Ca\textsuperscript{2+} Handling and Sarcoplasmic Reticulum Ca\textsuperscript{2+} Content in Isolated Failing and Nonfailing Human Myocardium

Burkert Pieske, Lars S. Maier, Donald M. Bers, Gerd Hasenfuss

Abstract—Disturbed sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} content may underlie the altered force-frequency and postrest contractile behavior in failing human myocardium. We used rapid cooling contractures (RCCs) to assess SR Ca\textsuperscript{2+} content in ventricular muscle strips isolated from nonfailing and end-stage failing human hearts. With an increase in rest intervals (1 to 240 s; 37°C), nonfailing human myocardium (n=7) exhibited a parallel increase in postrest twitch force (at 240 s by 121\pm44%; \(P<0.05\)) and RCC amplitude (by 69\pm53%; \(P<0.05\)). In contrast, in failing myocardium (n=30), postrest twitch force decreased at long rest intervals and RCC amplitude declined monotonically with rest (by 25\pm9% and 53\pm9%, respectively; \(P<0.05\)). With an increase in stimulation frequencies (0.25 to 3 Hz), twitch force increased continuously in nonfailing human myocardium (n=7) by 71\pm17% (at 3 Hz; \(P<0.05\)) and RCC amplitude increased in parallel by 247\pm55% (\(P<0.05\)). In contrast, in failing myocardium (n=26), twitch force declined by 29\pm7% (\(P<0.05\)) and RCC amplitude increased only slightly by 36\pm14% (\(P<0.05\)). Paired RCCs were evoked to investigate the relative contribution of SR Ca\textsuperscript{2+} uptake and Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange to cytosolic Ca\textsuperscript{2+} removal during relaxation. SR Ca\textsuperscript{2+} uptake (relative to the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange) increased significantly in nonfailing but not in failing human myocardium as stimulation rates increased. We conclude that the negative force-frequency relation in failing human myocardium is due to an inability of SR Ca\textsuperscript{2+} content to increase sufficiently at high frequencies and thus cannot overcome the frequency-dependent refractoriness of SR Ca\textsuperscript{2+} release. The rest-dependent decay in twitch force in failing myocardium is due to rest-dependent decline in SR Ca\textsuperscript{2+} content. These alterations could be secondary to depressed SR Ca\textsuperscript{2+}-ATPase combined with enhanced cytosolic Ca\textsuperscript{2+} extrusion via Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange. (Circ Res. 1999;85:38-46.)

Key Words: Ca\textsuperscript{2+} ▪ Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange ▪ myocardial contraction ▪ sarcoplasmic reticulum ▪ heart failure

Alterations in intracellular Ca\textsuperscript{2+} handling may play a critical role in the pathophysiology of myocardial failure. Intracellular Ca\textsuperscript{2+}-homeostasis and excitation-contraction (E-C) coupling are regulated mainly by the function of the sarcoplasmic reticulum (SR). On stimulation, Ca\textsuperscript{2+} enters the myocytes, thereby inducing the release of a larger amount of Ca\textsuperscript{2+} from the SR, which leads to activation of contractile proteins and contraction.1

Contractile dysfunction in end-stage human heart failure2–7 has been attributed to depressed myocardial Ca\textsuperscript{2+} sensitivity8 or reduced Ca\textsuperscript{2+} transients.6,9 Smaller Ca\textsuperscript{2+} transients could be due to either lower fractional SR Ca\textsuperscript{2+} release10 or lower SR Ca\textsuperscript{2+} content.11 Lower levels of SR Ca\textsuperscript{2+} uptake and reduced SR Ca\textsuperscript{2+}-ATPase gene and protein expression levels have been described in failing human myocardium.5,6,12–15 In addition, increased activity and gene expression of the sarcolemmal Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger were reported in failing human myocardium.16–18 A direct correlation between depressed SR Ca\textsuperscript{2+}-ATPase expression and depressed force-frequency response in failing myocardium was reported.5 In addition, it was observed that the ratio of Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger to SR Ca\textsuperscript{2+}-ATPase is considerably increased in failing human myocardium.19 Consequently, it was speculated that reduced SR Ca\textsuperscript{2+} reuptake and increased Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange might limit SR Ca\textsuperscript{2+} content, possibly leading to the observed negative force-frequency relation and blunted postrest potentiation in failing myocardium (with parallel changes in intracellular Ca\textsuperscript{2+} transients).6,7

