Alterations in Early Action Potential Repolarization Causes Localized Failure of Sarcoplasmic Reticulum Ca^{2+} Release

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Abstract—Depressed contractility of failing myocytes involves a decreased rate of rise of the Ca^{2+} transient. Synchronization of Ca^{2+} release from the junctional sarcoplasmic reticulum (SR) is responsible for the rapid rise of the normal Ca^{2+} transient. This study examined the idea that spatially and temporally dyssynchronous SR Ca^{2+} release slows the rise of the cytosolic Ca^{2+} transient in failing feline myocytes. Left ventricular hypertrophy (LVH) with and without heart failure (HF) was induced in felines by constricting the ascending aorta. Ca^{2+} transients were measured in ventricular myocytes using confocal line scan imaging. Ca^{2+} transients were induced by field stimulation, square wave voltage steps, or action potential (AP) voltage clamp. SR Ca^{2+} release was significantly less well spatially and temporally synchronized in field-stimulated HF versus control or LVH myocytes. Surprisingly, depolarization of HF cells to potentials where Ca^{2+} currents (I_{Ca}) were maximal resynchronized SR Ca^{2+} release. Correspondingly, decreases in the amplitude of I_{Ca} desynchronized SR Ca^{2+} release in control, LVH, and HF myocytes to the same extent. HF myocytes had significant loss of phase 1 AP repolarization and smaller I_{Ca} density, which should both reduce Ca^{2+} influx. When normal myocytes were voltage clamped with HF AP profiles SR Ca^{2+} release was desynchronized. SR Ca^{2+} release becomes dyssynchronized in failing feline ventricular myocytes because of reductions in Ca^{2+} influx induced in part by alterations in early repolarization of the AP. Therefore, therapies that restore normal early repolarization should improve the contractility of the failing heart. (***Circ Res*. 2005;96:543-550.**)

Key Words: heart failure ■ excitation contraction coupling ■ sarcoplasmic reticulum ■ calcium transients

Hemodynamic overload induces cardiac hypertrophy and alterations in the contractile properties of the resident cardiac myocytes, both of which can help the heart maintain cardiac output. However, when the hemodynamic stress is persistent, the heart usually makes a transition from compensated hypertrophy to a progressively deteriorating functional state termed congestive heart failure (CHF). This transition is known to be associated with alterations in the magnitude, duration, and kinetics of the systolic Ca^{2+} transient.

Multiple cellular and molecular changes are thought to underlie the abnormal Ca^{2+} transient of the hypertrophied/failing myocyte.[1–7] The reduced size and prolonged duration of the Ca^{2+} transient involves reduced sarcoplasmic reticulum (SR) Ca^{2+} stores[1,7–9] resulting from a slowed rate of SR Ca^{2+} uptake. This reduced SR function is thought to be caused by reductions in the density of the SR Ca^{2+} ATPase (SERCA)[10–12] and by reduced phosphorylation of the SERCA inhibitory protein phospholamban (PLB).[13–15] There is also some evidence for an increased rate of Ca^{2+}"leak" from the SR through "hyperphosphorylated" Ca^{2+} release channels (ryanodine receptors, RYR).[16,17]

Recently, it has been shown that in myocytes in the infarct border zone, the rate of rise of the Ca^{2+} transient is slowed because SR Ca^{2+} release from junctional SR is dyssynchronous.[18] In the normal cardiac myocyte, junctional release sites are located along each Z-line and these sites are triggered to synchronously release their Ca^{2+} during the early portion of the action potential by Ca^{2+} entry through the L-type Ca^{2+} channel (LTCC).[19,20] In normal myocytes, it appears that almost all SR storage sites release their Ca^{2+} with each heart beat.[19] Spatially dyssynchronous SR Ca^{2+} release would slow the rate of rise of the global cytosolic Ca^{2+} transient in diseased myocytes. This type of deranged SR Ca^{2+} release could result from fixed structural abnormalities including loss of T-tubules and/or junctional SR from some regions of the myocyte.[21,22] An alternative explanation would be failure of some SR sites to release their stored Ca^{2+} due to deranged excitation contraction (EC) coupling.

This study examined if SR Ca^{2+} release is spatially and/or temporally dysynchronous in either hypertrophied or failing feline myocytes and then went on to examine the bases for the dysynchronous release. Confocal line scan imaging was used to study the synchrony of Ca^{2+} release within single ventricular myocytes from feline hearts with left ventricular hypertrophy (LVH) induced by ascending aortic constriction (AC). AC induced two distinct phenotypes, concentric LVH or

Original received October 5, 2004; revision received January 28, 2005; accepted January 28, 2005.

