SINGLE-CHANNEL CALCIUM AND BARIUM CURRENTS OF LARGE AND SMALL CONDUCTANCE FROM SARCOPLASMIC RETICULUM

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ABSTRACT Two types of divalent cation conducting channels from rabbit skeletal muscle sarcoplasmic reticulum (SR) were incorporated into planar lipid bilayers. A high conductance (100 pS in 53 mM *trans* Ca^{2+}) Ca^{2+} channel was incorporated from heavy density SR fractions. The 100-pS channel was activated by adenine nucleotides and Ca^{2+} and inhibited by Mg^{2+} and ruthenium red. A 10-pS calcium and barium conducting channel could be incorporated into planar lipid bilayers from light, intermediate, and heavy density SR vesicles. 10-pS channel activity in bilayers was not dependent on *cis* Ca^{2+} and was only weakly dependent on adenine nucleotides. Ruthenium red at concentrations up to 1 mM had no effect and Mg^{2+} was only marginally effective in inhibiting macroscopic Ba^{2+} currents from this channel. Calcium releasing activity in intermediate and heavy density SR fractions was assayed according to a rapid quench protocol and compared with the results obtained in the bilayer. Results from this comparison indicate that the 10-pS channel is probably not involved in rapid Ca^{2+}- and adenine nucleotide-induced Ca^{2+} release from isolated SR vesicles.

INTRODUCTION

The sarcoplasmic reticulum (SR) in skeletal muscle is responsible for the release and reabsorption of calcium during excitation contraction coupling. Contraction occurs when SR releases its load of Ca^{2+} into the cytoplasmic space. Relaxation begins when the SR Ca^{2+} pump absorbs Ca^{2+} back into the SR, lowering the cytoplasmic Ca^{2+} to its precontractile level. The active transport of calcium into the SR through the action of the SR calcium pump has been extensively studied and well characterized (for review see reference 11); however, the mechanism of calcium release and the proteins involved in this process have remained subjects of much conjecture (3, 4).

Studies in isolated vesicles have shown that SR contains a calcium release channel that is regulated by Ca^{2+} and adenine nucleotides and inhibited by Mg^{2+} and ruthenium red (16, 13). In a recent report, we identified a large conductance (~100 pS in 50 mM Ca^{2+}), nucleotide-activated calcium channel present in heavy SR vesicle fractions (19). Based upon its activation by adenine nucleotides and inhibition by ruthenium red, we proposed that this channel was likely the mediator of calcium fluxes measured in isolated SR vesicles. In this report we identify an additional type of calcium conducting channel incorporated from SR into planar bilayers. We compare its properties with the high-conductance heavy SR calcium channel and with the properties of calcium release from isolated SR vesicles.

MATERIALS AND METHODS

Reagents

$^{45}CaCl_2$ was obtained from ICN Pharmaceuticals, Inc., Irvine, CA. Nucleotides including the ATP analog AMP-PCP were purchased from Sigma Chemical Co., St. Louis, MO. Ruthenium red (95%) was from Fluka Chemical Corp., Hauppauge, NY. Phospholipids were obtained from Avanti Polar Lipids, Inc., Birmingham, AL. All other chemicals were of reagent grade. Glass distilled deionized water was used to prepare all solutions. HEPES buffer solutions were prepared as in reference 20.

Preparation of SR Membranes

Intermediate and heavy density SR membranes were prepared according to the procedure of Meissner (13). Light and intermediate SR fractions (ISR) were recovered from the 30–32% and 36–40% region of sucrose gradients, respectively, containing rabbit skeletal muscle membranes obtained by differential pelleting at 105,000 *g*. Heavy SR (HSR) fractions were recovered from the 36–45% region of sucrose gradients that contained membranes sedimenting at 2,600–35,000 *g*.

Isotope Flux Measurements

$^{45}Ca^{2+}$ efflux from vesicles passively loaded with $^{45}Ca^{2+}$ was measured as described in Meissner et al. (12) using a Chemical Quench apparatus (1000; Update Instrument, Inc., Madison, WI) and by filtration system. Briefly, vesicles were incubated for 2 h at 22°C in a medium containing 20 mM K PIPES, pH 7.4, 0.1 M KCl, 0.1 mM EGTA, 5.1 mM $^{45}Ca^{2+}$. $^{45}Ca^{2+}$ efflux behavior of the vesicles was assessed by diluting vesicles into isoosmolal unlabeled media followed by filtration. Rapid $^{45}Ca^{2+}$ release was measured with a Chemical Quench apparatus (Update Instrument, Inc.), fitted with three syringes and two acrylic mixing chambers. One 0.5-ml syringe was filled with the vesicle suspension and two 2-ml syringes were filled with two isoosmolal mixing solutions to initiate and inhibit $^{45}Ca^{2+}$ release. One 2-ml syringe contained release media with varying concentrations of EGTA, Ca^{2+}, nucleotide, Mg^{2+}, and ruthenium red. Three examples are: (*a*) 6.25 mM EGTA plus 4.85 mM Ca^{2+}; (*b*) 6.25 mM EGTA plus 4.85 mM Ca^{2+} and 1.25 mM AMP-PCP; and (*c*) 6.25 mM EGTA plus 4.85 mM Ca^{2+}, 1.25 mM AMP-PCP, and 1.87 mM Mg^{2+}; so that after mixing the vesicles were present in a medium containing 4 μM free Ca^{2+} (5 mM EGTA plus 4.88 mM Ca^{2+}), 4 μM free Ca^{2+} plus 1 mM AMP-PCP or 4 μM free Ca^{2+}, 1 mM AMP-PCP, and 1.5 mM Mg^{2+}. $^{45}Ca^{2+}$ efflux was stopped by adding, in a second mixing step, a quench solution containing the two Ca^{2+} release inhibitors Mg^{2+} and ruthenium red, at concentrations of 22 mM and 22 μM, respectively. Reaction times were determined by changing the length of the tubing (aging hose) between the two mixing chambers. Untrapped as well as released $^{45}Ca^{2+}$ was separated from intravesicular $^{45}Ca^{2+}$ by rapidly rinsing the vesicles on a 0.45 μM filter (Millipore Corp., Bedford, MA).

