Transmural Heterogeneity and Remodeling of Ventricular Excitation-Contraction Coupling in Human Heart Failure

Qing Lou, MS; Vadim V. Fedorov, PhD; Alexey V. Glukhov, PhD; Nader Moazami, MD; Vladimir G. Fast, PhD; Igor R. Efimov, PhD

Background—Excitation-contraction (EC) coupling is altered in end-stage heart failure. However, spatial heterogeneity of this remodeling has not been established at the tissue level in failing human hearts. The objective of this article was to study functional remodeling of excitation-contraction coupling and calcium handling in failing and nonfailing human hearts.

Methods and Results—We simultaneously optically mapped action potentials and calcium transients in coronary perfused left ventricular wedge preparations from nonfailing (n=6) and failing (n=5) human hearts. Our major findings are the following. First, calcium transient duration minus action potential duration was longer at subendocardium in failing compared with nonfailing hearts during bradycardia (40 bpm). Second, the transmural gradient of calcium transient duration was significantly smaller in failing hearts compared with nonfailing hearts at fast pacing rates (100 bpm). Third, calcium transient in failing hearts had a flattened plateau at the midmyocardium and exhibited a 2-component slow rise at the subendocardium in 3 failing hearts. Fourth, calcium transient relaxation was slower at the subendocardium than at the subepicardium in both groups. Protein expression of sarcoplasmic reticulum Ca$^{2+}$-ATPase 2a was lower at the subendocardium than the subepicardium in both nonfailing and failing hearts. Sarcoplasmic reticulum Ca$^{2+}$-ATPase 2a protein expression at subendocardium was lower in hearts with ischemic cardiomyopathy compared with those with nonischemic cardiomyopathy.

Conclusions—For the first time, we present direct experimental evidence of transmural heterogeneity of excitation-contraction coupling and calcium handling in human hearts. End-stage heart failure is associated with the heterogeneous remodeling of excitation-contraction coupling and calcium handling. (Circulation. 2011;123:1881-1890.)

Key Words: action potentials | calcium | excitation contraction coupling | heart failure | ventricular remodeling

Congestive heart failure (HF) is one of the leading causes of death in Western countries. Depressed contractility during congestive HF is associated with altered excitation-contraction (EC) coupling in general and with calcium handling in particular. Investigating calcium handling, Laurita et al showed that the recovery of intracellular calcium in canine LV was slower in cells near the endocardium compared with cells near the epicardium. We have recently described spatial heterogeneity of action potential (AP) in human ventricle and its implication in the vulnerability to arrhythmias. However, spatial heterogeneity of EC coupling and intracellular calcium handling in human heart remains unclear.

It was suggested by a molecular study by Prestle et al that the transmural heterogeneity of calcium handling was enhanced in failing human hearts compared with nonfailing human hearts. In failing human hearts, the protein expression of the sarcoplasmic reticulum Ca$^{2+}$-ATPase 2a (SERCA2a) was reduced significantly in the subendocardium compared with the subepicardium, which might lead to the heterogeneous uptake of intracellular calcium and facilitate the induction of ventricular arrhythmias. Despite the molecular...
evidence, it remains unknown whether the heterogeneity of EC coupling and calcium handling is present, and how it is functionally remodeled in HF. Furthermore, it is unknown whether this remodeling could contribute to the increased ventricular arrhythmogenesis and mechanical dysfunction associated with human HF. We hypothesize that across the intact transmural wall, intrinsic heterogeneities of EC coupling and calcium handling exist, and thus that the susceptibility to remodeling during HF differs in different transmural layers of the LV. To test this hypothesis, dual optical mappings of AP and calcium transient (CaT) were conducted in LV wedge preparations from both failing and nonfailing human hearts.

**Methods**

**Experimental Protocol**

The study was approved by the Washington University Institutional Review Board. Both failing (n=5) and nonfailing (n=6) human hearts were optically mapped in this study. For Western blot assay, we used tissue from 18 hearts. Patient information is shown in the Table. The isolated LV wedge preparation has been described in our previous article. Briefly, a piece of LV wedge from the scar-free postlateral LV free wall perfused by the left marginal artery (Figure 1A) was isolated and cannulated. Tissue was immobilized by blebbistatin (10 to 20 μmol/L; Tocris Bioscience, Ellisville, MO) to suppress motion artifacts in optical recordings. The tissue was costained with RH237 and Rhod-2 AM for simultaneous mapping of voltage or calcium fluorescent signal. The AP duration (APD) was measured as the time from the upstroke to 80% repolarization (APD80; Figure 1D). Similarly, the CaT duration (CaTD) was measured as the time from the upstroke to 30% and 80% recovery (ie, CaTD30 and CaTD80; Figure 1D). The 10% to 90% rise time of CaT was measured as the time from 10% CaT (close to the baseline) to 90% CaT (close to the peak; Figure 1D). Relaxation of CaT was quantified by the time constant (τ) of a single exponential fit of the CaT tail, ie, the time from the minimum of d(CaT)/dt to the resting level of CaT. The subepicardium was defined as the region within 2 mm from the epicardial surface (see Figure 2B, right); the midmyocardium was the 2-mm-wide midmyocardial layer; and the subendocardium was the region within 2 mm from the endocardial surface.

