Heart Failure After Myocardial Infarction
Altered Excitation-Contraction Coupling

A.M. Gómez, PhD; S. Guatimosim, PhD; K.W. Dilly, PhD; G. Vassort, PhD; W.J. Lederer, MD, PhD

Background—Heart failure (HF) frequently follows the occurrence of myocardial infarction (MI). Questions about how HF develops and what cellular defects contribute to this dysfunction led to this study.

Methods and Results—MI was induced in rats by coronary artery ligation. Clinical examination of the post-MI (PMI) surviving animals indicated that they were in overt HF by all measures. Cellular examination of the cardiomyocytes by patch-clamp and confocal [Ca$^{2+}$], imaging methods indicated that cellular function was significantly compromised. At the single-cell level, [Ca$^{2+}$], transient amplitudes were reduced and contractions were decreased and slowed, although Ca$^{2+}$ current ($I_{Ca}$) remained unchanged. The excitation-contraction coupling (ECC) gain function measured as $\Delta$[Ca$^{2+}$]/$I_{Ca}$, was significantly decreased. Ouabain, a cardiotonic steroid that blocks the Na$^+$-K$^+$-ATPase and activates Ca$^{2+}$ entry via cardiac Na$^+$ channels, largely alleviated this defect.

Conclusions—After MI, $I_{Ca}$ becomes less able to trigger release of Ca$^{2+}$ from the sarcoplasmic reticulum. This failure of ECC is a major factor contributing to the development of contractile dysfunction and HF in PMI animals. The improved ECC gain, enhanced Ca$^{2+}$ entry, and augmented Ca$^{2+}$ signaling due to cardiotonic steroids contribute to the beneficial effects of these agents. (Circulation. 2001;104:688-693.)

Key Words: excitation □ myocardial infarction □ ouabain □ heart failure □ calcium
Signaling and Contractile Dysfunction Post-MI

Results

MI produces a dramatic change in cardiac output and may be lethal. In the animal model used here, ∼40% of the animals died during the first 2 days. We waited 6 months because in our hands, this is the time when consistent HF is presented in PMI rats. Visual inspection revealed large scars on the left ventricular free wall. Table 1 characterizes the cardiovascular properties of the surviving animals, indicating that PMI animals were suffering from HF. Table 2 shows that there was cellular hypertrophy.

Cellular Alterations

To investigate whether excitation-contraction coupling (ECC) was altered after MI, isolated cardiomyocytes were examined by electrical and optical methods. A loading protocol (see Methods) was used to normalize SR Ca^{2+} content. The similarity of the SR Ca^{2+} load was verified by a caffeine-release method. This procedure was carried out because SR Ca^{2+}-ATPase (SERCA) levels are decreased in MI and glucose 5.5; pH 7.4. Internal solution was the same as for caffeine-release method. This procedure was carried out, 0.05 was assumed to be statistically significant.

Statistics

Data are presented as mean±SEM. Two-sample comparison was made by paired or independent t test, as appropriate. A level of P<0.05 was assumed to be statistically significant.

BW indicates body weight; HW, heart weight; SBP, systolic blood pressure; EDP, end-diastolic pressure; and FS, fractional shortening.

*P<0.05; † P<0.001.

Average data are plotted as a function of voltage in Figure 2. The decreases in cell shortening (Figure 2A) and [Ca^{2+}], transient (Figure 2B) observed for PMI cells are statistically significant compared with control cells over a wide range of membrane potentials. I_{Ca} density was maintained (Figure 2C), however, suggesting that changes in I_{Ca} density cannot account for the contractile dysfunction observed in PMI cells. Additional explanations include the “myosin isoform shift hypothesis” and the “Ca^{2+}-signaling hypothesis” (Figure 2D and Discussion).

