Altered E-C coupling in rat ventricular myocytes from failing hearts 6 wk after MI

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Received 7 June 1999; accepted in final form 2 February 2000

Wasserstrom, J. Andrew, Even Holt, Ivar Sjaastad, Per Kristian Lunde, Annlaug Ødegaard, and Ole M. Sejersted. Altered E-C coupling in rat ventricular myocytes from failing hearts 6 wk after MI. Am J Physiol Heart Circ Physiol 279: H798–H807, 2000.—Excitation-contraction (E-C) coupling was investigated in rat hearts 6 wk after induction of myocardial infarction (MI) by ligation of the left coronary artery. Heart weight was increased by 74% and left ventricular end-diastolic pressure was 23 ± 2 mm Hg in MI compared with 8 ± 2 mm Hg in sham-operated controls (Sham, P < 0.001). Cell shortening was measured in voltage-clamped myocytes at 36°C. In solutions where Cs+ had been replaced by K+, the voltage dependence of contraction was sigmoidal between −20 and +100 mV in Sham and MI cells. Verapamil (20 μM) blocked L-type Ca2+ current and reduced contraction in Sham cells by ∼50% (P < 0.01) but did not decrease contraction significantly in MI cells at test potentials above +10 mV. Verapamil-insensitive contractions were blocked by Ni2+ (5 mM). Na+/Ca2+ exchange current was doubled in MI compared with Sham cells at test potentials between −20 and +80 mV (P < 0.05), whereas mRNA and protein expression increased by 30–40%. Finally, voltage dependence of contraction was bell shaped in Na+-free solutions, but contraction was significantly increased in MI cells over a wider voltage range (P < 0.05). The insensitivity to Ca2+ channel block in MI cells may result from an increased contribution of the Nai/Ca+ exchanger to triggering of E-C coupling. These results suggest significant changes in E-C coupling in the hypertrophy and failure that develop in response to extensive MI.

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The development of congestive heart failure (CHF) is one of the most serious outcomes of myocardial infarction (MI). CHF is also the end stage of a variety of other conditions, such as hypertension, valvular disease, and cardiomyopathies almost invariably associated with cardiac hypertrophy. It is unclear whether hypertrophy and CHF are associated with reduced cell shortening. Most studies, some of which are based on quite heterogeneous human material, have demonstrated a decrease in the magnitude of contractions and intracellular Ca2+ transients as well as a slowing in time to peak and relaxation of the mechanical and Ca2+ signals (6, 11, 14, 15). However, these changes in mechanical function are most obvious at elevated Ca2+ concentrations and heart rates (11). Other in vitro studies of heart failure do not show reduced contractility, including one of myocardial strips from explanted human hearts (34) and another on isolated cells from rats with MI (1). The Ca2+ current (ICa) through L-type Ca2+ channels seems to be unchanged in failing human (5, 6) and pacing-induced hypertrophy and CHF in dog (31) myocytes. One might therefore speculate that the coupling between ICa and Ca2+ release from the sarcoplasmic reticulum (SR) is highly variable depending on the specific disease conditions and choice of experimental models. Indeed, a recent report suggests a reduced efficiency of the coupling between the sarcolemmal trigger and the Ca2+ release response of the SR during hypertrophy and CHF in a strain of rats with salt-sensitive hypertension (14).

Other mechanisms might also be involved in alterations in excitation-contraction (E-C) coupling in hypertrophy and CHF. It has been proposed that Na+/Ca2+ exchange, working in its reverse (Ca2+ influx) mode, can trigger Ca2+ release from the SR (4, 23, 27, 32). The sarclemmal Na+/Ca2+ exchange protein and mRNA have been reported to be upregulated in CHF (35, 40). In line with this, enhanced Na+/Ca2+ exchange activity was observed in a genetic model of cardiomyopathy (17). However, in an MI model in which direct electrophysiological measurements were used, it was reported that Na+/Ca2+ exchange current (INaCaX) is decreased in CHF (47).

To examine these possible alterations in E-C coupling, we investigated changes in the contribution of ICa to E-C coupling in a well-controlled model of hypertrophy and CHF after MI in normal Wistar rats. The extent of the MI was such that acute cardiac insuffi-
ciency was avoided, but already after 1 wk there were signs of hypertrophy and failure (41) closely mimicking the disease state in humans. Furthermore, experimental conditions were chosen to ensure that normal temperature and transmembrane K+ gradient were maintained, since recent results have indicated that lower temperatures and substitution of Cs+ for K+ might have direct influences on E-C coupling (25, 42).