<table>
<thead>
<tr>
<th>No.</th>
<th>Group</th>
<th>Sex</th>
<th>Age, y</th>
<th>Diagnosis</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nonfailing</td>
<td>Male</td>
<td>55</td>
<td>Death resulting from stroke</td>
<td>Mapping, WB</td>
</tr>
<tr>
<td>2</td>
<td>Nonfailing</td>
<td>Female</td>
<td>59</td>
<td>Anoxic brain injury after cardiac arrest</td>
<td>Mapping, WB</td>
</tr>
<tr>
<td>3</td>
<td>Nonfailing</td>
<td>Male</td>
<td>53</td>
<td>Intracranial hematomata</td>
<td>Mapping, WB</td>
</tr>
<tr>
<td>4</td>
<td>Nonfailing</td>
<td>Male</td>
<td>56</td>
<td>Intracranial hematomata</td>
<td>Mapping, WB</td>
</tr>
<tr>
<td>5</td>
<td>Nonfailing</td>
<td>Female</td>
<td>47</td>
<td>Brain death caused by anoxia</td>
<td>Mapping, WB</td>
</tr>
<tr>
<td>6</td>
<td>Failing</td>
<td>Male</td>
<td>65</td>
<td>Ischemic cardiomyopathy</td>
<td>Mapping, WB</td>
</tr>
<tr>
<td>7</td>
<td>Failing</td>
<td>Male</td>
<td>63</td>
<td>Ischemic cardiomyopathy</td>
<td>Mapping, WB</td>
</tr>
<tr>
<td>8</td>
<td>Failing</td>
<td>Male</td>
<td>49</td>
<td>Idiopathic cardiomyopathy</td>
<td>Mapping, WB</td>
</tr>
<tr>
<td>9</td>
<td>Failing</td>
<td>Female</td>
<td>54</td>
<td>Idiopathic cardiomyopathy</td>
<td>Mapping, WB</td>
</tr>
<tr>
<td>10</td>
<td>Failing</td>
<td>Female</td>
<td>54</td>
<td>Idiopathic cardiomyopathy</td>
<td>Mapping, WB</td>
</tr>
<tr>
<td>11</td>
<td>Nonfailing</td>
<td>Female</td>
<td>50</td>
<td>Brain death caused by anoxia</td>
<td>Mapping</td>
</tr>
<tr>
<td>12</td>
<td>Nonfailing</td>
<td>Female</td>
<td>66</td>
<td>Brain death caused by hemorrhaging</td>
<td>WB</td>
</tr>
<tr>
<td>13</td>
<td>Failing</td>
<td>Male</td>
<td>61</td>
<td>Ischemic cardiomyopathy</td>
<td>WB</td>
</tr>
<tr>
<td>14</td>
<td>Failing</td>
<td>Male</td>
<td>64</td>
<td>Ischemic cardiomyopathy</td>
<td>WB</td>
</tr>
<tr>
<td>15</td>
<td>Failing</td>
<td>Female</td>
<td>49</td>
<td>Ischemic cardiomyopathy</td>
<td>WB</td>
</tr>
<tr>
<td>16</td>
<td>Failing</td>
<td>Male</td>
<td>50</td>
<td>Ischemic cardiomyopathy</td>
<td>WB</td>
</tr>
<tr>
<td>17</td>
<td>Failing</td>
<td>Male</td>
<td>47</td>
<td>Idiopathic dilated cardiomyopathy</td>
<td>WB</td>
</tr>
<tr>
<td>18</td>
<td>Failing</td>
<td>Female</td>
<td>44</td>
<td>Idiopathic cardiomyopathy</td>
<td>WB</td>
</tr>
<tr>
<td>19</td>
<td>Failing</td>
<td>Male</td>
<td>70</td>
<td>Idiopathic dilated cardiomyopathy</td>
<td>WB</td>
</tr>
</tbody>
</table>

Mapping indicates optical mapping experiments; WB, Western blotting assay.