$^{45}Ca^{2+}$ remaining inside the vesicles was determined by liquid scintillation counting.

Planar Bilayer Methods

SR vesicles were fused into Mueller-Rudin planar lipid bilayers containing 50:50 phosphatidylethanolamine and phosphatidylserine (50 mg/ml lipid in decane) according to the procedure given in Smith et al. (19). Briefly, 3 μg/ml vesicle protein was added to the *cis* chamber containing 250 mM choline chloride, 5 mM $CaCl_2$, 10 mM Tris/HEPES, pH 7.4. Fusion was monitored as discrete conductance increases that resulted in a Cl^- specific macroscopic current. Perfusion of both chambers was performed with 100 μM Ca EGTA (2 μM free Ca^{2+}), 125 mM Tris (base)/250 mM HEPES, pH 7.4, *cis* and 53 mM Ca or $Ba(OH)_2$/250 mM HEPES, pH 7.4 *trans*.

Bilayer currents were measured with a low noise operational amplifier (LF-157; National Semiconductor, Santa Clara, CA) and 10,000 MΩ feedback resistor as described by Smith et al. (20). Amplified bilayer currents were output to an FM tape recorder or filtered at 300 Hz (−3 dB points from a low-pass eight-pole Bessel filter) and digitized at 1 kHz for storage on hard disk.

RESULTS

$^{45}Ca^{2+}$ Release from SR Fractions

Fig. 1 (*top*) shows a thin-section electron micrograph of a heavy density SR (HSR) preparation used in our vesicle flux and bilayer experiments. A large proportion of the vesicles contain heavily stained intravesicular material, which is thought to be the low affinity calcium binding protein, calsequestrin. Many vesicles also show a thickening of the membrane marked by knob-like protuberances. Junctional feet structures similar to these have been observed in other SR preparations purported to be derived from the junctional terminal cisternae region of the SR (18). The vesicles were found to range in size from 1,000 to 3,000 Å with an average vesicle diameter of 2,800 Å. These heavy vesicle fractions were used for the incorporation of calcium-activated and nucleotide-activated channels because a large percentage of the vesicles contain one or more calcium release channels (see Fig. 2). Fig. 1 (*bottom*) is of an intermediate SR fraction, which was determined, from isotope flux experiments as in Fig. 2, to possess only half as much calcium-induced and nucleotide-induced calcium release activity as seen in the HSR release fractions. The vesicles in this fraction were on the average much smaller than those shown above. The average vesicle diameter here was found to be 1,500 Å. This fraction does, however, contain a small population of large vesicles similar to those seen in the HSR fractions.

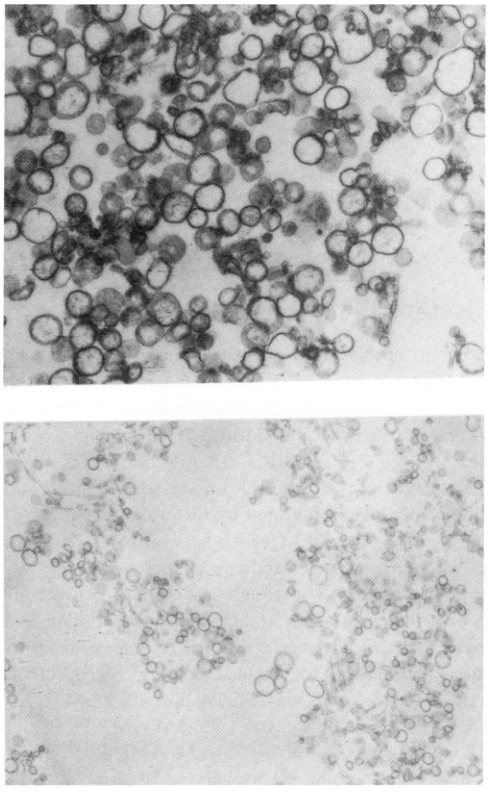

FIGURE 1 Electron micrograph of HSR and ISR vesicle preparations used in planar bilayer and isotope flux measurements. Samples were fixed with 2.5% glutaraldehyde and 0.5% tannic acid in 0.3 M sucrose, pH 7.2, for 15 min on ice and 45 min at 23°C, followed by sedimentation. Pellets were postfixed with 1% OsO_4, embedded, sectioned, and stained as previously described in reference 10. (*Top*) Heavy density SR, magnification × 10,000. (*Bottom*) Intermediate density SR vesicles, magnification × 10,000.