Recently, the first study that measured SR Ca\textsuperscript{2+} content in myocytes from failing human hearts showed reduced caffeine-induced contractures at steady-state conditions and a constant stimulation frequency compared with nonfailing myocardium.11 However, it is still unknown and controversial10 whether the inverse force-frequency relation and the blunted postrest behavior in failing human myocardium are related to parallel changes in SR Ca\textsuperscript{2+} content or to some other defect in Ca\textsuperscript{2+} handling. Accordingly, a major goal of the present study was to characterize the influence of stimulation frequency and rest intervals on SR Ca\textsuperscript{2+} content and relate it to contractile behavior in nonfailing and end-stage failing human myocardium. Changes in SR Ca\textsuperscript{2+} content were characterized by use of rapid cooling contractures (RCCs) as

Received August 11, 1998; accepted April 9, 1999.
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38
described and validated by us and others previously.\textsuperscript{1,21–26} RCCs have the advantage over SR Ca\textsuperscript{2+}-uptake measurements and caffeine-induced contractures because they can be performed in intact muscle under identical conditions as isometric twitches. The information about SR Ca\textsuperscript{2+} content also complements that obtained by the study of twitch contractions and Ca\textsuperscript{2+} transients, in which only a fraction of SR Ca\textsuperscript{2+} is released.\textsuperscript{1}

In addition, no information exists that concerns the relative contributions of SR Ca\textsuperscript{2+}-ATPase and Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange for cytosolic Ca\textsuperscript{2+} removal in nonfailing versus failing human myocardium. Paired RCCs have been used previously to evaluate this competition between the SR Ca\textsuperscript{2+}-ATPase and Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange during relaxation in rabbit and guinea pig ventricle.\textsuperscript{24,26} Thus, experiments were performed with nonfailing and failing human myocardium with the use of paired RCCs.

### Materials and Methods

#### Myocardial Tissue

Experiments were performed in 6 left and 1 right ventricular trabeculae obtained from 4 nonfailing donor hearts that could not be transplanted for technical reasons and in 30 trabeculae from 26 end-stage failing hearts with ischemic (n=10) or dilated (n=16) cardiomyopathy (with 22 of 30 trabeculae from the left ventricle). The mean age in the donor group was 49±4 years; 3 of the donors were men. None of the donors had a history of heart disease and all had normal left ventricular function. The mean age in the heart failure group was 55±3 years. Clinical data of these patients including medications are shown in the Table. There were no differences in contractile behavior with respect to the different medications (eg, digitalis pretreated and untreated patients). The study was approved by the Ethical Committee of the University Clinics of Freiburg, Freiburg, Germany.

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Mean±SEM: 54.9±2.7, 24.8±1.4, 22.5±1.0, 2.1±0.1

DCM indicates dilated cardiomyopathy; ICM, ischemic cardiomyopathy; PCW, pulmonary capillary wedge pressure (mm Hg); EF, ejection fraction; CI, cardiac index (L·min\textsuperscript{-1}·m\textsuperscript{2}); ACEI, ACE inhibitor; BBL, β-blocker; CANT, Ca\textsuperscript{2+} antagonist; CAT, catecholamine; DIG, digoxin/digitoxin; DIU, diuretics; NIT, nitrates; PDE, phosphodiesterase inhibitor; and NA, not available.
at both ends with loops of fine silk suture and stored in the dissection chamber for 10 minutes. Muscles were then transferred to the specially designed RCC chamber (0.2 mL bath volume) and fixed horizontally with 1 end attached to a hook that was connected to a force transducer. The other end was attached to a fixed pin in the RCC chamber. After the cardioplegic solution was washed out, the muscles were superfused with standard KHB (without 2,3-butanedione monoxime) at 37°C and stimulated (voltage 25% above threshold; 5 ms duration). After an equilibration period of 15 to 30 minutes, the muscles were stretched gradually (0.05- to 0.1-mm steps) to the length at which maximum steady-state twitch force was reached (Lmax). Force was amplified and recorded simultaneously with the bath temperature on a strip chart recorder (Graphtec Linearcorder Mark VII, Hugo Sachs Elektronik). The flow rate in the chamber was 15 to 20 mL/min. The diameter of the preparations ranged between 0.15 to 0.65 mm, which was measured with a micrometer mounted in the microscope eyepiece with the muscle in the bath at Lmax (accuracy ±10 μm).