Data Analysis

All signals were low-pass filtered at 60 Hz. The voltage–calcium delay was defined as the delay between the upstrokes of AP and CaT (Figure 1D). Each upstroke was defined at (dF/dt)max, where F is the voltage or calcium fluorescent signal. The AP duration (APD) was measured as the time from the upstroke to 80% repolarization (APD80; Figure 1D). Similarly, the CaT duration (CaTD) was measured as the time from the upstroke to 30% and 80% recovery (ie, CaTD30 and CaTD80; Figure 1D). The 10% to 90% rise time of CaT was measured as the time from 10% CaT (close to the baseline) to 90% CaT (close to the peak; Figure 1D). Relaxation of CaT was quantified by the time constant (τ) of a single exponential fit of the CaT tail, ie, the time from the minimum of d(CaT)/dt to the resting level of CaT. The subepicardium was defined as the region within 2 mm from the epicardial surface (see Figure 2B, right); the midmyocardium was the 2-mm-wide midmyocardial layer; and the subendocardium was the region within 2 mm from the endocardial surface.

Statistical Analysis

For statistical analysis, we used ANOVA. Specifically, we fit a linear mixed-effects repeated measures model in which the patient was a random effect and other factors (failing/nonfailing, tissue layers, and BCL) were fixed effects. Models contained parameters that allow heterogeneous variance among levels of the failing/nonfailing by tissue-layer interaction and repeated measures correlation among tissue layers. We compared models by the small-sample-size–corrected version of Akaike information criterion. Contrasts were used to test the significance of differences between the failing and nonfailing groups within different tissue layers (subendocardium/midmyocardium/subepicardium). Bonferroni adjustment was used to
account for multiple comparisons. Detailed specifications of statistical analysis for individual figures are provided in the online-only Data Supplement. Values of $P<0.05$ were considered statistically significant. Values are given as mean±SD.

Results

Voltage-Calcium Delay (Action Potential—Calcium Transient Delay)

To quantify the EC coupling, the delay between the AP upstroke and CaT rise was measured. As expected, the upstroke of the AP was always followed by the rise of CaT (Figure 1D). To quantify the transmural heterogeneity, this delay was measured and averaged at all 3 tissue layers (subendocardium, midmyocardium, and subepicardium). Figure 2A and 2B display AP and CaT activation maps and the voltage-calcium delay. This delay is summarized in Figure 2C for the BCL of 1500 milliseconds. We observed a transmural gradient of this delay within the failing group. That is, the delay was significantly larger at the subendocardium than the subepicardium ($P<0.015$; see Figure 2B as an example).

Action Potential Duration and Calcium Transient Duration

We quantified APD, CaTD, and the difference between the 2 (ie, CaTD—APD). Figure 3 shows 1 example from a nonfailing heart (Figure 3A) and 1 example from a failing (Figure 3B) heart. Maps of APD80 and CaTD80 are shown for both hearts. It should be noted that the color scales for APD and CaTD maps are different. It can be seen that APD80 was mildly increased in this failing heart compared with the nonfailing heart, whereas CaTD80 was increased in a more substantial manner. Because of the disproportionate prolongation of CaTD relative to APD, CaTD80—APD80 in the failing heart was larger than in the nonfailing heart.

APD80, CaTD80, and CaTD80—APD80 for both failing and nonfailing groups are shown in Figure 4A for the BCL of 1500 milliseconds and are summarized in Figure 4B for multiple BCLs. The transmural APD and CaTD gradients represented by the subendocardium and subepicardium duration differences are shown in Figure 4C. Transmural APD gradients are present in both failing and nonfailing human hearts. Similar to our previous study, at slow heart rates, this gradient was less pronounced in the failing group compared with the nonfailing group (Figure 4A and 4C, left). CaTD80 (Figure 4A, middle) also exhibited gradients from the endocardium to the epicardium. Interestingly, this gradient (Figure 4C) was significantly smaller in the failing group compared with the nonfailing group at fast heart rates (eg, 100 bpm or BCL of 600 milliseconds) but not at slow heart rates (eg, 40 bpm or BCL of 1500 milliseconds).

The duration difference (CaTD80—APD80) was significantly increased at the subendocardium in the failing hearts during bradycardia ($P=0.022$; Figure 4A, right). As the BCL decreased, the duration difference was significantly decreased within the failing group but remained unchanged in the nonfailing group (Figure 4B, right). Both APD80 and CaTD80 were decreased as the BCL was decreased (Figure 4B, left and middle).