Fig. 2 illustrates the differences seen when the two vesicle fractions are assayed for calcium release activity according to the rapid quench protocol described in Methods. Fig. 2 *A* shows the time course of calcium release from heavy and intermediate SR fractions in the presence of the Ca^{2+} release channel inhibitors Mg^{2+} and ruthenium red as well as an activating concentration of Ca^{2+} (4 μM). The time course in the presence of micromolar Ca^{2+} is biphasic with both rapid and slow components. This biphasic behavior has been interpreted previously to represent two subpopulations of vesicles; a permeable fraction containing the calcium-gated calcium release channel and an impermeable fraction devoid of the channel (13). The heavy fraction in Fig. 2 *A* releases ~90% of its passively loaded calcium within a few seconds, whereas the intermediate fraction loses only 50% of its $^{45}Ca^{2+}$ within the same period. This result suggests that the rapidly permeable vesicle population in the heavy fraction is nearly twice as large as in the intermediate fraction. Light fractions isolated from low density (30–32%) regions of sucrose gradients exhibit little if any calcium-induced and nucleotide-induced calcium releasing activity and appear to contain only a small (5–10%) population of vesicles rapidly permeable to Ca^{2+} (13). Fig. 2 *B* shows the time course of calcium release from the rapidly permeable vesicle populations in both heavy and intermediate SR fractions. The kinetics for calcium- and calcium-plus-nucleotide–induced calcium release in each fraction are virtually identical, which suggests that both contain the same calcium release channel. A summary of the effects of Ca^{2+}, Mg^{2+}, adenine nucleotide, and ruthenium red on the calcium-releasing behavior of SR Ca^{2+} release vesicles is shown in Table I. There are three important aspects of passive $^{45}Ca^{2+}$ efflux

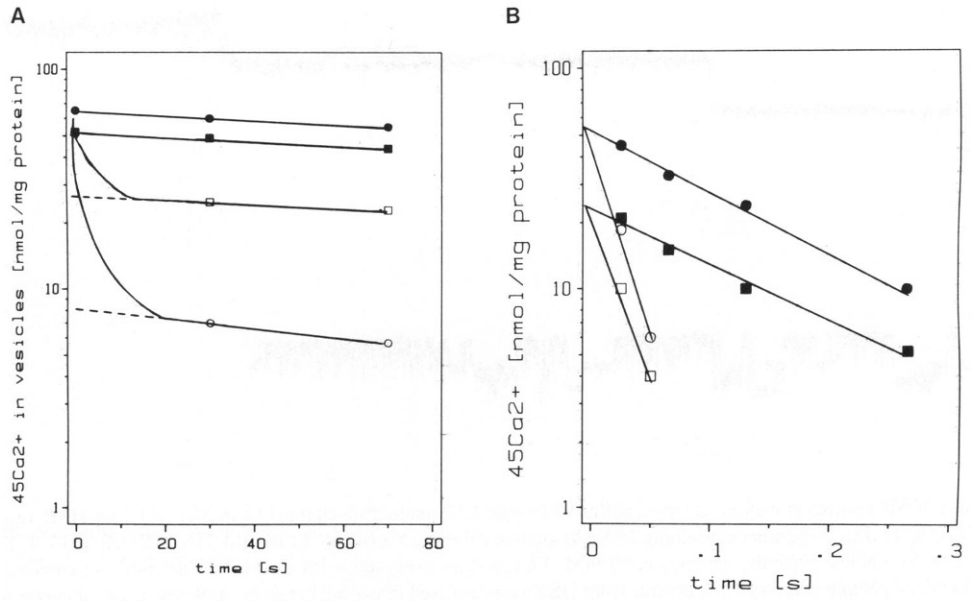

FIGURE 2 $^{45}Ca^{2+}$ efflux from passively loaded ISR and HSR vesicles. (*A*) Ca^{2+} induced $^{45}Ca^{2+}$ efflux from ISR (■, □) and HSR (•, ○). SR vesicles passively loaded with 5 mM $^{45}CaCl_2$ were diluted into media containing 10 mM $MgCl_2$ and 10 μM ruthenium red (■, •) or 4 μM free Ca^{2+} (○, □). The fraction of vesicles not rapidly permeable to Ca^{2+} was found by back extrapolation to the ordinate (*dashed lines*). (*B*) Time course of Ca^{2+}- and nucleotide-induced $^{45}Ca^{2+}$ efflux from the rapidly permeable fraction in ISR (■, □) and HSR (•, ○). Symbols indicate $^{45}Ca^{2+}$ efflux measured in the presence of 4 μM free Ca^{2+} (■, •) or 4 μM Ca^{2+} plus 1 mM AMP-PCP (□, ○).

that we have focused on in our companion bilayer studies. (*a*) Ca^{2+} release from SR seems to predominate in the terminal cisternae derived (heavy) SR fractions; (*b*) $^{45}Ca^{2+}$ efflux from passively loaded vesicles is optimal in the presence of micromolar external Ca^{2+} and millimolar adenine nucleotide; and (*c*) Mg^{2+} and ruthenium red added to the dilution buffer inhibit Ca^{2+}- and nucleotide-induced $^{45}Ca^{2+}$ efflux.

