SINGLE CHANNEL AND $^{45}Ca^{2+}$ FLUX MEASUREMENTS OF THE CARDIAC SARCOPLASMIC RETICULUM CALCIUM CHANNEL

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ABSTRACT Purified canine cardiac sarcoplasmic reticulum vesicles were passively loaded with $^{45}CaCl_2$ and assayed for Ca^{2+} releasing activity according to a rapid quench protocol. Ca^{2+} release from a subpopulation of vesicles was found to be activated by micromolar Ca^{2+} and millimolar adenine nucleotides, and inhibited by millimolar Mg^{2+} and micromolar ruthenium red. $^{45}Ca^{2+}$ release in the presence of 10 μM free Ca^{2+} gave a half-time for efflux of 20 ms. Addition of 5 mM ATP to 10 μM free Ca^{2+} increased efflux twofold ($t1/2$ = 10 ms). A high-conductance calcium-conducting channel was incorporated into planar lipid bilayers from the purified cardiac sarcoplasmic reticulum fractions. The channel displayed a unitary conductance of 75 ± 3 pS in 53 mM *trans* Ca^{2+} and was selective for Ca^{2+} vs. $Tris^+$ by a ratio of 8.74. The channel was dependent on *cis* Ca^{2+} for activity and was also stimulated by millimolar ATP. Micromolar ruthenium red and millimolar Mg^{2+} were inhibitory, and reduced open probability in single-channel recordings. These studies suggest that cardiac sarcoplasmic reticulum contains a high-conductance Ca^{2+} channel that releases Ca^{2+} with rates significant to excitation-contraction coupling.

INTRODUCTION

Calcium efflux from the sarcoplasmic reticulum (SR) in cardiac and skeletal muscle is necessary for activating the myofilaments during excitation-contraction coupling. This calcium efflux is thought to occur via calcium-release channels located in the junctional SR membrane (4, 6, 8, 17). In skeletal muscle, a calcium and adenine nucleotide stimulated calcium channel was found to be active in skinned fibers (4, 5), isolated SR vesicles (11, 13, 14, 16), and planar bilayers (18). The channel was optimally stimulated by micromolar Ca^{2+} and millimolar adenine nucleotides (18) to an extent where half-times for $^{45}Ca^{2+}$ efflux for vesicles approached 5 ms (10, 13, 16). Calcium and nucleotide-stimulated calcium flux through the skeletal muscle calcium release channels was also distinguished by sensitivity to inhibition with Mg^{2+} and ruthenium red (10, 11, 13, 14, 15, 18). The presence of a similar release mechanism in cardiac muscle has been well documented in skinned fiber studies (6, 7), however, comparable data from isolated cardiac vesicles or planar bilayers are essentially lacking. There have been a few reports of a calcium and ruthenium red-sensitive Ca^{2+} release system in isolated cardiac SR vesicles, (2, 3, 17) however, the release rates reported were several orders of magnitude slower than what is expected to occur in intact muscle.

Here we report on the observation of a calcium and adenine nucleotide-activated calcium release channel present in a subpopulation of purified cardiac SR vesicles. $^{45}Ca^{2+}$ release rates from passively loaded vesicles are more than 100-fold greater than any previously reported and are of the magnitude to be significant to excitation-contraction coupling in vivo. A high-conductance calcium channel similar to that described previously in skeletal muscle SR (18, 19) was incorporated into planar lipid bilayers. The channel in bilayers is stimulated by *cis* calcium and adenine nucleotides, and inhibited by ruthenium red and Mg^{2+}.

MATERIALS AND METHODS

Reagents

$^{45}Ca^{2+}$ was obtained from ICN Pharmaceuticals (Irvine, CA), ATP and the ATP analog AMP-PCP from Sigma Chemical Co. (St. Louis, MO), and ruthenium red (95%) from Fluka Chemical Corp. (Hauppauge, NY). Phospholipids were purchased from Avanti Biochemicals (Birmingham, AL). All other chemicals were of reagent grade. HEPES buffer solutions were prepared as in references 18 and 19, using glass-distilled water.

Preparation of Membranes

Canine cardiac muscle sarcoplasmic reticulum vesicles were prepared by differential- and sucrose-gradient centrifugation as described (12). Cardiac SR Ca^{2+}-release vesicle fractions were recovered from the 28–40%

region of sucrose gradients that contained membranes sedimenting at 10,000–100,000 *g*. Heavy rabbit skeletal muscle SR Ca^{2+}-release vesicles were prepared as previously described (13).