M-cell islands, which contain prolonged APDs and are surrounded by large APD gradients, were observed previously in nonfailing human hearts. In the majority of the hearts in this study, APD decreased gradually from the endocardium to epicardium without the presence of M cells. However, M cells were observed in 1 nonfailing heart in which we specifically searched for them (Figure 5). As shown on the map of APD80 (Figure 5B), the M-cell area was in the form of an isolated island rather than a continuous layer. This region exhibited a delayed repolarization (Figure 5E), and was surrounded by steep local APD gradients (Figure 5C). As
shown in Figure 5D and 5E, the M-cell island had longer CaTD compared with neighboring regions. Other M-cell island parameters (AP-CaT delay, CaTD/H11002 APD, CaT rise time, CaTD30/CaTD80, and /H9270) were not different from neighboring midmyocardium regions (Figure II in the online-only Data Supplement).

Morphological Changes in Calcium Transients
There were 2 morphological changes in CaT in the failing hearts compared with the nonfailing hearts. Figure 6A shows 2 representative examples of CaT recorded at the subendocardium of a failing (top) and a nonfailing (bottom) heart. The first change was a 2-component rising phase, including an initial fast rising phase (labeled I in Figure 6A) and a subsequent second slow rising phase (labeled II in Figure 6A). This was observed in 3 of 5 failing hearts, but not in any of the nonfailing hearts, and it was present only at the subendocardium. The second slow component resulted in a significant increase in the rise time of CaT (P<0.001) from 26±3 milliseconds (nonfailing and 2 failing hearts) to 49±12 milliseconds (3 failing hearts) at the subendocardium. However, if a comparison is made between the nonfailing and failing groups, this increase would not be statistically significant (see Figure 6B). The second morphological difference was a more flattened plateau of CaT within the failing group, which is reflected by an increased ratio of CaTD30 to CaTD80. Figure 6C shows that this ratio was significantly increased in failing hearts compared with nonfailing hearts at the midmyocardium.

Relaxation of Calcium Transients
The time constant of CaT relaxation reflects the rate of Ca2+/H11001 reuptake from the cytoplasm by SERCA2a and Na+/Ca2+/H11001 exchanger. We observed a gradient of the relaxation time constant of CaT from the endocardium to epicardium in both failing and nonfailing hearts. Figure 7A and 7B shows representative examples of the τ measurement in a failing human heart. It is evident that τ was larger at the subendocardium than at the subepicardium. The difference between the failing and nonfailing groups was not statistically significant, although there was a trend of an increase in τ within the failing group (Figure 7C).

Protein Expression of Sarcoplasmic Reticulum
Ca2+/H11021-ATPase 2a and Phospholamban
To determine the molecular mechanism of the observed gradient of τ presented above, we quantified the protein expressions of SERCA2a and phospholamban. In Figure 8, representative bands and the statistical summary are shown for SERCA2a (Figure 8A) and phospholamban (Figure 8B). We divided our samples into 3 groups (Table): nonfailing, failing with ischemic cardiomyopathy, and failing with nonischemic/idiopathic cardiomyopathy. Each group was subdivided into subendocardium and subepicardium. For SERCA2a, there was a significant difference between the subendocardium and subepicardium (P<0.001; Figure 8A); interaction between tissue layers and patient groups was not significant (P=0.295). SERCA2a expression at the subendocardium in the ischemic group was significantly lower compared with that in the nonischemic group (P=0.023; Figure 8A). For phospholamban, we did not observe any differences between the subendocardium and subepicardium or among any of the 3 groups (Figure 8B).

Discussion
In the present study, we conducted for the first time the simultaneous mapping of both voltage and calcium in LV wedge preparations from failing and nonfailing human hearts. We found that HF-induced remodeling consists of (1) increased differences in APD and CaTD at the subendocardium during bradycardia (40 bpm), (2) decreased transmural CaT gradients at fast pacing rates (100 bpm), (3) a slow component of rise and a dome-shaped plateau in CaT, and (4) a lowered level of SERCA2a expression at the subendocardium in failing human hearts with ischemic cardiomyopathy. We also found that there existed transmural gradients of CaTD80,
CaT relaxation time constant ($\tau$), and protein expression of SERCA2a in both failing and nonfailing human hearts.

