Contribution of a voltage-sensitive calcium release mechanism to contraction in cardiac ventricular myocytes

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Howlett, Susan E., Jie-Quan Zhu, and Gregory R. Ferrier. Contribution of a voltage-sensitive calcium release mechanism to contraction in cardiac ventricular myocytes. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H155–H170, 1998.—The contribution of a voltage-sensitive release mechanism (VSRM) for sarcoplasmic reticulum (SR) Ca2+ to contraction was investigated in voltage-damped ventricular myocytes at 37°C. Na+ current was blocked with lidocaine. The VSRM exhibited steady-state inactivation (half-inactivation voltage: −47.6 mV; slope factor: 4.37 mV). When the VSRM was inactivated, contraction-voltage relationships were proportional to L-type Ca2+ current (ICa,L). When the VSRM was available, the relationship was sigmoidal, with contractions independent of voltage positive to −20 mV. VSRM and ICa,L contractions could be separated by activation-inactivation properties. VSRM contractions were extremely sensitive to ryanodine, thapsigargin, and conditioning protocols to reduce SR Ca2+ load. ICa,L contractions were less sensitive. When both VSRM and ICa,L were available, sigmoidal contraction-voltage relationships became bell-shaped with protocols to reduce SR Ca2+ load. Myocytes demonstrated restitution of contraction that was slower than restitution of ICa,L. Restitution was a property of the VSRM. Thus activation and recovery of the VSRM are important in coupling cardiac contraction to membrane potential, SR Ca2+ load, and activation interval.

excitation-contraction coupling; calcium current; cardiac muscle; sarcoplasmic reticulum; ryanodine

IT IS WELL ESTABLISHED that contraction in heart is initiated by an increase in free intracellular Ca2+. This increase in activator Ca2+ is derived from two sources, Ca2+ influx and Ca2+ release from intracellular stores. It is generally believed that the main routes for Ca2+ entry are voltage-gated Ca2+ channels and reverse-mode Na+/Ca2+ exchange, whereas the main source for Ca2+ release is the sarcoplasmic reticulum (SR) (3, 15, 19). Fabiato (11) has shown that a rapid rise in intracellular free Ca2+ can cause Ca2+ release from cardiac SR. This phenomenon has been called Ca2+-induced Ca2+ release (CICR) (11).

Identification of the trigger or triggers for SR Ca2+ release continues to be a subject of intense research. Evidence has been presented for at least three mechanisms that trigger CICR. These are 1) Ca2+ influx via L-type Ca2+ channels (4, 7, 22), 2) elevation of intracellular Ca2+ via reverse Na+/Ca2+ exchange in response to a rapid rise in intracellular Na+ during the upstroke of the action potential (15, 19), and 3) elevation of intracellular Ca2+ via reverse Na+/Ca2+ exchange in response to depolarization, although this last mechanism may depend on intracellular Na+ concentration and temperature (6, 16, 17, 29, 30).

Recently we reported a study of excitation-contraction (EC) coupling in guinea pig ventricular myocytes at 37°C that used high-resistance microelectrodes to prevent intracellular dialysis (13). Under these conditions, the threshold for activation of contractions is much more negative than the threshold for activation of L-type Ca2+ current (ICa,L). In addition, the magnitude of contraction is not proportional to the magnitude of inward current, and contractions remain maximal at membrane potentials near or beyond the reversal potential for ICa,L (13). In that study we were able to separate a new component of contraction that was abolished by a low concentration of ryanodine, but not by L-type Ca2+ channel blockers or Na+ channel blockers, from contraction initiated by ICa,L (13). The new component of contraction exhibits a sigmoidal contraction-voltage relationship in contrast to the bell-shaped relationship observed for contractions initiated by ICa,L. It also is unlikely that this component of contraction is initiated by reverse Na+/Ca2+ exchange, because its voltage dependence is not affected by large changes in concentrations of extracellular Na+ or Ca2+ (12, 32). Because activation of this new component of contraction clearly is dependent on membrane potential but is not proportional to macroscopic transmembrane current, we have called this component a voltage-sensitive release mechanism (VSRM) for SR Ca2+ (13). The present study examines the contribution of this voltage-activated Ca2+ release mechanism to cardiac EC coupling.

Many previous studies have reported bell-shaped contraction-voltage relationships in which the amplitudes of contractions or Ca2+ transients were proportional to the magnitude of ICa,L (2, 3, 4, 9, 10, 20). However, many of these earlier studies of cardiac EC coupling in isolated myocytes have been conducted with conditions different from those in our study, including use of holding potentials near −40 mV (2, 3, 9, 10, 20). The absence of the VSRM in those studies might be explained if the VSRM has steady-state inactivation properties and is inactivated at −40 mV. Therefore, one of the goals of this study was to determine whether the VSRM exhibits steady-state inactivation and to determine the voltage range over which inactivation occurs.

Contractions initiated by the VSRM are abolished by 30 nM ryanodine, an agent that disrupts EC coupling at the level of SR release of Ca2+ (13). Thus SR Ca2+ stores are likely essential for initiation of contraction by the VSRM. Repetitive activation of ICa,L serves to load or maintain SR stores of Ca2+ (3), and altering the voltage of repetitive depolarizations can be used to manipulate SR Ca2+ load and the amount of releasable Ca2+ (14). Therefore, a second goal of the present study...
was to determine and compare the effects of protocols designed to alter SR Ca\textsuperscript{2+} load on the components of contraction initiated by the VSRM or by I_{Ca-L}. SR Ca\textsuperscript{2+} loading and recovery of SR Ca\textsuperscript{2+} release also are believed to be important components in restitution of contractility, which plays a major role in adjusting the magnitude of contraction in response to changes in the interval between contractions (3, 5). Therefore, the final goal of this study was to evaluate the role of the VSRM in restitution.

