Abnormal Intracellular Calcium Handling in Myocardium From Patients With End-Stage Heart Failure


Intracellular Ca\(^{2+}\) release and reuptake are essential for contraction and relaxation of normal heart muscle. Intracellular Ca\(^{2+}\) transients were recorded with aequorin during isometric contraction of myocardium from patients with end-stage heart failure. In contrast to controls, contractions and Ca\(^{2+}\) transients of muscles from failing hearts were markedly prolonged, and the Ca\(^{2+}\) transients exhibited 2 distinct components. Muscles from failing hearts showed a diminished capacity to restore low resting Ca\(^{2+}\) levels during diastole. These experiments provide the first direct evidence from actively contracting human myocardium that intracellular Ca\(^{2+}\) handling is abnormal and may cause systolic and diastolic dysfunction in heart failure. (Circulation Research 1987;61:70-76)

Becau se the calcium ion (Ca\(^{2+}\)) plays a central role in the process of excitation–contraction coupling in the heart, alterations from normal in the subcellular handling of Ca\(^{2+}\) may provide the basis for cardiac contractile failure. Although this hypothesis is supported by a large body of experimental evidence in animals,\(^1,2\) it has not been directly tested in humans. The purpose of the present study was to test this hypothesis in human working myocardium by comparing the intracellular Ca\(^{2+}\) transients and mechanical twitches of trabeculae carneae isolated from patients with end-stage heart failure with those of non-failing controls.

Materials and Methods

Specimens consisting of predominantly left and right ventricles without atria were obtained from 9 patients with end-stage biventricular failure undergoing cardiac transplantation for dilated cardiomyopathy (idiopathic, \(n = 3\); ischemic heart disease, \(n = 4\); myocardial deterioration after mitral valve replacement, \(n = 1\)) or hypertrophic cardiomyopathy \((n = 1)\). Dilated hypertrophic hearts \((\text{weight, } 300-620 \text{ g})\) were removed from 7 males and 1 female, aged 14–54 years. The heart with hypertrophic cardiomyopathy \((\text{not dilated; weight, } 400 \text{ g})\) was removed from a 44-year-old woman who had biventricular hypertrophy.

Received May 14, 1986, accepted February 27, 1987

From the Charles A Dana Research Institute and Harvard-Thorndike Laboratory of Beth Israel Hospital, the Consolidated Department of Medicine (Cardiovascular Divisions) and Department(s) of Pathology, Beth Israel and Brigham's Hospitals, the Divisions of Cardiovascular Surgery, Brigham and Women's Hospital and Children's Hospital Medical Center, and Harvard Medical School, Boston, Mass.

This work was supported in part by U.S. Public Health Service Grant HL 31117, HL 07374, and HL 36797, a Grant-in-Aid from the American Heart Association, Massachusetts Affiliate, and a Research Career Development Award (HL 01611) (J.P.M.)

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Table 1. Hemodynamic Values in Patients With End-Stage Heart Failure

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Etiology of heart failure</th>
<th>Days</th>
<th>RA (mm Hg)</th>
<th>PA (mm Hg)</th>
<th>PCW (mm Hg)</th>
<th>LV (mm Hg)</th>
<th>CI (L/min/mm²)</th>
<th>LVEF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>M</td>
<td>Ischemic</td>
<td>58</td>
<td>10</td>
<td>41/25</td>
<td>23</td>
<td>2</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>M</td>
<td>S/P valve replacement</td>
<td>92</td>
<td>13</td>
<td>39/29</td>
<td>29</td>
<td>89/29</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>F</td>
<td>Hypertrophic</td>
<td>97</td>
<td>19</td>
<td>31/21</td>
<td>20</td>
<td>90/18</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>M</td>
<td>Idiopathic</td>
<td>95</td>
<td>12</td>
<td>33/21</td>
<td>21</td>
<td>90/20</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>M</td>
<td>Idiopathic</td>
<td>27</td>
<td>10</td>
<td>32/10</td>
<td>16</td>
<td>80/12</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>M</td>
<td>Ischemic</td>
<td>50</td>
<td>15</td>
<td>72/36</td>
<td>25</td>
<td>85/32</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>M</td>
<td>Ischemic</td>
<td>95</td>
<td>17</td>
<td>48/29</td>
<td>25</td>
<td>88/30</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>M</td>
<td>Ischemic</td>
<td>60</td>
<td>6</td>
<td>31/16</td>
<td>14</td>
<td>98/17</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>M</td>
<td>Ischemic</td>
<td>60</td>
<td>14</td>
<td>63/38</td>
<td>30</td>
<td>90/12</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