Implications From Calcium Transient Morphology Changes
There was a 2-component rise of CaT at the subendocardium in 3 failing hearts, an initial fast component followed by a slow second component. This was previously observed in isolated myocytes from failing human and canine hearts.\(^2,21\) Piacentino et al\(^2\) suggested that this might result from increased Ca\(^{2+}\) entry during the AP plateau caused by less calcium-mediated inactivation of L-type calcium currents and increased activity of the Na\(^+/\)Ca\(^{2+}\) exchanger in the reverse mode (Ca\(^{2+}\) influx). The same mechanism could also explain the apparent dome shape of CaT observed in the failing hearts.

The morphological changes in CaT could also result from dyssynchronous Ca\(^{2+}\) release within a cell. The delayed release of Ca\(^{2+}\) in defective regions might be responsible for the slow component of rise and subsequent dome shape of CaT observed in the failing human hearts. Confocal line scan recordings in whole failing rat hearts revealed that the release of Ca\(^{2+}\) at some part within a cell does not occur at the time of initial depolarization but a short time after the depolarization.\(^22\) It is possible that the normal Ca\(^{2+}\) release corresponds to the first fast rising phase of CaT and that delayed Ca\(^{2+}\) release corresponds to the second slow component of CaT.

Interestingly, this slow secondary rise of CaT was observed only in the subendocardium region in 60% of failing hearts. Further studies are required to investigate the mechanisms of this remodeling at both the molecular and tissue structural levels.

The decay of CaT was markedly prolonged in isolated cells from failing human hearts.\(^9\) In contrast, CaTD in failing hearts was not statistically different from that in nonfailing hearts in our study. This is likely due to differences between isolated cell and tissue preparations, and to differences in the pacing cycle length. O'Rourke et al\(^21\) showed that CaTD was 3-fold longer in myocytes from failing hearts at a BCL of 6 seconds but was not significantly different at a 1-second interval compared with myocytes from nonfailing hearts. Another possible explanation for this discrepancy is the afterload dependence of CaT, which indicates that mechanical work and metabolic demand are crucial for inducing the pathological regulation of and morphological changes in CaT.\(^12\) Mechanical work was inhibited in our study by blebbistatin to eliminate motion artifacts, so changes in CaT might not be as evident.

CaT–APD was significantly increased at the subendocardium at a slow heart rate (40 bpm) in failing hearts compared with nonfailing hearts (Figure 4A, right). That is, CaT significantly outlasts AP and is elevated during phase 3 of the AP. This difference in duration was previously proposed to promote late phase 3 early afterdepolarization by the strong recruitment of electrogenic Na\(^+/\)Ca\(^{2+}\) exchanger currents.\(^23,24\) Although early afterdepolarization was not observed in this study, we speculate that it might contribute to the enhanced arrhythmogenesis in HF by promoting early afterdepolarizations under conditions such as metabolic inhibition.

Transmural Calcium Transient Duration Gradient
The transmural gradient of CaTD at fast heart rates (100 bpm) was significantly smaller in the failing group compared with
the nonfailing group. This might have important physiological implications relevant to the mechanical dysfunction of failing human hearts.

The transmural gradient of CaTD was 72 ± 10 and 81 ± 16 milliseconds at a slow rate (40 bpm) for failing and nonfailing groups during endocardial pacing, respectively (Figure 4C, right). The corresponding conduction time from the endocardium to epicardium was 49 ± 13 and 30 ± 5 milliseconds (40 bpm). Therefore, the transmural gradient of the time at 80% of CaT relaxation from endocardium to epicardium (endocardial to epicardial CaTD difference minus conduction time) was 23 ± 15 and 51 ± 19 milliseconds. The positivity of these values indicates that the sequence of relaxation of CaT was from the epicardium to the endocardium for both the failing and nonfailing groups at 40 bpm. This sequence is the same as the transmural sequence

Figure 4. Action potential duration (APD), calcium transient duration (CaTD), and the duration difference (APD − CaTD). A, APD at 80% repolarization (APD80), CaTD at 80% repolarization (CaTD80), and the duration difference at a basic cycle length (BCL) of 1500 milliseconds in failing (F; n = 5) and nonfailing (NF; n = 6) hearts at the subendocardium (sub-ENDO), midmyocardium (MID), and subepicardium (sub-EPI). B, Dynamics of APD80, CaTD80, and the duration difference at various cycle lengths at sub-ENDO, MID and sub-EPI. The top row is for nonfailing hearts; the bottom row, for failing hearts. C, Transmural APD and CaTD gradients. The gradient is calculated as the difference between the values of the subendocardium and subepicardium.
of myofiber relaxation measured in vivo in normal canine hearts during sinus rhythm.25