METHODS

Cell isolation. All experiments were performed in accordance with the guidelines published by the Canadian Council on Animal Care, and this investigation was approved by the Dalhousie University Committee on Animal Care. Most experiments were conducted on isolated guinea pig ventricular myocytes. Male guinea pigs (350–400 g, Charles River) were injected with heparin (3.3 IU/g) 30 min before anesthesia with pentobarbital sodium (80 mg/kg). The chest was opened, and the heart was rapidly cannulated in situ and immediately perfused retrogradically through the aorta (10–12 ml/min) with oxygenated (100% O\textsubscript{2}, 36°C) Ca\textsuperscript{2+}-free solution of the following composition (in mM): 120 NaCl, 3.8 KCl, 1.2 KH\textsubscript{2}PO\textsubscript{4}, 1.2 MgSO\textsubscript{4}, 10 Na-2-hydroxyethylpiperazine-N\textsuperscript{-2-ethanesulfonic acid (HEPES), and 11 glucose (pH 7.4 with NaOH). The heart was then removed from the chest during perfusion with Ca\textsuperscript{2+}-free solution for 7–8 min. Collagenase A (35–40 mg, Boehringer Mannheim) and protease (4.8–6 mg, Sigma type XIV; Sigma, St. Louis, MO) were then included in 65 ml of this Ca\textsuperscript{2+}-free solution, and the heart was perfused for an additional 5–8 min. After enzymatic dissociation, the ventricles were minced and washed in a substrate-enriched solution of the following composition (in mM): 80 KOH, 50 glutamic acid, 30 KCl, 30 KH\textsubscript{2}PO\textsubscript{4}, 20 taurine, 10 HEPES, 10 glucose, 3 MgSO\textsubscript{4}, and 0.5 ethylene glycol-bis-(β-aminoethyl ether)-N,N,N\textsuperscript{-2}-tetraacetic acid (pH 7.4 with KOH). In some experiments, rat ventricular myocytes were utilized. The isolation procedure was similar to that for guinea pig myocytes except that dissociation was accomplished with collagenase (20 mg, Worthington type 2) and trypsin (2 mg, Sigma). After 1–2 h of incubation at room temperature, myocytes were placed in a modified culture dish (approximate volume = 0.75 ml) in an open-perfusion microincubator (model PDMI-2, Medical Systems) on the stage of an inverted microscope. Cells were allowed to adhere to the bottom of the chamber for 15–20 min and were then superfused at 37°C with the HEPES-buffered solution described above, supplemented with 2.0 mM Ca\textsuperscript{2+}. After 10–15 min, cells were superfused with an oxygenated (100% O\textsubscript{2}, 37°C) solution of the following composition (in mM): 100 choline chloride, 45 NaCl, 10 glucose, 10 HEPES, 4 KCl, 2.0 CaCl\textsubscript{2}, 1.0 MgCl\textsubscript{2}, and 200 µM lidocaine (pH 7.4 with NaOH). Solutions were pumped through the chamber from a buffer reservoir at a rate of 3 ml/min and were changed by switching the inlet to the pump between buffer reservoirs. The time required to replace the bath solution was determined by measuring the membrane potential response to a step change in K\textsuperscript{+} concentration. Transit time to the bath was ~1 min and changeover time was ~90 s.

Experimental methods. Discontinuous single-electrode voltage-clamp recordings (sample rate 10–14 kHz) were made with an Axodamp 2A amplifier (Axon Instruments, Foster City, CA). Recordings were made with high-resistance microelectrodes (18–25 MΩ, filled with 2.7 M KCl) to reduce cell dialysis. A 2.7 M KCl-agar bridge was used as a bath ground to minimize liquid junction potential changes. Voltage-clamp protocols were generated with pCLAMP software (Axon Instruments); pCLAMP software also was used to acquire and analyze data on computer. Recordings were only made from rod-shaped myocytes with clear, well-organized striations and with resting potentials more negative than –65 mV. In all experiments, both current and transmembrane voltage were recorded. During discontinuous single-electrode voltage clamp, we continuously monitored the output of the switching circuit to ensure that adequate settling time for accurate voltage measurement was maintained.

Inward and delayed rectifier potassium currents were not blocked in this study because the effects of blocking agents on the VSRM are not known. Therefore, changes in steady currents at the ends of the activation steps represent steady-state current-voltage (I-V) relations. After trains of conditioning pulses, the membrane potential was usually repolarized to a postconditioning potential (V_{PC}) more positive than the holding potential. Thus, background currents during the V_{PC} period also reflect steady-state I-V relations.

I_{Ca-L} was measured as the difference between the peak inward current and a reference point at the end of the voltage step (normally 250 ms). Figure 1 demonstrates that current measured in this way is abolished by 2 µM verapamil, an established L-type Ca\textsuperscript{2+} channel blocker. The top trace in Fig. 1A shows the membrane potential response to a voltage-clamp step from –65 to –5 mV, measured by the current-passing electrode. In a previous study, we demonstrated that the voltage measured by the current-passing electrode is an accurate measurement of the membrane potential by monitoring membrane potential with a second independent electrode (13). The next two traces show the corresponding current records before and after exposure of the cell to 2 µM verapamil. The inward current deflection following the capacitive transient was completely blocked by verapamil. The bottom trace is a difference trace derived by subtracting the current trace in the presence of verapamil from the control trace. Figure 1B shows I-V relationships derived from voltage steps to different potentials before and after exposure to verapamil. Figure 1C shows the I-V relationship derived from the difference currents. The results indicate that verapamil completely blocked the current identified as I_{Ca-L}, and that this current was identical to the difference in current before and after drug treatment.

Cell images were monitored with a closed-circuit television camera with interlace defeat and partial scan capability (model 1-GP-CD60, Panasonic) and were displayed on a video monitor (model VM-1220C, Hitachi Densi). Unloaded cell shortening was sampled at 120 Hz with a video edge detector (Crescent Electronics, Sandy, UT) coupled to the television camera. Details of specific voltage-clamp protocols are provided in the appropriate sections in RESULTS. In most experiments, voltage-clamp protocols were repeated two to three times and the data were averaged. Current, voltage, and contractions were digitized with a Labmaster A/D interface at 125 kHz (TL1–125, Axon Instruments) and stored on hard disk for subsequent analysis.

Data analyses. Ionic current, voltage, and contraction were measured with pCLAMP analysis software. Significance of differences between population means was tested with a Student's t-test with a Bonferroni correction for multiple comparisons. I-V relationships, contraction-voltage relationships, and time courses were analyzed with a two-way repeated-measures analysis of variance. Post hoc comparisons were made with a Bonferroni test. All statistical analyses were performed with Sigma Stat (Jandel, version 1.02) or with SAS (SAS Institute). Nonlinear curve-fitting procedures...
were conducted with SigmaPlot (Jandel, version 2.0). Data are presented as means ± SE. The value of \( n \) represents the number of myocytes sampled; no more than two replicates (myocytes) were collected from the same heart.

Sources of drugs and chemicals. Lidocaine was purchased from Sigma, and ryanodine was purchased from Calbiochem (San Diego, CA). All drugs were dissolved in distilled water, except nifedipine stock, which was prepared in ethanol. Choline chloride was purchased from Fisher Scientific (Fairlawn, NJ).