The reduction of the [Ca^{2+}], transient for PMI cells shown in Figure 2 would not have been predicted if the only significant cause of the contractile dysfunction were changes in the contractile proteins. The Ca^{2+}-signaling hypothesis is consistent with the data in Figure 2 but requires further testing. To investigate the importance of Ca^{2+} signaling, an inotropic agent was applied. The cardiotoxic steroid ouabain was chosen because it has no known direct actions on contractile proteins and has established actions on Ca^{2+} signaling (see below). Figure 1C shows records obtained in the same PMI myocyte as displayed in Figure 1B, 1 minute after 100 mmol/L ouabain application. Ouabain clearly increased the [Ca^{2+}], transient and contraction. Averaged data obtained from PMI myocytes after ouabain perfusion are shown in Figure 2. Cell shortening and [Ca^{2+}], transients were restored to normal over voltage steps negative to +20 mV. No significant changes in I_{Ca} density were seen.

**TABLE 1. Animal Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>BW, g</th>
<th>HW, g</th>
<th>HW/BW, mg/g</th>
<th>SBP, mm Hg</th>
<th>EDP, mm Hg</th>
<th>FS, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats</td>
<td>559.7±16.4</td>
<td>2.2±0.2</td>
<td>4.0±0.2</td>
<td>111.2±3.7</td>
<td>2.5±0.5</td>
<td>47.9±4.2</td>
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<tr>
<td>(n=5)</td>
<td>(n=5)</td>
<td>(n=5)</td>
<td>(n=5)</td>
<td>(n=9)</td>
<td>(n=5)</td>
<td></td>
</tr>
<tr>
<td>PMI rats</td>
<td>564.4±12.6</td>
<td>2.8±0.2*</td>
<td>5.0±0.3*</td>
<td>109.0±3.5</td>
<td>17.1±1.3*</td>
<td>20.6±1.8†</td>
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<tr>
<td>(n=11)</td>
<td>(n=11)</td>
<td>(n=11)</td>
<td>(n=9)</td>
<td>(n=9)</td>
<td>(n=8)</td>
<td></td>
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</tbody>
</table>

BW indicates body weight; HW, heart weight; SBP, systolic blood pressure; EDP, end-diastolic pressure; and FS, fractional shortening.

*P<0.05; † P<0.001.

<table>
<thead>
<tr>
<th></th>
<th>C, pF</th>
<th>RP, mV</th>
<th>APD_{20}, ms</th>
<th>APD_{50}, ms</th>
<th>APD_{90}, ms</th>
</tr>
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<tr>
<td>Control cells</td>
<td>134.7±8.6</td>
<td>−77.9±2.6</td>
<td>2.6±0.3</td>
<td>7.1±0.5</td>
<td>29.7±4.9</td>
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<td>(n=28)</td>
<td>(n=7)</td>
<td>(n=7)</td>
<td>(n=7)</td>
<td>(n=7)</td>
<td></td>
</tr>
<tr>
<td>PMI cells</td>
<td>189.7±10.9*</td>
<td>−77.5±1.2</td>
<td>20.4±4.1*</td>
<td>52.3±10.8*</td>
<td>134.7±20.4*</td>
</tr>
<tr>
<td>(n=51)</td>
<td>(n=11)</td>
<td>(n=11)</td>
<td>(n=11)</td>
<td>(n=11)</td>
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</tr>
</tbody>
</table>

C indicates membrane capacitance; RP, resting potential; and APD_{20}, APD_{50}, and APD_{90}, APD at 20%, 50%, and 90% of repolarization, respectively.

*P<0.005.
The efficacy of $I_{Ca}$ to activate SR Ca$^{2+}$ release is measured as the ECC gain: [Ca$^{2+}$], transient normalized to $I_{Ca}$, [(F/F0)/$I_{Ca}$] (Figure 2D). It is best to measure gain at fairly negative potentials because the opening of a single L-type Ca$^{2+}$ channel at these negative potentials can trigger a single Ca$^{2+}$ spark.\textsuperscript{13} The decrease in gain seen in PMI cells was restored by ouabain application.