At fast heart rates, this sequence was maintained in nonfailing human hearts (as expected), but was reversed in failing human hearts. At 100 bpm in the failing group, the transmural gradient of CaTD was 25±11 milliseconds and conduction time increased to 57±13 milliseconds (Figure 4C, right). Therefore, the transmural gradient of the time at 80% of CaT relaxation was −29±15 milliseconds; the negativity indicates that the sequence of CaT relaxation for the failing group was from the endocardium to epicardium. This reversed sequence of relaxation at fast heart rates could be associated with poor mechanical function and might be one of the mechanisms underlying the higher risk for the primary composite end point in HF patients with higher heart rates.26

Heterogeneous Calcium Handling

The protein expression of SERCA2a was significantly lower in ischemic failing hearts than in nonischemic failing hearts at the subendocardium, but not the subepicardium. This indicates that the protein level of SERCA2a is dependent on both the cause of HF and the anatomic location of the myocardium. Previously, SERCA2a was found to be significantly downregulated in failing human hearts in some studies,27–30 but not in other studies.31–37 According to our results, the inconsistent observations might be related to the anatomic inconsistency within and across studies and could be due to the etiology dependence of downregulation.

In both failing and nonfailing hearts, the protein level of SERCA2a was less abundant at the subendocardium than that at subepicardium (Figure 8A). This difference was consistent with previous observations in canine and human hearts.15,17 The lower expression of SERCA2a was suggested to lead to the larger relaxation time constant of CaT in canine hearts.15 Our results suggest that this causal relationship might also exist in human hearts.

No significant increase in relaxation time constant was observed in failing human hearts in this study. Because our failing group consisted of 2 hearts with ischemic cardiomyopathy and 3 hearts with nonischemic/idiopathic cardiomyopathy for the functional part of this study, the lack of statistical significance could be explained by different types of HF. Indeed, the time constant in hearts with ischemic cardiomyopathy was longer than in hearts with nonischemic/idiopathic cardiomyopathy (ie, subendocardium, 170 versus 136 milliseconds; midmyocardium, 152 versus 124 milliseconds; subepicardium, 137 versus 114 milliseconds). Because of limited samples, future studies are needed to test this hypothesis. Nevertheless, this hypothesis is supported by a
study in isolated myocytes from human hearts with end-stage HF that showed that relaxation of CaT and contraction was significantly slower in hearts with ischemic cardiomyopathy than in hearts with dilated cardiomyopathy. This difference might be explained by the significant downregulation of SERCA2a protein expression in the ischemic failing group but no change within the nonischemic failing group, as shown in this study.

**M-Cell Island**

We have recently reported that M cells were present in the form of spatially discrete and isolated islands rather than a continuous layer in 3 of 5 nonfailing human hearts. Moreover, M cells were not observed in failing human hearts owing to nonhomogeneous APD prolongation and decreased transmural APD gradient. To compare failing and nonfailing hearts under the same pattern of APD distribution, we did not concentrate on searching for M-cell islands in nonfailing hearts. This explains why we present M cells in only 1 nonfailing heart (Figure 5). In this experiment, we specifically searched for M cells that were in the form of isolated islands rather than a continuous layer, as was previously described. The regions above and below the M-cell island had a continuous APD gradient from the endocardium to epicardium (similar to Figure 3). To compare failing and nonfailing hearts under the same pattern of APD distribution, data only in the region without M cells (eg, the top part of Figure 5B) were used in the statistical analysis.

As shown in Figure 5D, CaTD within the M-cell island was prominently longer compared with the neighboring region. However, CaTD within the M-cell island (698±9 milliseconds) was comparable to that at the subendocardium (711±15 milliseconds; see Figure 5D), whereas APD within the M-cell island (649±7 milliseconds) was longer than at the subendocardium (618±13 milliseconds; see Figure 5B). This difference is similar to the observation made in the canine study by Cordeiro et al. Interestingly, other parameters related to EC coupling and calcium handling (such as AP-CaT delay, CaT rise time, and CaT relaxation time constant) were not distinctly different from the surrounding region (Figure II in the online-only Data Supplement). The role of M-cell islands in the contraction and their nature in nonfailing hearts remain unclear.

**Limitations**

This study has several limitations. First, nonfailing donor hearts are not necessarily representative of healthy hearts. However, none of the donors has a history of HF and were...
thus the best controls available for this functional study. Second, because of technical limitations, only a limited transmural surface of LV with good perfusion was mapped.