RESULTS

Effects of vPC on contraction-voltage and I-V relations. Figure 2 (A and B) shows representative original recordings of membrane currents and contractions determined in the presence of 200 µM lidocaine to inhibit inward Na\(^+\) current. A schematic of the voltage-clamp protocol is illustrated at the top of each panel. The complete voltage-clamp protocol was repeated every 7 s. Each test step was preceded by a train of 10 200-ms conditioning steps to 0 mV to activate I\(_{\text{Ca-L}}\) and maintain SR Ca\(^{2+}\) loading. With this conditioning protocol, signs of Ca\(^{2+}\) overload were not observed. In Fig. 2A, the test step was separated from the last conditioning step by a 300-ms vPC of -40 mV. When the vPC was -40 mV, a test step to -10 mV initiated both inward current and a large contraction, whereas a test step to +80 mV activated no inward current and only a very small contraction. Traces in Fig. 2B were recorded with a similar voltage-clamp protocol, except that the vPC was -70 mV. Here a test step to -40 mV activated a small inward current and a large contraction, and a test step to -10 mV activated a larger inward current and contraction (Fig. 2B). However, in contrast to the results shown in Fig. 2A, a depolarizing step to +80 mV initiated a large contraction but did not activate inward current when the vPC was -70 mV.

The effect of vPC on contractions and inward currents is clearly seen when contraction-voltage and I-V relationships are plotted as shown in Fig. 2, C and D, respectively. These curves represent mean values ± SE for 12 cells studied with a vPC of -70 mV and 7 cells studied with a vPC of -40 mV. When the vPC was -40 mV, a bell-shaped contraction-voltage relation was observed (Fig. 2C). The magnitude of contractions increased to a peak at 0 mV and then declined at more positive potentials. These changes in the magnitudes of contractions were proportional to changes in Ca\(^{2+}\) current, as shown in Fig. 2D. Figure 2C also shows contraction-voltage relationships determined by voltage steps from a vPC of -70 mV. The threshold for activation of these contractions was about -60 mV, and contractions increased to a plateau at approximately -20 mV (Fig. 2C). Contractions remained large at very positive potentials, even though peak inward current decreased (Fig. 2D). We previously suggested that the additional component of contraction activated when the vPC is more negative than -40 mV may represent activation of a VSRM (13).

Steady-state inactivation properties of the VSRM. Our observation that the VSRM component of contraction was inhibited when test steps were made from a vPC of -40 mV suggests that the VSRM might show...
voltage-dependent inactivation. To determine whether the VSRM exhibits the property of steady-state voltage inactivation, we examined the effects of systematically changing the $V_{PC}$ on the magnitude of contractions activated by voltage steps to $-240$ mV. We have shown previously that contractions initiated by an activation step to $-240$ mV are entirely attributable to the VSRM (13). The schematic in Fig. 3A depicts the voltage-clamp protocol used in these studies. Each test step was preceded by 10 conditioning steps to $0$ mV followed by repolarization to the $V_{PC}$ for 700 ms. The $V_{PC}$ was changed in 5-mV steps, from $-35$ to $-70$ mV, with each repetition of the voltage-clamp protocol. Each 700-ms $V_{PC}$ was followed by a very brief (10 ms) step to $-65$ mV. The complete voltage-clamp protocol was repeated every 9 s. Representative original records of contraction and current also are shown in Fig. 3B. When the $V_{PC}$ was $-35$ mV, no contraction was observed with the step to $-40$ mV. However, when the $V_{PC}$ was changed to $-45$ mV, a small contraction was elicited by the activation step. Successively larger contractions were observed with $V_{PC}$ values of $-55$ and $-65$ mV (Fig. 3B). The activation step elicited very little inward current with any of the $V_{PC}$ values tested. Figure 3C shows a plot of mean magnitudes of peak contraction as a function of $V_{PC}$ for eight myocytes. These data were fitted with a Boltzmann equation of the following form: $y = (a - b)/(1 + \exp[(V_{PC} - v_h)/k]) + b$, where $a$ is the maximum contraction, $b$ is the minimum contraction, $v_h$ is the half-inactivation voltage, and $k$ is the slope factor. Mean data also were normalized to the maximum contraction and plotted as a function of $V_{PC}$ in Fig.
3D. The line in Fig. 3D represents a Boltzmann function fitted to the normalized data using the following equation: \( y = \frac{1}{1 + \exp[(v_{PC} - v_0)/k]} \). \( v_0 \) and \( k \) were calculated for each myocyte (n = 8). Mean \( v_0 \) was \(-47.6 \pm 1.0\) mV; mean \( k \) was \(4.37 \pm 0.65\) mV. These observations clearly show that contractions initiated by the step to -40 mV exhibit the property of steady-state inactivation.

Separation of contractions induced by the VSRM and \( I_{\text{Ca,L}} \) by voltage. The observation that contractions initiated by the VSRM exhibit the property of steady-state inactivation suggests that the activation and inactivation properties of contraction can be used to separate contractions initiated by the VSRM from those initiated by \( I_{\text{Ca,L}} \). Indeed, we previously have shown that contractions initiated by the VSRM can be separated from contractions initiated by \( I_{\text{Ca,L}} \) by sequential steps to -40 and 0 mV (13). Figure 4A shows records in which the VSRM was activated by a 250-ms step to -40 mV from a \( v_{\text{PC}} \) of -65 mV, and \( I_{\text{Ca,L}} \) was activated by the second step to 0 mV. Figure 4A shows contractions and currents recorded under control conditions. The step to -40 mV activated a large contraction and little if any inward current. The second step to 0 mV activated an additional contraction and \( I_{\text{Ca,L}} \). Figure 4C shows the effects of exposing the same cell to 2.5 \( \mu \)M nifedipine. Nifedipine selectively blocked \( I_{\text{Ca,L}} \) and the corresponding contraction. The VSRM contraction was only slightly reduced in amplitude. Mean results for five myocytes exposed to nifedipine are presented in Fig. 5. Nifedipine significantly inhibited \( I_{\text{Ca,L}} \) and the contraction elicited by the step to 0 mV (Fig. 5D and B, respectively) but had no significant effect on the current or contraction elicited by the step to -40 mV (Fig. 5C and A, respectively). Similar results were observed in an additional five cells exposed to verapamil (2 \( \mu \)M) (not illustrated).