Days, number of days between catheterization and transplantation, RA, right atrium; PA, pulmonary artery; PCW, pulmonary capillary wedge; LV, left ventricle; CI, cardiac index, LVEF, left ventricular ejection fraction. values from ventriculogram at time of catheterization or radionuclide study.

into control and myopathic muscles by a chemical technique; light and tension responses were recorded simultaneously. Muscles that recovered less than 70% of preload tension or twitch duration after loading were excluded from the study. After loading, peak tension returned to 93.7 ± 8.1% and 91.2 ± 5.8%, and the RT50 (time to 50% decline from peak tension) returned to 99 ± 3.7% and 96.9 ± 3.3% of preload values in the control and myopathic groups, respectively (p > 0.1). Light signals were recorded with an EMI 9635QA photomultiplier (EMI Gencom Inc., Plainview, N.Y.) and signals averaged to obtain a satisfactory signal-to-noise ratio. In general, we have found that the chemical loading technique provides brighter preparations than can be obtained routinely with microinjection of aequorin, especially in tissue such as human myocardium that is difficult to penetrate with fine-tipped glass micropipettes. Statistical comparisons were made by Student's t-test, and p values < 0.05 were considered significant.

Results

Figure 1 shows the light signals (i.e., intracellular Ca2+ transients) and isometric twitches recorded with aequorin-loaded cardiac muscle from a control patient without heart failure, a patient with dilated cardiomyopathy, and the patient with hypertrophic cardiomyopathy. The amplitudes and time courses of light and tension in 2 mM [Ca2+]0, are detailed in Table 2. Figure 2 shows action potential recordings in muscles from the same 3 groups of patients. Compared to the controls, the myopathic muscles showed a prolongation of isometric tension development with a marked delay in relaxation and a corresponding prolongation of their Ca2+ transients. Also, duration of the action potential in myopathic muscles was prolonged.

To test whether the prominent L2 in myopathic muscle reflects a diminished capacity to handle increases in cytoplasmic Ca2+, calcium dose-response curves were performed. In control and myopathic muscle, calcium produced a dose-related positive inotropic effect that was associated with a corresponding increase in the amplitude of the light signal. In the myopathic muscles, increasing doses of calcium produced a progressive increase in the amplitude of L2 relative to L1; this increase was particularly obvious in the muscle from the patient with hypertrophic cardiomyopathy. In myopathic muscles, the increased prominence of L2 was associated with a prolongation of the isometric twitch. In contrast, the time course of contraction in control muscles changed only at the 16 mM dose of Ca2+ where a slight prolongation was noted; in 1 of 9
Table 2. Characteristics of Light and Tension Responses in Control and Myopathic Human Myocardium

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>PT (mN/mm²)</th>
<th>TPT (msec)</th>
<th>TPL (msec)</th>
<th>RT₅₀ (msec)</th>
<th>RL₈₀ (msec)</th>
<th>L₂ / L₁ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>3.8 ± 1.4</td>
<td>283 ± 16</td>
<td>33 ± 6</td>
<td>258 ± 26</td>
<td>246 ± 37</td>
<td>0</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>9</td>
<td>3.9 ± 1.5</td>
<td>548 ± 45*</td>
<td>53 ± 5*</td>
<td>331 ± 40*</td>
<td>569 ± 48*</td>
<td>50 ± 12*</td>
</tr>
</tbody>
</table>

Muscles are perfused with 2 mM [Ca²⁺]₀, PO₄⁻ free solution. n, number of preparations. PT, peak tension. TPT, time to peak tension. TPL, time to peak light. RT₅₀, time to 50% relaxation from PT. RL₈₀, time to 80% decline from peak light. L₂ / L₁, ratio of peaks of 2 components. All values are mean ± SEM. *p < 0.05 compared with controls by Student’s t-test.

aequorin-loaded controls, a distinct second component appeared in the light signal (shown in Figure 3). As can be seen in Figure 3, higher concentrations of [Ca²⁺]₀ were associated with an increase in the end-diastolic levels of light and tension; this was observed in 5 of 9 myopathic muscles but did not occur in any of the 9 controls.