As a result of the anatomic heterogeneity of the heart itself, caution should be taken when extrapolating the results to the whole heart. Third, owing to the limited access to functional human hearts, the number of hearts for each group is small and might compromise the statistical significance of potential differences. Because all of the failing hearts with different cardiomyopathy were grouped together for the functional data analysis, only changes common to different types of cardiomyopathy could be revealed. Changes unique to individual cardiomyopathy could be masked. Fourth, several other important aspects of EC coupling such as the sarcoplasmic reticulum calcium content and the EC coupling gain were not examined in this study. Regional differences in these parameters need to be resolved in future studies. Fifth, application of blebbistatin in our study liberated ATP from mechanical contraction and thus allowed an ample supply of ATP for electrophysiological processes. Pathological changes could thus be less evident in the absence of metabolic disturbance that could be unmasked by mechanical work. Finally, as shown in neonatal rat myocyte cultures, the use of high-affinity dyes, including Rhod-2, may overestimate CaTD, which was about twice as large as APD. This was not likely the case in our measurements because CaTD in normal human hearts was comparable to APD. In addition, the main focus of the present study was on regional differences in CaTD rather than on its absolute value. Thus, potential systematic errors, if they existed, were likely to be subtracted or minimized.

Conclusion

We present for the first time the simultaneous transmural mapping of AP and CaT in LV wedge preparations in both failing and nonfailing human ventricles. Our results demonstrate that transmural heterogeneity of EC coupling and calcium handling are evident in failing human ventricles. Therefore, the transmurally heterogeneous remodeling of these properties resulting from HF.

Acknowledgments

We thank Ai-Li Cai, Christina Ambrosi, and Deborah Janks for their help with tissue collection. We also thank Dr Bum-Rak Choi for his valuable suggestions in developing the dual imaging system.

Sources of Funding

This work was supported by National Institutes of Health grants HL085369, HL067322, and HL074283.

Disclosures

None.

References

Excitation-contraction coupling is a complex process mediated by a network of proteins that control ionic currents, cell signaling, calcium handling, and sarcomeric mechanics. The 2 hallmarks of excitation-contraction coupling preceding mechanical contraction are the transmembrane action potential and intracellular calcium transient. Numerous studies in animal models have significantly advanced our understanding of the fundamental mechanisms of excitation-contraction coupling at the molecular, cellular, and whole-heart levels. However, the extrapolation of these findings to clinical practice is limited by the lack of functional human data. Our study provides, for the first time in medical history, such functional data: simultaneous recordings of action potentials and calcium transients from the human left ventricular tissue of nonfailing and failing hearts. We found that the nonfailing human left ventricle has a transmural gradient of calcium transient kinetics, with the calcium transient being longer at the endocardial than at the epicardial layers of the ventricular wall. A decrease in this gradient in the failing heart reversed the normal sequence of relaxation. We also found that heart failure leads to significant changes in calcium dynamics, manifesting as a biphasic calcium entry into the cytosol. Downregulation of sarcoplasmic reticulum Ca\(^{2+}\)-ATPase 2a depends on the origin and anatomic location, which might explain the inconsistency in the literature on the subject. Transmural heterogeneity indicates that the anatomic location must be considered in studies of molecular remodeling of excitation-contraction coupling. Our study confirms some of the earlier findings in animal models but contradicts others. Thus, extrapolation of findings from animal models to humans should be done with caution.

Go to http://cme.ahajournals.org to take the CME quiz for this article.
Transmural Heterogeneity and Remodeling of Ventricular Excitation-Contraction Coupling in Human Heart Failure
Qing Lou, Vadim V. Fedorov, Alexey V. Glukhov, Nader Moazami, Vladimir G. Fast and Igor R. Efimov

Circulation. published online April 18, 2011;
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/early/2011/04/18/CIRCULATIONAHA.110.989707

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2011/04/22/CIRCULATIONAHA.110.989707.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation 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 is online at:
http://circ.ahajournals.org//subscriptions/
SUPPLEMENTAL MATERIAL

Title: Transmural Heterogeneity and Remodeling of Ventricular Excitation-Contraction Coupling in Human Heart Failure

Authors: Qing Lou, MS; Vadim V. Fedorov, PhD; Alexey V. Glukhov, PhD; Nader Moazami, MD; Vladimir G. Fast, PhD; Igor R. Efimov, PhD

SUPPLEMENTAL METHODS

Tissue Preparation

Failing human hearts were obtained during heart transplantation from Barnes-Jewish Hospital of Washington University in St. Louis, MO. Donor hearts, which were not suitable for heart transplantation due to age, were provided by Mid-America Transplant Service (St. Louis, MO) and used as control. For Western blot assay, we used tissue from 18 hearts (i.e., 6 hearts for every group including nonfailing heart, failing heart with ischemic cardiomyopathy, and failing heart with nonischemic cardiomyopathy (Table 1)).