In contrast to \( \text{Ca}^{2+} \) channel blockers, ryanodine (30 nM) strongly inhibited the VSRM contraction, rather than the \( I_{\text{Ca,L}} \) contraction. Figure 4B shows control recordings of currents and contractions initiated by sequential steps to -40 and 0 mV. Fig. 4D shows that exposure of the same cell to ryanodine abolished the contraction initiated by the step to -40 mV but had little effect on current and contraction initiated by the step to 0 mV. Mean data for ryanodine also are presented in Fig. 5. Ryanodine virtually abolished contractions initiated by the VSRM (from 2.2 ± 0.2 to 0.10 ± 0.03, \( P < 0.001 \)). In contrast, contractions initiated by the second step to 0 mV were only moderately decreased by exposure to ryanodine (from 1.64 ± 0.2 to 1.06 ± 0.14, \( P < 0.05 \)). Treatment with ryanodine did not affect inward currents associated with steps to either -40 or 0 mV (Fig. 5C and D).

These observations indicate that sequential steps to -40 and 0 mV separate two types of contractions with
clearly different pharmacological sensitivities. These observations also suggest that VSRM contractions should be very sensitive to manipulations designed to alter SR Ca\(^{2+}\) load, whereas contractions initiated by I\(_{\text{Ca-L}}\) should be much less sensitive to these manipulations.

Effects of conditioning pulses designed to alter SR load on contractions initiated by L-current and VSRM. To increase SR loading, we used trains of conditioning pulses to 0 mV to repetitively activate I\(_{\text{Ca-L}}\). Figure 6A shows representative recordings of voltages and contractions with conditioning pulses to 0 mV, which is close to the peak of the I-V relation for I\(_{\text{Ca-L}}\). After the first contraction, which is a rest contraction, a positive staircase was observed for the next nine conditioning pulses. In this example, the train of conditioning pulses was followed by repolarization to a v\(_{\text{PC}}\) of -70 mV without a test step. To reduce SR load, trains of conditioning pulses to -40 mV were used to activate release of SR Ca\(^{2+}\) with minimal activation of I\(_{\text{Ca-L}}\) (Fig. 6B). With this protocol, the rest contraction had the same magnitude as the corresponding rest contraction in Fig. 6A, but there was no positive staircase. Clearly, conditioning pulses to 0 or -40 mV had very different effects on magnitude of contraction during the conditioning trains.

Next we determined the effects of conditioning pulses to 0 and -40 mV on contractions initiated by sequential test steps to -40 and 0 mV. Figure 7A was recorded when the activation steps were preceded by a series of 10 conditioning pulses to 0 mV. With this protocol, the step to -40 mV activated a large contraction and little if any inward current, whereas the second step to 0 mV activated an additional contraction and I\(_{\text{Ca-L}}\) (A). C: 2.5 µM nifedipine selectively blocked I\(_{\text{Ca-L}}\) and the corresponding contraction, with little effect on the VSRM contraction. To maintain SR Ca\(^{2+}\) stores in the presence of Ca\(^{2+}\) channel blockade, conditioning pulses to positive potentials (e.g., +80 mV) were used to cause Ca\(^{2+}\) entry via Na\(^+\)/Ca\(^{2+}\) exchange. B: control recordings of currents and contractions initiated by sequential steps to -40 and 0 mV in a separate cell. Exposure of this cell to 30 nM ryanodine inhibited VSRM contraction but had little effect on current and contraction initiated by the step to 0 mV (D).
associated with the steps to −40 and 0 mV were not affected by changing the conditioning-pulse voltage in this example. Mean data for the effects of conditioning-pulse voltage are shown in Fig. 5. Contractions initiated by the VSRM were significantly reduced in amplitude (from 2.2 ± 0.2 to 0.3 ± 0.1 µm, P < 0.001, Fig. 5A), whereas the amplitude of I_{Ca-L} contractions was not significantly decreased by conditioning pulses to −40 mV (from 1.6 ± 0.2 to 1.5 ± 0.3 µm, NS, Fig. 5B). The small inward current initiated by the step to −40 mV was not significantly affected (Fig. 5C); however, there was a significant increase in peak I_{Ca-L} when conditioning-pulse voltage was changed from 0 to −40 mV (Fig. 5D, P < 0.05). The effects of changing conditioning-pulse voltage to −40 mV on the VSRM contraction were very similar to those of ryanodine.

Comparison of the effects of conditioning pulses to −40 mV with effects of ryanodine on contraction-voltage and I-V relationships. The results of the preceding experiments (Fig. 5) show that the VSRM is affected much more than I_{Ca-L}-induced contractions by conditioning steps to −40 mV or by exposure to ryanodine. Thus one would predict that changing conditioning-pulse voltage might also affect the contribution of the VSRM to contractions initiated when both mechanisms are available. Therefore, we determined the effects of conditioning-pulse amplitude on contraction-voltage relationships determined with voltage steps to a wide range of potentials. First, we determined the effects of conditioning-pulse voltage on I-V and contraction-voltage relationships when the VSRM was inactivated by a V_{PC} of −40 mV (Fig. 8, A and B). I-V and contraction-voltage relationships determined from a V_{PC} of −40 mV were both bell shaped. When conditioning-pulse voltage was changed from 0 to −40 mV, I-V relationships were not affected; however, the magnitudes of the contractions were significantly decreased at all voltages (P < 0.05).

Figure 8C shows the difference between contraction-
We also determined the effects of 30 nM ryanodine on contraction-voltage and I-V relations initiated by activation steps from \( V_{PC} \) values of −40 and −70 mV. In these experiments, all conditioning pulses were to 0 mV (Fig. 9). Ryanodine significantly reduced (\( P < 0.05 \)) inward current determined with either \( V_{PC} \) but did not shift the voltage dependence (Fig. 9, A and D). Figure 9B shows that ryanodine significantly decreased (\( P < 0.05 \)) the amplitudes of contractions initiated from a \( V_{PC} \) of −40 mV. The contraction-voltage relationship in the presence of ryanodine remained bell shaped. Figure 9C shows the difference between the contraction-voltage relationships determined in the absence and presence of ryanodine. When contraction-voltage relationships were determined from a \( V_{PC} \) of −70 mV (Fig. 9E), ryanodine changed the shape of the contraction-voltage relationship from sigmoidal to bell shaped. Ryanodine also significantly decreased (\( P < 0.05 \)) the amplitudes of contractions initiated at virtually all test voltages (Fig. 9E). The component of contraction inhibited by ryanodine showed a sigmoidal voltage dependence (Fig. 9F). These effects of ryanodine on contraction-voltage relationships were very similar to those of changing conditioning-pulse voltage from 0 to −40 mV.