Planar Bilayer Experiments

One of the major problems encountered when trying to observe calcium channels from SR in planar bilayers has been the presence of other ion channels in the SR membrane. The SR membrane is lavishly endowed with a full complement of ion conducting pathways among which K^+ and Cl^- specific channels predominate (14). In the bilayer experiments whole SR vesicles are incorporated into the bilayer so all of the ion channels present in the native membrane are also present in the artificial bilayer. Our approach has been to remove all of the ions permeant in the contaminating channels and to leave only those permeant in the SR calcium channels. In our system we fused SR vesicles into planar bilayers formed in asymmetric choline chloride buffer. Fusion of SR vesicles in chloride containing buffers may be observed as discrete current steps that give rise to a chloride specific conductance (Fig. 3 *A*). Occasionally, Cl^--specific single-channel currents may be measured in the bilayers (Fig. 3 *B*). These occur as multiples of unitary conductance states of ~60 and 95 pS in our choline chloride buffer. The SR chloride channels can be partially inhibited by the stilbene derivatives SITS (4-acetamido-4′-isothio-cyanatostilbene-2,2′-disulfonic acid) and DIDS; (4,4′,diisothiocyanato-stilbene-2,2′-disulfonic acid) however, even when present at several millimolar, these compounds do not seem to produce a complete channel blockade (not shown). We have incorporated chloride channels from light, intermediate, and heavy density SR fractions and they seem to be distributed fairly evenly throughout the SR membrane. To separate Ba^{2+} or Ca^{2+} currents from the Cl^- specific conductance, we perfused away the Cl^--containing buffers and replaced them with HEPES/Tris or Cs (*cis*) and HEPES/Ba or Ca (*trans*). The large organic anion HEPES does not pass

TABLE I
Ca^{2+} RELEASE PROPERTIES OF Ca^{2+} RELEASE VESICLES

Additions to release medium				$^{45}Ca^{2+}$ Efflux
Free Ca^{2+}	Nucleotide	Mg^{2+}	Ruthenium red	
M	*M*	*M*	*M*	$k_1(s^{-1})$
$\sim 10^{-8}$	—	—	—	0.4
$\sim 10^{-8}$	10^{-3}	—	—	19
4×10^{-6}	—	—	—	7
4×10^{-6}	10^{-3}	—	—	58
4×10^{-6}	10^{-3}	1.5×10^{-3}	—	12
4×10^{-6}	10^{-3}	2.5×10^{-3}	—	1
4×10^{-6}	10^{-3}	—	10^{-6}	0.03
4×10^{-6}	—	5×10^{-3}	10^{-5}	0.004

$^{45}Ca^{2+}$ release was assayed according to reference 12 and the protocol given in Methods. Free Ca^{2+} was determined using the binding constants given in reference 5.

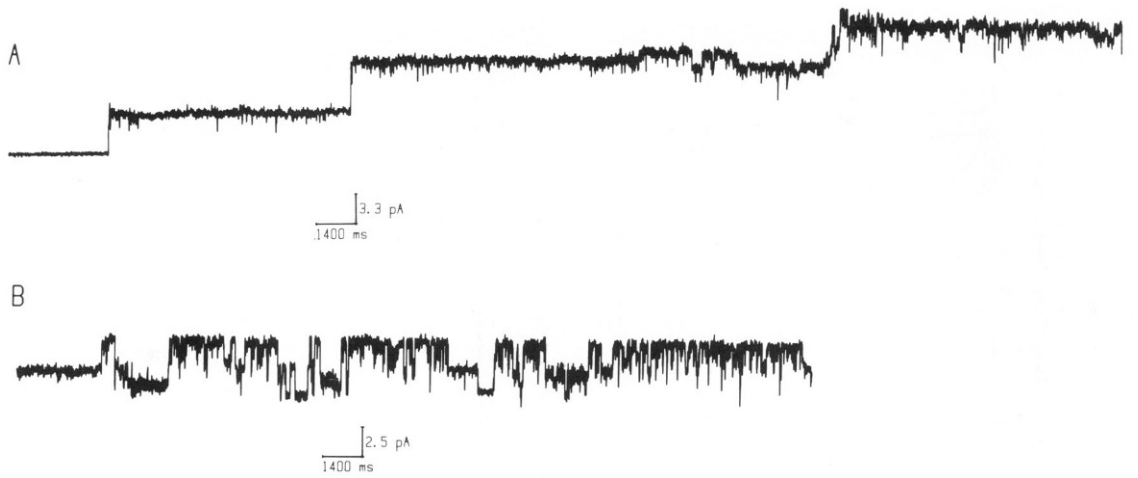

FIGURE 3 Fusion of SR vesicles in choline chloride buffer and single Cl^- conducting channel from SR. (*A*) 1 μg HSR vesicle protein/ml was added with stirring to the *cis* chamber containing 250 mM choline chloride, 5 mM $CaCl_2$, 10 mM Tris/HEPES, pH 7.4. The *trans* buffer was identical except for choline chloride, which was 50 mM. Fusion steps averaged 6 pA at 0 mV. The resulting current had a reversal potential at +25 mV. (*B*) Single-channel Cl^- currents from HSR vesicles fused into a bilayer as in *A*. Single-channel currents at 0 mV were 1.66 pA and 2.33 pA.

through the SR Cl^- channel (14), and the SR K^+ channel has been found to be generally impermeable to $Tris^+$ and Cs^+ (2).

Fusion of light- and intermediate-density SR fractions was tried first in the bilayer experiments because these fractions contain less Ca^{2+}- and nucleotide-induced Ca^{2+} releasing activity and it was thought that if fewer calcium release channels were present it might make recording of single calcium channels easier. In Fig. 4 *A* (*top*), ISR vesicles were fused into the bilayer followed by perfusion with 100 μM Ca EGTA, 125 mM Tris/250 mM HEPES, pH 7.4, *cis* and 53 mM Ca/250 mM HEPES, pH 7.4 *trans*. Note that there is only baseline current with no obvious open channel fluctuations. When 1 mM of the nonhydrolyzable ATP analog AMP-PCP was added to the *cis* chamber with stirring, as in the second trace, a macroscopic current (5 pA at 0 mV) was induced. This current had a reversal potential of +50 mV, which indicates the current was carried by the *trans* Ca^{2+}. The magnitude of this macroscopic conductance varied from one experiment to another and seemed dependent on the number of fusions observed in the Cl^--containing fusion buffer. If, instead, heavy density (HSR) vesicles were incorporated into the bilayer, after perfusion we most often saw large single channel currents as in Fig. 4 *B* (*top*). The unitary conductance of these transitions was 100 pS in 53 mM Ca^{2+}. In this situation if nucleotide was added in the lower trace, the channels began to burst continuously and single events became difficult to distinguish. In experiments where high conductance channels were not seen after incorporation of HSR vesicles, we found that currents identical to that described in Fig. 4 *A* could be induced by added nucleotide and sometimes by rapid stirring alone. After careful examination of our experiments at heavier filtering (20 Hz) and increased gain (0.1 V/pA), we found that this