Explanted hearts were cardioplegically arrested in the operating room immediately after removal from the chest. The heart was then bathed in the cold cardioplegic solution for transportation, dissection and cannulation. During dissection, a piece of LV wedge from the scar-free post-lateral LV free wall supplied by left marginal artery was isolated and cannulated. Major arteries were ligated to maintain an arterial pressure of 60-70 mmHg.

Simultaneous Mapping of Voltage and Calcium

After cannulation, the LV wedge was both coronary-perfused and superfused by 37°C Tyrode solution. The initial 20-30 minutes of perfusion were used to allow the tissue to stabilize. Tissue was then immobilized by 10–20 μM blebbistatin (Tocris Bioscience, Ellisville, MO) to suppress motion artifacts in optical recordings. The tissue was co-stained via coronary perfusion with
voltage sensitive dye RH237 (30μL of 1.25mg/ml solution in DMSO, Invitrogen, Carlsbad, CA) and calcium indicator Rhod-2 AM (0.4-0.6mL of 1mg/ml solution in DMSO, Invitrogen, Carlsbad, CA).

Two halogen lamps (Newport Oriel Instruments, Stratford, CT; SciMedia, Costa Mesa, CA) equipped with 520 ± 45nm bandpass filters were used for excitation. Fluorescent voltage and calcium signals were simultaneously collected from the same field of view. Fluorescence emission was separated by a dichroic mirror (635nm cutoff, Omega Optical, Brattleboro, VT), and filtered by a 700nm longpass filter (Thorlabs, Newton, New Jersey) for voltage signals and by a 590 ± 15nm bandpass filter (Omega Optical, Brattleboro, VT) for calcium signals. The two signals were then recorded by a dual CMOS camera system (ULTIMA-L, SciMedia, Costa Mesa, CA).

**Western Blot**

Immediately after the delivery of the heart to our research lab, tissue samples at sub-endocardium (sub-ENDO, 2 mm to the endocardium) and sub-epicardium (sub-EPI, 2 mm to the epicardium) were dissected from LV, frozen in liquid nitrogen, and stored at -80°C until use. Standard Western blot procedures were used. We used the anti-SERCA2a monoclonal antibody and an anti-phospholamban monoclonal antibody (Affinity BioReagent, Golden, CO) as well as anti-GAPDH monoclonal antibody (Sigma, St. Louis, MO). Chemiluminescence was measured by luminescent image analyzer LAS-4000 (Fujifilm, Tokyo, Japan). Protein bands were quantified by software Multi-Gauge 3.0 (Fujifilm, Tokyo, Japan). Protein expression was analyzed in three groups of hearts (Table 1): nonfailing hearts (n=6), and failing hearts due to different etiologies: nonischemic/idiopathic cardiomyopathy (n=6) and ischemic cardiomyopathy (n=6).
Supplemental Statistical Analysis Method

We analyzed data using ANOVA with linear mixed effects repeated measures models. We evaluated repeated measures covariance structures by the small-sample-size corrected version of Akaike information criterion (AICc) model comparison and found that for tissue layer (sub-ENDO/MID/sub-EPI) an unstructured covariance fit best with ratio data in Figure 6C. All other models were independence models. We used models with variance parameters that vary among levels of basic cycle length (BCL) for APD80 and CaTD80 (Figures 4B top), and APD80 (Figure 4B bottom). We used models with variance parameters that vary among levels of tissue layer in Figure 6B and Figure 8.
Supplemental Figures and Figure Legends

Supplemental Figure 1. Representative simultaneous optical recordings of action potential (AP, blue) and calcium transient (CaT, red). Signals are taken from the evenly spaced local regions (labeled by the brown squares) which span the entire mapping field of view of a left ventricular wedge preparation. The endocardium (ENDO) and epicardium (EPI) are labeled by the white lines.

Supplemental Figure 2. Quantifications of nonfailing heart #11 with an M-cell island. Here shows the maps for APD80, AP-CaT delay, CaTD80-APD80, rise time of CaT, CaTD30/CaTD80, and the CaT relaxation time constant (τ).
Supplemental Figure 1:
Supplemental Figure 2: