Cellular Basis of Abnormal Calcium Transients of Failing Human Ventricular Myocytes

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Abstract—Depressed contractility is a central feature of the failing human heart and has been attributed to altered $[Ca^{2+}]_i$. This study examined the respective roles of the L-type Ca^{2+} current (I_{Ca}), SR Ca^{2+} uptake, storage and release, Ca^{2+} transport via the Na^+-Ca^{2+} exchanger (NCX), and Ca^{2+} buffering in the altered Ca^{2+} transients of failing human ventricular myocytes. Electrophysiological techniques were used to measure and control V_m and measure I_m, respectively, and Fluo-3 was used to measure $[Ca^{2+}]_i$ in myocytes from nonfailing (NF) and failing (F) human hearts. Ca^{2+} transients from F myocytes were significantly smaller and decayed more slowly than those from NF hearts. Ca^{2+} uptake rates by the SR and the amount of Ca^{2+} stored in the SR were significantly reduced in F myocytes. There were no significant changes in the rate of Ca^{2+} removal from F myocytes by the NCX, in the density of NCX current as a function of $[Ca^{2+}]_i$, I_{Ca} density, or cellular Ca^{2+} buffering. However, Ca^{2+} influx during the late portions of the action potential seems able to elevate $[Ca^{2+}]_i$ in F but not in NF myocytes. A reduction in the rate of net Ca^{2+} uptake by the SR slows the decay of the Ca^{2+} transient and reduces SR Ca^{2+} stores. This leads to reduced SR Ca^{2+} release, which induces additional Ca^{2+} influx during the plateau phase of the action potential, further slowing the decay of the Ca^{2+} transient. These changes can explain the defective Ca^{2+} transients of the failing human ventricular myocyte. **(*Circ Res*. 2003;92:651-658.)**

Key Words: excitation-contraction coupling ■ sarcoplasmic reticulum ■ Na^+-Ca^{2+} exchanger ■ congestive heart failure

Congestive heart failure (HF) is the leading cause of death in Western civilization.[1] Although this syndrome has many different and distinct causes, all forms share a number of common features, which include prolongation of the QT interval,[2,3] progressive depression of basal cardiac contractility,[4,5] and loss of inotropic reserve.[6] Whereas these changes in the physiological properties of the heart have been described in HF animal models[7] and in failing human hearts, muscle strips,[8] and isolated myocytes,[9] their cellular basis is still not well understood and is the topic of this article.

Contraction of human cardiac myocytes is a Ca^{2+}-dependent process. During diastole, the intracellular $[Ca^{2+}]_i$ is maintained at sufficiently low levels to prevent activation of contractile proteins.[10] With each heartbeat, Ca^{2+} influx via the L-type Ca^{2+} channel triggers release of Ca^{2+} from the sarcoplasmic reticulum (SR).[11] These two sources combine to elevate $[Ca^{2+}]_i$, which promotes Ca^{2+} binding to troponin and activation of the contractile process. Contraction is terminated as Ca^{2+} is transported back into the SR by the SR Ca^{2+}-ATPase (SERCA) and out of the cell via the sarcolemmal Na^+-Ca^{2+} exchanger (NCX).[10] The rate, intensity, and duration of contraction are largely determined by the amount of Ca^{2+} delivered to the cytoplasm, the Ca^{2+} binding properties of troponin and other Ca^{2+} binding proteins,[12] and the rate of Ca^{2+} removal from the cytoplasm by the SR and from the cell via the NCX.[10]

The depressed contractility of the failing heart is thought to involve alterations in myocyte Ca^{2+} regulation[8] and the isoforms and regulation of thin and thick filament contractile proteins.[13] This article will focus on the role of altered Ca^{2+} regulation in the depressed contractility of the failing human ventricular myocyte. Only two studies[14,15] have shown alterations in the amplitude and duration of the Ca^{2+} transients of failing myocytes, and these have not established the underlying cellular basis. Alterations in SERCA mRNA, protein, or function have been reported, but these SERCA changes have not been uniformly observed or well characterized in isolated myocytes.[8] Significant abnormalities in EC coupling, in the properties of SR Ca^{2+} release channels (ryanodine receptors, RYR)[16] or in NCX abundance have also been reported.[8]

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These findings suggest that whereas dysregulated myocyte Ca^{2+} may be a common feature of HF, the cellular basis may be highly variable. This could reflect fundamental species-specific differences in Ca^{2+} regulation[10] and the complex interaction of different Ca^{2+} regulatory processes in a given species.[7]

The objective of this study was to perform an in-depth evaluation of Ca^{2+} regulatory processes in nonfailing (NF) and failing (F) human ventricular myocytes to determine the cellular basis of deranged Ca^{2+} transients in HF. The aim was to first determine the changes in Ca^{2+} transient characteristics in F human myocytes and then to determine the respective roles of alterations in Ca^{2+} current, SR Ca^{2+} storage and release, Ca^{2+} buffering, and Ca^{2+} transport by the SR and NCX in these changes. Our results show that the altered Ca^{2+} transients of the F human myocyte are largely dependent on reduced SR Ca^{2+} uptake, storage, and release without significant alterations in Ca^{2+} current, Ca^{2+} buffering, or the abundance or properties of the NCX. These changes reduce peak systolic Ca^{2+} and contribute to the slow decay of the Ca^{2+} transient in HF. We also show that during the action potential (AP) in HF myocytes, there can be a slow secondary increase in Ca^{2+} (after SR Ca^{2+} release) or a slow Ca^{2+} transient decay rate that is caused by increased late Ca^{2+} influx and slow SR Ca^{2+} uptake. These results show that Ca^{2+} influx during the AP makes a larger than normal contribution to the Ca^{2+} transient of F human ventricular myocytes and that this behavior is dependent on reduced Ca^{2+} release from a dysfunctional SR.