$^{45}Ca^{2+}$ Flux Measurements

$^{45}Ca^{2+}$ efflux from vesicles passively loaded with $^{45}Ca^{2+}$ was measured using an Update System 1000 Chemical Quench apparatus (Update Instrument Inc., Madison, WI) and by Millipore filtration (11, 13). Briefly, vesicles (2–10 mg protein/ml) were incubated for 60 min at 22°C in a medium containing 30 mM K PIPES, pH 7.0, 100 mM KCl, 1 mM diisopropyl fluorophosphate, a protease inhibitor, 0.1 mM EGTA, and 1.1 mM $^{45}Ca^{2+}$. $^{45}Ca^{2+}$ efflux was initiated by diluting vesicles into isoosmolal, unlabeled release media containing varying concentrations of free Ca^{2+}, adenine nucleotide, and Mg^{2+}. Untrapped as well as released $^{45}Ca^{2+}$ was separated away by placing the vesicles on 0.45 μ HAWP Millipore filters, followed by rapid rinsing to remove extravesicular $^{45}Ca^{2+}$. $^{45}Ca^{2+}$ radioactivity retained by the vesicles on the filters was determined by liquid scintillation counting.

Planar Bilayer Measurements

Cardiac SR vesicles were fused into Mueller-Rudin planar lipid bilayers containing phosphatidylethanolamine (bovine brain), phosphatidylserine (bovine brain), and dioleoyl-phosphatidylcholine (synthetic), 50:30:20 (50 mg/ml phospholipid in decane) according to the procedure given in references 18 and 19. Briefly, 10 μg/ml vesicle protein was added to the *cis* chamber containing 250 mM choline Cl, 5 mM $CaCl_2$, 10 mM Tris/HEPES, pH 7.4. Fusion was monitored as steplike conductance increases that resulted in a Cl^- specific macroscopic current. After fusion, the *cis* chamber was perfused with 100 μM CaEGTA (2.5 μM free Ca^{2+}), 125 mM Tris(base)/250 mM HEPES, pH 7.4; followed by *trans* perfusion with 53 mM $Ca(OH)_2$/250 mM HEPES, pH 7.4.

Bilayer currents were measured with amplifier circuits as in reference 19 and filtered at 4 kHz (−3 dB point from an 8-pole Bessel low-pass filter) for storage on videotape using a modified digital audio processor and videotape recorder (Unitrade Inc., Philadelphia, PA) as described by Bezanilla (1).

RESULTS

Fig. 1 shows that cardiac SR vesicle preparations contain a Ca^{2+}-release channel that is activated by 10 μM extravesicular Ca^{2+}. SR vesicles were passively loaded with 1 mM $^{45}Ca^{2+}$ and then either diluted into a medium that inhibited or activated the release of the intravesicular $^{45}Ca^{2+}$ stores. $^{45}Ca^{2+}$ efflux was slow in a medium that contained 10 mM Mg^{2+} and 10 μM ruthenium red. About 22 nmol $^{45}Ca^{2+}$/mg protein were retained by the vesicles in the Ca^{2+} release inhibiting medium. Of this amount about half was released in <30 s in a 10-μM free Ca^{2+}-release medium. Fig. 1 (*inset*) shows the time course of $^{45}Ca^{2+}$ efflux from the Ca^{2+} permeable vesicle population. At 10 μM external Ca^{2+}, calcium permeable vesicles released half their $^{45}Ca^{2+}$ stores within 15–30 ms. A biphasic behavior consisting of a rapid and slow release component has indicated the presence of two subpopulations of vesicles (11), a permeable fraction containing a Ca^{2+}-release channel, and an impermeable fraction lacking the channel.

The rate of $^{45}Ca^{2+}$ efflux from the Ca^{2+} permeable vesicle population was dependent on extravesicular Ca^{2+} concentration and on the presence or absence of Mg^{2+} and ATP (AMP-PCP) (Table I). At 10^{-9} M Ca^{2+}, Ca^{2+} release was slow, requiring a half-time of ~30 s. Increase in