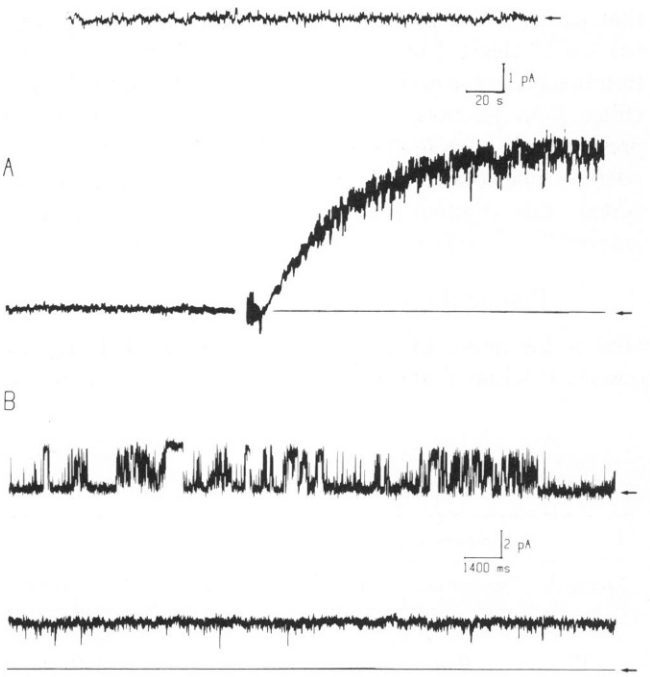

FIGURE 4 Recordings of calcium channels from ISR and HSR. (*A*) ISR vesicles were incorporated into bilayers as described in Methods. Upper trace is bilayer current after perfusion with 100 μM Ca EGTA (2 μM free Ca^{2+}), 125 mM Tris(base)/250 mM HEPES, pH 7.4, *cis* and 53 mM $Ca(OH)_2$/250 mM HEPES, pH 7.4, *trans*. Lower trace shows current increase upon addition of 1 mM AMP-PCP. Solid line indicates baseline current level before added nucleotide. Filter is 20 Hz. (*B*) *Upper* trace shows single channel fluctuations after incorporation of HSR vesicles and perfusion as in *A*. *Lower* trace is the result of 1 mM AMP-PCP added to the *cis* chamber. Solid line indicates baseline current level. Filter is 300 Hz.

smaller calcium current could be measured in many instances from all of the SR fractions tested. For the remainder of our experiments in which we study the smaller calcium conducting channel, we used ISR vesicles because the frequency of incorporating high conductance Ca^{2+} channels is low in these fractions.

We have resolved single-channel Ba^{2+} currents mediated by ion channels incorporated from ISR and have determined the single-channel conductance and a permeability ratio for Ba^{2+} vs. $Tris^{+}$. Single-channel records in Fig. 5 were obtained several minutes after the addition of 1 mM nucleotide *cis*. AMP-PCP in this instance appeared to activate only one small unit conductance barium conducting channel, whereas in Fig. 4 *A* as many as 10 small channels may have been in the bilayer. The resulting current voltage relation had a slope of 10 pS in 53 mM Ba^{2+}. A 5-pS substate may also be seen in some of the current traces; however, the 10-pS state is much more common. In the presence of 1 mM ATP and 2 μM Ca^{2+} the open time was 65–80%. Increasing *cis* nucleotide above 1 mM had little or no effect on the open time of the 10-pS channel. Ruthenium red also had no effect on *cis* or *trans* at concentrations up to 1 mM. Using an expression derived from constant field theory which considers both divalent and monovalent ion permeability (9), the current reversal at +50 mV gave a permeability ratio of 35 for $Ba^{2+}/Tris^{+}$.

Fig. 6 *A* demonstrates the effect of nanomolar *cis* Ca^{2+} and micromolar ruthenium red on nucleotide activated calcium current from the heavy SR Ca^{2+} channel. The top trace is a recording of a single channel in the presence of 1