Materials and Methods

Cell Isolation, Electrophysiology, and $[Ca^{2+}]_i$ Measurements

Myocytes were isolated from F and NF human hearts as described previously.[17] Membrane voltage and current were controlled and recorded using discontinuous, single-electrode voltage clamp techniques, respectively.[18] pClamp8 software (Axon Instruments) was used to control the patch clamp amplifier. $[Ca^{2+}]_i$ was measured with fluo-3 (K salt) loaded through patch pipettes. A typical AP, recorded in current clamp with physiological solutions at 1 Hz from a F human myocyte and 37°C, was used as a template for AP clamp. Myocytes were conditioned with ten 500-ms square wave voltage steps to +30 mV. SR Ca^{2+} content was assessed with rapid application of 10 mmol/L caffeine (10 seconds) in place of an AP clamp (1 Hz, $E_{hold}=-70$ mV).[19] All measurements were at 37°C.

An expanded Materials and Methods section can be found in the online data supplement available at http://www.circresaha.org.

Results

Patient Characteristics

Eleven F hearts were obtained at the time of transplantation, and 7 NF hearts that were unsuitable for transplantation were studied. In the F group, 5 had ischemic heart disease and 6 had idiopathic/nonischemic dilated cardiomyopathies. Other patient characteristics are listed in Table 1.

TABLE 1. Patient Characteristics

	Nonfailing	Failing	*P*
n	7	11	...
Age, y	66±3	49±4	0.01
Sex	5 male, 2 female	9 male, 2 female	...
Heart weight, g	468.9±33.4	583.4±51.8	0.12
HW/BW ratio	6.4±0.3	7.6±0.6	0.18
Inotropic support	1/7	7/11	...
Ejection fraction, %	54.1±4.6	17.5±4.9	0.002

Inotropic support includes patients receiving either β-adrenergic receptor agonists or phosphodiesterase inhibitors.

Action Potential and Contractions

AP and contraction durations were longer in F versus NF myocytes paced at 0.5 Hz (Figure 1). The amplitude of contraction was also smaller in F versus NF, but these differences were not statistically significant. These results confirm those we have reported previously[15] and show that the myocytes used in the present experiments have the electrophysiological and contractile alterations characteristic of the failing human heart. The experiments performed in the remainder of the study examined the role of abnormal myocyte Ca^{2+} regulation in the depressed contractility of the F myocytes. AP or standard voltage clamp techniques were used to eliminate the effects of differences in AP wave shape in F myocytes on the Ca^{2+} transient.

Ca^{2+} Transients and SR Ca^{2+} Load

There was no significant difference in the diastolic $[Ca^{2+}]_i$ in the F versus NF myocytes paced at 1 Hz with AP clamp (Table 2). However, the amplitude of Ca^{2+} transient was significantly smaller in F versus NF human myocytes (Figures 2A and 2D, Table 2). Because the amount of Ca^{2+} in the SR is a critical determinant of Ca^{2+} transient amplitude, SR Ca^{2+} content was assessed by rapid application of caffeine and measurement of the resulting Ca^{2+} transient (Figure 2B). The mean caffeine-induced $\Delta[Ca^{2+}]_i$ in F was 49% of that in NF. After converting $\Delta[Ca^{2+}]_i$ to a change in total cytosolic $[Ca^{2+}]$ ($\Delta[Ca^{2+}]_{Total}$, using cytosolic Ca^{2+} buffering as measured as described) the SR Ca^{2+} content in F was 58% of that in NF ($P<0.05$). SR Ca^{2+} load can also be measured by integrating I_{NCX} during caffeine-induced SR Ca^{2+} release

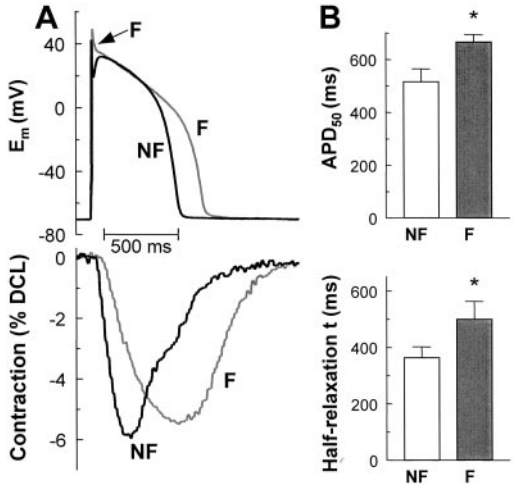

Figure 1. A, Representative action potentials and contractions from NF and F myocytes are shown. B, AP and contraction duration were significantly longer in F versus NF myocytes. All myocytes were paced at 0.5 Hz.