Because the decrease in gain appears to play a role in the contractile dysfunction in PMI cells, we were interested in determining why the [Ca$^{2+}$], transient was reduced. The Ca$^{2+}$ influx ($I_{Ca}$) activates Ca$^{2+}$ sparks that sum to produce the [Ca$^{2+}$], transient.\textsuperscript{14} To investigate whether alterations in Ca$^{2+}$-spark properties may lead to the reduction of [Ca$^{2+}$], transient, Ca$^{2+}$ sparks were analyzed. Signal-averaged Ca$^{2+}$ sparks from control and PMI cells look alike (Figure 3A and 3B). They have similar magnitudes (F/F0: 1.70 $\pm$ 0.02 versus 1.72 $\pm$ 0.02 in 141 Ca$^{2+}$ sparks in control versus 338 Ca$^{2+}$ sparks in PMI myocytes) and decay kinetics ($\tau$, obtained by fitting the decay phase to a single exponential: 21.87 $\pm$ 1.30 versus 24.20 $\pm$ 0.85 ms in control versus PMI Ca$^{2+}$ sparks). Counting the number of Ca$^{2+}$ sparks triggered at different membrane potentials in the presence of a submaximal concentration of nifedipine\textsuperscript{13} revealed that fewer Ca$^{2+}$ sparks were activated in PMI cells even though $I_{Ca}$ was similar. Because Ca$^{2+}$-spark characteristics in control and PMI cells are similar, these results suggest that the reduction in [Ca$^{2+}$], transient is due to a decreased ability of Ca$^{2+}$ current to trigger Ca$^{2+}$ sparks.

Changes in contractile protein isoforms or properties could also contribute to the observed dysfunction. To investigate this, the relationships between the [Ca$^{2+}$], transient and cell shortening were examined at different voltages (Figure 4A). The shapes of these plots (phase-plane loops) reflect the kinetic response of the contractile proteins to changes in [Ca$^{2+}$],.\textsuperscript{15} During the rapid [Ca$^{2+}$], rise, there is little contractile response. Contraction begins in earnest after [Ca$^{2+}$], is near its peak and continues even as [Ca$^{2+}$] falls. Finally, both [Ca$^{2+}$] and contraction decline together. [Ca$^{2+}$], transient reduction in PMI cells leads to small contraction and different loop trajectory (Figure 4B) that is restored back to control by ouabain (Figure 4C). There is, however, a small residual rounding of these trajectories that may reflect alterations in the contractile proteins (see Discussion). Figure 4D shows plots of peak contraction versus peak [Ca$^{2+}$], transient and is consistent with data shown in Figure 4A through 4C.

In vivo, the decreases of [Ca$^{2+}$], transients in PMI cells will be influenced by changes in action potential duration (APD), which is prolonged in HF. APs were recorded in control and PMI myocytes and used to control voltage. Figure 5 shows that the PMI cells had smaller contractions and [Ca$^{2+}$], transients than control cells despite their longer APD. In addition, ouabain increased both contraction and [Ca$^{2+}$], transient to normal. Blockade of Na$^+$ channel by tetrodotoxin (TTX) (10 $\mu$mol/L) reduced the [Ca$^{2+}$], transient back to the level of PMI cells before the ouabain addition (Figure 5B). Importantly, Santana et al\textsuperscript{16} showed in normal rat cells that TTX has no immediate effect on the [Ca$^{2+}$], transient in the absence of ouabain, that ouabain acutely increases the [Ca$^{2+}$], transient, and that TTX can block the immediate ouabain-dependent increase in the [Ca$^{2+}$], transient.

**Discussion**

Changes that contribute to contractile dysfunction in ventricular myocytes after MI were examined in this article. The reduction of SERCA$^{2,12}$ must play an important role in the altered Ca$^{2+}$ signaling observed in this model of HF. Data presented here, however, suggest that when SR Ca$^{2+}$ content is normalized, PMI cells still present a significant defect in Ca$^{2+}$ signaling: the ability of Ca$^{2+}$ influx (through L-type Ca$^{2+}$ channels) to trigger SR Ca$^{2+}$ release is reduced. Under our experimental conditions, changes in contractile proteins\textsuperscript{4} do not appear to be a major factor. The importance of defective Ca$^{2+}$ signaling in the cellular pathophysiology of

Figure 1. Contractile dysfunction examined in single cardiac myocytes after MI. A, Representative signals recorded from control cell. Top to bottom: Voltage protocol, $I_{Ca}$ trace, shortening, [Ca$^{2+}$], transient, and line-scan image. Bottom of image, reveals contraction. Ca$^{2+}$ signal is shown as fluorescence ratio (F/F0), with fluorescence signal (F) being normalized to signal before depolarization (F0). B, Data from PMI cell displayed as in A. C, Data from same PMI cell as in B, 1 minute after 100 nmol/L ouabain application.

Figure 2. Ca$^{2+}$ signaling after MI. A, Voltage dependence of unloaded cell shortening in control (○, n = 11), PMI (●, n = 26), and PMI after 100 nmol/L ouabain application (■, n = 9). Lines are drawn by eye. B, Peak [Ca$^{2+}$], transient (F/F0) plotted against voltage for control, PMI, and PMI after ouabain application. C, Current-voltage relationship for control, PMI, and PMI after ouabain application. D, ECC gain function [(F/F0)/$I_{Ca}$] measured at $\approx$ 30 mV under 3 conditions: control (open bar), PMI (solid bar), and PMI + ouabain 100 nmol/L (hatched bar). *P<0.05, **P<0.005 vs control myocytes.
PMI myocytes shown here has many implications for medical therapy and challenges our understanding of HF.

The contractile dysfunction and altered Ca\(^{2+}\) signaling that develop after MI are similar to those observed in other models of HF.\(^{8,9,15,17}\) To date, 2 molecular interventions that alleviate HF have been identified: restoration of \(\beta\)-adrenergic receptor function\(^{15,18}\) and augmented SERCA function.\(^{19,20}\) These approaches restore or improve Ca\(^{2+}\) signaling. Three issues are raised by studies of cellular Ca\(^{2+}\)-signaling defects in HF: (1) How do the molecular/drug therapies work? (2) What are the cellular defects contributing to the altered Ca\(^{2+}\) signaling? (3) What is the instigating cause of the Ca\(^{2+}\)-signaling defect?

**Molecular/Drug Therapies**

How can 100 nmol/L ouabain improve the defective Ca\(^{2+}\) signaling in PMI cells? There are at least 4 possible explanations. (1) Inhibition of the Na\(^{+}\) pump (Na\(^{+}\)/K\(^{+}\)-ATPase) decreases Na\(^{+}\) extrusion and, through the Na\(^{+}\)/Ca\(^{2+}\) exchanger, increases [Ca\(^{2+}\)]\(_i\). We cannot rule out a contribution by this mechanism to our findings if the changes in SR Ca\(^{2+}\) content were small (within the statistical error). These effects require time (minutes), however, and do not occur within a single heartbeat. (2) There may be sufficient elevation of subcellular [Na\(^{+}\)], during a single depolarization for Na\(^{+}\)/Ca\(^{2+}\) exchanger to trigger Ca\(^{2+}\) release (see Leblanc and Hume\(^{21}\)). This possibility is examined in Figure 3. (3) Ca\(^{2+}\) flux through Na\(^{+}\) channels occurs (ie, through slip-mode conductance). This does not apply to the data in Figures 1 to 3 because \(I_{\text{Na}}\) is inactivated, but it probably applies in part to Figure 5, because the effect is blocked by TTX. (4) There may be a novel action of ouabain to directly activate the ryanodine receptors (RyRs).\(^{22}\)

Leblanc and Hume\(^{21}\) suggested that \(I_{\text{Na}}\) could provide sufficient Na\(^{+}\) influx to activate Ca\(^{2+}\) entry through the Na\(^{+}\)/Ca\(^{2+}\) exchanger even during a single AP. To enable the brief Na\(^{+}\) influx to significantly elevate [Na\(^{+}\)], a subsarcolemmal region around Na\(^{+}\) channels with reduced diffusion, the “fuzzy space,” was hypothesized.\(^ {23}\) To test this possibility, we examined the actions of instantaneous Na\(^{+}\)-pump blockade on [Ca\(^{2+}\)]\(_i\), transients and contraction on control cardiac myocytes. We produced a maximal Na\(^{+}\) influx by applying an AP clamp to activate \(I_{\text{Na}}\) after removal of extracellular Cs\(^{+}\) (in K\(^{-}\)-free solutions) to completely block the Na\(^{+}\) pump (Figure 6). If the mechanisms of Leblanc and Hume\(^{21}\) and of Arnon et al\(^{25}\) were relevant to the enhanced contractions produced by ouabain (Figure 5), then an increase in [Ca\(^{2+}\)]\(_i\), transients and contraction should be seen after complete Na\(^{+}\)-pump inhibition in control cells. There was no statistical difference, however, in the measured [Ca\(^{2+}\)]\(_i\), transients (Figure 6B and 6C). This finding is similar to that reported by Su et al\(^{26}\) under similar conditions. From this and the fact that the effects in Figure 5 are blocked by TTX, we conclude that the positive inotropic effects of ouabain occur at least in part through the activation of...
slip-mode conductance of the Na\(^+\) channel. When [Na\(^+\)]\(_i\) is normal to high (10 to 15 mmol/L), however, in addition to slip-mode–dependent Ca\(^{2+}\) entry, 2 mechanisms will contribute to cardiotoxic steroids-increased Ca\(^{2+}\) signaling: the mechanism of Leblanc and Hume\(^{21}\) and the increase in SR Ca\(^{2+}\) content after Na\(^+\)-pump inhibition.\(^{26}\)

Cellular Defects That May Contribute to the Altered Ca\(^{2+}\) Signaling
The efficacy of I\(_{\text{Ca}}\) to activate SR Ca\(^{2+}\) release is reduced in PMI cells. In the absence of changes in other elements in the Ca\(^{2+}\)-signaling cascade, a spatial change in the organization of the ECC elements is the simplest explanation. If the L-type Ca\(^{2+}\) channels, on average, were more distant from the RyRs, then the probability that the opening of a Ca\(^{2+}\) channel would activate Ca\(^{2+}\) release would decrease.\(^{8}\) There are several topologically equivalent changes in cellular structures that could lead to such changes: reorganizing the distribution of the L-type calcium channels or the RyRs with respect to each other (“mismatch”), increasing the separation of the transverse tubules from the SR (“gap”), or T-tubule remodeling (“orphan”) (Figure 7). Some recent evidence favors the third possibility.\(^{27,28}\) It is thus possible that the ECC defect reflects T-tubule remodeling that cuts off, or orphans, SR from the triggering Ca\(^{2+}\) signal.

During the early phase of such hypothesized cellular remodeling, the increased distance between triggering Ca\(^{2+}\) influx and RyRs makes activation of SR Ca\(^{2+}\) release less likely. By itself, however, such a change would lead to only
transient changes in [Ca\(^{2+}\)], transient amplitude, because higher than normal levels of SR Ca\(^{2+}\) would result. Indeed, the orphaned SR phenomenon may also explain the large Ca\(^{2+}\) sparks reported in the early phases of hypertensive cardiomyopathy.\(^{20}\) An agent that increases Ca\(^{2+}\) entry or the sensitivity of the RyRs to be triggered by Ca\(^{2+}\) would effectively reverse or reduce this effect, as has been observed with \(\beta\)-adrenergic receptor activation or ouabain application.

**What Is the Initiating Cause of the Ca\(^{2+}\)-Signaling Defect?**

It is clear from the discussion above that \(\geq 2\) defects are involved in the development of the Ca\(^{2+}\)-signaling defect in HF: SERCA is expressed at a lower level, and the efficacy of calcium-induced calcium release is reduced. It is not clear which of these 2 events precedes the other or whether there is any causal relationship.

**Conclusions**

We conclude that PMI cells have a contractile defect that involves altered Ca\(^{2+}\) signaling as a major component. Changes in AP shape may serve as a means of slightly increasing Ca\(^{2+}\) entry and hence contractility, but compensation is not complete. Increased contractions with the cardiac Na\(^{+}\) channel by ouabain can improve the Ca\(^{2+}\) signaling in PMI cells and that Na\(^{+}\)-pump inhibition produced by ouabain will provide a further beneficial effect that depends on [Na\(^{+}\)], and increased SR Ca\(^{2+}\) content.

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**References**


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