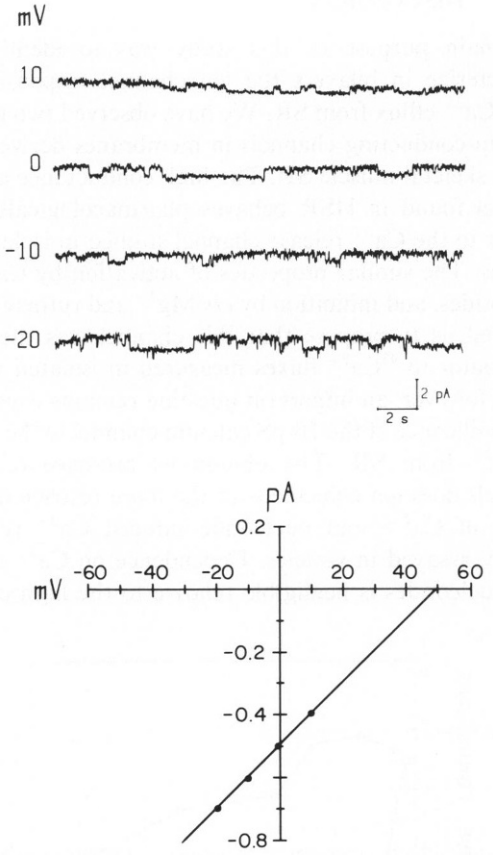

FIGURE 5 Single-channel current voltage relationship for Ba^{2+} conducting channels from ISR. Single-channel fluctuations were recorded at 20 Hz filter in 1 mM AMP-PCP, 100 μM Ca EGTA (2 μM free Ca^{2+}), 125 mM Tris(base)/250 mM HEPES, pH 7.4, *cis*, and 53 mM $Ba(OH)_2$/250 mM HEPES, pH 7.4, *trans*. Unitary currents were: 0.40 pA at +10 mV, 0.49 pA at 0 mV, 0.62 pA at −10 mV, and 0.70 pA at −20 mV. The extrapolated equilibrium reversal potential at +50 mV gave a permeability ratio of $Ba^{2+}/Tris^{+}$ = 35 using the expression: $P_{Ba}/P_{Tris} = [Tris]/4[Ba]\,[(1 + e^{-FV/RT})/e^{FV/RT}]$.

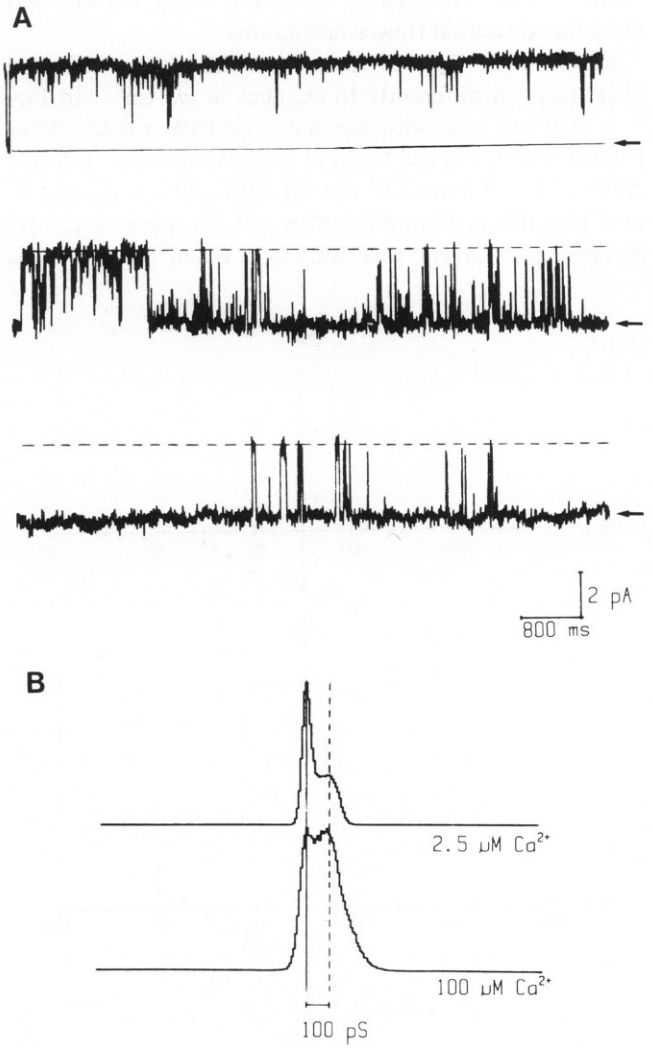

FIGURE 6 Ca^{2+} dependence and ruthenium red inhibition of the HSR calcium channel. (*A*) (*Top*) Nucleotide-activated single channel Ca^{2+} currents from HSR calcium channels in the presence of 1 mM ATP and 2 μM *cis* Ca^{2+} (P_o >0.99). (*Middle*) Currents after 4 mM EGTA *cis* (1 nM free Ca^{2+} in *cis* chamber) (P_o = 0.18). (*Bottom*) Single-channel currents after 1 μM ruthenium red *cis* (P_o = 0.01) (*B*) (*Top*) Current histogram made from single-channel records taken in the presence of 2 μM *cis* Ca^{2+}. (*Bottom*) Current histogram after increase of free *cis* Ca^{2+} to 100 μM. Solid line indicates baseline current. Dashed line represents open channel current (100 pS).

mM ATP and 2 μM Ca^{2+} *cis*. Open probability (P_o) in this trace was >0.99. When Ca^{2+} in the *cis* bath was lowered to 1 nM by the addition of 4 mM EGTA (Fig. 6, *middle*), P_o was reduced from 0.95 to 0.18. One micromolar ruthenium red was added in the lower trace, resulting in a further inhibition of P_o. The remaining single channel currents were infrequent and of brief duration. Increasing *cis* Ca^{2+} from 2.5 to 100 μM in the absence of nucleotide greatly stimulates heavy SR calcium channel activity. In Fig. 6 *B*, a current histogram was made from recordings of heavy SR calcium channels in the presence of 2 μM *cis* Ca^{2+}. A low shoulder to the right of the baseline current peak is due to open channel current and represents ~25% of total current. When *cis* Ca^{2+} was increased to 100 μM, total current noise was greatly increased. Open current was potentiated twofold (lower histogram).