The effects of thapsigargin on contraction-voltage and I-V relationships determined from a \( V_{PC} \) of −70 mV in rat ventricular myocytes. An alternate method of evaluating the contribution of SR Ca\(^{2+}\) release to contraction is to inhibit SR Ca\(^{2+}\) uptake with thapsigargin, an agent that blocks the SR Ca\(^{2+}\)-adenosinetriphosphatase. Figure 10, A and B, shows representative recordings from a rat ventricular myocyte before and after exposure to 0.2 µM thapsigargin. Contractions and currents initiated by sequential steps to −40 and 0 mV from a \( V_{PC} \) of −65 mV were very similar to those observed with guinea pig ventricular myocytes. Thapsigargin strongly inhibited the contraction initiated by the VSRM but only partially inhibited the contraction accompanying \( I_{Ca,L} \). Figure 10, C and D, shows mean contraction-voltage and I-V relationships recorded with voltage steps from a \( V_{PC} \) of −70 mV (\( n = 3 \)). Thapsigargin had no effect on \( I_{Ca,L} \), but significantly reduced the amplitudes of contractions (\( P < 0.05 \)). In addition, thapsigargin, like ryanodine or conditioning pulses to −40 mV, caused the contraction-voltage relationship to become bell shaped.

In rat myocytes there is clear separation between the threshold for activation of contraction and the threshold for activation of inward current (Fig. 10, C and D), probably because of the absence of T-type Ca\(^{2+}\) current in this species (28). Because rat myocytes only have L-type Ca\(^{2+}\) current, we were able to compare the steady-state inactivation properties of the VSRM to those of \( I_{Ca,L} \). The voltage-clamp protocol used in these experiments is similar to that shown in Fig. 3. The \( V_{PC} \) was changed in 5-mV steps with each repetition of the voltage-clamp protocol. Each 700-ms \( V_{PC} \) was followed by a very brief (10 ms) step to −65 mV, followed by a step to −35 mV to activate the VSRM, or a brief step to −50 mV followed by a step to 0 mV to activate \( I_{Ca,L} \). Figure 10E shows mean normalized steady-state inacti-

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The diagram shows the effects of conditioning pulse voltage on contractions initiated by the VSRM and \( I_{Ca,L} \). Sequential steps to −40 and 0 mV were used to activate VSRM and \( I_{Ca,L} \) contractions, respectively. A: recordings of contractions (top) and currents (bottom) when conditioning-pulse voltage was 0 mV. Each step initiated contractions; however, inward current was only observed with the step to 0 mV. B: when voltage of the conditioning pulses was changed to −40 mV, the contraction initiated by the VSRM was abolished, but \( I_{Ca,L} \) contraction was decreased only slightly.

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**Fig. 7.** Comparison of effects of conditioning pulse voltage on contractions initiated by the VSRM and \( I_{Ca,L} \). Sequential steps to −40 and 0 mV were used to activate VSRM and \( I_{Ca,L} \) contractions, respectively. A: recordings of contractions (top) and currents (bottom) when conditioning-pulse voltage was 0 mV. Each step initiated contractions; however, inward current was only observed with the step to 0 mV. B: when voltage of the conditioning pulses was changed to −40 mV, the contraction initiated by the VSRM was abolished, but \( I_{Ca,L} \) contraction was decreased only slightly.
voltage curves for the VSRM and $I_{Ca-L}$ determined in nine myocytes. The VSRM had a $v_h$ of $-53.2 \pm 0.4$ mV and a $k$ of $4.6 \pm 0.2$ mV, whereas the corresponding values for $I_{Ca-L}$ were $-25.3 \pm 0.9$ mV and $6.0 \pm 0.2$ mV, respectively. The $v_h$ of the VSRM was 28 mV negative to that of $I_{Ca-L}$ and was significantly different from $v_h$ of $I_{Ca-L}$ ($P < 0.001$). The values of $k$ for the VSRM and $I_{Ca-L}$ also were significantly different ($P < 0.001$). Thus, when the $v_{PC}$ was $-40$ mV, the VSRM was completely inactivated but $I_{Ca-L}$ was still fully available.

Comparison of the effects of conditioning pulses to $-40$ mV with effects of ryanodine on restitution of contraction. Contractions initiated by an early test stimulus following a previous activation are small, but show recovery or restitution when the test interval increases (5). We determined the time course of restitution of contraction in isolated myocytes as well as restitution of $I_{Ca-L}$. The voltage-clamp protocol used in these experiments is shown at the top of Fig. 11A. Test steps to 0 mV from a $v_{PC}$ of $-65$ mV were used to activate both VSRM and $I_{Ca-L}$ components of contraction (total contraction). The interval ($\Delta t$) between the last conditioning pulse and the test step was increased progressively from 6 to 246 ms, in 20-ms increments. Figure 11A shows representative original recordings of current and contraction for three selected test intervals (26, 106, and 186 ms). Contraction increased progressively as the interval was lengthened. In contrast, $Ca^{2+}$ current increased when the test interval was lengthened from 26 to 106 ms, but remained relatively constant with further increases in test interval. Mean data for restitution of contraction are shown in Fig. 11B. The line represents a single exponential fit to the mean data. The exponential function for restitution of contraction had a time constant of 57.8 ms. Mean data for recovery of $Ca^{2+}$ current are shown in Fig. 11C. The exponential fit to these data had a time constant of 31.1 ms. Thus the recovery of contraction followed a slower
time course than the recovery of peak inward Ca\(^{2+}\) current.

We next determined the effects of 30 nM ryanodine on restitution of total contraction determined with test steps to 0 mV from a \(v_{PC}\) of \(-65\) mV (Fig. 12A). The voltage-clamp protocols are shown at the far right of Fig. 12. For the ryanodine experiments, the conditioning pulses were to 0 mV. Ryanodine significantly \((P < 0.05)\) decreased the magnitude of contractions initiated by activation steps from \(-65\) to 0 mV (total contraction), and the plateau of the curve was reached at a shorter test interval than in control (Fig. 12A).