TABLE 2. Failing (F) Versus Nonfailing (NF) Myocyte Properties

	Nonfailing	n	Failing	n	F/NF	Statistically Significant
Twitch [Ca^{2+}]$_i$						
$\Delta[Ca^{2+}]_i$, nmol/L	804±197	11	398±58	22	49%	Yes
Diastolic [Ca^{2+}]$_i$, nmol/L	153±20	11	147±14	22	96%	No
$d[Ca^{2+}]_i/dt_{max}$, nmol/L per ms	29.2±8.6	11	15.2±2.7	22	52%	No
TTP, ms	188±38	11	192±19	22	102%	No
$\tau[Ca^{2+}]_i$ decline, ms	209±31	11	306±27	22	147%	Yes
Caffeine-induced Ca^{2+} transient						
$\tau[Ca^{2+}]_i$ decline, ms	870±133	8	915±230	8	105%	No
SR Ca^{2+} load, μmol/L cytosol						
I_{NCX} integral	112±12	8	65±15	6	58%	Yes
$\Delta[Ca^{2+}]_{Total}$ caffeine	85±11	8	49±11	8	58%	Yes
Rate twitch, s^{-1}	5.88±0.67	8	3.66±0.52	8	62%	Yes
Rate NCX, s^{-1}	1.38±0.21	8	1.32±0.31	8	95%	No
Rate SR, s^{-1}	4.50±0.60	8	2.54±0.34	8	57%	Yes
NCX % contribution	23±6.4	8	36±4.4	8	157%	Yes
SR % contribution	77±6.4	8	64±4.4	8	83%	Yes
Capacitance, pF	355±23	11	584±56	22	164%	Yes

(Figure 2C).[20] The SR Ca^{2+} load measured by integrated I_{NCX} is larger than the amount measured by $\Delta[Ca^{2+}]_{Total}$ (because some Ca^{2+} is extruded via I_{NCX} during the rising phase of the Ca^{2+} transient). However, the reduction in SR Ca^{2+} load in F myocytes measured by I_{NCX} was almost identical to that assessed by $\Delta[Ca^{2+}]_i$ (F was 58% of NF; Figure 2E, Table 2). Thus, reduced SR Ca^{2+} load may be largely responsible for the smaller Ca^{2+} transient in F myocytes. The ratio of twitch $\Delta[Ca^{2+}]_i$ to SR Ca^{2+} load (an index of fractional SR Ca^{2+} release[21]) was not significantly different in NF and F myocytes (Figure 2F). This is consistent with the notion that a lower SR Ca^{2+} load is the primary cause of the reduced Ca^{2+} transient amplitude in F.

In vivo the AP duration (QT interval) is prolonged in the failing heart by 15 to 40 ms (dependent on heart rate).[2] This would tend to increase Ca^{2+} influx and SR Ca^{2+} loading and limit the difference between F and NF myocytes (versus our case where AP clamps were identical). In separate controls, we found that prolonging depolarization by 120 ms increased SR Ca^{2+} load by 34%, but was still less than NF myocytes. Smaller, more physiological prolongations of depolarization (30 ms) did not significantly alter SR Ca^{2+} load. Thus, even with in vivo APs the SR Ca^{2+} content would be significantly smaller in F versus NF myocytes.

In principle, reduced L-type Ca^{2+} current ($I_{Ca,L}$) as a trigger could also cause reduced Ca^{2+} transient in F myocytes. In experiments where $I_{Ca,L}$ was studied with other currents blocked (Figure 3), $I_{Ca,L}$ density was not significantly different in F versus NF myocytes, particularly at positive voltages associated with the peak and plateau phase of the AP. There was a negative shift in the E_m dependence of $I_{Ca,L}$ activation in F myocytes (Figure 3), but this cannot account for the depressed Ca^{2+} transient observed in F myocytes in the present experiments. These findings do not rule out a role for altered Ca^{2+} influx via the L-type Ca^{2+} channel during increases in heart rate[22] or secondary to changes in shape of early portions of the AP[23] in the failing heart.

Contributions of SR Ca^{2+}-ATPase and NCX to [Ca^{2+}]$_i$ Decline

The function and competition between the SR Ca^{2+}-ATPase and NCX can be assessed by analyzing the rate of [Ca^{2+}]$_i$ decline during twitch and caffeine-induced Ca^{2+} transients.[24] The rate constant of [Ca^{2+}]$_i$ decline during a caffeine-induced Ca^{2+} transient largely reflects the function of NCX (k_{NCX}), and this was not different between F and NF myocytes (Figure 4A, Table 2). Thus, the intrinsic Ca^{2+} extrusion activity of NCX seems unaltered in the F myocytes studied here. Both NCX and the SR Ca^{2+}-ATPase contribute to twitch [Ca^{2+}]$_i$ decline, and the rate constant (k_{Twitch}) is significantly slower in F myocytes (Figure 4A, Table 2). The difference between k_{Twitch} and k_{NCX} can be taken as the rate constant of twitch [Ca^{2+}]$_i$ decline attributable to the SR Ca^{2+}-ATPase (k_{SR}). In F myocytes, this rate was only 57% of that in NF myocytes (Figure 4A, Table 2). This indicates a substantially weaker Ca^{2+} transport by the SR Ca^{2+}-ATPase in F myocytes.

We also assessed how NCX and SR Ca^{2+}-ATPase compete functionally during twitch [Ca^{2+}]$_i$ decline, by comparing the ratios k_{NCX}/k_{Twitch} and k_{SR}/k_{Twitch} (Figure 4B). Based on this analysis, in NF myocytes the contributions of NCX and SR Ca^{2+}-ATPase to [Ca^{2+}]$_i$ decline are 23% and 77%, respectively. In F, these values change to 36% and 64%. This indicates a 57% greater fractional contribution of NCX (driven mainly by weaker intrinsic SR Ca^{2+}-ATPase function).