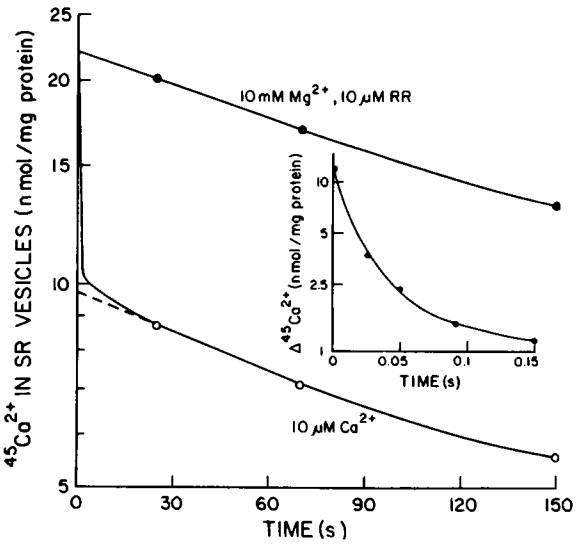

FIGURE 1 Measurement of $^{45}Ca^{2+}$ release rates. A canine cardiac SR Ca^{2+} release vesicle preparation (10 mg protein/ml) was incubated for 1 h at 22°C with 1 mM $^{45}Ca^{2+}$ in a medium containing 30 mM K PIPES, pH 7, and 0.1 M KCl. $^{45}Ca^{2+}$ efflux was initiated by diluting vesicles 100-fold into release media containing either 10 mM Mg^{2+}, 1 mM EGTA, and 10 μM ruthenium red (RR) (*solid circle*)) or 1 mM EGTA plus 0.96 mM Ca^{2+} (10 μM free Ca^{2+} after the addition of the vesicles) (*open circle*). $^{45}Ca^{2+}$ efflux was terminated by placing vesicles on 0.45 μ Millipore filters, followed by rapid rinsing with release medium to remove extravesicular $^{45}Ca^{2+}$. Amounts of $^{45}Ca^{2+}$ trapped by all intact vesicles in the incubation medium (22 nmol/mg protein) as well as amounts not readily released in the 10 μM Ca^{2+} release medium (10 nmol/mg protein) were obtained by back-extrapolation to the time of vesicle dilution. In the inset, an Update System 1000 Chemical Quench apparatus was used to determine the initial $^{45}Ca^{2+}$ efflux rate in the 10 μM Ca^{2+} release medium (13). Vesicles (2 mg protein/ml) passively loaded with 1 mM $^{45}Ca^{2+}$ were rapidly mixed with four volumes of release medium containing 0.5 mM EGTA and 0.24 mM Ca^{2+} (10 μM free Ca^{2+} after the addition of the vesicles). Rapid $^{45}Ca^{2+}$ efflux was inhibited at the indicated times by the addition of four additional volumes of a quench solution containing 22.5 mM Mg^{2+}, 11.25 mM EGTA, and 45 μM ruthenium red. Vesicles were subsequently placed on 0.45 μ Millipore filters and rinsed with a medium containing 10 mM Mg^{2+}, 0.1 mM EGTA, and 20 μM ruthenium red.

free Ca^{2+} to 10^{-5} increased the release rate by a factor of ~1,000 ($t^1/_2$ ~0.02 s). ATP or the nonhydrolyzable ATP analog AMP-PCP had an activating effect, increasing the release rates by a factor of ~2 at 10^{-9} and 10^{-5} M Ca^{2+}. $^{45}Ca^{2+}$ efflux was partially inhibited by the addition of 3 mM Mg^{2+} to the 10^{-5} M Ca^{2+} release medium or by increasing the extravesicular Ca^{2+} concentration to 10^{-3} M.

Heavy rabbit skeletal muscle SR vesicles resemble cardiac SR vesicles in that they contain a Ca^{2+} release channel that is activated by Ca^{2+} and ATP (AMP-PCP), and inhibited by Mg^{2+} (Table 1). In the presence of 10^{-5} M Ca^{2+} and 5 mM AMP-PCP, both membrane fractions released half of their $^{45}Ca^{2+}$ stores within ~10 ms. Several important differences were, however, noted to exist between the two membrane fractions. At 10^{-5} M external Ca^{2+}, the half-time of $^{45}Ca^{2+}$ release was 20 ms for cardiac vesicles, as compared to 600 ms for skeletal muscle. In

TABLE I
Ca^{2+} RELEASE PROPERTIES OF CANINE CARDIAC AND RABBIT SKELETAL MUSCLE Ca^{2+} RELEASE SR VESICLES

Additions to release medium	$^{45}Ca^{2+}$ efflux	
	Cardiac SR	Skeletal SR
	$t_{1/2}$s	$t_{1/2}$s
10^{-9} M Ca^{2+}	30	7
10^{-9} M Ca^{2+}, 5 mM ATP	17	0.06
10^{-5} M Ca^{2+}	0.02	0.6
10^{-5} M Ca^{2+}, 5 mM AMP-PCP	0.01	0.01
10^{-5} M Ca^{2+}, 3 mM Mg^{2+}	0.6	70
10^{-3} M Ca^{2+}	0.1	20

$^{45}Ca^{2+}$ efflux rates from Ca^{2+}-permeable vesicle fractions were determined as described in Fig. 1. $^{45}Ca^{2+}$ release in the presence of ATP at 10^{-9} M Ca^{2+} was determined by initially lowering the free Ca^{2+} concentration to 2×10^{-9} M Ca^{2+} before adding the nucleotide medium in a second mixing step. At 10^{-5} M Ca^{2+}, the nonhydrolyzable ATP analog AMP-PCP was used to avoid reuptake of the released $^{45}Ca^{2+}$ by the Ca^{2+} pump. Vesicles released half their $^{45}Ca^{2+}$ stores within the indicated times.

