Role of voltage-sensitive release mechanism in depression of cardiac contraction in myopathic hamsters

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Howlett, Susan E., Wei Xiong, Cindy L. Mapplebeck, and Gregory R. Ferrier. Role of voltage-sensitive release mechanism in depression of cardiac contraction in myopathic hamsters. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H1690–H1700, 1999.—We investigated excitation-contraction (EC) coupling in isolated ventricular myocytes from prehypertrophic cardiomyopathic (CM) hamster hearts. Conventional and voltage-clamp recordings were made with high-resistance microelectrodes, and cell shortening was measured with a video-edge detector at 37°C. Contractions were depressed in myocytes from CM hearts, whether they were initiated by action potentials or voltage-clamp steps. As in guinea pig and rat, contraction in hamster myocytes could be triggered by a voltage-sensitive release mechanism (VSRM) or Ca2+-induced Ca2+ release (CICR). Selective activation of these mechanisms demonstrated that the defect in EC coupling was primarily caused by a defect in the VSRM. However, activation and inactivation properties of the VSRM were not altered. When the VSRM was inhibited, the remaining contractions induced by CICR exhibited identical bell-shaped contraction voltage relations in normal and CM myocytes. Inward Ca2+ current was unchanged. Thus a defect in the VSRM component of EC coupling precedes the development of hypertrophy and failure in CM hamster heart.

Depressed contractility is observed at the level of individual myocytes has not been determined. Therefore, this study examines EC coupling in heart disease could reside in one or both of these mechanisms. The possibility that a defect in CICR might occur has been explored in several models of heart disease. Sen et al. (23, 24) showed that the magnitude of cell shortening is reduced in myocytes from failing hamster hearts, with no change in the magnitude of Ca2+ current. This suggests that the ability of Ca2+ current to trigger SR Ca2+ release (CICR) may be compromised in heart failure. Gomez et al. (12) also have demonstrated that contractions and Ca2+ transients are reduced in myocytes from rat hearts with hypertrophy and heart failure (12). This is associated with a decrease in the ability of Ca2+ current to activate SR Ca2+ release, measured as Ca2+ sparks (12). Thus the results of these studies suggest that a defect in CICR can contribute to contractile dysfunction in heart disease.

Whether defects in the VSRM may contribute to depression of contractility in heart disease has not been established. In fact, the VSRM is inhibited by experimental conditions widely used in studies of EC coupling in mammalian heart. Contractions initiated by the VSRM are inhibited when 1) studies are conducted at room temperature rather than at 37°C (7, 16), 2) holding or postconditioning potentials near −40 mV are used (17), and/or 3) cells are dialyzed with patch pipette solutions that do not contain cAMP or calmodulin to support phosphorylation (9, 29). As previous studies of EC coupling in heart disease have utilized one or more of these conditions, it is not known whether defects in the VSRM might contribute to contractile dysfunction in heart disease.

This study explores whether changes in the VSRM contribute to contractile dysfunction in the cardiomyopathic (CM) hamster. The CM hamster has been selected for study because it is a well-characterized genetic model of cardiomyopathy and congestive heart failure in which the disease progresses in a characteristic and reproducible fashion (1, 2, 26). Cardiac cell necrosis begins at 40 to 50 days of age (18, 19). This is followed by hypertrophy of remaining cells, which starts at about 120 days of age, and progresses to heart failure and premature death by about one year (18, 19). Previous studies (3, 15) in multicellular preparations have shown that depression of contractility in CM hamster heart precedes the onset of hypertrophy and failure. This suggests that the defect in contractility is an early event rather than a secondary response to advancing disease. Whether this early decrease in contractility is accompanied by a defect in EC coupling at the level of individual myocytes has not been determined. Therefore, this study examines EC coupling in

CONTRACTILE DYSFUNCTION occurs with many forms of heart disease and plays a major role in heart failure. Depressed contractility is observed at the organ and tissue levels (3–5, 10, 11, 22, 26, 28) and more recently has been identified in myocytes isolated from hypertrophied or failed hearts (12, 23, 24). The latter observations indicate that defects in contractile function may originate at the cellular level. Thus it is possible that depressed contractility may reflect, in part, abnormalities in the sequence of events that link myocyte membrane depolarization to contraction, a process known as excitation-contraction coupling (EC coupling).

