L-Type Ca\(^{2+}\) Channel Density and Regulation Are Altered in Failing Human Ventricular Myocytes and Recover After Support With Mechanical Assist Devices

Xiongwen Chen, Valentino Piacentino III, Satoshi Furukawa, Bruce Goldman, Kenneth B. Margulies, Steven R. Houser

Abstract—Ca\(^{2+}\) influx through the L-type calcium channel (LTCC) induces Ca\(^{2+}\) release from the sarcoplasmic reticulum (SR) and maintains SR Ca\(^{2+}\) loading. Alterations in LTCC properties, their contribution to the blunted adrenergic responsiveness in failing hearts and their recovery after support with LV assist devices (LVAD) were studied. L-type Ca\(^{2+}\) current (\(I_{\text{Ca,L}}\)) was measured under basal conditions and in the presence of isoproterenol (ISO), dibutryl-cAMP (db-cAMP), Bay K 8644 (BayK), Okadaic acid (OA, a phosphatase inhibitor), and phosphatase 2A (PP2A) in nonfailing (NF), failing (F), and LVAD-supported human left ventricular myocytes (HVMs). Basal \(I_{\text{Ca,L}}\) density was not different in the 3 groups but \(I_{\text{Ca,L}}\) was activated at more negative voltages in F- and LVAD- versus NF-HVMs (\(V_{\text{0.5}}\): −7.18±1.4 and −7.0±0.9 versus 0.46±1.1 mV). Both ISO and db-cAMP increased \(I_{\text{Ca,L}}\) in NF- and LVAD- significantly more than in F-HVMs (NF >LVAD> F: ISO: 90±15% versus 77±19% versus 24±12%; db-cAMP: 235%>172%>90%). ISO caused a significant leftward shift of the \(I_{\text{Ca,L}}\) activation curve in NF- and LVAD- but not in F-HVMs. After ISO and db-cAMP, the \(I_{\text{Ca,L}}\) activation was not significantly different between groups. BayK also increased \(I_{\text{Ca,L}}\) more in NF- (81±30%) and LVAD- (70±15%) than in F- (51±8%) HVMs. OA increased \(I_{\text{Ca,L}}\) by 85.6% in NF-HVMs but had no effect in F-HVMs, while PP2A decreased \(I_{\text{Ca,L}}\) in F-HVMs by 35% but had no effect in NF-HVMs. These results suggest that the density of LTCC is reduced in F-HVMs but basal \(I_{\text{Ca,L}}\) density is maintained by increasing in LTCC phosphorylation. (Circ Res. 2002;91:517-524.)

Key Words: L-type calcium channel ■ heart failure ■ left ventricle assist device ■ PKA-dependent phosphorylation

Basal contractility of the failing human heart is depressed\(^1\) and that contractile reserve via β-adrenergic stimulation is blunted.\(^2,3\) These abnormalities are thought to contribute to the progressive decay of cardiac pump function that characterizes the failing human heart and can be at least partially reversed by left ventricular assist device (LVAD) support.\(^4-6\)

The role of abnormalities in the density and regulation of L-type Ca\(^{2+}\) channels (LTCCs) in the deranged Ca\(^{2+}\)-homeostasis of the failing human ventricular myocyte (F-HVM) and its recovery after LVAD support is the topic of this investigation.

Alterations in Ca\(^{2+}\) handling are thought to be centrally involved in heart failure contractile dysfunction.\(^7\) Reduced SR Ca\(^{2+}\) storage and release\(^8,9\) and slow removal of Ca\(^{2+}\) from the cytoplasm\(^1,10\) appear to be contributors to the reduced rate and magnitude of force generation and prolongation of systole that are key features of the failing human heart. Reduction in the abundance and activity of the sarcoplasmic reticulum (SR) Ca\(^{2+}\) ATPase (SERCa) and increased abundance and/or activity of the sarcolemmal Na\(^{+}\)·Ca\(^{2+}\) exchanger (NCX) appear to be critical contributors to the abnormal Ca\(^{2+}\) handling in the failing human heart.\(^7\)

The blunted contractile reserve of the failing heart is thought to involve alterations in the abundance and activity of molecules that participate in the adrenergic signaling cascade. Activation of this signaling pathway increases contractility in the normal heart by phosphorylation of Ca\(^{2+}\) regulatory proteins.\(^2,3,11\) Normalization of the abundance of some of these proteins has been observed in LVAD-supported human hearts\(^12\) and is likely to be involved in the associated restitution of contractility and adrenergic responsiveness.\(^4,12\)