METHODS

Induction of MI. Methods for induction of MI and hypertrophy have been published elsewhere (41), as have the characteristics of the resulting cardiac disease (38). Extensive MI was induced by ligature of the left coronary artery in male Wistar rats weighing ~300 g under halothane anesthesia according to a protocol approved by the Norwegian National Committee for Animal Care and Use conforming to the Norwegian Animal Welfare Act and National Institutes of Health guidelines. Sham animals were treated identically, except no ligature was placed on the left coronary artery. Animals were kept for 6 wk before isolation of myocytes, during which time they developed a well-defined and organized scar area. The surviving myocardium showed no inflammatory reaction or collagen deposit (38). At this time the animals were again anesthetized with halothane, and left ventricular end-diastolic pressure (LVEDP) was measured by means of a 2-F micromanometer-tipped catheter (model Ventriculat end-diastolic pressure (LVEDP) was measured by adding saxitoxin (3 μM, VNa = 40 mV) and lidocaine (150 μM, VCa = 40 mV). All experimental conditions used for measuring Ica gave similar results, so data were pooled. 3) ICaox was measured using a modification of the methods described by Zhang et al. (47) to increase the rate of reverse-mode exchange. The minor modifications included a normal Tyrode solution containing 5 mM Ca2+ and internal solution containing 25 mM Na+. In control experiments, extracellular Ca2+ concentration was 0.5 mM and intracellular Na+ was 0 mM to abolish reverse-mode Na+/Ca2+ exchange. Internal and external solutions were K− free (Cs− substitution). Verapamil (5 μM) and ryanodine (5 μM) were present in the external solution. Three prepulses to 0 mV were delivered for 50 ms at 1 Hz before activation of the test pulse (1,000 ms). Vt was −40 mV, and test potential (VT) ranged up to +80 mV. Current measurements were taken as the average of the last 300 ms of each VT step. Nickel chloride (10 mM) was added to the superfusate after completion of control recordings, and protocols were repeated to obtain Ni2+-sensitive current. 4) Contractions in the absence of Na+ were performed with the solutions described for the first type of experiments (Cs− free), but with tetramethylammonium replacing Na+ in the external and internal solutions.

Northern blot analysis of mRNA for the Na+/Ca2+ exchanger. Poly(A)+ RNA was extracted from homogenized left ventricular tissue from MI and Sham rats. The infarct area was carefully removed, together with the right ventricle and the atria, leaving only viable tissue from the left ventricle. Oligo(dT)-conjugated paramagnetic beads were used to extract poly(A)+ RNA according to the manufacturer’s instructions (Dynal, Oslo, Norway). The poly(A)+ RNA was size fractionated on a formaldehyde-agarose gel by use of 10 μg of poly(A)+ RNA per lane, transferred to a Biotrans nylon membrane (ICN Biomedicals), and hybridized at 42°C first for 3 days with a cDNA probe for the NCX (500 bp, kindly provided by Dr. K. D. Phillipson, University of California at Los Angeles) and after stripping for 1 day with a probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 1,300 bp, kindly provided by Dr. H. Prydz, University of Oslo, Norway). After hybridization, the Na+/Ca2+ exchanger and GAPDH membranes were washed four times at 35°C in 0.1× standard saline-sodium citrate (SSC)-0.1% SDS and 2× saline-sodium citrate-0.1% SDS at room temperature for 10
and 15 min and then washed twice for 15 min at 60°C. cDNA probes were randomly primed with [32P]dCTP and [32P]dATP, and stripping was carried out at 65°C for 1 h in a buffer containing 10 mM NaH2PO4 and 50% formamide at pH 6.5. The membranes were scanned and analyzed using PhosphorImager and ImageQuant software (both from Molecular Dynamics, Queensland, Australia). To estimate the Na+/Ca2+ exchanger mRNA tissue level, the Na+/Ca2+ exchanger transcript level-to-GAPDH transcript level ratios were calculated in each sample. For more precise quantification and to ensure linearity of transcript signal, a slot-blot analysis was carried out by applying 0.5, 1, and 2 μg of poly(A) RNA to a nylon membrane with use of a filtration manifold (Minifold II, Schleicher & Schuell, Dassel, Germany). The slot-blot membranes were subjected to the same procedure as the Northern membranes, and the Na+/Ca2+ exchanger transcript signal was normalized to the signal of an oligo(dT)18 probe (Eurogentec, Seraing, Belgium) for each sample.

Western blot analysis. A Western blot analysis was performed on a crude membrane fraction isolated from homogenates of left ventricles from Sham and MI hearts, with care taken to exclude the infarct area (16, 30, 38). Protein concentration in the membrane preparation was determined by a micro-bicinchoninic acid protein assay (Pierce Chemical, Rockford, IL) with BSA as standard. The proteins were size fractionated by SDS-PAGE on 8% polyacrylamide gels as described by Laemmli (22) and electrophoretically transferred to nitrocellulose membranes. After they were blocked with 10% nonfat dry milk in Tris-buffered saline with 0.1% Tween 20 (TBS-T buffer) for 1 h at room temperature, the membranes were incubated for 1 h with a rabbit anti-Na+/Ca2+ exchanger polyclonal antibody (code p11–13, Swant, Bellinzona, Switzerland) diluted 1:1,000 in blocking solution. The membranes were incubated with anti-rabbit IgG conjugated to horseradish peroxidase (catalog no. NA 934, Amersham, Oakalnd, ON, Canada) as secondary antibody. Again, repeated washing was carried out in TBS-T buffer. The immunoreactive bands were detected by the enhanced chemiluminescence method (RPN 2106, Amersham). The image was scanned and staining density was quantified with Image Quant.