A central step in cardiac EC coupling is the rapid release of Ca2+ from the sarcoplasmic reticulum (SR). Membrane depolarization can initiate SR Ca2+ release by two mechanisms: 1) Ca2+-induced Ca2+ release (CICR), whereby a small influx of Ca2+ through the sarcolemma leads to a larger release of Ca2+ from the SR (6); and 2) a voltage-sensitive release mechanism (VSRM) by which SR Ca2+ release is linked to membrane depolarization and which operates indepen-
ventricular myocytes from 80- to 90-day-old normal and CM hamster hearts. The objectives were 1) to determine whether the early depression in contractility is accompanied by a defect in EC coupling in myocytes isolated from CM hearts and 2) to evaluate whether depressed contractility occurs as a result of changes in the VSRM, CICR, or both mechanisms of EC coupling.

METHODS

Myocyte isolation. Experiments were conducted on ventricular myocytes isolated from 80- to 90-day-old normal (CHF 146) and genetically matched normal (CHF 148) male hamsters purchased from Canadian Hybrid Farms (Halifax, Nova Scotia, Canada). Animals were weighed, injected with heparin (3.3 IU/g), and anesthetized with pentobarbital sodium (80 mg/kg). The heart was rapidly cannulated in situ and perfused, retrogradely through the aorta (8–10 ml/min), with oxygenated (100% O2; 36.5°C) Ca2+-free solution containing (in mM) 120 NaCl, 3.8 KCl, 1.2 KH2PO4, 1.2 MgSO4, 10 HEPES, and 11 glucose (pH 7.4 with NaOH). The heart was removed from the chest and perfused with Ca2+-free solution for 7–8 min. Then the heart was perfused with the same solution supplemented with collagenase A (35 mg/50 ml; Boehringer-Mannheim), trypsin (1 mg/50 ml; Sigma type II), dispase (20 mg/50 ml; neutral protease, Grade II, Boehringer Mannheim), and 50 µM CaCl2 for 10–15 min. When the ventricles had become soft, they were minced and washed in a substrate-rich dissociation solution containing (in mM) 80 KOH, 50 glutamic acid, 30 KCl, 30 KH2PO4, 20 taurine, 10 HEPES, 10 glucose, 3 MgSO4, and 0.5 EGTA (pH 7.4 with KOH). With this method, ~60–70% of the dissociated cells were Ca2+-tolerant, rod-shaped myocytes. There were no apparent differences in cell survival between normal and CM hearts.

Ventricular myocytes were placed in a modified culture dish (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 an Axoclamp 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 and to avoid buffering intracellular Ca2+ levels. The bath was grounded with a 2.7 M KClagar bridge to minimize liquid junction potential changes. Voltage-clamp protocols were generated with pCLAMP software (Axon Instruments), which also was used to acquire and analyze data on computer. The output of the switching circuit was continuously monitored during discontinuous single-electrode voltage clamp to ensure that adequate settling time for accurate voltage measurement was maintained. Current and transmembrane voltage were recorded in all experiments. We had previously confirmed 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. Cells were visualized with a closed-circuit television camera with interlace defeat and partial scan capability (Panasonic, model 1-GP-CD60), and were displayed on a video monitor (Hitachi Densi, model VM-1220C). Unloaded cell shortening was sampled at 120 Hz with a video edge detector (Crescent Electronics, Sandy, UT) coupled to the camera. Current, voltage, and contractions were digitized with a Labmaster analog-to-digital interface at 125 kHz (TL1–125, Axon Instruments) and stored on hard disk for subsequent analysis. Detailed descriptions of specific voltage-clamp protocols are provided in the appropriate results sections.

Data measurement and analyses. Current, voltage, and contraction were measured with pClamp analysis software. The magnitude of inward L-type Ca2+ current (I_L) was measured as the difference between the peak inward current and the reference point at the end of the voltage step. Li et al. (20) have demonstrated previously that inward current measured in this way provides an accurate measurement of the magnitude of I_L. Cell capacitance was estimated by integrating the capacitive transients with pCLAMP analysis software. Contraction was measured as the difference between the baseline preceding contraction and the peak of the contraction.

Differences between means were tested either with a Student’s t-test (with a Bonferroni correction for multiple comparisons) or with a two-way repeated-measures ANOVA. Post hoc comparisons were made with a Tukey’s Studentized range test. All statistical analyses were performed with SigmaStat (Jandel) or with SAS (SAS Institute). Nonlinear curve-fitting procedures were conducted with Sigmaplot (Jandel). Data are means ± SE. The value n represents the number of myocytes sampled; no more than two replicates (myocytes) were collected from cells from the same heart.