Figure 4 shows the effects of ryanodine and verapamil on the 2 components of the light response in myopathic muscle. Ryanodine alone was able to completely suppress L₁, but a combination of ryanodine plus verapamil was necessary to suppress completely both L₁ and L₂.

Table 3 documents that the myopathic muscles used in these experiments were significantly hypertrophied compared with the controls, and Figure 5 shows the effects of changes in frequency of stimulation on [Ca²⁺]₀, and tension development in a myopathic trabecula.

Discussion

The light signal recorded from the control muscle in Figure 1 consisted of a single component that rose to a peak and declined towards baseline before peak tension was reached. Similar aequorin signals have been recorded from nonfailing human atrial and ventricular muscle and appear to be typical for mammalian working myocardium. In contrast, the light signals recorded from myopathic muscle were not only prolonged compared with the controls but consisted of 2 temporally distinct components (L₁ and L₂ in Figure 1). L₂ was diminutive or absent in control preparations but was prominent in all of the myopathic muscles. These data indicate that the prolonged contraction of myopathic muscle in vitro as well as the myocardial relaxation abnormalities observed in patients with dilated cardiomyopathy appear to correlate with changes in intracellular Ca²⁺ handling. Similar results have been reported in normal canine Purkinje strands, which typically display two temporally distinct components in their Ca²⁺ transients. However, the time courses of our myopathic aequorin signals are much longer than those recorded in canine Purkinje strands.

Diastolic [Ca²⁺], in myocardial cells is generally estimated to be on the order 10⁻⁷ M, while concentrations in the extracellular space are in the range of 10⁻⁴ M; the normally functioning heart, therefore, is able to maintain a 10,000-fold concentration gradient for Ca²⁺ across the sarcolemma. Our data indicate that muscles from myopathic hearts have a lesser capacity to maintain Ca²⁺ homeostasis in the presence of normal and increased transsarcolemmal gradients. The single component of light signals recorded from normal mammalian working myocardium predominantly reflects Ca²⁺ handling by the sarcoplasmic reticulum. Since Ca²⁺ uptake by sarcoplasmic reticulum vesicles isolated from failing human hearts is depressed, we postulated that L₂ in intact myocardial fibers might reflect dysfunction of this organelle. To determine the sources of Ca²⁺ responsible for L₁ and L₂, the effects of ryanodine, an alkaloid that blocks release of Ca²⁺ from the sarcoplasmic reticulum, was studied. In control muscle, ryanodine decreased the amplitude and prolonged the time course of the monophasic Ca²⁺ signal. However, in myopathic muscle, ryanodine had little effect on the amplitude or time course of the L₁ component but had a more pronounced effect on L₂.

Figure 2. Action potentials recorded in trabeculae carnea from control patient (Panel A) and patients with dilated cardiomyopathy (Panel B) and hypertrophic cardiomyopathy (Panel C). Recordings were made under steady-state conditions at 30°C, 0.33 Hz with muscles stretched to L₉₀. Fine-tipped straight glass microelectrodes containing 3 M KCl were used for impalement. Recordings A and B were obtained from same control and myopathic trabeculae carnea. Figure 1 C was recorded in different trabecula from same hypertrophic heart for which aequorin signals are illustrated in Figure 1.
transient, as has been reported in animal studies. In myopathic muscle, a relatively low dose of ryanodine abolished L, without significantly affecting the amplitude or time course of L, (Figure 4A), indicating that the L, component of the Ca$^{2+}$ transient arises from sarcoplasmic reticular Ca$^{2+}$ release. As shown in Figure 4B, a maximally effective dose of ryanodine prolonged but did not suppress completely the Ca$^{2+}$ transient, suggesting that this residual component of L, arises from some source other than the sarcoplasmic reticulum. These results could be explained by Ca$^{2+}$ release from a ryanodine-insensitive store, but no definitive experimental evidence supports the existence of such a mechanism in normal or failing myocardium. The addition of verapamil, a Ca$^{2+}$ channel blocker, suppressed the light and tension remaining after ryanodine, indicating that a significant component of L, reflects Ca$^{2+}$ entry through voltage-dependent sarcolemmal channels. Since the combination of ryanodine plus verapamil completely suppressed the Ca$^{2+}$

![Figure 3](image-url)  
**Figure 3.** Representative Ca$^{2+}$ dose-response curves in muscles from control and patients with dilated or hypertrophic cardiomyopathy. [Ca$^{2+}$]$_{i}$ is shown in each panel. A rise in end-diastolic light (upper trace) or tension (lower trace) in 16 mM [Ca$^{2+}$]$_{o}$ compared with 2 mM [Ca$^{2+}$]$_{o}$. Middle trace, stimulus artifact.

![Figure 4](image-url)  
**Figure 4.** Effects of ryanodine (ryan) and verapamil (verap) on L, and L, of myopathic muscles. Panel A. Muscle from patient with hypertrophic cardiomyopathy. Panel B. Muscle from patient with dilated cardiomyopathy. In Panel B, 10$^{-6}$ M ryanodine was maximally effective dose. Concentrations expressed in mol/l.
 transient, other sources of Ca$^{2+}$, such as Na$^+$/Ca$^{2+}$ exchange, do not appear to contribute significantly to generation of either L$\text{t}$ or L$\text{a}$ on a beat-to-beat basis. These results suggest that L$\text{a}$ in myopathic muscle reflects dysfunction of both the sarcolemma and sarcoplasmic reticulum. The former may cause increased Ca$^{2+}$ entry, possibly via voltage-dependent channels, and the latter may cause slowed restoration of low resting tone during diastole due to a decreased rate of Ca$^{2+}$ resequestration.

The force per unit cross-sectional area generated by both normal and myopathic muscles in our experiments (Table 2) was greater than reported in other studies of human myocardium but lower than expected on the basis of animal data. The reason for these apparent discrepancies remains unclear but may reflect differences in structure or connective tissue content among the muscles used in these various studies. We were surprised to find that isometric tension development in muscles from the failing hearts was not depressed significantly compared with the controls (Table 2); moreover, maximal activation in 16 mM Ca$^{2+}$ yielded tensions in the control (13.8 ± 4.1 mN/mm$^2$; n = 8) and myopathic (11.0 ± 3.3 mN/mm$^2$; n = 5) groups that were not statistically different (p > 0.1). Similar results have been reported by other investigators using human muscle and may reflect the selection for study of relatively viable trabeculae from the failing hearts since areas of dense necrosis or fibrosis were avoided, as well as considerable variation among preparations. These findings raise the possibility that the peak isometric tension generated by our muscles in vitro may not be reflective of the contractile reserve of the failing heart in vivo. Of interest, all of the myopathic hearts from which muscles were obtained had significant biventricular hypertrophy noted grossly and microscopically (see Table 3); as expected, the heart from the patient with hypertrophic cardiomyopathy showed the most pronounced hypertrophic change. This raises the possibility that force/mm$^2$ of contractile protein actually may be decreased since the ratio of myofibrils:average cell cross-sectional area increases with hypertrophy. Thus, the alterations from normal in the time courses of the Ca$^{2+}$ transients and contractions in these experiments may reflect the hypertrophic response of human working myocardium rather than "heart failure," per se. On the other hand, these results may depend on experimental conditions. As shown in Figure 5 for a myopathic muscle, a faster, more physiologic rate of stimulation than that routinely used in our experiments (0.33 Hz) resulted in fusion of the Ca$^{2+}$ transients and twitches with increases in both end-diastolic [Ca$^{2+}$], and tension. Moreover, as end-diastolic tone increased, peak active tension development declined. Under the same conditions with control muscles, fusion phenomena were not observed to occur. Fusion could be prevented by pretreating myopathic muscles with positively lusitropic agents, such as forskolin and isoproterenol, which enhance Ca$^{2+}$ sequestration by the sarcoplasmic reticulum and abbreviate the Ca$^{2+}$ transient and the twitch. Fusion was facilitated in myopathic muscles by drugs that increase [Ca$^{2+}$],, including digitalis, and by elevations in extracellular Ca$^{2+}$. Assuming that similar fusion phenomena may occur under some circumstances in vivo and at normal body temperature, these results indicate that both the systolic and diastolic dysfunction of end-stage heart failure may be due to abnormal Ca$^{2+}$ handling.