We then determined whether contractions initiated by the VSRM exhibited restitution. VSRM contractions were elicited by a test step to \(-30\) mV from a \(v_{PC}\) of \(-65\) mV. Figure 12B shows changes in magnitude of VSRM contractions with progressive increase in test interval. In the absence of ryanodine, restitution of VSRM contractions (Fig. 12B) followed a time course similar to that for total contractions (Fig. 12A). Ryanodine had virtually the same effect on restitution of VSRM contractions as on total contraction (Fig. 12B, \(P < 0.05\)). Thus restitution of total contraction could be accounted for almost entirely by restitution of the VSRM component of contraction. We also evaluated restitution of contractions initiated at different test intervals by test steps to 0 mV from a \(v_{PC}\) of \(-35\) mV to inactivate the VSRM. With this protocol, contractions were smaller and exhibited very little restitution (Fig. 12C). In addition, with this protocol, contractions at all test intervals were insensitive to 30 nM ryanodine.

We also examined the effects of conditioning-pulse voltage on restitution of contraction. Figure 12D shows the effects of changing the voltage of conditioning pulses from 0 to \(-40\) mV on total contraction. When test steps were preceded by conditioning pulses to \(-40\) mV, restitution was inhibited \((P < 0.05)\). Figure 12E shows the effects of changing conditioning-pulse amplitude on contractions initiated by the VSRM. The magnitudes of contractions also were decreased, although the
Fig. 10. Currents and contractions in rat ventricular myocytes. A and B: representative recordings of currents (top) and contractions (bottom) elicited by sequential voltage steps to −40 and 0 mV (shown schematically above A). Thapsigargin (0.2 µM) almost abolished the contraction initiated by the step to −40 mV but only partially inhibited the contraction initiated by the step to 0 mV. C: mean contraction-voltage relationships determined from a vPC of −70 mV. Thapsigargin significantly reduced the amplitudes of contraction and converted the contraction-voltage relationship from sigmoidal to bell shaped. Current was unaffected by thapsigargin (D). *P < 0.05 with respect to control. E: mean steady-state inactivation curves for the VSRM contraction and ICaL determined in 9 rat myocytes. Curves were normalized to maximum contraction or inward current. Voltage-clamp protocols were similar to that shown in Fig. 3. Specific voltage steps are given in RESULTS. vh for VSRM (−53.2 ± 0.4 mV) was significantly different from that of ICaL (−25.3 ± 0.9 mV, P < 0.001). Values for k also were significantly different (4.6 ± 0.2 mV for VSRM and 6.0 ± 0.2 mV for ICaL, P < 0.001).
Fig. 11. Time course of restitution of contraction and restitution of inward current. A schematic of the voltage-damp protocol is shown at top of A. Test steps to 0 mV from a $V_{PC}$ of $-65$ mV were preceded by 10 conditioning pulses to 0 mV to maintain SR Ca$^{2+}$ load. The interval between the last conditioning pulse and the test step ($\Delta t$) was progressively lengthened from 6 to 246 ms. A: representative original records of contraction (top trace in each pair) and current (bottom trace in each pair) for 3 test intervals, 26, 106, and 186 ms. Contractions increased in magnitude with increases in test interval. However, inward current did not increase in magnitude at test intervals beyond 106 ms. Mean data for the time course of restitution of contractions are shown in B, and corresponding data for restitution of current are shown in C. Recovery of contraction followed a slower time course than recovery of current. $\tau$, Time constant of a single exponential fitted to the mean data.

DISCUSSION

The objectives of this study were 1) to determine whether the VSRM exhibits steady-state inactivation and to determine the voltage range over which inactivation occurs, 2) to determine and compare the effects of protocols designed to alter SR Ca$^{2+}$-load on components of contraction initiated by the VSRM or by $I_{Ca-L}$, and 3) to evaluate the role of the VSRM in restitution. Our observations demonstrate that the VSRM does show steady-state inactivation, is exquisitely sensitive to conditioning-pulse voltage and drugs that disrupt SR function, and is an important determinant of restitution of cardiac contraction.

One of the central observations of this study was that the VSRM exhibits steady-state inactivation properties. The VSRM was fully available when activation steps were made from membrane potentials more negative than $-60$ mV. The $V_i$ of the VSRM was found to be approximately $-48$ mV in guinea pig and $-53$ mV in rat. Complete inactivation occurred near $-35$ and $-40$ mV in the two species, respectively. Clearly, earlier studies of cardiac EC coupling, which utilized holding or conditioning voltages near $-40$ mV, would have almost completely inactivated the VSRM (2, 3, 9, 10, 20). Indeed, the bell-shaped contraction-voltage or Ca$^{2+}$ transient-voltage relationships reported in those studies likely reflects activation of contraction by $I_{Ca-L}$ in the absence of the VSRM.

The steady-state inactivation properties of the VSRM help distinguish it from CICR coupled to $I_{Ca-L}$. The steady-state inactivation curve for the VSRM was found to be significantly different statistically from that for $I_{Ca-L}$, with respect to both $V_i$ and $k$ values. The steady-state inactivation curves for the VSRM and $I_{Ca-L}$ were separated by 28 mV at $V_{PC}$. Because of this wide separation, the VSRM was completely inactivated when $V_{PC} = -40$ mV but $I_{Ca-L}$ was still fully available. Because the inactivation properties of the VSRM and $I_{Ca-L}$ are widely divergent, it is very unlikely that the VSRM represents CICR initiated by $I_{Ca-L}$.

The steady-state inactivation properties of the VSRM allowed us to utilize two sequential activation steps to $-40$ and 0 mV to separate VSRM and $I_{Ca-L}$-induced contractions within a single voltage-damp protocol. The ability of nifedipine to selectively inhibit $I_{Ca-L}$ and the $I_{Ca-L}$-induced contraction with minimal effect on the VSRM contraction confirmed this separation. This selective effect of nifedipine also indicates that initiation of the VSRM contraction cannot be attributed to a very small influx of Ca$^{2+}$ through L-type Ca$^{2+}$ channels on the step to $-40$ mV, because inhibition of the large Ca$^{2+}$ current on the step to 0 mV resulted in strong inhibition of the $I_{Ca-L}$-induced contraction.

The steady-state inactivation properties of the VSRM also serve to distinguish it from contractions triggered by reverse Na$^+$/Ca$^{2+}$ exchange. Contractions attributed to reverse Na$^+$/Ca$^{2+}$ exchange can be elicited by activation steps from either $-70$ or $-40$ mV (30). This indicates that contractions initiated by Na$^+$/Ca$^{2+}$ ex-
change do not show inactivation at −40 mV. Indeed, Na⁺/Ca²⁺ exchange is not known to exhibit voltage-dependent inactivation. Furthermore, contractions attributed to reverse Na⁺/Ca²⁺ exchange demonstrate sigmoidal, N-shaped, or progressively increasing contraction-voltage relations with activation steps from −40 mV, depending on the intracellular concentration of Na⁺ (16, 23, 29, 30). The present study was conducted with high-resistance microelectrodes, which would minimize intracellular dialysis and which did not contain Na⁺. Under these conditions the contraction-voltage relationships were always bell shaped when the VPC was −40 mV.

Steady-state inactivation is generally described as a property of voltage-gated ion channels. In skeletal muscle, which also shows voltage-dependent release of SR Ca²⁺, the voltage sensor for EC coupling is believed to be the sarcolemmal L-type Ca²⁺ channel (24). The L-type Ca²⁺ channel is believed to be linked physically to the Ca²⁺ release channel (ryanodine receptor) in junctional SR (24). Interestingly, the release mechanism in skeletal muscle exhibits voltage-dependent inactivation (8, 24). Thus demonstration that the VSRM in cardiac myocytes also exhibits steady-state inactivation suggests that the voltage sensor for the VSRM may be a voltage-gated ion channel. The identity of the voltage sensor in cardiac myocytes is unknown. It would seem unlikely that the L-channel serves as the voltage sensor because the Vh of the cardiac L-channel is near −25 mV under our conditions as well as in other

Fig. 12. Effects of ryanodine and conditioning-pulse voltage on restitution of contraction. Experiments were conducted as described in Fig. 11. Schematics illustrating voltage-clamp protocols are shown at right. A: mean contraction-interval relationship for contractions initiated by a test step from −65 to 0 mV in the absence and presence of ryanodine. Ryanodine decreased the magnitude of contractions at all test intervals. B: contraction-interval relations determined with a test step from −65 to −30 mV to selectively activate VSRM. Restitution of VSRM followed a similar time course to that of restitution of total contraction and was similarly sensitive to ryanodine. C: contraction-interval relationships for contractions initiated by depolarizing steps from −35 to 0 mV to selectively activate ICa-L contractions. Contractions initiated by ICa-L showed minimal restitution and were virtually insensitive to ryanodine. D: effects of changing conditioning-pulse voltage on restitution of contractions initiated by a test step from −65 to 0 mV. Conditioning pulses to −40 mV greatly reduced the magnitude of contractions at all test intervals and virtually eliminated restitution. E: conditioning pulses to −40 mV inhibited restitution of contractions initiated by the VSRM. F: contractions initiated by ICa-L were not affected by conditioning pulses to −40 mV. Curves represent mean data from 5–7 experiments. *Statistically significant with respect to corresponding controls, P < 0.05.
studies (21). However, we cannot completely exclude this possibility because the voltage sensitivity of L-type Ca\(^{2+}\) channels might change when they are coupled to another protein such as Ca\(^{2+}\) release channels in the SR. Of the known voltage-sensitive channels in the cardiac cell membrane, only the T-type Ca\(^{2+}\) channel has a steady-state inactivation curve that closely matches that of the VSRM. The \(v_h\) of the T-type Ca\(^{2+}\) channel has been reported to be near \(-50\) mV, with a \(k\) value of \(-5\) mV (27, 31). These values correspond closely to the \(v_h\) and \(k\) values determined for the VSRM in the present study. However, in the present study we also were able to demonstrate the VSRM in rat ventricular myocytes, which have been reported not to have T-type Ca\(^{2+}\) current (28). This clearly excludes CICR in response to T-type Ca\(^{2+}\) current as the trigger for the VSRM, but does not necessarily exclude a role for T-channels as voltage sensors. T-channels might serve as voltage sensors in rat, but one would have to postulate that the T-channels have lost their current carrying capacity but retained their gating properties.

The intracellular Ca\(^{2+}\) transient that initiates cardiac contraction is derived from release of SR Ca\(^{2+}\), as well as influx of Ca\(^{2+}\) through the sarcolemma. The magnitude of Ca\(^{2+}\) released from the SR varies with SR load (14). Han et al. (14) demonstrated that test pulses to 0 mV activate only a small Ca\(^{2+}\) transient when preceded by conditioning pulses to \(-30\) mV, to provide a low SR Ca\(^{2+}\) load. However, the same test pulse initiated a much larger Ca\(^{2+}\) transient, when preceded by conditioning pulses to 0 mV, to increase SR Ca\(^{2+}\) load. In the present study, contractions initiated by the VSRM and \(I_{\text{Ca-L}}\) were affected differently by similar changes in conditioning-pulse voltage. VSRM contractions were greatly reduced in amplitude by conditioning-pulse protocols designed to reduce SR Ca\(^{2+}\) loading. This is compatible with the VSRM contraction, depending on release of SR Ca\(^{2+}\). In contrast, contractions initiated by activation of \(I_{\text{Ca-L}}\) were much less affected by conditioning-pulse voltage. These observations suggest that the \(I_{\text{Ca-L}}\) contraction, unlike the VSRM contraction, depends on both SR Ca\(^{2+}\) release and Ca\(^{2+}\) influx. This interpretation is also supported by the effects of ryanodine and thapsigargin, which interfere with SR function. Both agents strongly inhibited VSRM contractions but had much less effect on \(I_{\text{Ca-L}}\) contractions. Our observations with 30 nM ryanodine imply that, in undialyzed guinea pig cells at 37°C, part of the \(I_{\text{Ca-L}}\) contraction may be mediated by direct activation of myofilaments by Ca\(^{2+}\) influx. There are conflicting reports as to whether influx of Ca\(^{2+}\) via \(I_{\text{Ca-L}}\) is sufficient to initiate contraction directly (3). Effects of ryanodine vary widely depending on temperature, duration of exposure, concentration, stimulation interval, atrial versus ventricular tissue, and species (3). Thus in some species virtually all contraction can be eliminated by ryanodine (e.g., adult rat ventricle), whereas in others a ryanodine-resistant component has been observed (e.g., guinea pig ventricle) (3). For example, in field-stimulated guinea pig ventricular myocytes (18) and multicellular preparations (26) studied at physiological temperatures, 25–80% of contraction amplitude was retained in the presence of ryanodine and/or thapsigargin. However, these earlier studies did not separate effects of ryanodine or thapsigargin on the two components of EC coupling examined in this study.