This analysis can be made more rigorous using the entire [Ca^{2+}]$_i$ dependence of NCX and SR Ca^{2+}-ATPase function.[24] Figure 4C shows the [Ca^{2+}]$_i$ dependence of NCX flux (obtained from $d[Ca^{2+}]_{Total}/dt$ versus [Ca^{2+}]$_i$ during caffeine exposure). Then we can subtract this from the overall twitch

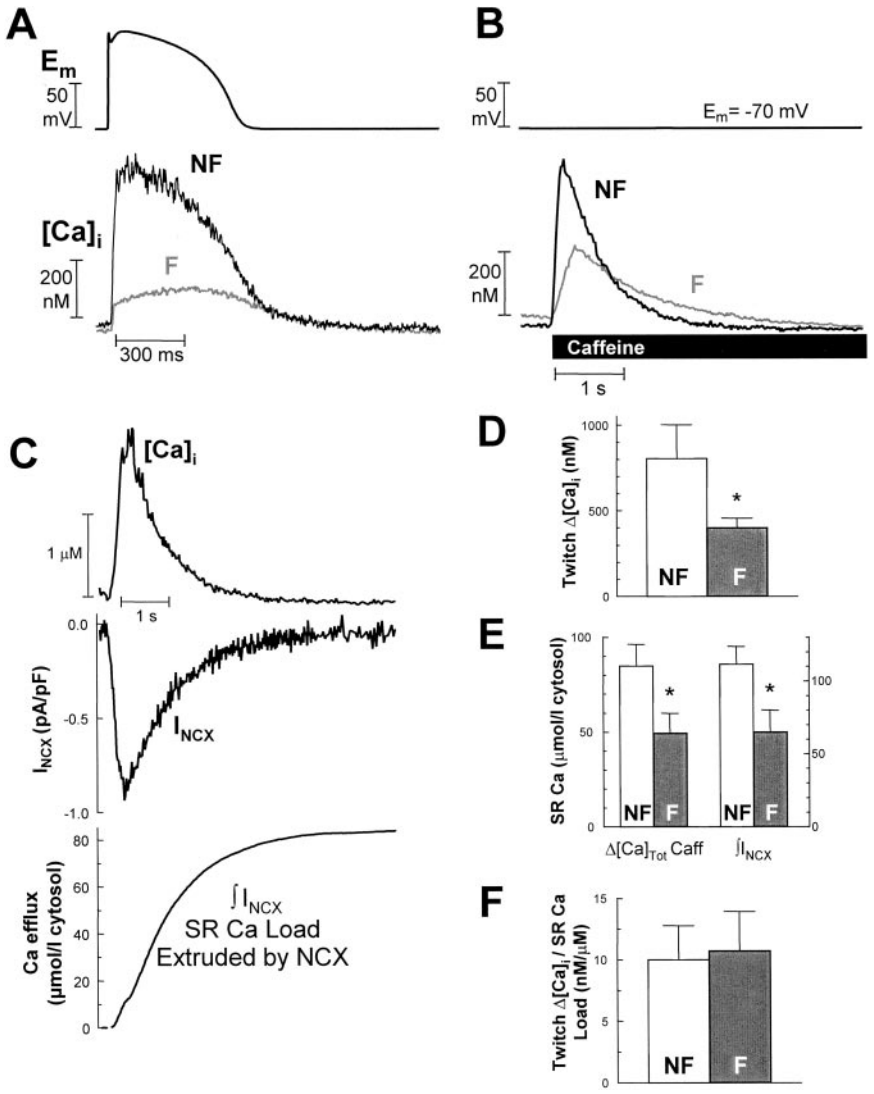

Figure 2. Twitch $\Delta[Ca^{2+}]_i$ and SR Ca^{2+} load. A, Representative Ca^{2+} transients from NF and F myocytes under AP clamp conditions (1 Hz.). B, Example of caffeine-induced Ca^{2+} transients in NF and F myocytes. C, Simultaneous $[Ca^{2+}]_i$ and I_{NCX} measured and calculated I_{NCX} integral ($\int I_{NCX}$, using 13 pF/pl cytosol). D, Average values for twitch $\Delta[Ca^{2+}]_i$. E, Mean SR Ca^{2+} load based on $\Delta[Ca^{2+}]_{Total}$ and $\int I_{NCX}$ during caffeine-induced Ca^{2+} transients. F, Mean twitch $\Delta[Ca^{2+}]_i$/SR Ca^{2+} load (an index of fractional SR Ca^{2+} release). *$P<0.05$ NF versus F.

$d[Ca^{2+}]_{Total}/dt$ curve to infer SR Ca^{2+}-ATPase function (Figure 4C). This allows calculation of NCX and SR Ca^{2+}-ATPase mediated Ca^{2+} flux during the twitch (Figure 4D) in NF and F myocytes (using the measured $[Ca^{2+}]_i$ to calculate flux). The integrated Ca^{2+} flux analysis gives similar, but not identical results as the simpler rate constant analysis in Figures 4A and 4B. In NF myocytes, SR Ca^{2+}-ATPase flux is 3 times that of NCX, whereas in F myocytes, the SR Ca^{2+} flux is only ≈2 times higher. We conclude that SR Ca^{2+}-ATPase function is depressed in F, whereas NCX function is unchanged. However, this results in greater reliance on NCX function during $[Ca^{2+}]_i$ decline, and this tends to decrease SR Ca^{2+} load.