Statistics. Values are means ± SE. Comparisons between means were made using Student’s t-test with the Bonferroni correction for multiple comparisons when appropriate. A minimum of three animals was used in each group. Comparisons of averaged ICa,L were accomplished with multivariate ANOVA followed by Scheffe’s test. Differences between sample means were considered significant when P < 0.05 unless specified otherwise.

RESULTS

Characteristics of the rat MI model. Six weeks after ligation of the left coronary artery, heart weight was 74% higher in MI than in Sham rats (Table 1). Heart weight-to-body weight ratios were also significantly increased, showing that a significant hypertrophy had developed in the remaining viable part of the left ventricle mainly localized to the septal region (Table 1). Left ventricular systolic pressure was significantly lower and LVEDP significantly higher in MI than in Sham rats. Together with an increased lung weight (data not shown), this indicates that the animals had developed CHF.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sham (n = 14)</th>
<th>MI (n = 24)</th>
<th>P</th>
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<tbody>
<tr>
<td>Body wt, g</td>
<td>386.1 ± 6.4</td>
<td>402.6 ± 7.2</td>
<td>NS</td>
</tr>
<tr>
<td>Heart wt, g</td>
<td>1.22 ± 0.05</td>
<td>2.12 ± 0.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart wt/body wt μg</td>
<td>3.16 ± 0.11</td>
<td>5.32 ± 0.32</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>117 ± 4</td>
<td>98 ± 3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>8 ± 1</td>
<td>23 ± 2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SE. Sham, sham-operated rats; MI, rats in which myocardial infarction had been induced 6 wk previously; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; NS, not significant.

Cells from MI rats were 14% longer than Sham cells, and cell capacitance was 32% larger in accordance with a 30% larger cell volume, as reported elsewhere (38) (Table 2). The within-group variation was about the same in the two groups. Cells from all 14 Sham and 24 MI rats were used in the patch-clamp experiments.

Voltage dependence of contraction in Sham and MI. The characteristics of contraction and changes in E-C coupling after MI were investigated under voltage-clamp conditions. Contractions were elicited by voltage steps to Vt of −30 to +100 mV from a V0 of −40 mV, at which cell length was stable and not different from the length at −80 mV throughout the 1-s period between the last prepulse and the test pulse. Maximum shortening was greater at a Vt of +80 than 0 mV by 47 ± 10 and 37 ± 11% in Sham and MI, respectively (P < 0.05 in both groups). This is exemplified by the two experiments shown in Fig. 1 (cf. A and C with B and D). The voltage dependence of contraction in each of these two voltages was shown in Fig. 1A and B. However, contractions were significantly slower in MI than in Sham, since time to peak contraction and time to 50% relaxation were increased at both Vt (Table 2).

Figure 1 also shows that, in the Sham and the MI cell at both Vt, a slowly decaying outward current was activated (see below).

Figure 2 shows the contractions at all voltages normalized to the contraction amplitude at 0 mV, the voltage at which peak ICa,L occurs. Contraction was activated at about −30 mV in the Sham cells, reached a maximum value at about +40 mV, and remained fairly constant up to +100 mV, giving a sigmoidal dependence of contraction on Vt (Fig. 2A). The voltage dependence of contraction was nearly identical in MI cells under the same control conditions (Fig. 2B).

Verapamil sensitivity of contraction. The effects of verapamil and Ni2+ on voltage dependence of contraction in Sham and MI cells are illustrated by the two experiments in Fig. 1. Verapamil (20 μM) reduced the contraction at a Vt of 0 mV by 66% and at +80 mV by 48% in a Sham cell (Fig. 1, A and B). Subsequent addition of Ni2+ (4 mM) abolished the remaining verapamil-insensitive contraction at both voltages. Exposure of an MI cell to verapamil reduced contraction by 26% at 0 mV and by 19% at +80 mV (Fig. 1, C and D).
In contrast, exposure to verapamil had only modest effects on cell shortening at all $V_t$ in MI cells. The verapamil-sensitive component of cell shortening is compared in Fig. 2C for Sham and MI myocytes. This drug produced a significant negative inotropic effect at nearly all $V_t$ above $-30$ mV in Sham cells. In contrast, the negative inotropic effect of verapamil in MI cells was not significant, except between $-20$ and $+10$ mV. Verapamil caused no significant change in shortening above and below this voltage range. These results demonstrate that cardiomyocytes isolated from the viable part of the left ventricle of rats with MI show a significantly diminished sensitivity to the effects of Ca$^{2+}$ current blockade compared with age-matched, sham-operated controls.