Macroscopic Ba^{2+} currents from channels in ISR and HSR respond differently to changes in *cis* Ca^{2+}. In Fig. 7 *A*, channels were incorporated from ISR vesicles. After perfusion with 100 μM Ca EGTA (2 μM free Ca^{2+}), 1 mM ATP, 125 mM Tris/250 mM HEPES, pH 7.4, *cis* and 53 mM $Ba(OH)_2$/250 mM HEPES, pH 7.4, *trans*, a 125-pS macroscopic current was obtained. When *cis* Ca^{2+} was reduced to 1 nM by the addition of 4 mM EGTA, no reduction in current was observed. A considerably larger Ba^{2+} current was obtained in Fig. 7 *B*, when HSR vesicles were fused into a bilayer under similar conditions as in Fig. 7 *A*. In this case, when *cis* Ca^{2+} was lowered to 1 nM, the current was substantially reduced. This decrease in macroscopic current was due to an increase in the amount of time the channels spent in the closed state (see Fig. 6). The current remaining was still Ba^{2+}- specific as judged by no change in the reversal potential.

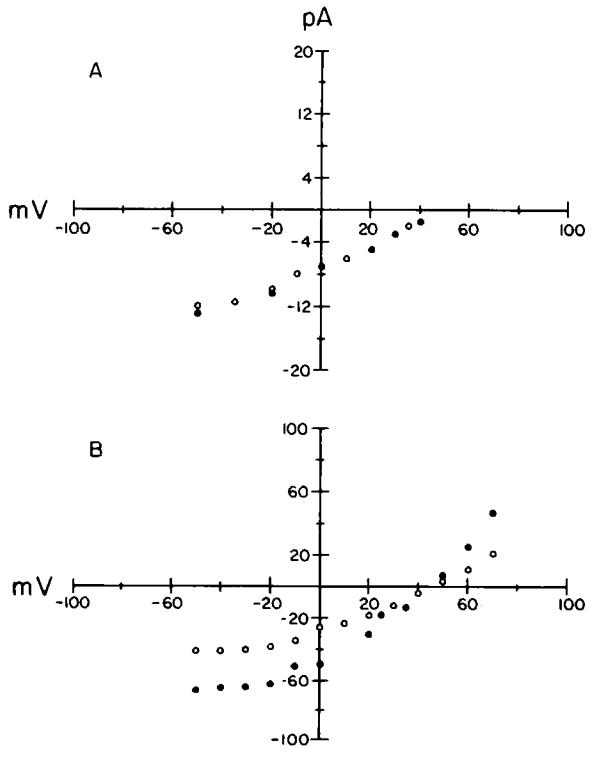

FIGURE 7 Macroscopic current voltage behavior for Ba^{2+} conducting channels from ISR and HSR. (*A*) Macroscopic Ba^{2+} current from ISR calcium channels in 100 μM Ca EGTA, 1 mM ATP, 125 mM Tris(base)/250 mM HEPES, pH 7.4, *cis* and 53 mM $Ba(OH)_2$/250 mM HEPES, pH 7.4, *trans* with (○) or without (●) added 4 mM EGTA *cis*. (*B*) Macroscopic Ba^{2+} current from HSR channels in 100 μM Ca EGTA, 1 mM ATP, 80 mM Cs(OH)/250 mM HEPES, pH 7.4, *cis* and 53 mM $Ba(OH)_2$/250 mM HEPES, pH 7.4, *trans* with (○) or without (●) added 4 mM EGTA *cis*.

Cis Mg^{2+} inhibits Ca^{2+}- and nucleotide-induced $^{45}Ca^{2+}$ efflux (Table I). In contrast, Mg^{2+} has very little effect on macroscopic currents mediated by the 10-pS calcium channel. In Fig. 8, macroscopic Ba^{2+} current due to calcium- and nucleotide-activated channels incorporated from HSR and ISR is plotted vs. total Mg^{2+} added to the *cis* buffer. The Ba^{2+} current from HSR channels was half-maximally inhibited at 1.5 mM free Mg^{2+}, whereas the current obtained from ISR channels was not inhibited at all below 10 mM free Mg^{2+}.

DISCUSSION

The main purpose of this study was to identify and characterize in bilayers the ion channel responsible for rapid Ca^{2+} efflux from SR. We have observed two types of calcium-conducting channels in membranes derived from rabbit skeletal muscle SR. The high conductance calcium channel found in HSR behaves pharmacologically most similar to the Ca^{2+} release channel studied in isolated SR vesicles. The similar properties of activation by Ca^{2+} and nucleotides, and inhibition by *cis* Mg^{2+} and ruthenium red have led us to propose that this channel was the major contributor to $^{45}Ca^{2+}$ fluxes measured in isolated vesicles (19). However, an important question remains concerning the significance of the 10-pS calcium channel to the release of Ca^{2+} from SR. The ubiquitous presence of 10-pS channels does not coincide with the more restricted distribution of Ca^{2+}- and nucleotide-induced Ca^{2+} releasing activity assayed in vesicles. Dependence on Ca^{2+} or adenine nucleotides is negligible relative to the high conduc-

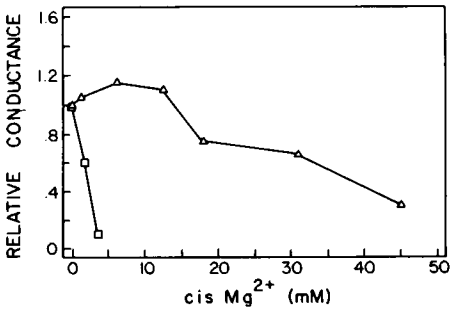

FIGURE 8 Effect of Mg^{2+} on macroscopic Ba^{2+} currents from ISR and HSR. Ba^{2+} current in the presence of 2 μM *cis* Ca^{2+} and 1 mM ATP is plotted vs. total Mg^{2+} in the *cis* chamber (Mg^{2+} was added as the hemi-gluconate salt). (□) Indicates current derived from HSR channels and (△) is current from ISR.