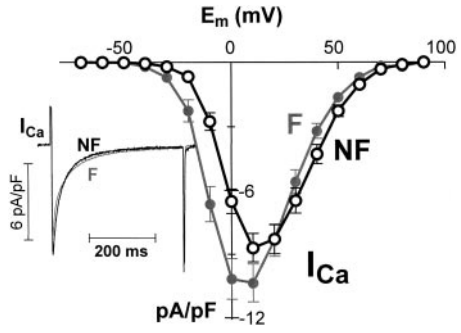

Figure 3. $I_{Ca,L}$-voltage relationship from NF (n=25) and F (n=21) myocytes. Representative current traces at +10 mV are shown in the inset. Peak $I_{Ca,L}$ density was not significantly different.

NCX Surface:Volume Ratio and Ca^{2+} Buffering

The analysis above suggests that NCX Ca^{2+} extrusion properties are unchanged in F myocytes (based on $[Ca^{2+}]_i$ decline). We also assessed NCX function directly as I_{NCX}. Figure 5A shows that inward I_{NCX} density as a function of $[Ca^{2+}]_i$ (at $E_m=-70$ mV) was not significantly different in F versus NF myocytes. This confirms that NCX characteristics are unaltered in F human ventricular myocytes.

NCX function was unchanged whether measured as a function of cytosolic volume ($\Delta[Ca^{2+}]_i$ in mol/L cytosol) or surface area (I_{NCX} in A/F). This suggests that there is no major change in the surface to volume ratio in F myocytes. Indeed, the 64% increase in surface area in F versus NF based on cell capacitance (Table 2) is comparable to the increase in cell volume that we previously measured by flow cytometry