Sources of drugs and chemicals. Lidocaine, 4-AP, tetrodotoxin, and cadmium were purchased from Sigma Chemical (St. Louis, MO). Concentrated stock solutions of all drugs were dissolved in distilled water.

RESULTS

Electrophysiological and contractile properties of ventricular myocytes from normal and CM hamsters. Initially, conventional (non-voltage clamp) recordings were made to examine the characteristics of contractions initiated by action potentials in cells from normal and CM hearts. Figure 1 shows representative original recordings from normal (Fig. 1A) and CM (Fig. 1B) myocytes. Mean data are shown in Table 1. Resting membrane potentials (RMP) near −88 mV were recorded from both normal and CM myocytes (Table 1). Action potentials were characterized by a rapid upstroke, a marked first phase of repolarization followed by a relatively brief plateau at negative membrane potentials (Fig. 1, A and B). Action potential duration (APD) was not significantly different at −30 mV (APD30mV) or −80 mV (APD80mV) between myocytes from normal and CM hearts (Table 1). In contrast, mean peak amplitude of contraction recorded from CM myocytes was only 21% of the amplitude observed in normal myocytes (Table 1, P < 0.05). Although the
magnitude of contraction was depressed in CM myocytes, there were no significant differences in time to peak contraction, half-relaxation time, or cell length (Table 1).

Differences in EC coupling might be related to differences in electrical activity or to differences in subsequent events coupling electrical activity to contraction. In the present case, it is unlikely that the difference in contractions between normal and CM myocytes is related to changes in action potential configuration because we observed no significant differences in this parameter. To eliminate the effects of minor differences in action potential configuration, additional experiments were conducted under voltage-clamp conditions. Figure 1, C and D, shows representative recordings of membrane currents and contractions from normal and CM myocytes. Sodium currents and transient outward currents were inhibited with lidocaine (200 µM) and 4-AP (2 mM), respectively. A schematic of the voltage-clamp protocol is illustrated in Fig. 1, C and D. Each test step was preceded by a train of 10 conditioning pulses to 0 mV to provide a consistent history of regular activation. After the last conditioning pulse, the cell was repolarized to a postconditioning potential (VPC) of −60 mV for 300 ms. Then a test step to 0 mV was delivered to activate contraction under voltage-clamp conditions. Recordings of current are shown at top and contraction below (Fig. 1, C and D). Our results show that under voltage-clamp conditions the difference in contraction between normal and CM myocytes persisted. Mean peak contraction in CM myocytes was only 30% of the magnitude observed in normal cells (Table 1, P < 0.05). Time to peak and half-relaxation times were not significantly different under voltage-clamp conditions, despite the large change in peak contraction. Thus depression of contraction persisted in myocytes from CM hamsters when membrane potential was controlled by voltage-clamp. Furthermore, reduction in peak contraction is not likely caused by changes in the magnitude of I_{Ca,L}, because peak inward current was not significantly different (Table 1).

Initiation of contraction by the VSRM and CICR in hamster ventricular myocytes. These observations demonstrate that before the onset of hypertrophy and heart failure, myocytes from CM hamsters exhibit a defect in EC coupling. This defect might be related to changes in the VSRM, CICR, or both mechanisms of contraction. The VSRM has not previously been measured in hamster myocytes. Therefore, we first established whether a VSRM with characteristics similar to those described in other species (8, 17) is present in hamster ventricular myocytes. We used a voltage-clamp protocol which we have shown in previous studies with guinea pig and rat myocytes to separate CICR and VSRM components of EC coupling (8, 17). This protocol, shown in Fig. 2, utilized sequential test steps from −70 mV to −40

