ca, affinity sufficiently
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high to remain saturated at Ca\textsubscript{a} = 4 \mu M, the lowest level achieved in this study. In view of the evidence discussed above, which indicates that higher levels of Ca\textsubscript{a} reduce calcium efflux rate, it appears more likely that this finding arises from a direct effect of Ca\textsubscript{a} to reduce calcium permeability. Furthermore, the Ca\textsuperscript{2+} sensitivity of the receptor inside the vesicles which mediates the inhibitory effects of Ca\textsubscript{a} on the calcium pump is very low (6).

Calcium permeability, as estimated from Equation 1, bears a close relationship to the Ca\textsuperscript{2+} concentration gradient, Ca\textsubscript{a}/Ca\textsubscript{i} (Figs. 6 and 7). This relationship holds over a greater than 3000-fold range of Ca\textsubscript{a}/Ca\textsubscript{i}, in experiments where Ca\textsubscript{a} ranges between 4 and 750 \mu M, and Ca\textsubscript{i} varies between 0.1 and 3.3 \mu M. Substitution of phosphate for oxalate as the calcium-pumping anion is without significant effects on this relationship, showing that these effects do not arise from changing Mg\textsuperscript{2+} concentration such as might result from complexation of this ion by oxalate.

Findings similar to those described here have been reported by Portzig (9), who studied passive calcium influx into human erythrocyte ghosts. Portzig found that passive calcium influx into these cells, which is in some ways analogous to passive calcium efflux from the sarcoplasmic reticulum, is reduced by high levels of Ca\textsuperscript{2+} outside the cells and increased by high levels of internal Ca\textsuperscript{2+}. These findings were interpreted as reflecting a role of membrane-bound calcium in regulating the passive flux of calcium across these membranes (9).

The present findings can be interpreted in terms of an hypothetical calcium carrier whose mobility is determined by the Ca\textsuperscript{2+} concentrations on both sides of the sarcoplasmic reticulum membrane. Under conditions where Ca\textsubscript{a} and Ca\textsubscript{i} are approximately equal, the calcium binding site on this carrier would be able to approach the inner and outer faces of the membrane with similar probabilities, whereas when Ca\textsubscript{a} is much greater than Ca\textsubscript{i}, the ability of the carrier to carry calcium from the inner surface of the membrane might be impaired by the tendency of the calcium binding site to remain in the region of higher Ca\textsuperscript{2+} concentration. However, the finding that the calcium influx which is mediated by this permeability control mechanism can effect spontaneous changes in the total calcium content of the vesicles (Figs. 1 and 2), and that the calcium release which begins after addition of EGTA slows significantly with time (Fig. 8) indicate that these phenomena are not due simply to a variable rate of calcium-calcium exchange, as has been described recently by Beirão and de Meis (10). Instead, this permeability control mechanism is able to mediate a large net calcium movement out of the sarcoplasmic reticulum. As the retention of a large portion of the calcium stores of sarcoplasmic reticulum vesicles is influenced by the permeability control mechanism described in this report, these studies provide evidence that the calcium permeability of a significant fraction of the sarcoplasmic reticulum is sensitive to Ca\textsubscript{a} and Ca\textsubscript{i}. Although the calcium release in activated muscle may begin at specialized sites adjacent to the t-system (11), it is possible that other surfaces of the sarcoplasmic reticulum participate in a Ca\textsuperscript{2+}-controlled excitation process.

The findings that the calculated calcium permeability coefficient is inversely proportional to Ca\textsubscript{a} and is markedly reduced after the sudden calcium release caused by EGTA (Fig. 8), or caffeine (5) are similar to observations with partially "skinned" muscle fibers where sudden elevation of Ca\textsubscript{a} ("calcium-triggered calcium release," Refs. 12 and 13), or caffeine (14) causes a transient calcium release. The ability of elevated Ca\textsubscript{a} to promote calcium release in "skinned fibers" may reflect the relationship between Ca\textsubscript{a} and both calcium efflux (Fig. 4) and the calculated calcium permeability coefficient (Fig. 5), while the transient nature of both calcium- and caffeine-induced calcium release seen in "skinned" fibers may reflect time-dependent changes in calcium permeability such as shown in Figs. 2 and 8. Caffeine-induced oscillations in the tension developed by intact muscle fibers (15) may also be manifestations of these time-dependent permeability changes.

Calcium release from "skinned" muscle fibers (13, 14) and sarcoplasmic reticulum vesicles (16) has been observed after replacement of a nonpermeant anion with a permeant anion. This calcium release, which is believed to be associated with the entry of anions into the interior of the sarcoplasmic reticulum, has been postulated to result from depolarization of a positive potential within this structure that maintains calcium permeability at a low level. The present finding that calcium permeability coefficients correlate with the calcium concentration gradient (Figs. 6 and 7) might be interpreted to reflect an effect of an electrical potential associated with the Ca\textsuperscript{2+} concentration gradient across these membranes. Recently, however, we have found that the ability of increasing Ca\textsubscript{a} to enhance calcium efflux rate at a given Ca\textsubscript{i} is limited, and that this effect of Ca\textsubscript{a} resembles a saturable process. The relationships shown in Figs. 6 and 7, therefore, probably reflect the operation of opposing effects of Ca\textsubscript{a} and Ca\textsubscript{i} on calcium permeability, rather than a direct response of the vesicles to the calcium concentration gradient per se.

The maximum calcium efflux rate observed in this study was ~400 nmol/mg/min, which corresponds to a rate of ~2 x 10\textsuperscript{-15} mol/cm\textsuperscript{2}/s as each milligram of these vesicles contains approximately 3000 cm\textsuperscript{2} of membrane surface. This value is more than 10\textsuperscript{2} to 10\textsuperscript{3}-fold greater than the rates of passive calcium efflux from phospholipid vesicles (16, 17) and similar to that of the passive calcium efflux from reconstituted ATPase and sarcoplasmic reticulum vesicles (18).

The maximum calcium permeability of ~100 nmol/mg/min/\mu M Ca\textsubscript{a} (Fig. 7) would allow a calcium efflux rate of ~10\textsuperscript{-10} mol/cm\textsuperscript{2}/s if this high permeability became manifest in an intact muscle where the Ca\textsuperscript{2+} concentration within the sarcoplasmic reticulum was 100 \mu M. This value is approximately an order of magnitude less than that of 10\textsuperscript{-9} mol/cm\textsuperscript{2}/s calculated for calcium efflux from the sarcoplasmic reticulum of activated skeletal muscle in vivo (19-21). Furthermore, the observed rate of change in the calcium permeability coefficient following a decrease in Ca\textsubscript{a} (Fig. 8) is quite slow. However, the ability of the calcium permeability control mechanism observed in the present study to effect a 1000-fold change in the calculated calcium permeability coefficient suggests that it may be of some physiological significance in the intact muscle.

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