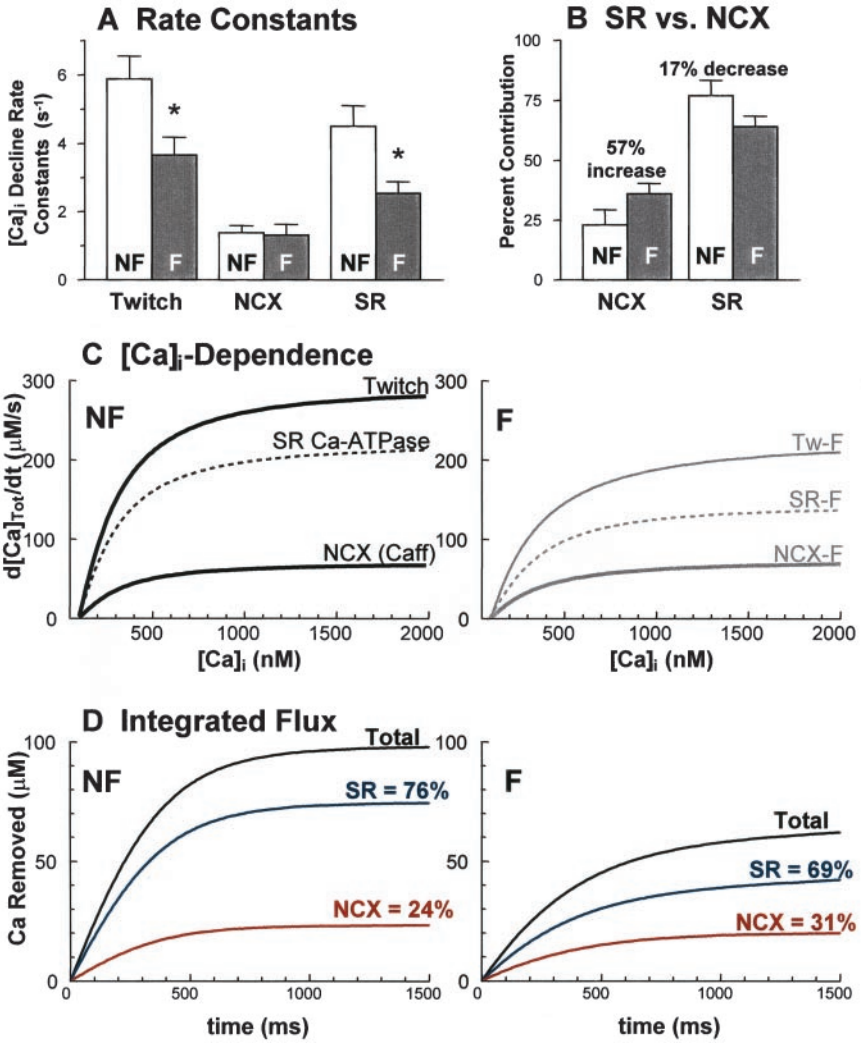

Figure 4. Ca^{2+} removal during relaxation. A, Rate constants of $[Ca^{2+}]_i$ decline during twitches, caffeine-induced Ca^{2+} transients attributable mainly to NCX, and the difference that reflects SR Ca^{2+}-ATPase function (n=8 for each). B, Percent contribution of NCX and SR Ca^{2+}-ATPase to $[Ca^{2+}]_i$ decline during twitches (based on k_{NCX}/k_{Twitch} and k_{SR}/k_{Twitch}). C, $[Ca^{2+}]_i$ dependence of Ca^{2+} transport by NCX (based on caffeine-induced Ca^{2+} transients), overall twitches (SR+NCX), and the difference (SR Ca^{2+}-ATPase).[11] V_{max} values (in μmol/L cytosol/second) were for SR Ca^{2+}-ATPase 168 (F) versus 280 (NF) and for NCX 96 (F) versus 88 (NF). K_m values (in nmol/L) were for SR Ca^{2+}-ATPase 268 (F) versus 224 (NF) and for NCX 241 (F) and 230 (NF). Hill coefficients were 1.6 for all (except 1.4 for F-NCX), and Y-offsets were included to produce a 0 net flux at 100 nmol/L $[Ca^{2+}]_i$. D, Integrated Ca^{2+} removal flux, based on measured twitch $[Ca^{2+}]_i$ and the $[Ca^{2+}]_i$-dependent rates of Ca^{2+} transport by NCX and SR Ca^{2+}-ATPase.

(85%), albeit from different hearts.[25] Because the surface:volume of a cylinder decreases with increasing size, there must be increased membrane area in transverse tubules (or other infoldings) to maintain total surface:volume relatively unchanged (see online data supplement).

We also measured cytosolic Ca^{2+} buffering as described by Trafford et al.[26] This is essentially a back-titration using $[Ca^{2+}]_i$ and $[Ca^{2+}]_{Total}$ from Figure 2C. Figure 5B shows that there was no difference in the cytosolic Ca^{2+} buffering characteristics in F versus NF myocytes. The mean Ca^{2+} buffering relationship, for both cells types (used also in other analyses) was as follows: $\Delta[Ca^{2+}]_{Total}=\{231/(1+833\ nmol/L/[Ca^{2+}]_i)\}-24$ (N.B. units are μmol/L cytosol and $\Delta[Ca^{2+}]_{Total}$ is the change in $[Ca^{2+}]_{Total}$ with respect to that at 100 nmol/L $[Ca^{2+}]_i$). This is similar to myocyte Ca^{2+} buffering measured in other species (dashed curves).[10]

Ca^{2+} Entry During the AP

The foregoing analysis focused mainly on Ca^{2+} extrusion from the cytosol during relaxation and $[Ca^{2+}]_i$ decline, especially after AP repolarization. However, during the AP plateau there could also be changes in Ca^{2+} influx (via $I_{Ca,L}$ or I_{NCX}) or even SR Ca^{2+} release. In particular, the smaller $[Ca^{2+}]_i$ transient in F myocytes may increase Ca^{2+} influx via both $I_{Ca,L}$ and NCX during the AP. This could further slow $[Ca^{2+}]_i$ decline. Overall, the rate of $[Ca^{2+}]_i$ decline during the late AP plateau was significantly slower (44%) in F versus NF myocytes (Figure 6A), consistent with 56% slower SR Ca^{2+} uptake (Figure 4C) and less complete Ca^{2+}-ATPase activation (due to lower $[Ca^{2+}]_i$). However, part of the slower $[Ca^{2+}]_i$ decline in F myocytes might also be due to late Ca^{2+} influx (especially when there is a slowly rising phase as in Figure 2A).

To explore whether NCX may contribute to the slow $[Ca^{2+}]_i$ decline in F myocytes, we measured the E_m dependence of $[Ca^{2+}]_i$ late in the AP using a two-step protocol (Figure 6B). After 5 conditioning beats, an E_m step to +10 mV initiated Ca^{2+} transients. The second step to +80 mV should reduce Ca^{2+} entry via $I_{Ca,L}$, but increase Ca^{2+} entry via NCX and reduce Ca^{2+} efflux via NCX. The second step caused a significant E_m-dependent increase in $[Ca^{2+}]_i$ in F, but not in NF myocytes (Figures 6B and 6C). These results are consistent with the possibility that changes in NCX activity during the AP contributes to slowing $[Ca^{2+}]_i$ decline in F myocytes. This hypothesis was tested more directly in further studies (C.R. Weber, V.I. Piacentino, S.R. Houser, D.M. Bers, unpublished data, 2003).

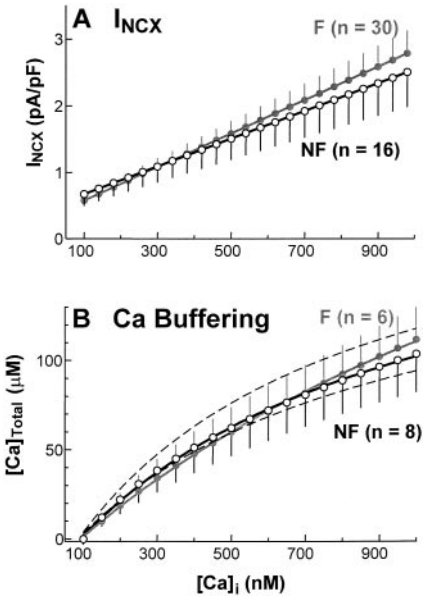

Figure 5. I_{NCX} and cytosolic Ca^{2+} buffering. A, $[Ca^{2+}]_i$ dependence of I_{NCX} in NF and F myocytes (during caffeine application). B, $[Ca^{2+}]_{Total}$ versus $[Ca^{2+}]_i$ in NF and F myocytes, with dashed curves based on data from animal myocytes.[10]

Discussion

Alterations in the size and shape of the systolic Ca^{2+} transient are characteristic phenotypic alterations of the failing human ventricular myocyte.[15] In the present experiments, we studied the cellular basis of these altered Ca^{2+} transients. Our major findings are as follows: (1) reduced peak systolic Ca^{2+} and slow decay of the Ca^{2+} transient are observed in F human myocytes when the AP wave shape is identical in NF and F; (2) under these conditions, there is reduced SR Ca^{2+} content and rate of SR Ca^{2+} uptake in F versus NF myocytes; (3) Ca^{2+} buffering, fractional SR Ca^{2+} release, and $I_{Ca,L}$ density are unchanged in F myocytes; (4) the $[Ca^{2+}]$ dependence of I_{NCX} is unchanged in F myocytes but the contribution of NCX to Ca^{2+} removal is increased; (5) the slower rate of decay of the Ca^{2+} transient during the AP in F myocytes is caused by decreased SR Ca^{2+} transport and possibly changes in NCX function.

Ca^{2+} Handling in the Failing Human Heart

Depressed cardiac contractility and diminished contractility reserve are important phenotypic abnormalities of the failing human heart that have been appreciated for more than 100 years. In the past two decades, it has been shown that alterations in myocyte Ca^{2+} regulation are centrally involved in deranged contractility but the cellular bases have not been well established, in large part because it is difficult to obtain high-quality human heart tissue for thorough in vitro evaluation. Although some aspects of Ca^{2+} regulation have been examined in F human myocytes, to our knowledge, ours is the first in which there has been an in-depth evaluation of the respective contributions of SR, NCX, Ca^{2+} buffers, and $I_{Ca,L}$ to defective Ca^{2+} regulation. Our results, consistent with results of others,[14,27] point to abnormal SR function as the primary basis for the deranged Ca^{2+} transients we observed in

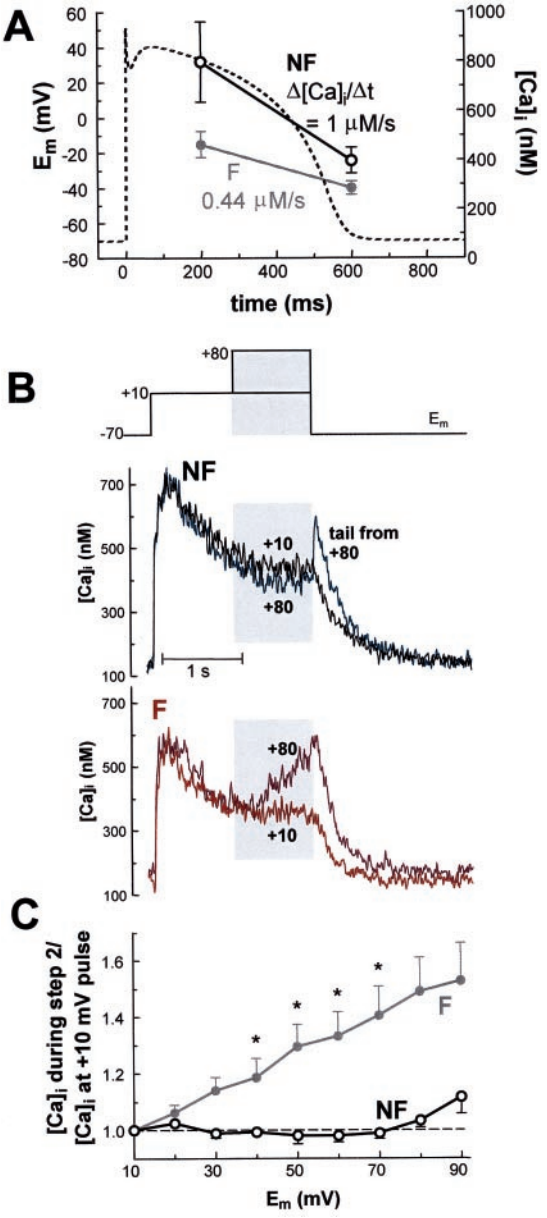

Figure 6. E_m dependence of $[Ca^{2+}]_i$ changes late in the AP. A, Average rate of $[Ca^{2+}]_i$ decline between 200 and 600 ms is indicated. B, Examples of $[Ca^{2+}]_i$ changes induced by further depolarization after 1 second at $E_m = +10$ mV. Note in the nonfailing myocyte a "tail" transient during repolarization from +80 mV. C, Fractional change in $[Ca^{2+}]_i$ during the second step [protocol as in panel B; NF (n=4) and F (n=5) myocytes].

human F myocytes. Depressed SR function would account for the slow rate of decay of the Ca^{2+} transient and the reductions in SR Ca^{2+} storage and release that reduce the magnitude of the Ca^{2+} transient.

The molecular bases for depressed SR function in F human myocytes was not examined in these experiments but has been studied before by us (in tissue samples from the same hearts used to obtain the isolated myocytes used in the present study)[15] and others.[8] Our previous study showed a smaller SERCA protein and no difference in the NCX protein abundance in NF versus F hearts.[15] These molecular measurements correlate well with the biophysical assessments of

Ca^{2+} regulation reported in the present study. Reduction in the abundance of SERCA protein, increased abundance of phospholamban (PLB), decreased PLB phosphorylation, and an increased rate of Ca^{2+} leak from the SR have all been described in the failing human heart by others and may all play some role.[7,8,15,28] Future studies will need to focus on the respective quantitative contribution of each of these changes to depressed SR function. The most important point here is that slower SR Ca^{2+} transport is not exclusively dependent on a reduced abundance of SERCA protein, but could also result from altered SERCA regulation via PLB[29] or because of an increased leak rate, eg, through a hyperphosphorylated Ca^{2+} release channel.[30]

The reduced SR Ca^{2+} content in HF is consistent with data in human, rabbit, and canine HF models.[27,31,32] In the rabbit HF model, SR Ca^{2+} content was reduced by a combination of large increase in NCX function and a modest decrease in SR Ca^{2+}-ATPase function (the canine model was similar[33]). Both of these changes unload the SR and depress systolic function, but they can be offsetting in terms of relaxation and diastolic function. Similar detailed analysis has not previously been done in human HF, but work from Hasenfuss and coworkers[34,35] suggested a similar combination of enhanced NCX and reduced SR Ca^{2+}-ATPase function. Moreover, in one subset of human HF (with relatively preserved diastolic function), they found greatly enhanced NCX expression and modestly reduced SR Ca^{2+}-ATPase expression, functionally like the rabbit HF model described earlier. However, another group had no significant increase in NCX, marked downregulation of SR Ca^{2+}-ATPase expression, and slower relaxation (resembling the ensemble human HF myocytes studied here). Importantly, we found some heart to heart heterogeneity, but there was no clear segregation of phenotypes. The reason for the difference in human HF phenotype between these studies is not clear. We speculate that the failing human hearts studied here are at a more uniformly advanced stage of HF (evidenced by the mean ejection fraction of 17.5% versus 24.2%[34]). We hypothesize that there is an increase in the abundance of NCX in earlier, more compensated forms of heart failure, and that a shift from a high NCX expression (with modest SR Ca^{2+}-ATPase decrease) to marked downregulation of SR Ca^{2+}-ATPase function (with NCX returning to nearly normal) is associated with HF progression.

Our results do not indicate significant intrinsic changes in EC coupling in F human myocytes (similar to the rabbit and dog studies).[31,32] Some rat and mouse studies of hypertrophy and failure[36] found reduced ability of $I_{Ca,L}$ to trigger SR Ca^{2+} release (reduced EC coupling gain), without altered SR Ca^{2+} load. Our results show no significant alteration in $I_{Ca,L}$ density in F myocytes and normal fractional SR Ca^{2+}, despite the reduced SR Ca^{2+} loading. These finding are inconsistent with large reductions in EC coupling "gain" in human F myocytes, at least under our conditions. Whereas dysregulated Ca^{2+} is central to depressed contractility in failing hearts of both large and small animals, the precise cellular basis for the abnormalities might differ. Given the fundamental differences in normal Ca^{2+} regulation in large and small mammals,[10] this may not be surprising.

Ca^{2+} Influx During the AP

In large mammals, the AP duration lasts for hundreds of milliseconds. It is well appreciated that Ca^{2+} influx early in the AP triggers SR Ca^{2+} release.[7,10] Less is known about the sources and amounts of Ca^{2+} that enter the cell during the later portions of the AP (as the $[Ca^{2+}]_i$ declines) and the influence of this influx on the decline of $[Ca^{2+}]_i$. In the present experiments, we show that peak $[Ca^{2+}]_i$ is reduced and the $[Ca^{2+}]_i$ declines more slowly during the AP in F myocytes. These findings are largely explained by reduced SR Ca^{2+} loading, release, and reuptake by the SR. However, in some cells, we observed a slow secondary rise in $[Ca^{2+}]_i$ during the AP plateau (Figures 2A and 6B), suggesting Ca^{2+} entry during the latter portions of the AP. Increased Ca^{2+} entry during the plateau is predicted when the size of the Ca^{2+} transient is reduced, because there should be less Ca^{2+}-mediated inactivation of the L-type Ca^{2+} current[37,38] and because the NCX is biased more toward reverse mode (Ca^{2+} influx) NCX.[39] We have proposed previously[40] that Ca^{2+} influx via the NCX can occur during the AP plateau in failing human ventricular myocytes. To explore this possibility, we abruptly made E_m more positive during the AP plateau period and measured the effect on $[Ca^{2+}]_i$. The fact that $[Ca^{2+}]_i$ increased in F but not in NF myocytes is most consistent with a role for Ca^{2+} influx via the NCX. However, the approaches we used do not rule out a role for the L-type Ca^{2+} current and do not exclude the possibility that positive E_m simply reduced forward mode NCX. This important topic is beyond the scope of the present investigation (C.R. Weber, V.I. Piacentino, S.R. Houser, D.M. Bers, unpublished data, 2003).

Limitations

All studies that use cells and tissues from NF and F human hearts should be interpreted cautiously. Human HF is a complex syndrome and treatments are not uniformly applied. Therefore, substantial heterogeneity in myocyte properties is expected. In addition, nonfailing hearts are not necessarily representative of the normal human population. In addition, these hearts must be protected from ischemic injury.[17] In spite of these limitations, we contend that novel insights into the bases of cardiac dysfunction have been obtained in the present experiments. These insights should form the bases of new hypotheses that can be best tested in appropriate animal models of human HF.

Summary and Conclusions

The present results suggest that reduced SR Ca^{2+} uptake, storage, and release are the primary causes of depressed contractility in failing human myocytes. These changes reduce the size of the Ca^{2+} transient, which should promote additional Ca^{2+} influx during the AP plateau, which would further slow the rate of Ca^{2+} transient decay.

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