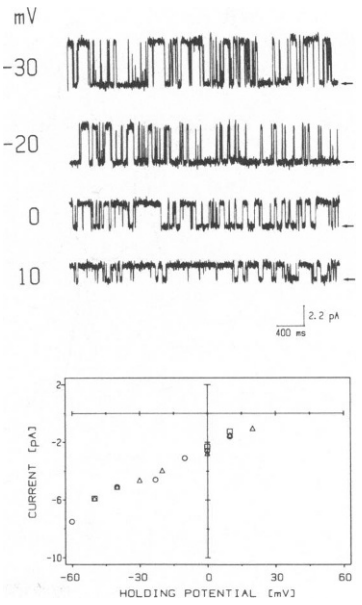

FIGURE 2 Current voltage behavior of single cardiac SR calcium release channels. (*Upper*) Single channel currents, shown as upward deflections, were recorded in 53 mM $Ca(OH)_2$/250 mM HEPES, pH 7.4 *trans* and 125 mM Tris(base)/250 mM HEPES, pH 7.4, and 2.5 μM free Ca^{2+} (100 μM EGTA and 100 μM Ca^{2+}) *cis*. (*Lower*) Unitary currents of cardiac calcium release channels recorded as a function of holding potential in the presence of (*open triangle*) 2.5 μM free Ca^{2+} *cis*, (*open circle*) 0.5 μM free Ca^{2+} *cis* (1.25 mM EGTA and 1.1 mM Ca^{2+}), or (*open square*) 2.5 μM free Ca^{2+} and 1 mM ATP *cis*. Slope conductance from linear regression was determined to be 75 ± 3 pS. The equilibrium reversal potential at +30 mV gave a permeability ratio $Ca^{2+}/Tris^+$ = 8.74 using the expression P_{Ca}/P_{Tris} = [Tris]/4[Ca] $[(1 + e^{-FV/RT})/e^{FV/RT}]$. Filter = 300 Hz. Sampling = 1 kHz.

contrast, adenine nucleotides were more effective in stimulating $^{45}Ca^{2+}$ release from skeletal than cardiac vesicles. Another significant difference was that $^{45}Ca^{2+}$ efflux from cardiac SR vesicles was only partially inhibited in media containing 10^{-3} M Ca^{2+} or 10^{-5} M Ca^{2+} plus 3×10^{-3} M Mg^{2+}, whereas the Ca^{2+} release channel of skeletal SR was essentially fully inhibited by millimolar Ca^{2+} or Mg^{2+}.

Fig. 2 shows single-channel recordings of cardiac calcium-release channels recorded in 100 μM Ca EGTA (2.5 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*. The cardiac calcium-release channels are very active in the presence of micromolar *cis* calcium. Single-channel fluctuations appear in bursts of activity composed of both long and short lived events. Some open events may be as long as several seconds, especially at more positive potentials, where P_0 is greater. At negative holding potentials open events become more brief in duration with inactivation occurring after prolonged application of negative voltages. A single-channel current-voltage relation was constructed from single-channel fluctuations recorded in the above *cis* buffer (2.5 μM free Ca^{2+}), 0.5 μM free Ca^{2+} *cis*, or with 1 mM ATP and 2.5 μM free Ca^{2+}. The slope conductance was found to be 75 ± 3 pS with 53 mM Ca^{2+} as the *trans* current carrier. A reversal potential of +30 mV gave a permeability ratio $Ca^{2+}/Tris^+$ = 8.74.

In Fig. 3 *A* a single cardiac calcium-release channel was recorded in 53 mM $Ca(OH)_2$/250 mM HEPES, pH 7.4 *trans* and 125 mM Tris(base)/250 mM HEPES, pH 7.4 *cis*, plus 1 mM Ca^{2+} *cis*, 0.5 μM free Ca^{2+} *cis* or 0.5 μM free Ca^{2+} plus 1 mM ATP *cis*. In the presence of 1 mM Ca^{2+} (*top*), the channel was found to be continuously bursting, such that open probability (P_0) was near unity. When free Ca^{2+} *cis* was reduced to 0.5 μM by the addition of 1.1 mM EGTA, P_0 was reduced to 0.25 (*middle*). In this situation the fluctuations were grouped into clusters or appeared as single events of variable duration. 1 mM ATP was added to the *cis* chamber (*bottom*) resulting in a restoration of continuous bursting activity as in the top recording.