The possibility must be considered that the chemical loading procedure might damage human trabecular canine and produce as an artifact the abnormal calcium transients observed in myopathic muscles. We do not believe that this is the case for several reasons. First, as indicated above, peak tension and the RT returned toward preload levels after loading. However, since the preload and postload tensions were not exactly the same in all experiments, the argument could be made that a small number of damaged cells.

---

**Table 3.** Histologic Features of Control and Myopathic Muscles

<table>
<thead>
<tr>
<th>Group</th>
<th>Fiber diameter (µm)</th>
<th>Nuclear width (µm)</th>
<th>Nuclear length (µm)</th>
<th>Nuclear area (µm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.0 ± 0.4</td>
<td>4.3 ± 0.2</td>
<td>10.1 ± 0.4</td>
<td>34.5 ± 2.3</td>
</tr>
<tr>
<td>Cardiomyopathic</td>
<td>23.1 ± 0.9*</td>
<td>6.9 ± 0.3*</td>
<td>15.7 ± 0.6*</td>
<td>84.2 ± 4.9*</td>
</tr>
</tbody>
</table>

*p < 0.0001, n, number of preparations, measurement not available from 1 control and 2 myopathic fibers.

---

**Figure 5.** Response of hypertrophied trabecula from patient with end-stage heart failure to increased rates of stimulation at 30°C. (Ca$^{2+}$), 16 mM Upper trace, aequorin signal (i.e., [Ca$^{2+}$]), middle trace, tension development, lower trace, stimulus artifact Interval between stimuli noted at bottom of figure.
might be responsible for the biphasic aequorin signals observed in the myopathic muscles. On the other hand, as shown in Figure 6, L, and L, were present in myopathic muscles in which preload and postload tension development were exactly equal. Moreover, control muscles were subjected to the same loading conditions as the myopathic muscles, and none showed evidence of a second component in normal [Ca\(^{2+}\)]. Second, one author (J.P.M.) has reported calcium transients from human myocardium microinjected with aequorin that are qualitatively similar to those reported in the present study. The aequorin signals of microinjected muscles from patients without heart failure consisted of a single component, the signals of a muscle from a patient with hypertrophic cardiomyopathy showed both L, and L,\(^{2}\). The similarity of results obtained with these 2 different methods of aequorin loading makes selective damage by the chemical technique unlikely. Third, the effects of interventions on the twitch of loaded and unloaded muscles were similar within the control and myopathic groups, as indicated in the present study for [Ca\(^{2+}\)]. Damage from the chemical loading technique might be expected to produce a "chemically skinned," maximally activated muscle under control conditions. Figure 3 shows that this was not the case even in the presence of markedly elevated [Ca\(^{2+}\)].

In conclusion, these experiments demonstrate that the Ca\(^{2+}\) transient in myopathic human cardiac muscle consists of 2 components that appear to reflect abnormal Ca\(^{2+}\) handling by the sarcolemma and sarcoplasmic reticulum. The inability of myopathic muscle to maintain Ca\(^{2+}\) homeostasis may be a primary cause of contractile dysfunction in heart failure.

Acknowledgments

The authors gratefully acknowledge the contributions of Drs. Richard Jonas, John Mayer, and Aldo Castenada of Children’s Hospital Medical Center, who provided the normal myocardium from organ donors. We also thank Drs. Lawrence Cohn, John J. Collins, and James Marsh of Brigham and Women’s Hospital, who provided the myopathic muscle. Finally, we thank Drs. Kathleen Morgan, Meei Jiang, and Maurice Briggs of Beth Israel Hospital for their critical comments.

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Key Words: • calcium indicators • aequorin • hypertrophic cardiomyopathy • dilated cardiomyopathy • cardiac hypertrophy • ryanodine
Abnormal intracellular calcium handling in myocardium from patients with end-stage heart failure.


_Circ Res._ 1987;61:70-76
doi: 10.1161/01.RES.61.1.70

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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