Effect of verapamil on $I_{Ca}$. The efficacy of Ca$^{2+}$ channel blockade by verapamil was confirmed in each cell type in a separate series of experiments. Figure 3A shows the effects of verapamil (20 μM) on Sham and MI cells. The $I_{Ca}$ density was nearly the same in the two cell types under control conditions. These values from rats are almost three times greater than in normal and failing human hearts (5, 31). Verapamil blocked inward current in both treatment groups, as shown by the two inset current traces ($V_t = 0$ mV) and by the summarized mean data for all $V_t$. Thus it is likely that $I_{Ca}$ was blocked to the same extent in both cell types, so that differential sensitivity to the Ca$^{2+}$ channel antagonist in the two cell types cannot underlie the resistance of MI cells to the negative inotropic effects produced in Sham cells.

Relationship between $I_{Ca}$ and contraction. Figure 3B summarizes the relationship between normalized current (from Fig. 3A) and normalized cell shortening under these conditions (data from Fig. 2, A and B).

<p>| Table 2. Characteristics of isolated cardiomyocytes from Sham and MI rats |
|-------------------------------|---|---|---|</p>
<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI</th>
<th>$P$</th>
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<tr>
<td>Cardiomyocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n$</td>
<td>13</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Resting length, μm</td>
<td>$121 \pm 3$</td>
<td>$138 \pm 7$</td>
<td>&lt;0.05</td>
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<tr>
<td>Capacitance, pF</td>
<td>$146.8 \pm 9.5$</td>
<td>$194.2 \pm 10.3$</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Shortening at 0 mV ($n$)</td>
<td>7</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Maximum shortening, %</td>
<td>$6.5 \pm 0.9$</td>
<td>$7.4 \pm 0.6$</td>
<td>NS</td>
</tr>
<tr>
<td>Time to peak, ms</td>
<td>$90.6 \pm 3.6$</td>
<td>$103.2 \pm 3.0$</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Time to 50% relaxation, ms</td>
<td>$31.0 \pm 1.8$</td>
<td>$36.1 \pm 1.1$</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Shortening at 80 mV ($n$)</td>
<td>7</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Maximum shortening, %</td>
<td>$8.5 \pm 0.9$</td>
<td>$10.4 \pm 0.5$</td>
<td>NS</td>
</tr>
<tr>
<td>Time to peak, ms</td>
<td>$94.8 \pm 4.6$</td>
<td>$114.9 \pm 4.5$</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Time to 50% relaxation, ms</td>
<td>$31.8 \pm 2.3$</td>
<td>$39.5 \pm 1.0$</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE.

Thus a high concentration of verapamil caused much less block of contraction at both $V_t$ in the MI than in the Sham cell. Again, exposure to Ni$^{2+}$ abolished the verapamil-insensitive component of contraction. Exposure to ryanodine (5 μM) blocked all verapamil-insensitive contraction in both cell types ($n = 5$; data not shown).

In both cell types, verapamil completely abolished the outward current at a $V_t$ of 0 mV (Fig. 1, A and C), whereas some outward current was still detectable at +80 mV (Fig. 1, B and D). It is possible that this verapamil-sensitive current might be a Ca$^{2+}$-activated K$^+$ current (19).

The effects of verapamil on the magnitude of contraction in Sham and MI cells over the full range of $V_t$ are summarized in Fig. 2, A and B, respectively. Exposure to verapamil blocked contraction by ~50% at all $V_t$ tested ($P < 0.05$ at all $V_t$ above $-30$ mV) in Sham cells.

Fig. 1. Transmembrane voltage, net ionic current, and cell shortening during depolarizations from a holding potential of $-40$ mV to test potentials ($V_t$) of 0 mV (A and C) and +80 mV (B and D) in a Sham cell (A and B) and in a cell 6 wk after myocardial infarction (MI, C and D) in control, in the presence of verapamil (20 μM), and after addition of Ni$^{2+}$ to the superfusate. Recordings were made 2 min after the addition of each drug. Inset: prepulse protocol used in all experiments unless otherwise indicated.
Fig. 2. Summary of voltage dependence of cell shortening in Sham (A, n = 7) and MI (B, n = 10) cells in the absence and presence of verapamil (20 μM). Cell shortening was normalized to 100% in control at 0 mV. C: verapamil-sensitive component of contraction in Sham (●) and MI (■) cardiomyocytes. In contrast to Sham cells, cell shortening in MI cells was not significantly different from zero, except in the voltage range of maximal Ca2+ current (*P < 0.05).

There was a close association between ICa and contraction amplitude over the voltage range of current activation (arrow) in the Sham cells until maximal current was reached (at 0 mV). Current declined with additional depolarization as the reversal potential for ICa was approached, but contraction continued to increase. Similar results were obtained in MI cells, demonstrating a close correlation between activation of ICa and contraction only up to the voltage range of peak current (~0 mV), above which current and contraction amplitudes became negatively correlated.