The effects of Ca^{2+}, inositol-1,4,5-triphosphate (IP_3) and ATP on single channel recordings of cardiac calcium release channels are illustrated in Fig. 3 *B*. In the top trace, a single channel was recorded in the presence of 2.5 μM free Ca^{2+} *cis*. P_0 in this instance was 0.95. Free Ca^{2+} was lowered to 18 nM by the addition of 4 mM EGTA to the *cis* chamber (*middle*), resulting in a P_0 of 0.005. Open events at 18 nM *cis* Ca^{2+} were few and of brief duration. Inositol-1,4,5-trisphosphate (IP_3) was added to the *cis* chamber in the presence of 18 nM *cis* Ca^{2+} to test its effectiveness as an activator of the cardiac calcium release channel. IP_3 at concentrations as high as 125 μM had no effect on single-channel conductance or P_0 in single-channel recordings (not shown). When 5 mM ATP was added *cis* (*bottom*) P_0 was also not affected. ATP does not seem to be a good activator of the cardiac calcium release channel at nanomolar free Ca^{2+}. In the lowermost trace, channel activity was restored by the addition of 10 μM free Ca^{2+} to the *cis* chamber. Therefore, there appears to be a

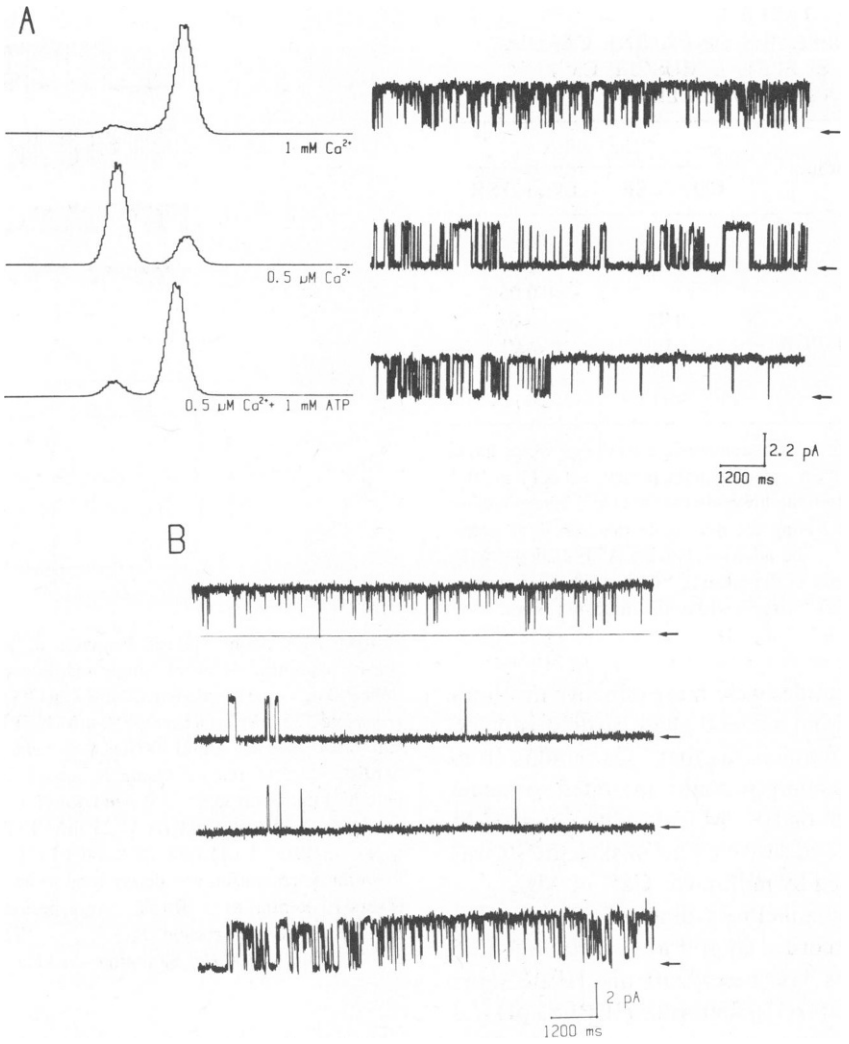

FIGURE 3 Effect of Ca^{2+}, inositol-1,4,5-triphosphate and adenine nucleotide on single-channel recordings of cardiac calcium release channels. (*A*) A single cardiac calcium channel was recorded at 0 mV in 53 mM $Ca(OH)_2$/250 mM HEPES, pH 7.4 *trans* and 125 mM Tris(base)/250 mM HEPES, pH 7.4 *cis* plus 1 mM free Ca^{2+} (100 μM EGTA and 1.1 mM Ca^{2+}) (*top*), 0.5 μM free Ca^{2+} (1.25 mM EGTA and 1.1 mM Ca^{2+}) (*middle*), or 0.5 μM free Ca^{2+} plus 1 mM ATP (*bottom*). Current histograms to the left of each record indicate the relative contribution of open and closed states. Open probability in each record was 0.98 (*top*), 0.25 (*middle*), and 0.90 (*bottom*). (*B*) Cardiac calcium-release channels were recorded as in *A* except with 2.5 μM free *cis* Ca^{2+} (*top*), 18 nM free *cis* Ca^{2+} (1.1 mM EGTA and 100 μM Ca^{2+}) (*second trace*), 18 nM free *cis* Ca^{2+}, 125 μM inositol-1,4,5-trisphosphate and 5 mM ATP (*third trace*), or 10 μM free Ca^{2+} (1.1 mM EGTA and 1.1 mM Ca^{2+}) plus 5 mM ATP (*bottom*). P_0 in each trace was determined from data stored in 50-s files and was from top to bottom 0.95, 0.005, 0.005, and 0.98. Filter = 300 Hz. Sampling = 1 kHz.

strict requirement for Ca^{2+}, which is necessary to activate the cardiac calcium-release channel.

Cardiac calcium-release channels are inhibited by micromolar *cis* ruthenium red. Fig. 4 illustrates the effect of ruthenium red on single channel fluctuations recorded in the presence of 2.5 μM free Ca^{2+} plus 0.375 mM ATP *cis*. The current histograms to the left of each single-channel recording represent the relative contributions of the open- and closed-channel current levels before 1 μM ruthenium red and at 12-s intervals after addition of ruthenium red to the *cis* chamber. The open-channel current level is diminished with time until, at 36 s after ruthenium red addition, open current has been reduced by fourfold. The single-channel traces show the same effect in a more informative manner. Before ruthenium red, P_0 in the presence of 2.5 μM free Ca^{2+} plus 0.375 mM ATP was 0.99. After ruthenium red, P_0 was decreased in a time-dependent manner, due to more frequent channel closings. Inhibition by 1 μM ruthenium red was not complete, however, as evidenced by a constant $P_0 = 0.25$ for several minutes after ruthenium red addition. At 10 μM ruthenium red (*lowermost trace*), P_0 was reduced further to 0.02. Events became extremely brief and were separated by long periods of quiescence.

Mg^{2+} is an effective inhibitor of Ca^{2+} flux through the calcium-release channel in isolated cardiac and skeletal SR vesicles (Table I). Mg^{2+} likewise exerts an inhibitory effect on single-channel fluctuations from cardiac calcium-

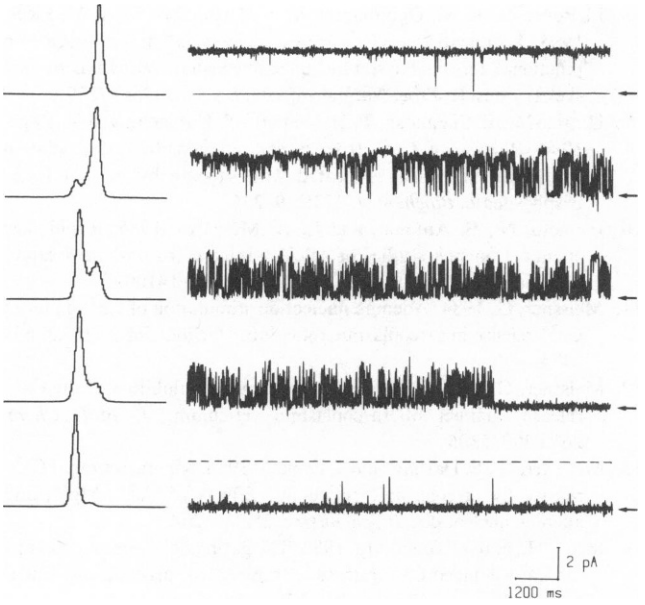

FIGURE 4 Ruthenium red inhibition of cardiac calcium release channels. Channels were recorded in 53 mM $Ca(OH)_2$/250 mM HEPES, pH 7.4 *trans* and 2.5 μM free Ca^{2+} (100 μM CaEGTA), 0.375 mM ATP, 125 mM Tris(base)/250 mM HEPES, pH 7.4 *cis*. Current histograms to the left of each record indicate the relative contributions of open and closed current levels to the total current profile. Single-channel records from top to bottom were taken at 0 mV before 1 μM ruthenium red, 0–12 s, 12–24 s, and 24–36 s after 1 μM ruthenium red, and 38–50 s after 10 μM ruthenium red *cis*. P_0 from top to bottom was 0.99, 0.85, 0.34, 0.25, and 0.02. Filter = 300 Hz. Sampling = 1 kHz.

release channels in bilayers (Fig. 5). The presence of multiple current levels after *cis-trans* perfusion (not shown) indicated that at least 3 channels had been incorporated into the bilayer. Free *cis* Ca^{2+} was decreased from 2.5 to 0.25 μM by the addition of 25 μM EGTA to the *cis* chamber to reduce channel open-time to a point where only

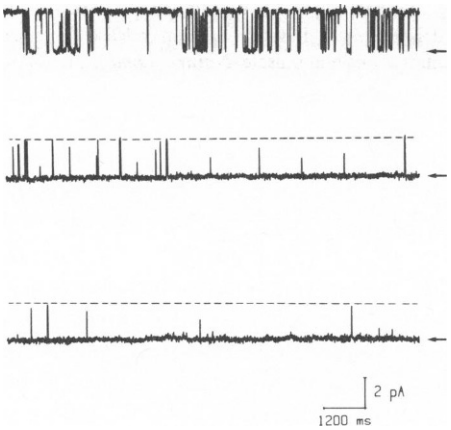

FIGURE 5 Mg^{2+} inhibition of single channel currents from cardiac calcium release channels. Channels were recorded as in Fig. 3 except free *cis* Ca^{2+} was 0.25 μM (125 μM EGTA, 100 μM Ca^{2+}). P_0 at 0.25 μM Ca^{2+} was 0.66 (*top*), 1 mM Mg^{2+} (added to *cis* chamber as the gluconate salt) lowered P_0 to 0.02 (*middle*). 10 mM Mg^{2+} (*bottom*) gave P_0 = 0.004. Filter = 300 Hz. Sampling = 1 kHz.

one current level was detectable (Fig. 5, *top*). The resulting single channel record had a P_0 of 0.66 before the addition of Mg^{2+}. When *cis* Mg^{2+} was increased to 1 mM the P_0 was reduced to 0.02 with no apparent effect on the single-channel conductance. Single-channel events at 1 mM *cis* Mg^{2+} were short in duration, and separated by long closed intervals. >90% of the open events could be fit with a time constant τ = 12 ms. 10 mM *cis* Mg^{2+} lowered P_0 to 0.004 again with no significant effect on single-channel conductance.

DISCUSSION

The calcium-release channels from canine cardiac SR and rabbit skeletal SR appear to be regulated in a similar manner. Both channels are activated by Ca^{2+} and adenine nucleotides, and can be inhibited by Mg^{2+} and ruthenium red. They differ, however, in their sensitivity to activation or inhibition by these various ligands. The canine cardiac channel is more strictly regulated by Ca^{2+}. Ca^{2+} seems to be an essential requirement for opening the cardiac channel, whereas the skeletal channel may be partially activated by ATP at nanomolar Ca^{2+}. In Table I, the half-time for $^{45}Ca^{2+}$ efflux from skeletal SR vesicles is decreased more than 100-fold when 5 mM ATP is added to a release medium containing nanomolar free Ca^{2+}. In contrast, the cardiac preparation shows only a twofold decrease in the half-time under the same release conditions.

In the bilayer, the cardiac channel shows no significant activation by ATP when *cis* free Ca^{2+} is in the nanomolar range (Fig. 3). In contrast, the skeletal muscle calcium-release channel may be activated by ATP alone to an extent similar to that obtained with high (1 mM) *cis* Ca^{2+} (19). In the presence of 2.5 μM free *cis* Ca^{2+}, the cardiac channel in bilayers routinely exhibits single channel P_0 near 0.9. By comparison, single channel P_0 = 0.9 is rare for the skeletal calcium-release channel unless both ATP and micromolar Ca^{2+} are present in the *cis* chamber. With both activators present, the skeletal channel routinely displays single-channel P_0 near unity (18). The cardiac channel also exhibits this fully activated single-channel behavior in the presence of ATP and Ca^{2+}, however, P_0 near unity may also be recorded in the presence of micromolar *cis* Ca^{2+} alone.

The cardiac calcium-release channel is less sensitive to inhibition by Mg^{2+} and ruthenium red. Ca^{2+}-induced $^{45}Ca^{2+}$ efflux from cardiac SR vesicles is only partially inhibited by 3 mM Mg^{2+}, while in skeletal vesicles complete inhibition is observed. In planar bilayers in the presence of 0.25 μM free Ca^{2+}, 1 mM *cis* Mg^{2+} decreases single-channel P_0 from ~0.65 to 0.02 for the cardiac calcium channel. The corresponding data for the skeletal channel is not yet available. 1 μM ruthenium red reduces single-channel activity of the cardiac channel by about fourfold (Fig. 4), while the skeletal channel in bilayers is nearly completely inhibited at 1 μM ruthenium red (18).

The strict regulation of the cardiac calcium release

channel by *cis* (cytoplasmic) Ca^{2+} is in accord with other reports, mostly from skinned fibers (6, 7), which have also suggested an important role for Ca^{2+}-induced Ca^{2+} release in cardiac muscle. In addition to Ca^{2+}, inositol-1,4,5-trisphosphate has been implicated as a chemical messenger in excitation-contraction coupling in skeletal and cardiac muscle (9, 20, 21). As suggested by one study with permeabilized cardiac myocytes and isolated SR (15), we were unable to detect any effect of inositol-1,4,5-trisphosphate on single channel activity. This finding does not, however, rule out the possibility that an effect of IP_3 may require some intact structure that is lost during vesicle preparation or bilayer reconstitution.

This research was supported by U.S. Public Health Service grants HL 27430 and AM 18687.

Received for publication 6 February 1986 and in final form 20 May 1986.