![Fig. 1. Electrophysiological and contractile recordings from normal and cardiomyopathic (CM) hamster myocytes. A: action potential (top) and contraction (bottom) recorded from a normal hamster ventricular myocyte. Action potentials were initiated by trains of 5 stimuli (1–4 nA) delivered at a frequency of 2 Hz. Recordings shown are average of 5 traces. B: representative action potential and contraction recorded from a CM myocyte. Action potential configuration appeared similar in normal and CM cells. However, magnitude of contractions was greatly reduced in CM cells compared with normal. C: recordings of membrane current (top) and contraction (bottom) under voltage-clamp conditions in a normal myocyte. Voltage-clamp protocol is above (inst). A 200-ms voltage step from −60 to 0 mV initiated both inward current and contraction. D: membrane current and contraction recorded from a CM myocyte. Contraction was reduced in amplitude although magnitude of inward current appeared similar in normal and CM myocytes. Mean data are tabulated in Table 1.](http://ajpheart.physiology.org/)

| Table 1. Electrophysiological and contractile properties of ventricular myocytes from normal and CM hearts |
|-----------------|-----------------|
|                 | Normal          | CM              |
| Action potentials and contractions (conventional recording) |                 |                 |
| RMP, mV         | −88.6±1.1       | −88.3±1.3       |
| APD_{30 mV}, ms | 37.1±7.2        | 42.1±11.6       |
| APD_{80 mV}, ms | 94.2±13.3       | 99.4±20.4       |
| Cell length, µm | 151±5.0         | 148±8.4         |
| Contraction amplitude, µm | 2.4±1.0 | 0.5±0.1*       |
| Time to peak contraction, ms | 39.8±4.1 | 36.5±2.2 |
| Half-relaxation time, ms | 25.8±2.4 | 27.8±1.9 |
| Inward current and contractions (voltage clamp) |                 |                 |
| I_{Ca,L}, pA/pF | −7.92±1.71      | −5.86±0.85      |
| Contraction amplitude, µm | 2.7±0.6    | 0.8±0.2*        |
| Time to peak contraction, ms | 39.3±5.8    | 29.1±2.8        |
| Half-relaxation time, ms | 25.0±2.2    | 24.4±3.5        |

Values are means ± SE; n = 12 normal and 16 cardiomyopathic (CM) myocytes/group. RMP, resting membrane potential; APD_{30 mV}, action potential duration at −30 mV; APD_{80 mV}, action potential duration at −80 mV; I_{Ca,L}, L-type Ca²⁺ current. *Significantly different from normal, P < 0.05.
then 0 mV to activate VSRM and CICR contractions respectively. Test steps were preceded by a series of 10 conditioning pulses to 0 mV, followed by a 4-s step to a $v_{PC}$ of $-70$ mV. During the 4-s interval, extracellular solution was changed rapidly at 37°C by a computer-controlled switching device. When myocytes were exposed to control solution, steps to $-40$ and 0 mV each elicited contractions (Fig. 2A). The step to 0 mV activated $I_{Ca,L}$, however, the step to $-40$ mV activated little inward current. Figure 2B shows the effects of a rapid change to solution containing 100 µM Cd$^{2+}$, 3 s in advance of the test steps. Cd$^{2+}$ had no effect on contraction activated by the step to $-40$ mV but strongly inhibited both current and contraction initiated by the step to 0 mV (Fig. 2B). These results closely resemble results previously shown for guinea pig and rat ventricular myocytes (8, 17); contractions activated by a step to $-40$ mV are initiated by the VSRM and are not affected by $I_{Ca,L}$ blockade, whereas contractions initiated by the step to 0 mV represent CICR and are abolished by inhibition of Ca$^{2+}$ current.

In guinea pig and rat ventricular myocytes, phasic VSRM contractions initiated by a step to $-40$ mV exhibit steady-state inactivation (17). In the present study, we determined the steady-state inactivation properties of VSRM contractions in hamster ventricular myocytes. The voltage-clamp protocol is shown schematically in Fig. 3A. The protocol utilized a train of 10 conditioning pulses followed by repolarization to a $v_{PC}$ for 600 ms. A test step to $-35$ mV was utilized to activate the VSRM. The test step was preceded by a 10-ms repolarization to $-65$ mV. Steady-state inactivation was assessed by changing the $v_{PC}$ in 5-mV steps from $-70$ to $-35$ mV. Representative recordings of contraction were obtained in normal myocytes.