Measurements of INaCaX. One possible contributor to activation of contraction in the absence of ICa could be reverse-mode Na+/Ca2+ exchange. We measured INaCaX in Sham and MI cells to determine any changes induced by MI. Under control conditions, successive depolarization ranging from −40 to +100 mV caused a progressively larger outward current, as seen in a Sham cell in Fig. 4A. Addition of Ni2+ (10 mM) to the superfusate reduced the magnitude of the outward currents at all Vt (Fig. 4B). The Ni2+-sensitive current, which is a measure of INaCaX, is shown in Fig. 4C. When the identical protocol was applied to an MI cell (Fig. 4, D and E), INaCaX was greater in the MI cell, as evidenced by the magnitude of the Ni2+-sensitive current (Fig. 4F), whereas Ni2+-insensitive currents were nearly the same. In control experiments with 0 mM Na+ in the pipette, Na+/Ca2+ exchange was inactive and Ni2+ had no detectable effect on current in either cell type.

The results of nine experiments from each cell type are summarized and the magnitude of INaCaX in Sham and MI cells is compared in Fig. 5. There is nearly a doubling in current magnitude at all Vt above −20 mV (P < 0.05). These results demonstrate an increase in the amount of outward INaCaX, corresponding to Ca2+ influx, produced by Na+/Ca2+ exchange in MI cells compared with Sham controls under conditions that favor reverse-mode operation of the exchanger.

Quantification of the Na+/Ca2+ exchanger mRNA signal and protein. An important question is whether this increased outward INaCaX represents an increase in the exchanger protein or whether exchange kinetics are altered. Figure 6A shows a representative Northern blot. A strong signal was detected at 7 kb, and weaker signals appeared at ~4.7 and 1.5 kb in accordance with previous reports (9). When normalized against the GAPDH signal, the transcript signal from MI (n = 13) hearts exceeded the Sham (n = 7) signal by 30–40% (P < 0.05). Figure 6A also shows a strong transcript signal for the Na+/Ca2+ exchanger in the infarct area. Figure 6B shows the summarized results from a separate slot-blot analysis. Again, the signal from MI hearts (n = 13) exceeded that from the Sham hearts (n = 7, P < 0.05) by 37 ± 10%. The signal in the infarct area was more than twice as strong as in Sham hearts.

Figure 7A shows a representative Western blot prepared from a membrane fraction from whole hearts. Immunoreactive bands were detected at 120 and 160 kDa, as reported previously (8). Immunostaining with a specific antibody revealed an upregulation of the Na+/Ca2+ exchanger protein by 33.6 ± 10.8% (Fig. 7B). These results cannot explain the much larger observed increase in INaCaX and, therefore, suggest that some

Fig. 3. A: effects of verapamil on Ca2+ current measured in Cs+-containing internal and external solutions after three 50-ms prepulses to 0 mV at 1 Hz. Insets: original current recordings at Vt = 0 mV (vertical bar is 1 nA). Ca2+ current is shown under control conditions in Sham (n = 11) and MI (n = 7) and during exposure to verapamil (Vp). B: relationship between the magnitude of Ca2+ current (data from A) normalized to 100% at maximal current at 0 mV and cell shortening (data from Fig. 2, A and B). Arrow, direction of increasing depolarization.
other characteristics of exchange activity, such as kinetics, may be altered in MI hearts.

**Cell contractions in the absence of Na\(^+\).** To confirm that \(I_{\text{Ca}}\) can serve as an effective trigger for E-C coupling in the absence of Na\(^+\)/Ca\(^{2+}\) exchange, we performed a separate series of experiments in the absence of Na\(^+\)/Ca\(^{2+}\) exchange. Figure 8 shows the results of experiments performed in a Sham and MI cell in the absence of Na\(^+\). The magnitude of net outward current in the Na\(^+\)-free solutions increased with depolarization in the Sham cells; the magnitude of the outward current was dramatically increased at all \(V_t\) in the MI cells. The nature of this outward current was not examined further, but it could be a chloride current; K\(^+\) currents are reduced in cardiac hypertrophy and failure (7, 36), whereas there seems to be sustained activation of chloride channels (3, 10).

A voltage step to \(-20\) mV activated a phasic contraction that increased as \(V_t\) was raised to 0 mV but then declined at \(+30\) mV in Sham cells. Similar results were obtained in the MI cell, in that cell shortening increased to a maximum at 0 mV and then declined at \(+30\) mV.