Contraction-voltage relationships determined from a \(V_{\text{PC}}\) of \(-70\) mV include both VSRM and \(I_{\text{Ca-L}}\) components of contraction. Both changes in conditioning-pulse voltage and ryanodine caused very large reductions in amplitudes of contractions initiated by steps from \(-70\) mV. The component of contraction inhibited by both of these manipulations had a sigmoidal voltage dependence. The magnitude of the component that was inhibited was large even at very positive membrane potentials near the reversal potential of \(I_{\text{Ca-L}}\). In contrast, the component that was inhibited was very small at positive membrane potentials when the VSRM was inactivated by a \(V_{\text{PC}}\) of \(-40\) mV. Thus, when both the VSRM and \(I_{\text{Ca-L}}\) are available, most of the contraction elicited at potentials corresponding to the action potential peak and plateau appears to be attributable to the VSRM.

Our studies also demonstrated an inhibitory effect of ryanodine on \(I_{\text{Ca-L}}\) that was apparent in the I-V relationships. It is unlikely that ryanodine directly inhibits the L-type Ca\(^{2+}\) channel, because experiments by others in which intracellular Ca\(^{2+}\) levels were strongly buffered showed no effect of ryanodine on \(I_{\text{Ca-L}}\) (1). In our experiments, in which intracellular Ca\(^{2+}\) was not buffered, inhibition of \(I_{\text{Ca-L}}\) by ryanodine may reflect Ca\(^{2+}\)-mediated inhibition of L-type Ca\(^{2+}\) channels (21). Low concentrations of ryanodine lock the SR Ca\(^{2+}\) release channel in an open subconducting state; however, the SR can still take up Ca\(^{2+}\) (25). This Ca\(^{2+}\) can gradually leak out of the SR over a period of several hundred milliseconds (3, 26). In the protocols used to determine I-V relationships, test steps were preceded by conditioning pulses and a postconditioning period of 500 ms. A possible explanation for the effect of ryanodine on \(I_{\text{Ca-L}}\) is that the L-type Ca\(^{2+}\) channels may have been inhibited by Ca\(^{2+}\) leaking from the SR during the period between the last conditioning pulse and the test step. At present we have no direct evidence supporting or refuting this explanation. However, it is interesting to note that thapsigargin, which does not lock the release channel in an open subconducting state, had no effect on \(I_{\text{Ca-L}}\).

The effects of ryanodine and conditioning-pulse voltage on \(I_{\text{Ca-L}}\) and on \(I_{\text{Ca-L}}\) contractions were slightly different depending on the voltage-clamp protocol used. For example, the \(I_{\text{Ca-L}}\) contraction was not significantly inhibited by conditioning pulses to \(-40\) mV when sequential activation steps to \(-40\) and 0 mV were used, but was significantly inhibited when contraction-voltage relations were determined. This difference might be attributable to differences in the activation sequence between the two protocols. With the sequential steps, the VSRM is activated immediately before the \(I_{\text{Ca-L}}\) contraction. Because the VSRM contraction is not accompanied by \(I_{\text{Ca-L}}\) in this protocol, activation of the
VSRM might partially reduce SR Ca\textsuperscript{2+} and decrease the sensitivity of I\textsubscript{Ca-L} contractions to changes in SR load.

The third goal of this study was to examine the role of the VSRM in recovery of contraction after a previous activation, a process termed restitution of contraction (5). In the present study we were able to demonstrate restitution of contraction in isolated myocytes under voltage-clamp conditions. Restitution was measured as the change in magnitude of test contractions initiated at different intervals after a previous activation. We found that the magnitude of the test contraction was negligible at short intervals but gradually increased with longer test intervals. Contractions initiated by depolarizing steps from −65 to 0 mV, to activate both VSRM and I\textsubscript{Ca-L} components of contraction, showed restitution of contraction with a time constant near 60 ms. In contrast, the time constant of restitution of I\textsubscript{Ca-L} was near 30 ms, which is similar to previous reports when one takes temperature into account (21). Thus the time course of restitution of contractions was slower than restitution of I\textsubscript{Ca-L}. Therefore, restitution of contraction is not limited by recovery of I\textsubscript{Ca-L}.

We found that contractions initiated by the VSRM, selectively activated by steps from −65 to −30 mV, also exhibited restitution. The magnitude of restitution of VSRM contractions was similar to that observed when both VSRM and I\textsubscript{Ca-L} components of contraction were activated by steps from −65 to 0 mV. Thus recovery of the VSRM could account for much of restitution of contraction. This observation further demonstrates that restitution of contraction is not limited by recovery of I\textsubscript{Ca-L}, because the VSRM contractions were elicited by voltage steps that did not activate measurable I\textsubscript{Ca-L}. Indeed, I\textsubscript{Ca-L} contractions initiated from a V\textsubscript{PC} of −35 mV showed only minimal restitution and only during the first 25–50 ms after the previous activation.

Manipulations to decrease SR Ca\textsuperscript{2+} load greatly attenuated restitution of total contractions initiated by voltage steps from −65 to 0 mV. The same manipulations, conditioning pulses to −40 mV or exposure to ryanodine, also reduced restitution of contractions initiated by the VSRM. These observations indicate that the VSRM plays an important role in restitution of cardiac contraction and that restitution of the VSRM is very sensitive to manipulations designed to reduce SR Ca\textsuperscript{2+} load.

The component of EC coupling that determines the time course of restitution of contraction has yet to be identified. It is very unlikely that the time course of restitution is limited by replenishment of SR Ca\textsuperscript{2+} stores because this is believed to be completed very quickly and in advance of recovery of SR Ca\textsuperscript{2+} release channels (3). Because of these considerations, restitution generally has been attributed to recovery of the SR release channels (3). However, our results suggest that the recovery process could be either at the level of the SR release channel or at the voltage sensor. Additional studies are needed to identify the precise component that determines restitution.

Our observations demonstrate that a component of contraction, which we have called a VSRM, plays an important part in EC coupling in heart. When the VSRM is inactivated, the maximum magnitude of contraction is reduced and contraction is weak at membrane potentials corresponding to the peak and plateau phases of the ventricular action potential. However, when the VSRM is available for activation, the magnitude of contraction is greater and becomes independent of voltage at potentials positive to approximately −20 mV. The VSRM then operates as a trigger mechanism that initiates maximal contractions over the entire range of potentials corresponding to the overshoot, initial repolarization, and plateau of the action potential. The VSRM also plays an important role in determining restitution of contraction following a previous activation and is very sensitive to manipulations that alter SR Ca\textsuperscript{2+} loading. I\textsubscript{Ca-L} activates a component of contraction that is less sensitive than the VSRM to loading of SR stores and also plays an important role in loading SR Ca\textsuperscript{2+} stores. Our observations support the hypothesis that the VSRM is a major link that couples cardiac contraction to membrane potential, activation interval, and SR Ca\textsuperscript{2+} loading.

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