![Fig. 2. Voltage-sensitive release mechanism (VSRM) contractions are resistant to blockade of L-type Ca$^{2+}$ current ($I_{Ca,L}$) by Cd$^{2+}$. Top inset: schematic of voltage-clamp protocol. A: representative traces of contraction (top) and membrane current (bottom) recorded from a normal hamster ventricular myocyte under control conditions. A voltage step to $-40$ mV elicited a VSRM contraction, and a second step to 0 mV elicited $I_{Ca,L}$ and a contraction. B: rapid application of 100 µM Cd$^{2+}$ 3 s in advance of activation steps strongly inhibited both $I_{Ca,L}$ and VSRM contraction but had no effect on VSRM contraction triggered by step to $-40$ mV. Data were recorded in presence of 50 µM tetrodotoxin and 2 mM 4-aminopyridine (4-AP) to inhibit sodium current and transient outward current, respectively. Similar results were obtained in 9 normal myocytes.](http://ajpheart.physiology.org/)

![Fig. 3. VSRM contractions in hamster ventricular myocytes exhibit steady-state inactivation. A: a schematic of voltage-clamp protocol. After last conditioning pulse, cells were repolarized to a postconditioning potential ($v_{PC}$) of $-35$ mV for 600 ms. The $v_{PC}$ was changed in 5-mV steps to potentials between $-70$ and $-35$ mV. After a 10-ms return to $-65$ mV, VSRM contractions were measured with a test step to $-35$ mV. B: representative recordings from a normal ventricular myocyte. Amplitude of contractions increased progressively as $v_{PC}$ was made more negative. C: a steady-state inactivation curve was constructed by plotting magnitude of contraction as a function of $v_{PC}$. Phasic VSRM contractions were fully available when steps were](http://ajpheart.physiology.org/)
made from −70 mV and almost completely inactivated at membrane potentials near −35 mV. The data in Fig. 3C were fitted with a Boltzmann function of the following form: $y = (a − b)/(1 + \exp[(v − v_i)/k]) + b$, where $a$ is maximum contraction, $b$ is minimum contraction, $v$ is the $V_{PC}$, $v_i$ is the half-inactivation voltage, and $k$ is the slope factor. In this example, from a normal hamster ventricular myocyte, $v_i$ and $k$ were −51.2 mV and 4.46 mV, respectively. These values are close to values reported previously for the VSRM in guinea pig and rat myocytes (9, 17). Thus the inactivation properties, negative activation voltage and $Cd^{2+}$ insensitivity indicate that a VSRM is operative in hamster ventricular myocytes.

Roles of the VSRM and CICR in contraction of CM hamster ventricular myocytes. We then determined whether contractions initiated by the VSRM and CICR differed between myocytes from normal and CM hearts. Figure 4 shows representative recordings of contractions and membrane currents. In Fig. 4A, the traces recorded from a normal myocyte demonstrate that sequential test steps to −40 and 0 mV activated VSRM and $I_{Ca,L}$ contractions, respectively. Figure 4B shows recordings from a CM myocyte. The magnitude of the VSRM contraction initiated by the step to −40 mV was much smaller in the CM myocyte. The contraction initiated by the step to 0 mV was also smaller in this example, but the relative difference was not as great as for the VSRM contraction. The magnitude of peak inward current with the step to 0 mV was slightly larger in the CM myocyte.

Mean data for contractions and currents from normal and CM myocytes are shown in Fig. 5. The data are from experiments conducted with the voltage-clamp protocol shown in Fig. 4. Figure 5A shows that VSRM contractions were markedly reduced in myocytes from CM hamsters compared with myocytes from normal hamsters ($P < 0.05$). The amplitude of contraction in CM cells was 33% of the contraction in normal myocytes. The mean data in Fig. 5B show that $I_{Ca,L}$ contractions also were reduced in CM cells, but only to 63% of the amplitude of myocytes in normal animals. This difference was not statistically significant. Figure 5, C and D, shows that there were no significant differences in inward current between normal and CM myocytes for either step.

Inactivation and activation properties of VSRM in normal and CM hamster myocytes. A possible explanation for the reduction in amplitude of VSRM contractions in CM cells would be a shift in the voltage dependence of steady-state inactivation. Therefore, we determined steady-state inactivation curves for the VSRM in myocytes from normal and CM hearts. Experiments were conducted with the voltage-clamp protocol described for Fig. 3. Figure 6 shows mean steady-state inactivation curves. The curves show that the maximum amplitude of VSRM contractions was significantly reduced in CM myocytes compared with normal (Fig. 6A). When the data were normalized as shown in Fig. 6B, it was clear that there was no shift in the steady-state inactivation properties. Curves were fitted with Boltzmann functions as described for Fig. 3. Mean values for $k$ were $4.16 \pm 0.49$ mV in normal myocytes and $4.65 \pm 1.1$ mV in CM myocytes ($n = 6–8$). Mean values for $v_i$ were $−49.9 \pm 1.3$ mV ($n = 8$) in normal and $−49.4 \pm 0.7$ mV in CM myocytes ($n = 6$). Neither $v_i$ nor $k$ values were significantly different between normal and CM myocytes. These data show that the change in magnitude of VSRM contractions in CM myocytes cannot be attributed to a change in steady-state inactivation properties.