The voltage dependence of contractions in the absence of Na\(^+\) is summarized over a wide voltage range for Sham and MI cells in Fig. 9A. Both demonstrate the

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**Fig. 4.** Na\(^+\)/Ca\(^{2+}\) exchange current (\(I_{\text{NaCaX}}\)) in Sham and MI cells. Currents were measured in Cs\(^+\)-containing solutions with stepping from a holding potential of \(-40\) mV to \(V_t\), ranging from \(-40\) to \(+80\) mV in a Sham (A–C) and an MI (D–F) cell. Test potentials were applied after 3 conditioning pulses to 0 mV (50 ms). Currents were recorded before (A and D) and after (B and E) exposure to Ni\(^{2+}\) (10 mM), and Ni\(^{2+}\)-sensitive currents were calculated (C and F).

**Fig. 5.** Summary of \(I_{\text{NaCaX}}\) measurements in Sham and MI cells. Ni\(^{2+}\)-sensitive currents normalized for cell capacitance are summarized for all voltages in Sham (\(n = 9\)) and MI (\(n = 9\)) cells (\(\ast P < 0.05\), for each data point). Open symbols show corresponding control measurements made in the absence of \(I_{\text{NaCaX}}\) (0 mM intracellular Na\(^+\) and 0.5 mM extracellular Ca\(^{2+}\)).

**Fig. 6.** Northern blot of Na\(^+\)/Ca\(^{2+}\) exchange (NCX) expression in Sham and MI hearts. A: mRNA was isolated from the tissue surviving the infarct (MI) and from the infarct area (IA). GAPDH, glycer-aldehyde-3-phosphate dehydrogenase; SH, Sham. B: quantitative slot-blot results. mRNA (0.5, 1.0, and 2.0 pg) extracted from 7 Sham and 13 MI hearts and 4 infarct areas were analyzed.
typical bell shape normally ascribed to the triggering of contraction via Ca\textsuperscript{2+} influx through Ca\textsuperscript{2+} channels; the threshold for activation of contraction coincides with activation of inward $I_{\text{Ca}}$ at $-30 \text{ mV}$, maximal contraction and inward current were achieved at $-0 \text{ mV}$, and further depolarization caused diminished contraction as the apparent reversal potential for $I_{\text{Ca}}$ was approached (at about $+60 \text{ mV}$). Fractional contraction was not significantly different in MI and Sham cells at 0 mV (5.0 $\pm$ 1 and 4.6 $\pm$ 1% in Sham and MI, respectively). In contrast to Na\textsuperscript{+}-containing solutions, verapamil blocked the contraction at all $V_t$ (open symbols). Thus Sham and MI cell types demonstrate typical triggering of E-C coupling as a result of activation of L-type Ca\textsuperscript{2+} channels. However, a closer examination of the voltage dependencies of contraction reveals subtle but important differences in the coupling between $I_{\text{Ca}}$ and contraction in the MI cells; shortening is greater at $-20$, $+30$, and $+40 \text{ mV}$ in the MI than in the Sham cells. The result is that nearly maximal activation occurs over a broader range of $V_t$, from $-20$ to $+30 \text{ mV}$, in the MI compared with the narrow peak of activation in Sham cells ($-10$ to $+10 \text{ mV}$). These results demonstrate that the coupling between $I_{\text{Ca}}$ and SR Ca\textsuperscript{2+} release is not suppressed in MI under our experimental conditions. On the contrary, there was a greater contraction over a wider range of potentials positive and negative to the peak at 0 mV, which might compensate for the other alterations in shortening activation and relaxation kinetics.

DISCUSSION

We found that, in a model of experimentally induced MI with resulting hypertrophy and failure in rat
hearts, isolated ventricular myocytes surviving the infarct show altered structure, physiological function, and pharmacological responses. Most importantly, the sensitivity of contraction to L-type \( \text{Ca}^{2+} \)-channel blockade with verapamil was greatly reduced in MI compared with Sham cells, and the verapamil-insensitive contractions were blocked by \( \text{Ni}^{2+} \). Finally, there was an increased \( I_{\text{NaCaX}} \), which might be responsible for a more prominent role of reverse-mode \( \text{Na}^{+}/\text{Ca}^{2+} \) exchange in triggering contraction in MI, thus explaining the relative insensitivity of MI cells to the effects of verapamil.