Ca\(^{2+}\) influx through the LTCC is essential for triggering the SR Ca\(^{2+}\) release (excitation-contraction coupling) that determines the rate and magnitude of contraction and is the major source of Ca\(^{2+}\) to load the SR.\(^13\) Therefore, abnormalities in LTCC density or regulation should have profound effects on cardiac function. Although most previous studies have not found substantial changes in whole-cell L-type calcium cur-
rent \( I_{\text{Ca,L}} \) density in heart failure,\textsuperscript{14,15} two recent investigations suggest that LTCC density may be reduced\textsuperscript{16} and that phosphorylation state\textsuperscript{17} may be increased. The present study was designed to explore these possibilities.

The objectives of the present experiments were to determine (1) if the density and properties of \( I_{\text{Ca,L}} \) are altered in F-HVMs; (2) by what mechanisms the regulation of \( I_{\text{Ca,L}} \) by \( \beta \)-adrenergic agonists is abnormal in F-HVMs; and (3) if LVAD support of the failing heart reverses LTCC abnormalities. Our results suggest that LTCC density is reduced in LVAD support of the failing heart reverses LTCC abnormalities, and that these alterations are partially reversed with LVAD support. These findings suggest that a reduction in LTCC density and aberrant channel regulation are centrally involved in the defective Ca\textsuperscript{2+} homeostasis of the F-HVMs and that reversing these defects is associated with recovery toward normal myocyte contractility.

**Materials and Methods**

**Myocyte Isolation**

Human ventricular myocytes (HVMs) were isolated from 7 nonfailing (NF), 15 failing (F), and 14 LVAD-supported failing human hearts as described previously.\textsuperscript{18} Failing and LVAD-supported failing human hearts were obtained from the Temple Cardiac Transplant Team at the time of cardiac transplantation. Nonfailing hearts were donor hearts unsuitable for transplantation. Our protocol was reviewed by Temple University Institutional Review Board and was determined to be exempt. Patient characteristics are presented in Table 1 with detailed information in an expanded Materials and Methods section, which can be found in the online data supplement available at http://www.circresaha.org.

**Ca\textsuperscript{2+} Current Measurement**

Whole-cell Ca\textsuperscript{2+} currents \( (I_{\text{Ca,L}}) \) were measured in Na\textsuperscript{+}- and K\textsuperscript{+}-free solutions at 37\textdegree{}C using techniques described in detail previously (see online data supplement). To define the \( I_{\text{Ca,L}} \)-voltage relationship, the membrane potential was held at \(-70\) mV and then depolarized in \( 10\)-mV increments. Myocytes were then exposed to various activators of the Ca\textsuperscript{2+} current (described below) and the effects were monitored by recording \( I_{\text{Ca,L}} \) during steps to \( +10\) mV. When effects were stable, the \( I_{\text{Ca,L}} \)-voltage relationship was again determined. Only myocytes with minimal (<10\%) rundown of \( I_{\text{Ca,L}} \) were included in the data sets.

**Pharmacology**

To activate protein kinase A (PKA) signaling pathways, myocytes were exposed to the nonselective \( \beta \)-adrenergic receptor agonist ISO (1 \( \mu \)mol/L, Sigma). PKA was also activated with a nonhydrolyzable form of cAMP (dibutyryl-cAMP [db-cAMP], 10 \( \mu \)mol/L, Sigma) in the pipette solution. Some cells were also exposed to the dihydropyridine Ca\textsuperscript{2+} channel agonist Bay K 8644 (1 \( \mu \)mol/L, Sigma) to increase \( I_{\text{Ca,L}} \) independently of PKA.\textsuperscript{20} These concentrations were used because they produced maximal stable effects on \( I_{\text{Ca,L}} \) in preliminary experiments. The phosphatase inhibitor Okadaic acid (OA, Calbiochem, 1 \( \mu \)mol/L in pipette) and phosphatase 2A1 (10 U/mL in pipette, Sigma) were tested as described.

**Statistics**

Data in the text and tables are reported as mean±SEM. Paired and unpaired \( t \) tests were used to compare the effects of drugs before and after their applications. Differences among multiple groups were tested with ANOVA or ANOVA for repeated measures with SAS (SAS Institute Inc). A value of \( P=0.05 \) was considered significant.

### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Etiology</th>
<th>Duration of LVAD, d</th>
<th>Age, y</th>
<th>CHF Duration, mo</th>
<th>LVEF, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF (N=7)</td>
<td>...</td>
<td>...</td>
<td>59±8</td>
<td>0</td>
<td>54.0±2.6</td>
</tr>
<tr>
<td>F (N=15)</td>
<td>Idiopathic (3)</td>
<td>...</td>
<td>54±3</td>
<td>70±14</td>
<td>12.2±1.3</td>
</tr>
<tr>
<td></td>
<td>Ischemic (10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hypertension (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Valvular (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVAD (N=14)</td>
<td>Idiopathic (4)</td>
<td>164±53</td>
<td>53±3</td>
<td>51±9</td>
<td>12.5±0.7</td>
</tr>
<tr>
<td></td>
<td>Ischemic (8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hypertension (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dilated (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data from individual patients, including medications, are presented in the online data supplement.
The average cell capacitance was significantly greater in F- versus LVAD-HVMs. Consistent with a larger cell size for failing myocytes and a reduction in myocyte size after LVAD support, as we have shown previously. The average cell capacitance of the nonfailing group was larger than has been reported in other studies. This likely reflects the larger than normal size of the nonfailing hearts used in our experiments (see online data supplement).

The most significant difference in \( I_{Ca,L} \) characteristics under basal conditions was that the voltage dependence of \( I_{Ca,L} \) activation \( (d_{v}) \) was more negative in F- and LVAD- versus NF-HVMs (Figure 1A and 1B). The membrane potentials at which one-half of the maximal \( I_{Ca,L} \) was activated \( (V_{0.5}) \) in F, LVAD, and NF were \(-7.18 \pm 1.4, -7.0 \pm 0.9, \) and \(0.46 \pm 1.1\) mV in F-, LVAD-, and NF-HVMs, respectively.

A negative shift in the voltage dependence of \( I_{Ca,L} \) activation can be induced by PKA-mediated phosphorylation (usually through \( \beta \)-adrenergic signaling pathway) of the LTCC complex. Therefore, one interpretation of our finding is that the basal level of LTCC phosphorylation is increased in F- and LVAD- versus NF-HVMs. This idea is consistent with the results of a recent study that found an increased open probability of single LTCCs in failing human myocytes, a behavior caused by channel phosphorylation. Because the whole-cell current density \( (I) \) is determined by the product of the LTCC density \( (N) \), open probability \( (P_{o}) \), and single channel current \( (i) \), ie, \( I=NP_{o}\cdot i \), our results would suggest that in F-HVMs, a reduced LTCC density \( (N) \) is offset by an increase in \( P_{o} \) so that the basal \( I_{Ca,L} \) density \( (I) \) is maintained.

**Effects of ISO on \( Ca^{2+} \) Current**

If LTCC phosphorylation state is increased in F-HVMs, then the effects of agents that promote PKA-mediated phosphorylation of the LTCC in F-HVMs should be less than in NF-HVMs, ie, a smaller percent change in \( I_{Ca,L} \) after the application of PKA activators (ISO). ISO increased \( I_{Ca,L} \) in every group of myocytes studied (Figure 2). However, the effects on peak \( I_{Ca,L} \) density were significantly smaller in F-versus NF- and LVAD-HVMs (Figures 2 and 3). ISO also caused a significant acceleration of the kinetics of \( I_{Ca,L} \) inactivation in NF- and LVAD-HVMs but not in F-HVMs (Figure 4), consistent with the larger increases in \( I_{Ca,L} \) in these myocytes. ISO also caused the voltage dependence of \( I_{Ca,L} \) activation to shift to more negative potentials in NF- and LVAD-HVMs but had no significant effect in F-HVMs (Figure 3). It is noteworthy that the voltage dependence of \( I_{Ca,L} \) activation was already shifted to the left in F-HVMs under our basal conditions, and after ISO exposure, there was no significant further shift. After ISO, there were no significant differences in \( V_{0.5} \) between any groups (Figure 3). These results suggest that there was an equivalent level of LTCC phosphorylation after ISO in all myocytes. Our observations also suggest that the blunted response to \( \beta \)-adrenergic agonists in failing myocytes involves the persistent phosphorylation of PKA target proteins under our basal conditions.

**Effects of db-cAMP**

A common explanation for reduced adrenergic effects in heart failure is that adrenergic signaling abnormalities pro-
duce reduced cytosolic cAMP concentration and thus reduced phosphorylation of PKA target proteins. Abnormal adrenergic signaling in human heart failure is well supported by many studies and is thought to involve changes in β-receptor density and subtypes, G-protein abundance and subtypes, phosphatase abundance, and activity of β-adrenergic receptor kinase. In general, these signaling alterations would reduce phosphorylation of PKA target proteins, and in this regard, there is evidence for reduced phosphorylation of both phospholamban and troponin-I in the failing human heart.

However, there is also evidence that the ryanodine receptor (RYR, also known as the Ca$^{2+}$ release channel) and the LTCC are hyperphosphorylated in human heart failure. If all of these results are correct, then it would indicate that a larger than normal fraction of the LTCC is phosphorylated under basal conditions in failing human myocytes. It is noteworthy that this idea is difficult to test directly with biochemical techniques because the phosphorylation state of the LTCC in cardiac myocytes is not easily measured.

**Effects of BAY K8644**

If the LTCC density and phosphorylation state are identical in our 3 populations of myocytes, then BAY K8644, which increases $P_s$ of the LTCC independent of PKA-

To determine if the reduced effects of ISO on $I_{Ca,L}$ in F-HVMs results from either defective cAMP generation that limits LTCC phosphorylation or from increased basal LTCC phosphorylation, myocytes were exposed to db-cAMP. Peak $I_{Ca,L}$ was significantly greater than control in the presence of db-cAMP in all 3 groups of myocytes (Figure 5). However, db-cAMP effects on peak $I_{Ca,L}$ in F-HVMs were significantly smaller than in either NF- or LVAD-HVMs (Figure 5C). The fact that the voltage dependence of $I_{Ca,L}$ activation was not different in any of the 3 groups after db-cAMP (Figure 5E) is consistent with a similar phosphorylation state of the LTCC. These results support the idea that a larger than normal fraction of the LTCC is phosphorylated under basal conditions in failing human myocytes. It is noteworthy that this idea is difficult to test directly with biochemical techniques because the phosphorylation state of the LTCC in cardiac myocytes is not easily measured.

**Figure 3.** Effect of 1 μmol/L ISO on $I_{Ca,L}$ in the test voltage relationship in NF- ($n=6$, N=3), F- ($n=14$, N=7), and LVAD- ($n=10$, N=5) HVMs. A, ISO increased $I_{Ca,L}$ significantly in NF and LVAD but had little effect in F. $V_{Ca,L}(d)$: −9.9 mV; $P<0.05$ and LVAD- (change of $V_{Ca,L}(d)$: −5.8 mV; $P<0.05$) HVMs but no significant shift in F. B, ISO caused a significant leftward shift of the voltage dependence of $I_{Ca,L}$ activation in NF- (change of $V_{Ca,L}(d)$: −9.9 mV; $P<0.05$) and LVAD- (change of $V_{Ca,L}(d)$: −5.8 mV; $P<0.05$) HVMs. After exposure to ISO, $V_{Ca,L}(d)$ was not significantly different in the 3 groups (NF vs F vs LVAD: −6.03±3.4 vs −8.4±3.0 vs −9.9±1.7 mV). C indicates control, open symbols; ISO, 1 μmol/L isoproterenol, filled symbols. *Significant increase in $I_{Ca,L}$ at these voltages ($P<0.05$, repeated ANOVA).
dependent signaling pathways, should have equivalent effects on ICa_L in all groups. If LTCC density is reduced but the phosphorylation state and P, are increased in F-HVMs versus NF- and LVAD-HVMs, then BAY K8644 should have a smaller effect in F-HVMs. We found that BAY K8644 increased ICa_L in all myocytes; however, the magnitude of the effect was significantly smaller in F-versus NF- and LVAD-HVMs (Figure 5). These results are also consistent with the idea that the number of LTCCs is reduced in failing myocytes and that there is an increase in channel phosphorylation. Our results also show that these changes are largely reversed in LVAD-HVMs.

Effect of Okadaic Acid and Phosphatase 2A
Alterations in the basal phosphorylation state of LTCC are likely to reflect changes in the balance of activities of PKA and phosphatases. An increase in basal LTCC phosphorylation could result from an increased activity of PKA and/or a decreased activity of phosphatases. In a small series of experiments to begin to explore these issues, we found that there was no significant change in peak ICa_L when NF- and F-HVMs were exposed to the PKA inhibitors H-89 (in NF [n=4], control versus H-89: −6.78±0.86 versus 6.51±0.50 pA/pF; in F [n=3], control versus H-89: −7.16±0.74 versus 6.93±0.64 pA/pF) and Rp-cAMP (in F cells [n=5], control versus Rp-cAMP: 6.45±0.61 versus 7.66±0.82 pA/pF). Schroder et al have suggested that the endogenous phosphatase activity associated with LTCCs is decreased in failing HVMs. We found that the phosphatase inhibitor OA increased ICa_L in NF-HVMs (control [n=5] −6.34±0.43 versus OA [n=3] −11.78±0.58 pA/pF; P<0.05) by 85.6% but had no significant effect in F-HVMs (control [n=3] −9.31±2.11 versus OA [n=3] −9.39±3.92 pA/pF). Next, we examined the effects of PP2A, which has been shown to dephosphorylate LTCCs in a previous study. PP2A caused a 35% decrease in the peak ICa_L in F-HVMs (Control [n=4, N=2] versus PP2A [n=4, N=2]: −11.32±0.66 versus −7.34±1.23 pA/pF; P<0.05), whereas PP2A did not significantly change ICa_L in NF-HVMs (Control [n=5, N=2] versus PP2A [n=5, N=2]: −6.22±0.38 versus −7.28±1.09 pA/pF; P<0.05). It is important to point out that these more mechanistic experiments were performed in a smaller number of myocytes than in those experiments shown in Figures 1 through 5 and these results will need to be confirmed in a broader sample set. Nevertheless, these findings suggest that in NF-HVMs (under our basal conditions), the endogenous phosphatase activity outweighs a low level of endogenous PKA activity so that there is little phosphorylation of LTCCs. In the few F-HVMs that were studied, there appears to be little or no endogenous phosphatase activity and the addition of PP2A is required to reduce ICa_L. These results suggest that under our conditions the basal phosphorylation state of LTCCs is largely determined by activity of protein phosphatases.

Evidence for Hyperphosphorylation of PKA Target Proteins in Human Heart Failure
Schroder et al have suggested that the biophysical properties of single LTCCs from failing human hearts are consistent with a higher than normal phosphorylation state (higher open probability). One of the limitations of this study is that LTCCs in the t-tubules were not studied. Our experiments using whole-cell techniques suggest that the phosphorylation state of the entire population of LTCCs is likely to be increased in human heart failure. Our results are also consistent with a recent study in failing canine myocytes, suggesting that reduced LTCC density is a general feature of failing myocardium. Our results are also in general agreement with those studies of RYR in failing human heart. These composite results suggest that the cellular microdomain between the t-tubule and the junctional SR can locally regulate the phosphorylation of PKA target proteins and that the phosphorylation state of these molecules is increased in failing human ventricular myocytes. We further suggest that a decreased activity of phosphatases in this microdomain may be responsible for our results.

As discussed earlier, there is also good evidence for decreased phosphorylation of phospholamban and troponin-I (Tn-I) in the failing human heart. Reduced PLB and Tn-I phosphorylation are thought to be partly
responsible for the depressed SR function and increased myofilament Ca\(^{2+}\) binding affinity that have been observed in the failing heart.\(^{31,36,37}\) The bases of uncoordinated phosphorylation states of PKA target proteins in human heart failure are yet to be resolved. High tonic levels of circulating catecholamines in end stage human heart failure\(^{38}\) would predispose toward increased phosphorylation of PKA target proteins whereas adrenergic signaling defects would blunt the effects of this chronically heightened adrenergic state\(^{29}\) and predispose toward a lower phosphorylation state. We speculate that different portions of the same myocyte can respond to persistent \(\beta\)-adrenergic activation in different fashions by locally regulating the activity of PKA and phosphatases rather than by changing the global concentration of cAMP.

We provide 3 independent observations consistent with an increased phosphorylation state of LTCC in F- versus NF-HVMs. These are as follows: (1) the leftward shift of \(I_{Ca,L}\) in F- versus NF-HVMs under basal condition and similar \(I_{Ca,L}\) curves after ISO and db-cAMP application; (2) smaller effects of isoproterenol, db-cAMP, and Bay K8644 on \(I_{Ca,L}\) in F-HVMs; and (3) a decrease in \(I_{Ca,L}\) by PP2A in F-HVMs. It is important to point out that other factors are likely to be involved in the differences in \(I_{Ca,L}\) observed in our experiments. The fact that LVAD-HVMs respond to ISO like NF-HVMs yet their basal \(G_{Ca,L}\) relationship is shifted to the left like F-HVMs attests to this idea. Other factors that will need to be considered to resolve these issues include the types of accessory (\(\alpha\)) LTCC subunits,\(^{34}\) different isoforms of the \(\alpha_{1c}\) subunit of LTCC,\(^{43}\) and the possibility that different phosphorylation sites are responsible for regulating different properties\(^{39}\) of LTCC, like \(P_{o}\) and the voltage-dependence of activation.

**Figure 5.** Effect of db-cAMP and Bay K 8644 on \(I_{Ca,L}\) in NF-, LVAD-, and F-HVMs. 10 \(\mu\)mol/L db-cAMP caused a greater increase in \(I_{Ca,L}\) in NF- \((n=6, N=2)\) and LVAD- \((n=7, N=2)\) than in F- \((n=6, N=2)\) HVMs. 1 \(\mu\)mol/L Bay K 8644 increased \(I_{Ca,L}\) more in NF- \((n=11, N=3)\) and LVAD- \((n=5, N=2)\) HVMs than in F- \((n=18, N=5)\) HVMs. Because these drugs shift the G-V curve to left, the maximal \(I_{Ca,L}\) was determined by examining the full I-V relationship of each cell before and after drug application. A, Representative current traces showing maximal effects of db-cAMP on \(I_{Ca,L}\). B, Representative current traces showing maximal effects of BAYK 8644 on \(I_{Ca,L}\). C, Maximal \(I_{Ca,L}\) under control, ISO, db-cAMP, and Bay K 8644. Maximal \(I_{Ca,L}\) after db-cAMP application was significantly greater \((P<0.05,\) ANOVA) in NF \((-27.14 \pm 4.41\) pA/pF) and LVAD \((-28.07 \pm 1.72\) pA/pF) than in F \((-15.94 \pm 5.11\) pA/pF). In addition, the maximal \(I_{Ca,L}\) after Bay K 8644 was significantly greater in NF- \((-14.77 \pm 1.45\) pA/pF) and LVAD \((-15.26 \pm 2.01\) pA/pF) than in F- \((-11.43 \pm 0.52\) pA/pF) HVMs. Numbers over the bars are the ratio of maximal \(I_{Ca,L}\) after drug application to before drug application under each condition. *Significant differences \((P<0.05,\) ANOVA) between NF vs F or F vs LVAD. D, Data were normalized for comparisons of treatment effects in the different groups. Percentage change of each treatment on maximal \(I_{Ca,L}\) in NF, F, and LVAD was normalized by the percentage change in NF-HVMs. E, Voltage dependence of \(I_{Ca,L}\) activation in NF, F, and LVAD after db-cAMP. \(V_{0.5}\) was not significantly different \((ANOVA, P=0.05)\) in NF \((-10.6 \pm 3.2\) mV), F \((-11.6 \pm 2.0\) mV), and LVAD \((-14.3 \pm 2.5\) mV).
Evidence for a Decreased Density of L-Type Ca\textsuperscript{2+} Channels in Failing Ventricular Myocytes

The density of L-type Ca\textsuperscript{2+} channels can be directly studied in intact myocytes with voltage clamp techniques. Most of the previous studies using these assays have shown that there is either no change or a small decrease in the LTCC current density in the hypertrophied and failing myocytes (see review by Mukherjee et al\textsuperscript{33}). However, one of these studies recently showed that although $I_{\text{Ca,L}}$ density was unchanged in failing canine myocytes the charge movement associated with channel activation (an assay for the total number of channels) was significantly reduced,\textsuperscript{16} in agreement with our present observations in human myocytes.

Changes in LTCC density in the failing human heart have also been evaluated using biochemical and molecular techniques. These studies have shown that the mRNA abundance of the $\alpha_1$ subunit of the LTCC is either unchanged\textsuperscript{40,41} or reduced in the failing heart.\textsuperscript{32} Quantifying LTCC number with the dihydropyridine binding method, Rasmussen et al\textsuperscript{40} found that the number of DHP binding sites was unchanged in failing hearts, whereas Takahashi et al\textsuperscript{32} found the $\alpha_1$ subunit decreased in abundance at both the mRNA and protein (DHP binding sites) level. These studies generally support our contention that the density of LTCC is decreased in human heart failure. It is important to keep in mind however that extrapolation of mRNA and DHP binding data to the functional status of LTCCs in intact cells should be done cautiously because it is known that LTCC accessory proteins,\textsuperscript{43} channel modifications (phosphorylation is one example), and localization can alter the function of the LTCC without requiring a change in mRNA or DHP binding.

Normalization of LTCCs in LVAD-HVMs

Recently, we\textsuperscript{4} and others\textsuperscript{5} have shown that many of the abnormalities in cardiac contractility and gene expression in the failing human heart are at least partially reversed if these hearts are supported with mechanical assist devices. The present results suggest that increases in the sarcolemmanal density of LTCCs and a reduction in the level of basal channel phosphorylation are involved in the improved basal contractility and improved $\beta$-adrenergic responsiveness observed in the LVAD-supported heart.

Functional Significance of Reduced LTCC Density With Increased Phosphorylation

The mechanisms responsible for increased LTCC phosphorylation in F-HVMs appear to involve alterations in phosphorylation activity. These LTCC alterations have predictable effects on the function of failing heart. A reduction in the density of LTCC should eventually produce alterations in EC coupling.\textsuperscript{44} Defective EC coupling would reduce the amount of Ca\textsuperscript{2+} released from the SR and contribute to systolic dysfunction. A higher level of basal channel phosphorylation would also limit the ability of additional $\beta$-adrenergic activation to further augment the $I_{\text{Ca,L}}$. This would explain the inability of $\beta$-agonists to rescue defects in EC coupling in animal models of CHF.\textsuperscript{44} Because $\beta$-agonist–induced augmentation of the $I_{\text{Ca,L}}$ is largely responsible for enhancing SR Ca\textsuperscript{2+} loading in normal myocytes,\textsuperscript{19} increased basal phosphorylation of LTCC is likely to be involved in the blunted inotropic effects of adrenergic stimulation in the failing human heart.

Limitations

All studies that utilize tissues and myocytes from end stage failing human hearts are limited by the fact that there is tremendous patient-to-patient variability in disease etiology and treatment (and a host of other factors). It is also important to point out that our nonfailing (control) group may not reflect normal human tissue. Therefore, all studies of this type need to be interpreted cautiously, with results used to point the way to more mechanistic studies under more controlled conditions in appropriate model systems. Finally, our experiments that examined the roles of PKA and PP2A in altered LTCC properties were performed in a small number of myocytes from a few hearts. The clues provided by these experiments will need to be confirmed in an expanded study.

Conclusion

Our results suggest that previously unrecognized alterations in the density and regulation of the L-type Ca\textsuperscript{2+} channel contribute to the abnormal contractility and blunted adrenergic responsiveness of the failing human heart.

Acknowledgments

This research was supported by grants from the National Institutes of Health (HL33920 and HL66415 to S.R.H., HL03560 and AG17022 to K.B.M.), and the Southeastern Pennsylvania Affiliate of the American Heart Association (0110091U to X.C.). We acknowledge Temple Cardiac Transplant Team for their assistance with this study.

References

9. Beuckelmann DJ. Contributions of Ca\textsuperscript{2+}–influx via the L-type Ca\textsuperscript{2+}–current and Ca\textsuperscript{2+}–release from the sarcoplasmic reticulum to [Ca\textsuperscript{2+}]-transients in human myocytes. Basic Res Cardiol. 1997;92(suppl 1):105–110.

13. Fabiato A. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. Am J Physiol. 1983;245:C1–C14.


L-Type Ca\(^{2+}\) Channel Density and Regulation Are Altered in Failing Human Ventricular Myocytes and Recover After Support With Mechanical Assist Devices

Xiongwen Chen, Valentino Piacentino III, Satoshi Furukawa, Bruce Goldman, Kenneth B. Margulies and Steven R. Houser

*Circ Res.* 2002;91:517-524; originally published online August 15, 2002;
doi: 10.1161/01.RES.0000033988.13062.7C
*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/91/6/517

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2002/09/14/91.6.517.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation Research* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation Research* is online at:
http://circres.ahajournals.org//subscriptions/