Another possible mechanism for the reduction in amplitude of VSRM contractions in CM cells is a shift in the voltage dependence of activation of the VSRM. Activation of VSRM contractions occurs at more negative membrane potentials than $I_{Ca,L}$ contractions. Therefore, the initial rising phase of the contraction-voltage relationship is determined primarily by the VSRM. Figure 7 shows mean contraction-voltage relationships for normal and CM myocytes determined from a $V_{PC}$ of −60 mV. In normal myocytes, contractions were first observed with steps to −50 mV, increased to reach a peak at 0 mV and decreased only slightly at membrane potentials as positive as +80 mV (Fig. 7A). The magni-
tude of contractions was greatly reduced in CM myocytes \((P, 0.05)\), however, contraction first appeared and reached a maximum over the same range of membrane potentials as in normal myocytes (Fig. 7A). This suggests that there is little difference in voltage dependence of activation of the VSRM between normal and CM myocytes. Indeed, when contraction voltage relationships were normalized as shown in Fig. 7B, the curves for normal and CM myocytes were superimposable between \(-60\) and \(-20\) mV. These potentials include the range over which the VSRM activates.

The contraction-voltage relationships determined from a \(v_{PC}\) of \(-60\) mV include components of contraction initiated both by the VSRM and by CICR linked to \(I_{Ca,L}\). To evaluate the contribution of CICR alone, we also determined contraction-voltage relations from a \(v_{PC}\) of \(-40\) mV to inactivate the VSRM. Figure 8A shows that identical, bell-shaped contraction-voltage relations were observed in myocytes from normal and CM hearts. Figure 8B shows that normalized curves also were superimposable. These data demonstrate that the voltage dependence of activation of CICR contractions also is not altered in CM cells. Thus CICR does not contribute to the decrease in magnitude of contractions in curves determined from a \(v_{PC}\) of \(-60\) mV (Fig. 7A).

Figure 9 shows current-voltage (I-V) relations corresponding to the experiments shown in Figs. 7 and 8. Current data have been normalized to cell capacitance, which was not significantly different between the two groups \((capacitance = 172.2 \pm 7.6 \text{ pF}, 153.0 \pm 13.7 \text{ pF for } n = 11 \text{ normal and } n = 14 \text{ CM cells, respectively})\). Figure 9A shows that I-V relationships were identical in normal and CM myocytes when the \(v_{PC}\) was \(-60\) mV. I-V relationships also were identical when the \(v_{PC}\) was \(-40\) mV as shown in Fig. 9B. There was a small increase in inward current observed when I-V relations were determined from a \(v_{PC}\) of \(-60\) mV compared with a \(v_{PC}\) of \(-40\) mV. This current might represent T-type \(Ca^{2+}\) current and/or \(Na^{+}\)-\(Ca^{2+}\) exchange current in response to release of SR \(Ca^{2+}\). We assessed the magnitude of this current by subtracting I-V relations determined with a \(v_{PC}\) of \(-40\) mV from those determined with a \(v_{PC}\) of \(-60\) mV. Figure 9C shows that magnitude and voltage dependence of the difference current was the same in normal and CM cells.

The results shown in Figs. 7 and 8 demonstrate that the amplitudes of VSRM contractions, but not CICR contractions, are decreased in myocytes from CM hearts. This suggests that the defect in contraction observed when both mechanisms are available is mediated primarily by the VSRM. If this is correct, selective inhibition of the VSRM should eliminate the difference in contraction between myocytes from CM and normal hearts. We have shown previously that VSRM contractions are inhibited selectively when preceded by conditioning pulses to \(-40\) mV, rather than \(0\) mV (17). Both CICR and the VSRM contribute to contraction when contraction-voltage relations are determined from a \(v_{PC}\) of \(-60\) mV. We therefore determined the effects of
changing conditioning pulse voltage on these contraction-voltage relations in cells from normal and CM hearts. Figure 10 shows mean contraction-voltage and I-V relations. Figure 10A shows that contraction-voltage relations in normal cells were greatly reduced when conditioning pulse amplitude was changed from 0 mV to −40 mV (P < 0.05). In myocytes from CM hamsters (Fig. 10B), contractions with conditioning pulses to 0 mV were smaller than in normal myocytes. In addition, changing the conditioning pulse voltage to −40 mV had much less effect (Fig. 10B). Figure 10, A and B, also show that contraction amplitudes were almost identical in myocytes from normal and CM animals, when VSRM contractions were inhibited by conditioning pulses to −40 mV. Figure 10, C and D, show I-V relations for normal and CM myocytes, respectively. In myocytes from both normal and CM hearts, peak inward current was larger with conditioning pulses to −40 mV than 0 mV. For each conditioning pulse voltage, the magnitudes of currents were similar in normal and CM myocytes.