Voltage dependence of E-C coupling after MI. E-C coupling has been studied extensively in isolated cardiac myocytes and has revealed that \( \text{Ca}^{2+} \)-induced \( \text{Ca}^{2+} \) release is activated by \( \text{Ca}^{2+} \) influx through the L-type \( \text{Ca}^{2+} \) channel (2, 44). The resulting voltage dependence demonstrates a clear reliance of E-C coupling on \( I_{\text{Ca}} \) giving a characteristic bell shape in which intracellular \( \text{Ca}^{2+} \) transients and contraction follow the voltage dependence of \( I_{\text{Ca}} \). Thus it is somewhat surprising that contractions from Sham and MI cardiomyocytes demonstrate a sigmoidal dependence on \( V_c \). The explanation for this result is likely to derive from recent reports suggesting that voltage-dependent activation of the reverse (\( \text{Ca}^{2+} \) influx) mode of \( \text{Na}^{+}/\text{Ca}^{2+} \) exchange might contribute to triggering in a number of mammalian species including the rat (21, 23, 24, 26, 42). However, in normal cardiomyocytes the importance of the reverse-mode exchange for normal contraction has been questioned (39). The difference between these and many other reports demonstrating an exclusive reliance of activation on \( I_{\text{Ca}} \) is most likely the result of differences in experimental conditions; substitution of the primary physiological monovalent cation \( \text{K}^+ \) with \( \text{Cs}^+ \) is usually used to block \( \text{K}^+ \) currents and thus isolate \( I_{\text{Ca}} \). However, low temperatures and \( \text{Cs}^+ \) replacement appear to suppress the apparent contribution of the exchanger to activation of contraction (25, 42, 43). The net result is that when the normal \( \text{K}^+ \) gradient and physiological temperatures are used, the voltage dependence of contraction is indeed sigmoidal. The present study reveals that this is true for Sham and MI cells, suggesting that the exchanger contributes to triggering in normal myocytes and in myocytes surviving MI.

On the other hand, we also confirmed that there was clearly a direct reliance of contraction on \( I_{\text{Ca}} \) when there was no \( \text{Na}^+ \) in internal or external solutions; indeed, all contraction was blocked by verapamil in Sham and MI cells under these conditions. This experiment serves as an important control for the effects of verapamil; if some minor fraction of \( I_{\text{Ca}} \) remained unblocked by verapamil, it is possible that even a small \( \text{Ca}^{2+} \) influx could be sufficient to activate significant contraction, which might subsequently be blocked completely by the addition of \( \text{Ni}^{2+} \). This was clearly not the case, as demonstrated by complete block of contractions by verapamil when \( I_{\text{Ca}} \)-activated contractions were isolated in \( \text{Na}^+ \)-free solutions.

These experiments also revealed subtle differences between Sham and MI, in that there was a greater contraction across a wider voltage range in the MI than in the Sham cells, despite similar peak shortening. Under conditions where \( \text{Na}^+/\text{Ca}^{2+} \) exchange is inoperative, we do not see the suppression of E-C coupling that was reported recently by Gomez et al. (14). The differences in results are likely to arise from differences in experimental conditions, including substitution of intracellular \( \text{Cs}^+ \) vs. intracellular \( \text{K}^+ \) and temperature. Furthermore, Gomez et al. used a salt-sensitive rat strain that develops hypertension and concentric ventricular hypertrophy and another inbred rat strain that develops signs of heart failure. In our rat model, echocardiography revealed significant myocardial failure at LVEDP > 15 mmHg, as judged by the reduced velocity of shortening of the surviving posterior wall of the left ventricle (unpublished results).

Altered sensitivity to verapamil in MI cells. The most striking observation in this study was that MI cells showed reduced sensitivity to block of contraction by verapamil compared with Sham cells, despite the demonstration that the same concentration of verapamil was highly effective in blocking \( I_{\text{Ca}} \) in both cell types. Our observation that Sham cells showed a ~50% decrease in contraction with verapamil over most of the voltage range tested is consistent with a previous finding that nifedipine blocked contraction to the same extent in normal rat myocytes under similar experimental conditions (42). Other reports have demonstrated that, in addition to verapamil, \( I_{\text{Ca}} \) block with nifedipine and cadmium had similar effects to reduce contraction in cat and guinea pig myocytes (24, 43). In contrast, we found that verapamil caused only a modest but not statistically significant negative inotropic effect in MI cells, except in the voltage range where \( I_{\text{Ca}} \) was maximal, suggesting that E-C coupling in MI cells is less sensitive to \( I_{\text{Ca}} \) block by verapamil than in Sham cells. In all cases, the remaining contraction was also blocked by \( \text{Ni}^{2+} \), suggesting a common mechanism underlying contractions activated in the absence of functional L-type \( \text{Ca}^{2+} \) channels.

Changes in \( I_{\text{NaCaX}} \) after MI. There have been several recent but conflicting reports of measurements of \( I_{\text{NaCaX}} \) in cells isolated from animal models of hypertrophy and CHF. One recent report found a decrease in \( I_{\text{NaCaX}} \) in a similar model of MI in rat heart (47). The cells were studied 3 wk after the MI, selection of animals was not made on the basis of LVEDP, the cellular hypertrophy was significantly less than in the present study, and the cells were studied at a lower temperature. Thus the data are not directly comparable to the present study. Litwin and Bridge (28) found that \( I_{\text{NaCaX}} \) was increased in a model of MI in rabbit heart. In addition, O’Rourke and co-workers (33, 46) found that a 28% reduction in sarco(endo)plasmic reticulum \( \text{Ca}^{2+}-\text{ATPase} \) subunit 2a (SERCA2a) and phospholamban in the pacing-induced model of heart failure in dog was accompanied by an increase in \( \text{Na}^+/\text{Ca}^{2+} \) exchange protein of 100%. Schwinger et al. (37) recently reported no change in exchanger protein lev-
els, despite evidence of an increase in activity in failing human heart (35).

We found a near doubling in $I_{\text{NaCaX}}$ in the rat model of MI under our experimental conditions. This was accompanied by a modest (30–40%) upregulation of exchanger mRNA and protein levels. We carefully selected viable tissue from the left ventricle for analysis, which was important since there was a strong upregulation of the $\text{Na}^+$/Ca$^{2+}$ exchanger signal in the scar tissue. Thus it appears that species differences or differences in experimental model of CHF cause a similar final outcome via different mechanisms. In the rat MI model, our observations suggest that the exchange rate is increased, although the mechanism for this increase is not clear from the present results but may involve altered regulation of the exchanger in addition to a small increase in protein expression. In either model, however, it is not at all surprising that the failing heart might increase the capacity for Ca$^{2+}$ removal from the cytoplasm via the exchanger in response to a diminished SR uptake rate (33, 46). Thus the increase in $\text{Na}^+$/Ca$^{2+}$ exchange, however it is achieved, may ultimately prove to be a compensatory mechanism to overcome a defect in cytoplasmic Ca$^{2+}$ removal.

Such a compensatory response of increasing $\text{Na}^+$/Ca$^{2+}$ exchange rate might also explain the observation that MI cells have a reduced sensitivity to $I_{\text{Ca}}$ blockade. Because the characteristics of $I_{\text{Ca}}$ are unchanged, as is its ability to trigger contraction in the absence of a contribution of the exchanger (0 mM Na$^+$ conditions), there may simply be a greater contribution of the exchanger to serve as a more effective trigger in MI than in Sham cells. An increased role of the $\text{Na}^+$/Ca$^{2+}$ exchanger, particularly in relaxation, was also recently reported on the basis of experiments on cardiomyocytes from human failing hearts (12, 13). Estimated equilibrium potential for $\text{Na}^+$/Ca$^{2+}$ exchange is $-10$ mV under these experimental conditions (with the assumption of an internal Ca$^{2+}$ concentration of 100 nM). Finally, the fact that all remaining verapamil-insensitive contraction was blocked by Ni$^{2+}$ supports this idea, since Ni$^{2+}$ is an effective blocker of the exchanger in this concentration range (20, 45).

In conclusion, the present results demonstrate the following: 1) Two mechanisms appear to participate in the activation of contraction in Sham and MI cells. Both cause an influx of Ca$^{2+}$ that is capable of triggering Ca$^{2+}$-induced release of Ca$^{2+}$ from stores in the SR and resulting in contraction. 2) One mechanism is the influx of Ca$^{2+}$ by voltage-gated, L-type Ca$^{2+}$ channels, which is blocked by organic and inorganic (divalent cation) antagonists. 3) A second mechanism also appears to be involved in triggering E-C coupling in Sham and MI cells that may involve reverse-mode $\text{Na}^+$/Ca$^{2+}$ exchange, which is activated by depolarization and blocked by Ni$^{2+}$. 4) The relative contributions of the two mechanisms to E-C coupling are altered in hypertrophied cells surviving MI where $I_{\text{NaCaX}}$ is nearly doubled, thus increasing the potential efficacy of this mechanism to trigger contraction. 5) The doubling of $I_{\text{NaCaX}}$ was much greater than the increase of exchanger protein content, suggesting changes in the regulation of exchange kinetics in MI.

The involvement of these mechanisms in the triggering of contraction in normal and diseased myocardium remains to be clarified, as does their relative contribution to normal and abnormal E-C coupling. One intriguing suggestion is that the two mechanisms may sum in a nonlinear fashion because of the sigmoidal relationship between the open probability of the SR Ca$^{2+}$ release channel and changes in internal Ca$^{2+}$, such that the two triggers may act synergistically in the modulation of SR release (29). It must also be considered that there may be pronounced differences in phenotypes between rat and human models of MI and heart failure. Even so, this study points to the considerable clinical importance of determining whether the increased activity of the exchanger and the concomitant increased role in E-C coupling in MI cells is the result of MI, hypertrophy, or CHF or some critical combination of these clinically relevant conditions. In addition to its contribution to E-C coupling, the potential for an increased $I_{\text{NaCaX}}$ contribution to inward current and, therefore, to arrhythmogenesis after MI indicates another important consequence of the upregulation in exchanger activity found in this disease model.

We thank Bjørn Amundsen, Morten Eriksen, Unni Lie Henriksen, Bjørn Kristiansen, Severin Leraand, Thea S. Solum, and Gerd Torgersen for expert technical assistance.

This study was supported by the Research Council of Norway, Anders Jahre’s Fund for Promotion of Science, the Laerdal Foundation, Rakel and Otto C. Bruun’s Fund, and National Heart, Lung, and Blood Institute Grant HL-30724 (to J. A. Wasserstrom).

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