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Circulation Research is available at http://www.circresaha.org DOI: 10.1161/01.RES.0000158966.58380.37

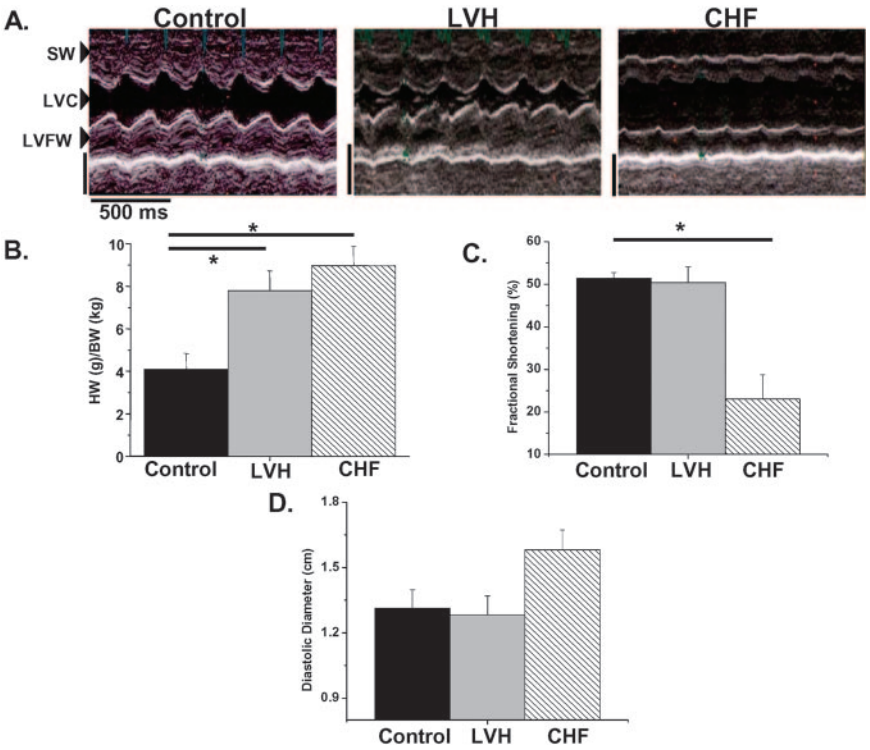

Figure 1. Morphology and function of LVH and CHF hearts. A, Representative echocardiographic images from control (n=5), LVH (n=5), and CHF (n=5) hearts (SW indicates septal wall; LVC, left ventricular cavity; LVFW, left ventricular free wall). B, Heart weight to body weight ratio (g/kg) was increased significantly in LVH and CHF vs control hearts. C, Fractional shortening [(Diastolic diameter−Systolic diameter)/Diastolic Diameter×100%], (*$P<0.05$, Vertical bars=1 cm) was significantly smaller in CHF vs control. D, Mean diastolic diameter (cm) measured with m-mode echocardiography was greatest in the CHF hearts.

hypertrophy with dilatation and signs and symptoms of early CHF. Field stimulation–induced SR Ca^{2+} release was significantly less synchronous in CHF versus normal and LVH myocytes. This dyssynchrony was associated with changes in the early repolarization phases of the AP and was eliminated by inducing maximal Ca^{2+} influx through the LTCC with voltage clamp techniques. These results show that failure of a fraction of junctional SR sites to release their Ca^{2+} contributes to the abnormal cytosolic Ca^{2+} transients of failing myocytes. Dyssynchronous release appears to be due to alterations in Ca^{2+}-induced SR Ca^{2+} release rather than from loss of release units, making these abnormalities viable targets for CHF inotropic therapy.

Materials and Methods

LVH was induced by aortic constriction as described in detail previously. Before euthanasia, echocardiography was used to assess in vivo cardiac function. Myocytes were isolated using standard techniques. Ca^{2+} transient were measured with line scan confocal imaging, and action potential and whole-cell currents were recorded with patch clamp techniques. Full details can be found in an expanded Materials and Methods section in the online data supplement available at http://circres.ahajournals.org.

Results

Characteristics of Pressure-Overloaded Hearts

Aortic constriction induced significant hypertrophy in all animals with two distinct structural and functional phenotypes. Representative echocardiographic analyses are shown in Figure 1A. Half of the banded animals (n=5) developed concentric LV hypertrophy (LVH) with significant increases in LV wall thickness and a small decrease in diastolic chamber dimensions. The remaining banded animals (n=5) developed LV dilatation (CHF) with thinner walls than the LVH group, but thicker than controls (n=5). There was a significant increase in the heart weight to body weight ratio (HW/BW ratio) in the LVH (7.79±0.93) and CHF groups (8.97±0.91) versus controls (4.10±0.45) (Figure 1B). CHF hearts had significant smaller fractional shortening (23.7±5.7%) versus LVH (50.5%±3.7) and controls (51.4%±1.63) (Figure 1C). CHF hearts also tended to have a greater diastolic diameter (1.58±.09 cm) versus control (1.31±0.11 cm) and LVH (1.28±.09 cm) groups (Figure 1D). CHF animals had shortness of breath with activity and ascites. This in vivo analysis suggests that baseline cardiac function is maintained in compensated LVH hearts and is depressed in those with dilatation and signs and symptoms of early heart failure.

Field Stimulation

Cytosolic Ca^{2+} transients were measured with line scan confocal imaging in myocytes isolated from normal and hypertrophied hearts paced at 0.2 and 1.0 Hz. (Figure 2A). Control myocytes had synchronous SR Ca^{2+} release at all pacing rates, whereas SR Ca^{2+} release in CHF myocytes was spatially and temporally less well organized. LVH myocytes had visually synchronous SR Ca^{2+} release; however, the rate of rise of the global Ca^{2+} transients (dCa^{2+}/dt) decreased to the greatest extent in these myocytes when their stimulation rate was increased (Figure 2B).

The spatial uniformity (synchrony) of Ca^{2+} release along the scan line was calculated as described in the expanded Materials and Methods. An example is shown in Figure 3A and spatial fluorescence profiles are shown in Figure 3B. The dotted lines (10 and 40 ms) represent the times chosen for analysis. At 10 ms, there are fewer regions of the cytosol with fluorescence above 50% of the peak value than at later times.

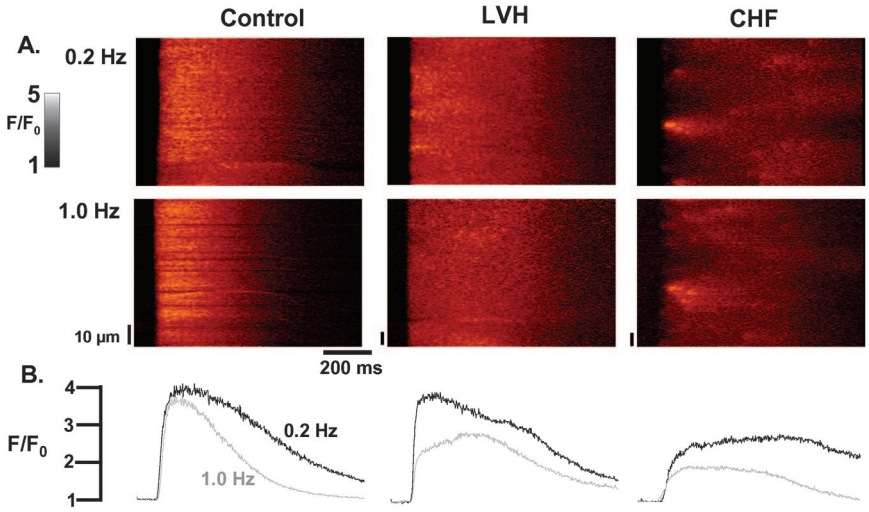

Figure 2. Dyssynchronous Ca^{2+} release in field stimulated CHF myocytes. A, Confocal line scan images from control (left), LVH (middle), and CHF (right) cells that were field stimulated at 0.2 Hz (top) and 1.0 Hz (bottom). B, Respective Ca^{2+} transients (0.2 Hz: black and 1.0 Hz: gray) from line scan images.

Spatial uniformity occurred earlier in control versus LVH and CHF myocytes. This analysis showed that control (n=9) and LVH cells (n=15) had similar spatial synchrony at 0.2 Hz (Figure 3C), but Ca^{2+} release became less well organized in LVH myocytes when the pacing rate was increased to 1.0 Hz. SR Ca^{2+} release was significantly less synchronous in CHF (n=12) versus control and LVH myocytes at both 0.2 Hz and 1.0 Hz.

Decreases in the synchrony of SR Ca^{2+} release was associated with slowing of dCa^{2+}/dt (Figure 3D). At 0.2 Hz, dCa^{2+}/dt was significantly slower in CHF versus control and LVH myocytes. Increasing the stimulation rate to 1.0 Hz caused dCa^{2+}/dt to decrease significantly in both LVH and CHF but not in control myocytes.

The global peak systolic Ca^{2+} (average fluorescence along the line scan) at 0.2 Hz was significantly smaller in CHF (n=12) versus control (n=9) and LVH (n=15) cells (Figure 3E). These results are consistent with our previous studies in which we measured whole-cell Ca^{2+} transients with indo-1.[2]

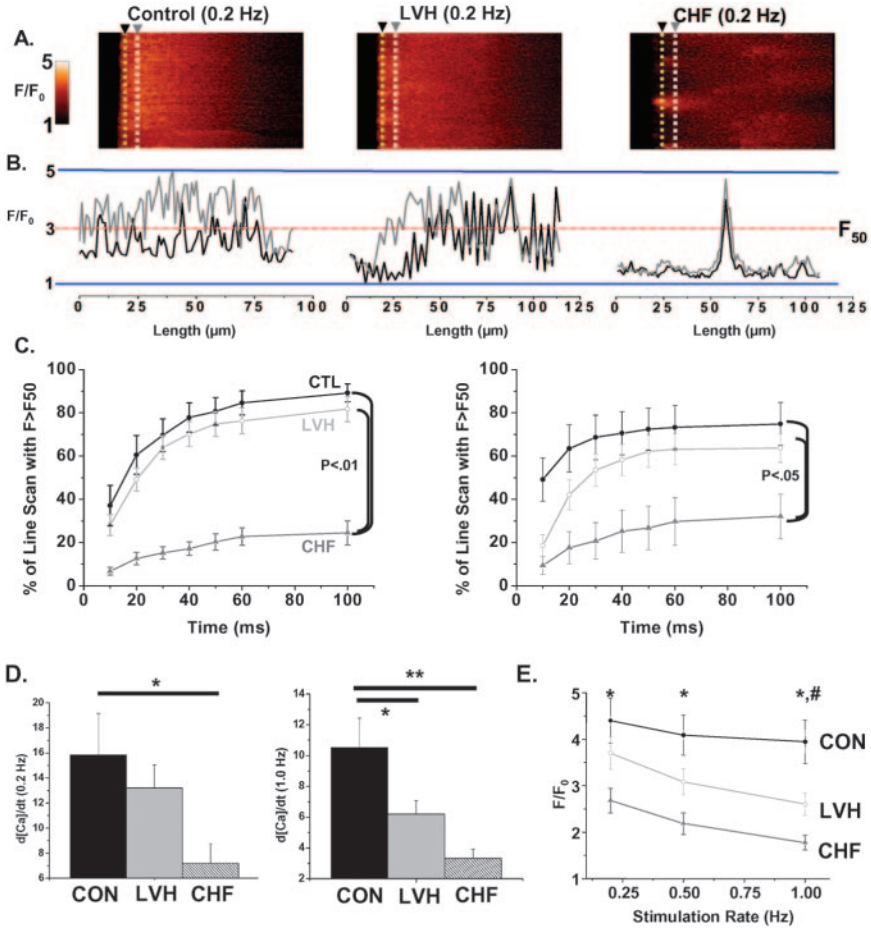

Figure 3. Ca^{2+} release synchrony. A, Confocal line scan images in representative control (left), LVH (middle), and CHF (right) myocytes. Spatial profiles at 10 (yellow dotted line) and 40 ms (white dotted line) are shown. B, Respective spatial profiles from the line scan images. Percentage of the line scan with an $F>F_{50}$ was used as an index of spatial synchrony of Ca^{2+} release. C, Mean data using the percentage of line scan with $F<F_{50}$ at 0.2 Hz (left) and 1.0 Hz (right) in CON (black), LVH (light gray), and CHF (dark gray) myocytes at 10, 20, 30,40 50, 60, and 100 ms (*$P<0.05$, ***$P<0.001$). D, Mean peak $d[Ca^{2+}]/dt$ at 0.2 Hz (left) and 1.0 Hz (right) (control, n=10; LVH, n=15; CHF, n=12). E, Whole-cell peak $[Ca^{2+}]_i$ (F/F0) at 0.2, 0.5 and 1.0 Hz (*$P<0.05$, **$P<0.01$, #$P<0.05$ vs LVH).

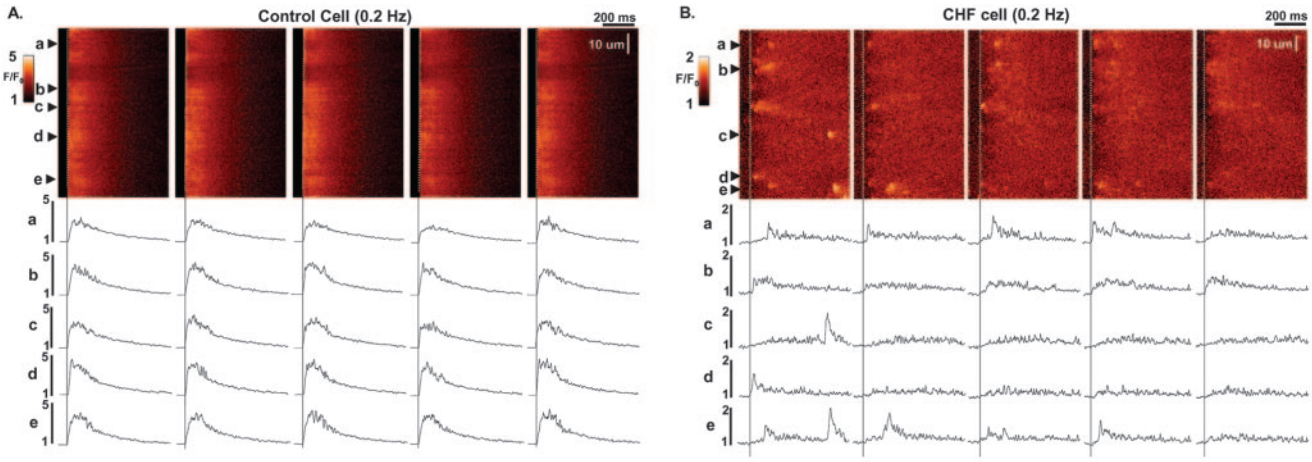

Figure 4. Five consecutive field stimulated line scan images (0.2 Hz, top) from representative control (A) and CHF (B) myocytes. Five areas (a through e) were chosen for analysis in each image (bottom). Dotted line represents the time at which the cell was field stimulated. In the control cell (A) localized Ca^{2+} transients were similar in each of the five beats. Localized Ca^{2+} release in the CHF cell (B) was spatially and temporally variable from beat to beat.

At 1.0 Hz, the peak Ca^{2+} was significantly greater in control myocytes versus LVH and CHF myocytes (Figure 3E).

Poorly organized SR Ca^{2+} release could result from physical loss of release units (couplons), which we loosely term structural defects (loss of T-tubules or junctional SR) and/or failure of existing couplons to release Ca^{2+}, which represent defective EC coupling. The local absence of SR Ca^{2+} release resulting from the absence of a couplon should produce spatial Ca^{2+} release profiles that are consistent from beat to beat, whereas those resulting from defective triggering of release should vary in time (from the stimulus) and location on a beat to beat basis. To evaluate these possibilities we measured local Ca^{2+} release from specific sites within representative normal and CHF myocytes during consecutive field stimulated beats at 0.2 Hz (Figure 4). In the normal (control) myocyte, the spatial and temporal characteristics of local Ca^{2+} transients were nearly identical in every beat (Figure 4A). A distinctly different pattern was observed in failing myocytes (Figure 4B). SR Ca^{2+} release was spatially and temporally variable from beat to beat. Similar results were observed in all failing myocytes. These results strongly support the hypothesis that defects in EC coupling cause either delayed or intermittent loss of SR Ca^{2+} release in feline CHF myocytes.

Voltage Clamp

To further test the idea that defects in EC coupling cause dyssynchronous SR Ca^{2+} release in CHF, myocytes voltage clamp techniques were used to control and vary the magnitude of the triggering LTCC current ($I_{Ca\text{-}L}$). Line scan images from representative control and CHF myocyte at 0 and ± 30 mV are shown in Figure 5A. SR Ca^{2+} release was not well synchronized at negative (-30 mV) and strongly positive voltages ($+30$ mV) where Ca^{2+} current is small (see later). Surprisingly, when the membrane potential was stepped to voltages that induce maximal $I_{Ca\text{-}L}$ ($+10$ mV), SR Ca^{2+} release was well organized in CHF myocytes (Figure 5C), even though the peak systolic [Ca^{2+}] was significantly smaller than in controls (Figure 5B and 5D). These studies show that the spatial and temporal synchrony of SR Ca^{2+} release varies with the amplitude of the initiating $I_{Ca\text{-}L}$ in all feline myocytes and that synchronous SR Ca^{2+} release can be induced in CHF myocytes, supporting the hypothesis that the disorganized release they exhibit during field stimulation is caused by factors that influence EC coupling.

The uniformity of SR Ca^{2+} release had a bell-shaped voltage dependence similar to the Ca^{2+} current-voltage relationship (Figure 5C) in control and CHF myocytes. There were no differences in the uniformity of SR Ca^{2+} release in CHF versus normal myocytes at potentials from 0 to $+60$ mV. At negative test potentials (near -30 mV), SR Ca^{2+} release was actually more uniform in CHF myocytes versus LVH and control (see Discussion). These results show that the differences in uniformity of SR Ca^{2+} release observed in field stimulated CHF versus normal myocytes are eliminated when release synchrony is compared under voltage clamp conditions. Such findings support the idea that synchronization abnormalities in CHF myocytes are caused by alterations in factors regulating triggered release of SR Ca^{2+} rather than from couplon loss. It is important to point out that the synchrony of SR Ca^{2+} release is steeply voltage dependent between 0 to $+60$ mV, with SR Ca^{2+} release becoming less uniform at more positive membrane potentials.

SR Ca^{2+} load was not measured directly in this study. However, its seems unlikely that it was significantly different in field stimulation and voltage clamp experiments given the fact that the induced Ca^{2+} transients were identical within each myocyte group under these two experimental settings (Figure 5D). Therefore, dyssynchronous SR Ca^{2+} release in field stimulated CHF myocytes might be caused by action potential alterations that reduce Ca^{2+} influx and induce defective EC coupling.

Changing the density of Ca^{2+} influx through LTCCs influences the efficacy of EC coupling and could contribute to the dyssynchronous SR Ca^{2+} release we have observed. $I_{Ca\text{-}L}$ was measured in myocytes from normal, LVH, and CHF animals (Figure 5E). Similar to what we found in previous studies,[23] $I_{Ca\text{-}L}$ density was smaller in LVH versus controls,

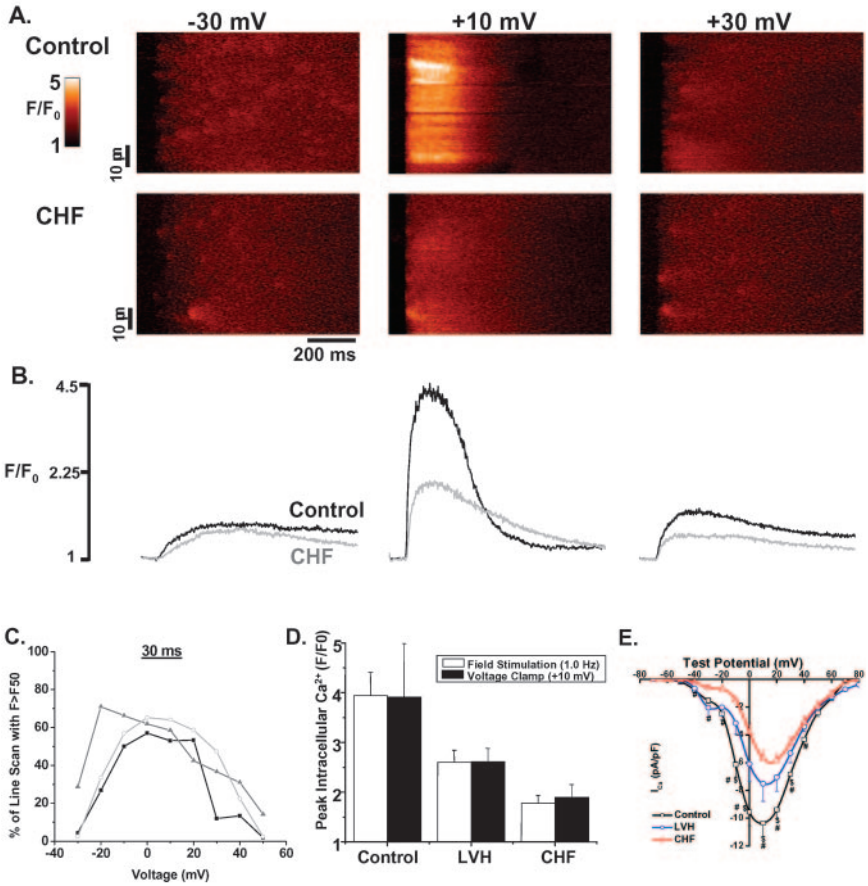

Figure 5. Effects of membrane potential on the synchrony of Ca^{2+} release. A, Control (top) or CHF (bottom) cells were voltage clamped from a holding potential of −70 to −50 mV (300 ms) before test steps (400 ms) in 10 mV increments were applied. Representative line scan images from test steps to −30 mV (left), +10 mV (middle), and +30 mV (right) are shown. B, Respective Ca^{2+} transients from line scan images from the control (black) and CHF (gray) cell. C, Synchrony of Ca^{2+} release 30 ms after the voltage step to +10 mV measured as the percent of line scan with $F>F_{50}$ at each voltage [control (black), n=6; LVH (light gray), n=4; CHF (dark gray), n=5]. There were no significant differences between groups except at −20 mV. Similar results were obtained at other times after the stimulus (data not shown). D, Peak whole-cell $[Ca^{2+}]_i$ in control, LVH, and CHF myocytes induced by field stimulation (1.0 Hz) and voltage steps (at +10 mV). E, Calcium current-voltage relationship between control (black, n=8), LVH (blue, n=8), and CHF myocytes (red, n=8) ($*P<0.05$ between control and LVH, $\#P<0.05$ between control and CHF).

and there was evidence that T-type Ca^{2+} current was present (see current at −30 mV) in LVH. $I_{Ca\text{-}L}$ density was further reduced in CHF. These experiments show that there is a progressive reduction of $I_{Ca\text{-}L}$ density in proportion to the severity of the hemodynamic stress. This reduction in Ca^{2+} influx is likely to be involved in the reduced synchrony of SR Ca^{2+} release in field stimulated CHF myocytes.

Action Potential Alterations in CHF Myocytes

The transient outward K^+ current (I_{to}) is decreased in hypertrophied myocardium.[24,25] In feline ventricular myocytes, I_{to} is responsible for a phase of rapid repolarization (phase 1) before the plateau portion of the action potential (AP). It is during this time period that LTCCs activate and the subsequent Ca^{2+} influx induces SR Ca^{2+} release.[26] Reduction in I_{to} should elevate the early portion of the AP to more positive potentials and, by reducing the electrochemical gradient, reduce the influx of trigger Ca^{2+}. APs measured in both LVH and CHF myocytes had significantly smaller amounts of early repolarization (Figure 6A), consistent with reductions in I_{to}. The average membrane potential at the base of the notch is significantly more positive in these hypertrophied myocytes versus control (Figure 6B).

To further explore the hypothesis that differences in the voltage profile of the AP plateau phase influence the uniformity of SR Ca^{2+} release, control myocytes were voltage clamped with AP (so called AP voltage clamp) profiles with either a normal or CHF (no phase 1 notch) wave shape (Figure 7A). In these experiments, AP clamp was preceded with five square wave voltage steps (250 ms) from −70 to +10 mV to provide uniform loading of the SR before the test AP clamp profiles. Figure 7A shows that when a control myocyte was clamped with a normal AP profile having the early phase 1 repolarization notch (left), there was uniform Ca^{2+} release. However, when a CHF AP profile without early rapid phase 1 repolarization was used, Ca^{2+} release was dyssynchronous. This dyssynchrony caused a slowing in the rate of rise of the Ca^{2+} transient and a loss of spatial and temporal release uniformity (Figure 7B). Similar results were obtained in five other control myocytes.

Discussion

Depressed basal contractility and reduced contractility reserve in heart failure are largely caused by a slowing of the rate of rise and a decrease in the magnitude of the systolic Ca^{2+} transient.[27] The objective of the present study was to determine whether alterations in the synchrony (uniformity) of SR Ca^{2+} release from the junctional SR contributes to defective Ca^{2+} regulation in CHF in either LVH or CHF myocytes. The major findings of our experiments were as follows: (1) only field-stimulated CHF myocytes had spatially and temporally disorganized Ca^{2+} release; (2) areas of Ca^{2+} release within CHF myocytes were spatially and temporally variable from beat to beat; (3) under voltage clamp conditions all myocytes had synchronous Ca^{2+} release at test potentials where Ca^{2+} current was largest, and all groups had

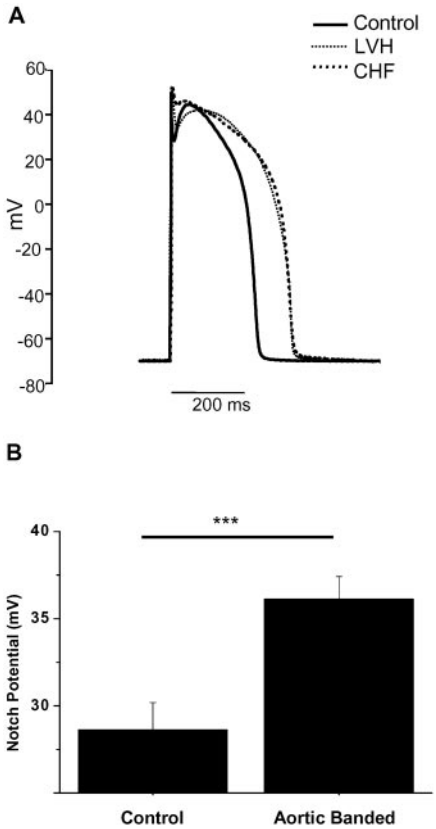

Figure 6. A, Representative action potentials from control (black, n=6) and LVH (small dots, n=9) and CHF (large dots, n=8). B, Mean data showing the membrane potential at the base of the notch in the groups (***$P<0.001$).

disorganized release when voltage clamped to more negative (−30 mV) and positive (+30 mV) potentials where Ca^{2+} current was smaller; (4) $I_{Ca\text{-}L}$ density was smaller in LVH and CHF versus control myocytes; (5) LVH/CHF myocytes had a more positive early portion of the AP (less notch in phase 1); and (6) a CHF action potential profile in control myocytes induced dyssynchronous SR Ca^{2+} release.

EC Coupling in Ventricular Myocytes

EC coupling in cardiac myocytes starts during the upstroke of the action potential, which causes the opening of LTCCs. The subsequent influx of Ca^{2+} elevates its subsarcolemmal concentration, which promotes binding to and opening of RYRs and initiates locally regenerative SR Ca^{2+} release. This process occurs at the t-tubule–SR junction (local control hypothesis),[28] and this region has been called the couplon.[29] Normal contraction requires that SR Ca^{2+} release is coordinated within the cell. A recent study in normal ventricular myocytes[19] showed that all regions of the junctional SR release their Ca^{2+} with each AP, producing a uniform Ca^{2+} activation wavefront. Because only a fraction of LTCCs open with each depolarization,[30] a sufficient number of channels must be present at each couplon to ensure a high "safety factor" for release in normal myocytes. In the present study, we demonstrate that in hypertrophied/failing feline myocytes there is intermittent failure or a large temporal delay of Ca^{2+} release from individual couplons, leading to a break down in the uniformity of SR Ca^{2+} release.

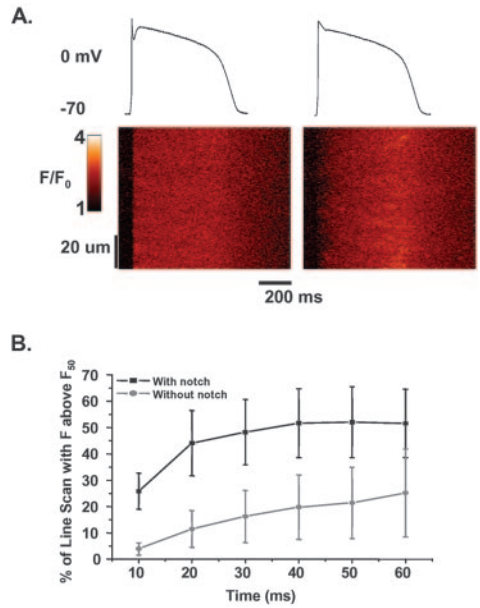

Figure 7. A, Representative line scan images from a control myocyte voltage clamped using a normal (left) and CHF AP profile. B, Mean data (n=5) showing synchrony of Ca^{2+} release using percentage of line scan with F<F50 at 10, 0, 30, 40, 50, and 60 ms.

I_{Ca2+} Amplitude Synchronizes SR Ca^{2+} Release

Our experiments suggest that local failure of EC coupling at individual couplons desynchronizes SR Ca^{2+} release and contributes to the slowly rising, slowly decaying Ca^{2+} transients of the CHF myocyte. These local release abnormalities can be rescued by optimizing Ca^{2+} influx through the LTCC, proving that the absence of release units (structural abnormalities)[21] is not the cause of dyssynchrony.

EC coupling defects that spatially and temporally desynchronize SR Ca^{2+} release are likely to involve changes in the number, properties, and localization of LTCCs and RYR within the junctional complex. Importantly, there is always some temporal variability in normal SR Ca^{2+} release. This normal temporal variability cannot be well resolved with the techniques used in the present experiments, and the temporal delays we have observed are well beyond those predicted by the properties of single LTCC in normal myocytes.[30] The time between depolarization and triggered release of Ca^{2+} from the junctional SR is determined by factors including the number of LTCCs within the couplon, LTCC properties, the morphology of the diffusion limiting space in which Ca^{2+} accumulates, the Ca^{2+} buffering within this space, the number of RYRs per couplon, and RYR properties. Alterations in any or all of these could produce the temporal delays and local failure of SR release we observed. However, little is known about many of these factors. We have shown that the density of LTCCs is reduced in LVH and further reduced in CHF myocytes. We speculate that this results in a decrease in the number of LTCCs per couplon that would increase temporal variability and reduce the safety factor for release. The

positive voltage shift in the early portion of the AP that we have observed would also reduce Ca^{2+} influx and should contribute to EC coupling defects. Our results suggest that a decreased LTCC density within the couplon and decreased Ca^{2+} flux through open channels (due to the AP alterations) produce a reduction in local submembrane Ca^{2+}, which is sufficient to cause intermittent localized failure of SR Ca^{2+} release. Importantly, our results show that there are a sufficient number of LTCCs per couplon in CHF myocytes to cause well-synchronized release, as evidenced by our voltage clamp studies. Our results are consistent with the hypothesis that the EC coupling safety factor is reduced in diseased myocytes and that this can lead to the release dyssynchrony we have observed.

Alterations in the relationships between Ca^{2+} influx and SR Ca^{2+} release (termed EC coupling "gain") is one possible explanation for our results.[31] We did not compute this parameter because $I_{Ca\text{-}L}$ density cannot be measured accurately with the conditions we used to study SR Ca^{2+} release. Our voltage clamp experiments show that when the membrane voltage is changed to potentials where maximal $I_{Ca\text{-}L}$ is induced (0 to +10 mV), synchronous SR Ca^{2+} release occurs in all myocytes (Figure 5), showing that failing myocytes are capable of having spatially uniformly release even with reduced $I_{Ca\text{-}L}$ density. Importantly, we show that spatially uniform SR Ca^{2+} release was induced even when the size of the Ca^{2+} transient was smaller, and SR Ca^{2+} stores are likely reduced.[2,7] Therefore, we do not believe that reduced SR Ca^{2+} loading is the principle cause of dyssynchronous release in field stimulated failing myocytes. Our composite results suggest that reduced Ca^{2+} influx, caused by reduced LTCC density and AP changes, produce dyssynchronous release in field stimulated, CHF myocytes.

We cannot infer the properties (opening probability) of LTCCs in failing feline myocytes from the present data. We and others[32,33] have shown that the phosphorylation state of the LTCC is likely to be increased in the failing cells. Our finding that the voltage dependence of SR Ca^{2+} release is shifted to negative potentials in failing myocytes is consistent with the effect of PKA-mediated phosphorylation on the voltage dependence of LTCC activation.[34,35] However, we did not see this leftward shift in the voltage dependence of $I_{Ca\text{-}L}$ activation when it was directly measured (Figure 5E).

AP Alterations Lead to EC Coupling Defects

Changes in AP wave shape of hypertrophied/failing ventricular myocytes have been studied in large and small animal models and in humans.[36] Although there are fundamental differences in the molecular bases of the AP in large and small mammals,[37] there some changes in AP morphology with hypertrophy and failure that are consistently observed in all species. Hypertrophy and failure are associated with prolongation of the QT interval, which results from a prolongation of the AP duration.[38] The ion channels density that changes most consistently among species in hypertrophy and failure is I_{to}.[24] This is the current that controls the rapid repolarization of the AP in small mammals (rats and mice) and the early repolarizing phase of the AP in large mammals (felines and humans). Reduction in I_{to} is likely to be responsible for the loss of early repolarization and the associated EC coupling defects we observed in failing myocytes. The channel responsible for I_{to} does not fully recover from inactivation at faster pacing frequencies, resulting in frequency-dependent loss of the early repolarization. We speculate that this could contribute to frequency-dependent EC coupling defects and the negative force frequency relationships in failing myocardium.[39]

Ca^{2+} Regulation in Heart Failure

In failing myocardium, the Ca^{2+} transient has a smaller than normal peak amplitude (at fast heart rates), a slower rate of rise, and a prolonged duration.[1,6,40,41] Alterations in SR Ca^{2+} uptake, storage, and release are contributing factors.[42] SR abnormalities are thought to be caused by reduced SERCA2 abundance,[10–12] decreases in PLB phosphorylation[13–15] and "hyperphosphorylation" of the RYR[16] with an associated increase in the SR Ca^{2+} leak rate. In addition, increased Na^+/Ca^{2+} exchanger activity and elevated cell $[Na^+]_i$ have been observed in failing myocytes[43,44] and these cause abnormalities in both systolic and diastolic Ca^{2+}. These changes in Ca^{2+} regulation in hypertrophied/failing myocytes lead to a reduction in the amount of Ca^{2+} stored in and released from the SR.[9,45] We have previously shown that SR Ca^{2+} stores are reduced in failing human myocytes[7] and the results of the present experiments (reduced peak Ca^{2+} under all conditions) strongly support the hypothesis that SR Ca^{2+} stores are smaller than normal in hypertrophied/failing feline myocytes. Although reduced SR Ca^{2+} stores can explain the reduced amplitude of the Ca^{2+} transient we observed, this abnormality does not appear to be the critical factor that intermittently causes a disorganized pattern of SR Ca^{2+} release in field stimulated myocytes.

Summary

The present study suggests that EC coupling defects contribute to abnormal Ca^{2+} transients of failing feline myocytes by decreasing the uniformity of local SR Ca^{2+} release. The fact that these defects can be rescued by optimizing Ca^{2+} influx through the LTCC shows that factors governing Ca^{2+} release within existing couplons rather than loss of couplons are responsible for disorganized SR Ca^{2+} release in CHF. Reduced $I_{Ca\text{-}L}$ density and the electrochemical driving force for Ca^{2+} entry (due to loss of phase 1 repolarization) appear to underlie these EC coupling abnormalities. Therapies that target these fundamental defects should help correct the defective contractility of the failing heart.

Acknowledgments

This study was supported by NIH HL33946 and HL66421 (to S.R.H.) and the American Heart Association (to D.H.).

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