**DISCUSSION**

The objectives of this study were 1) to determine whether there is a defect in EC coupling in myocytes isolated from CM hamsters before the development of hypertrophy and failure, and 2) to evaluate whether the VSRM, CICR or both mechanisms of EC coupling contribute to this defect. The present investigation demonstrates that a defect in EC coupling is present in isolated CM myocytes at this early stage of the disease. Furthermore, this malfunction in EC coupling originates primarily from a defect in the VSRM.

Previous studies in whole heart and in isolated cardiac tissues have shown that contractility is depressed in CM hamster heart (3–5, 10, 11, 15, 22, 26, 28). Furthermore, several studies in tissues from young (80–85 day old) CM hamsters indicate that depression of contractility precedes the onset of hypertrophy and heart failure (3, 15). The results of our study demonstrate that a corresponding defect is present in isolated
myocytes from 80- to 90-day-old CM hamsters. Although contractions were depressed in myocytes from CM heart, we found no significant change in action potential configuration. Furthermore, the difference in contraction persisted even when possible changes in action potential duration were eliminated with voltage-clamp techniques. These results indicate that a defect in EC coupling contributes to the decrease in contractility observed in myocytes from CM hearts.

Both CICR and the VSRM contribute to EC coupling in myocytes from normal heart. Therefore, a defect in EC coupling in CM myocytes could occur as a result of changes in either one or both mechanisms. Our results showed that when the VSRM was inactivated by depolarization, the remaining CICR contractions were of similar magnitude in normal and CM myocytes. Contraction-voltage relations for CICR were bell-shaped and reached similar peak amplitudes in normal and CM myocytes. Thus CICR was virtually unchanged in myocytes from young CM hamsters compared with normal.

We also investigated the role of the VSRM in EC coupling in CM heart. Our results showed that, when the VSRM was inhibited by preceding test steps with a

Fig. 8. Mean contraction-voltage relations determined from a $V_{PC}$ of $-40$ mV in myocytes from normal and CM hearts. Voltage-clamp protocol was similar to that described in legend to Fig. 7, except that test steps were made from a $V_{PC}$ of $-40$ mV. A: contraction-voltage relations determined from a $V_{PC}$ of $-40$ mV were bell-shaped with a peak near 0 mV in both normal and CM myocytes. Contraction-voltage relations were virtually identical in normal and CM cells. B: contractions normalized to amplitude of contraction at 0 mV also were similar in normal and CM myocytes.

train of conditioning pulses to $-40$ mV (17), contractions were of similar magnitude in normal and CM cells. In contrast, when the VSRM was available, contractions were much smaller in CM cells than in normal cells. These observations indicate that a defect in the VSRM is responsible for the decrease in contraction in myocytes from CM hearts. Our results also showed that normalized activation and inactivation

Fig. 9. Mean current-voltage (I-V) relations for inward currents in normal and CM hearts. Data are from experiments illustrated in Figs. 7 and 8. A: I-V relations determined with test steps from a $V_{PC}$ of $-60$ mV. In both normal and CM myocytes, inward current first appeared near $-40$ mV, increased to a maximum near 0 mV, and declined with steps to more positive voltages. I-V relations determined in normal and CM cells were similar. B: I-V relations determined with test steps from a $V_{PC}$ of $-40$ mV also were not significantly different in normal and CM cells. C: currents determined from a $V_{PC}$ of $-60$ mV were subtracted from currents determined from a $V_{PC}$ of $-40$ mV to illustrate difference currents. Difference currents were also of similar magnitude in normal and CM myocytes. Data have been normalized to cell capacitance.
curves from the two groups were superimposable. Thus the reduction in magnitude of VSRM contractions was not due to changes in voltage-dependent activation or inactivation of the VSRM. These results further suggest that the defect in the VSRM must be related to a change in the efficacy of the VSRM as a trigger, rather than changes in activation or inactivation.

We previously have reported that the VSRM is regulated by the adenylate cyclase-protein kinase A cascade and also by calcium-calmodulin-dependent kinase in guinea pig ventricular myocytes (9, 29). Thus it is possible that the defect in the VSRM in CM hamster myocytes might represent a defect in phosphorylation through one or both of these pathways. Therefore, it will be important to investigate the role of these and other regulatory mechanisms in causing the decreased effectiveness of the VSRM as a trigger for contraction in CM heart.

In the present study we found that I_{Ca,L} was of similar magnitude in myocytes from normal and CM heart. Previous studies have reported either no difference (23) or a decrease (13, 20) in the magnitude of I_{Ca,L} in CM myocytes compared with normal myocytes. It is not clear why different observations have been reported in these studies. However, the studies reporting a decrease in current were conducted with Ca^{2+} as the charge carrier and with holding potentials of −40 or −50 mV. With the use of depolarized holding potentials intracellular Ca^{2+} levels are expected to increase. Myocytes from CM hamsters also have been reported to have elevated Ca^{2+} levels (24, 28). It is possible that Ca^{2+} inhibition of Ca^{2+} current may have been greater in CM hamster myocytes, especially when depolarized holding potentials were utilized. This may have contributed to reduction of I_{Ca,L} in these studies. The study of Sen et al. (24) used Ba^{2+} as the charge carrier that would be expected to eliminate Ca^{2+} inhibition of I_{Ca,L}. The present study utilized a holding potential of −80 mV, which also may have minimized elevation of cytosolic Ca^{2+} levels and thereby inhibition of I_{Ca,L}. In addition, other differences in experimental conditions may have contributed to differences in observations in these studies.

In this study we also observed an increase in inward current in I-V relations determined from a V_{PC} of −60 mV compared with I-V relations determined from a V_{PC} of −40 mV. This might represent Ca^{2+} current through T-type Ca^{2+} channels (21). Previous studies have reported that the magnitude of T-type Ca^{2+} current is increased in myocytes from 200- to 300-day-old hamster heart compared with normal (25) myocytes. In the present study, we found no increase in the magnitude of putative T-type Ca^{2+} current in cells from young, prehypertrophic normal and CM hearts. Thus our results suggest that changes in contractile function develop before an increase in T-type Ca^{2+} current. Furthermore, it is highly unlikely that T-type Ca^{2+} current contributes to activation of VSRM contractions.
in the heart. We have observed VSRM contractions in myocytes from rat heart (17) where T-type Ca$^{2+}$ current is absent (27). Furthermore, VSRM contractions are not abolished by Ni$^{2+}$ (14), at concentrations in excess of those required to inhibit T-type Ca$^{2+}$ current (27).

Although the central question in this paper was to assess the contribution of the VSRM to contractile dysfunction in CM hamster heart, this study also characterizes the VSRM in hamster ventricular myocytes. We found that the VSRM in hamster myocytes exhibited characteristics very similar to those described in rat and guinea pig ventricular myocytes in previous studies (8, 9, 17). VSRM contractions were not blocked by 100 µM Cd$^{2+}$, a concentration of Cd$^{2+}$ which blocked both $I_{Ca,L}$ and CICR contractions. Furthermore, VSRM contractions in hamster myocytes exhibited steady-state inactivation properties with $v_C$ values near −50 mV and $v_K$ values near 4 mV. These values are similar to those reported previously for the VSRM in guinea pig and rat myocytes (17). In addition, when the VSRM was available ($v_C = −40$ mV), contraction-voltage relations became sigmoidal with a threshold near −60 mV and a plateau near −10 mV as in guinea pig and rat ventricular myocytes (8, 17). Thus the properties of the VSRM are very similar in ventricular myocytes from different species.

In summary, the results of this study demonstrate that a defect in EC coupling is present in CM myocytes early in the development of disease. This abnormality in EC coupling is not due to a change in CICR but originates primarily from a defect in the VSRM component of contraction. This raises the possibility that defects in the VSRM might contribute to the development of cardiac hypertrophy and/or heart failure.

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