tance channel or $^{45}Ca^{2+}$ release from vesicles. In addition, the lack of inhibition by Mg^{2+} and ruthenium red strongly suggests that the 10-pS channel is not involved in the Ca^{2+} and nucleotide-induced Ca^{2+} release process. However, it is entirely plausible that the 10-pS channel is regulated in some way other than by Ca^{2+}, Mg^{2+}, and adenine nucleotide. Several authors have suggested that protein phosphorylation (21), inositol-1,4,5-*tris*-phosphate (22, 23), or depolarization (3, 7, 15) may be involved in SR channel regulation. Preliminary data in the form of an abstract reported the presence of 5-pS Ca^{2+} and Ba^{2+} conducting channels in fragmented SR (17). The channels that were recorded in bilayers formed on patch pipettes were spontaneously active in symmetrical 200 mM $CaCl_2$ at potentials between +50 mV and +200 mV (inside pipette). The 10-pS channels described in this paper show no voltage dependence for activity similar to that reported in reference 17.

Calcium release in muscle is thought to occur in a matter of a few milliseconds and in isolated SR vesicles $^{45}Ca^{2+}$ release rates with half times for efflux approaching 5 ms have been measured (8, 12, 16). In light of this knowledge, it would be useful to have an estimate of the number of 10-pS channels or 100-pS channels necessary to accommodate such efflux rates. One approach to this question is to use an expression derived from constant field theory, which relates single-channel conductance to a time constant for ion flux from a vesicle (6).

$$\tau = 4\pi z^2F^2cr^3/3RTN\gamma p$$

In using this expression we assume that our SR vesicles are homogenous and that each vesicle contains N channels of γ conductance at calcium concentration c with a probability p of being open. To estimate the single-channel conductance at a more physiological Ca^{2+}, we must assume that the two channels display saturable conductance according to the equation: $g = g_{max}/(1 + K_m/c)$. This is not unreasonable since Ca^{2+}- and nucleotide-induced $^{45}Ca^{2+}$ efflux from passively loaded vesicles was shown to be saturable with a K_m for intravesicular Ca^{2+} equal to 20 mM (13). At 5 mM Ca^{2+} we estimate the single-channel conductances to be 2.8 and 28 pS for the 10-pS and 100-pS channels, respectively. Probability of opening in the presence of optimal nucleotide and Ca^{2+} is taken to be 1 for the 100-pS channel and 0.80 for the 10-pS channel. Table II compares the estimated time constants for the two calcium channels and the experimentally determined τ for $^{45}Ca^{2+}$ efflux from passively loaded vesicles as given in Table I.

Thus according to theoretical treatment, the presence of three 100-pS channels per vesicle should be sufficient to achieve flux rates comparable to those measured in vesicles. This is a reasonable number considering from the bilayer experiments the estimate is 2–3 per vesicle. The smaller channel would have to be present at >10 times the estimated density (~30 channels per vesicle) to permit efflux on a similar time scale. Although this treatment is valid only in the case of independent ionic movements, we feel it is useful in providing a rough estimate of the unit conductance and number of ion channels per vesicle necessary to permit ion flux in a reasonable period of time.

TABLE II
COMPARISON OF SINGLE-CHANNEL CONDUCTANCE AND $^{45}Ca^{2+}$ FLUX

	γ (at 5 mM Ca^{2+})	τ (for one channel)
	pS	*s*
10-pS channel	2.8	0.42
100-pS channel	28	0.033
Ca^{2+} release vesicles		0.012

Taus were calculated using the expression $\tau = 4\pi z^2F^2cr^3/3RNT\gamma p$ where c is the divalent cation concentration, r is the radius, N is the number of channels per vesicle, γ is the estimated single channel conductance at c, and p is the probability of finding the channel open. Average vesicle radius was assumed to be $r = 1.4 \times 10^{-5}$ cm. Probability of opening in the presence of optimal nucleotide and Ca^{2+} is taken to be 1 for the 100-pS channel and 0.80 for the 10-pS channel. (z^2, F^2, R, and T have the usual meanings).

At this point, we find it difficult to assign a role for the 10-pS channel in Ca^{2+}- and adenine nucleotide-induced Ca^{2+} release from SR. The channel in artificial bilayers does not fit the characteristics determined for Ca^{2+} release in isolated vesicles. There is no evidence that suggests that this channel is active in isolated SR fractions. If this channel did happen to be active in SR vesicles, then due to its lack of inhibition by Mg^{2+} and ruthenium red, the vesicles would not retain $^{45}Ca^{2+}$ during the course of an isotope flux assay (Fig. 2). Single-channel currents from the 10-pS channel appear kinetically very similar to the Cl^- selective channels, which are also distributed throughout the SR. One possibility might be that Cl^--selective channels conduct divalent cations in the absence of sufficiently permeable anions. This situation would be similar to the VDAC (voltage-dependent anion-selective channel) from mitochondrial membranes, which shows only a twofold selectivity for Cl^- over K^+ (personal communication from M. Colombini, cited in reference 1). Regardless of its true nature, we must conclude from our pharmacological evidence and our theoretical treatment, that the 10-pS calcium conducting channel does not appear to be important to Ca^{2+} release from SR.

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