REFERENCES

1. Bezanilla, F. 1985. A high capacity data recording device based on a digital audio processor and a video cassette recorder. *Biophys. J.* 47:437–441.
2. Chamberlain, B. K., P. Volpe, and S. Fleischer. 1984. Calcium-induced calcium release from purified cardiac sarcoplasmic reticulum vesicles. *J. Biol. Chem.* 259:7540–7546.
3. Chamberlain, B. K., P. Volpe, and S. Fleischer. 1984. Inhibition of calcium-induced calcium release from purified cardiac sarcoplasmic reticulum vesicles. *J. Biol. Chem.* 259:7547–7553.
4. Endo, M. 1977. Calcium release from the sarcoplasmic reticulum. *Physiol. Rev.* 57:71–108.
5. Endo, M., and T. Kitazawa. 1976. The effect of ATP on calcium release mechanisms in the sarcoplasmic reticulum of skinned muscle fibers. *Proc. Jpn Acad.* 52:595–606.
6. Fabiato, A. 1983. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. *Am. J. Physiol.* 245 (Cell Physiol. 14) :C1–C14.
7. Fabiato, A. 1981. Myoplasmic free calcium concentration reached during the twitch of an intact isolated cardiac cell and during calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned cardiac cell from the adult rat or rabbit ventricle. *J. Gen. Physiol.* 78:457–497.
8. Fleischer, S., E. M. Ogunbunmi, M. C. Dixon, and E. A. M. Fleer. 1985. Localization of Ca^{2+} release channels with ryanodine in junctional terminal cisternae of sarcoplasmic reticulum of fast skeletal muscle. *Proc. Natl. Acad. Sci. USA* 82:7256–7259.
9. Hirata, M., E. Suematsu, T. Hashimoto, T. Hamachi, and T. Koga. 1984. Release of Ca^{2+} from a non-mitochondrial store site in peritoneal macrophages treated with saponin by inositol 1,4,5-trisphosphate. *Biochem. J.* 223:229–236.
10. Ikemoto, N., B. Antoniu, and L. G. Mészáros. 1985. Rapid flow chemical quench studies of calcium release from isolated sarcoplasmic reticulum. *J. Biol. Chem.* 260:14096–14100.
11. Meissner, G. 1984. Adenine nucleotide stimulation of Ca^{2+}-induced Ca^{2+} release in sarcoplasmic reticulum. *J. Biol. Chem.* 259:2365–2374.
12. Meissner, G. 1986. Ryanodine activation and inhibition of the Ca^{2+} release channel of sarcoplasmic reticulum. *J. Biol. Chem.* 261:6300–6306
13. Meissner, G., E. Darling, and J. Eveleth. 1986. Kinetics of rapid Ca^{2+} release by sarcoplasmic reticulum. Effects of Ca^{2+}, Mg^{2+}, and adenine nucleotides. *Biochemistry.* 25:236–244.
14. Morii, H., and Y. Tonomura. 1983. The gating behavior of a channel for Ca^{2+}-induced Ca^{2+} release in fragmented sarcoplasmic reticulum. *J. Biochem. (Tokyo).* 93:1271–1285.
15. Movsesian, M. A., A. P. Thomas, M. Selak, and J. R. Williamson. 1985. Inositol trisphosphate does not release Ca^{2+} from permeabilized cardiac myocytes and sarcoplasmic reticulum. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 185:328–332.
16. Nagasaki, K., and M. Kasai. 1983. Fast release of calcium from sarcoplasmic reticulum vesicles monitored by chlortetracycline fluorescence. *J. Biochem. (Tokyo).* 94:1101–1108.
17. Seiler, S., A. D. Wegener, D. D. Whang, D. R. Hathaway, and L. R. Jones. 1984. High molecular weight proteins in cardiac and skeletal muscle junctional sarcoplasmic reticulum vesicles bind calmodulin, are phosphorylated and are degraded by Ca^{2+}-activated protease. *J. Biol. Chem.* 259:8550–8557.
18. Smith, J. S., R. Coronado, and G. Meissner. 1985. Sarcoplasmic reticulum contains adenine nucleotide-activated calcium channels. *Nature (Lond.).* 316:446–449.
19. Smith, J. S., R. Coronado, and G. Meissner. 1986. Techniques for observing calcium channels from skeletal muscle sarcoplasmic reticulum in planar lipid bilayers. *Methods Enzymol.* In press.
20. Vergara, J., R. Y. Tsien, and M. Delay. 1985. Inositol 1,4,5-trisphophate: a possible chemical link in excitation-contraction coupling in muscle. *Proc. Natl. Acad. Sci. USA* 82:6352–6356.
21. Volpe, P., G. Salviati, F. Di Virgilio, and T. Pozzan. 1985. Inositol 1,4,5-trisphosphate induces calcium release from sarcoplasmic reticulum of skeletal muscle. *Nature (Lond.).* 316:347–349.