Rapid Cooling Contractures
RCCs were elicited by a rapid decrease in the temperature of the muscle chamber from 37°C to 1°C as previously described. 22-24 This was achieved by switching from a warm to a cold solution with solenoid pinch valves at the bath inlet. The cold solution was maintained at −2°C by a cooling bath (RM20, Lauda), which cools the solution and surrounds the tubing that is connected to the chamber. All tubing was insulated to maintain a constant temperature. During the cooling period, the muscle was not stimulated. With this setup, it is possible to cool the surface of a muscle to <5°C in 300 ms and the core of a muscle with a diameter of 400 μm in <2 s. 25 Paired RCCs were elicited in the force-frequency experiments to investigate the competition between the SR Ca2+ pump and Na+/Ca2+ exchange for cytosolic Ca2+. The fraction of Ca2+ taken up by the SR is available for further release at RCC2, although the Ca2+ extruded from the cell by the Na+/Ca2+ exchange system cannot be reversibly loaded into SR Ca2+ stores. 26 The ratio of the RCC amplitudes (RCC2/RCC1) is an index of the fraction of Ca2+ taken up by the SR during relaxation of RCC1 (relative to that extruded by Na+/Ca2+ exchange). Indeed, when the Na+/Ca2+ exchange is blocked during the paired RCCs, the RCC2/RCC1 ratio is ∼1. 24,26

Experimental Protocol
Force-frequency relations were tested by increasing the stimulation rate in steps from 0.25 to 3 Hz (0.25, 0.5, 1, 1.5, 2, 2.5, and 3 Hz). Recordings of isometric force were obtained at steady-state conditions at each frequency, followed by RCCs. To investigate the influence of rest, rest intervals between 1 to 240 s (1, 5, 10, 30, 60, 120, and 240 s) were instituted from a basal stimulation rate of 1 Hz. Rest periods were repeated to measure postrest twitch and postrest RCC separately. The twitch or RCC amplitude was compared with control experiments were performed with the SR inhibited by either 20 mM/L caffeine (n=4) or 1 μM/L ryanodine (n=4), both of which can abolish RCCs. 24,25

Statistics
All data are expressed as mean±SEM. Statistical analysis was performed on the basis of muscle strip experiments with 1- or 2-way repeated measurements ANOVA followed by the Student-Newman-Keuls test when appropriate. However, similar statistical results were obtained by pooling the data from 1 heart and performing the statistical analysis on the basis of the number of hearts. Statistical significance was taken as P<0.05.

Results
Effects of Rest Intervals on Twitch Force and SR Ca2+ Content in Human Ventricle
Figure 1 shows representative isometric twitches and RCCs in muscle strips from a nonfailing (A) and a failing human heart (B). Records show steady-state twitches (at 1 Hz), as well as posttwitches and RCCs after 10 s and 120 s rest. Note that the muscle was reequilibrated at 1 Hz before the rest interval was repeated for the RCC measurements. When the muscles were rapidly cooled from 37°C to 1°C, the RCC developed to a plateau level over 15 to 30 s, and the amplitude of the contracture was used as an index of SR Ca2+ content. 24,26 When the muscles were rewarmed to 37°C, there was a prominent rewarming spike caused by the rapid increase in myofilament Ca2+ sensitivity, 28 which precedes the intracellular Ca2+ ([Ca2+]i) decline caused by reactivation of Ca2+ transport systems (which had been inhibited at 1°C).

In the nonfailing muscle, rest potentiation of twitch force is apparent after 10 s of rest and becomes even larger after 120 s of rest. RCC amplitudes are lower than the steady-state twitch force. This is typical of most mammalian ventricular muscle and is due to the reduced myofilament Ca2+ sensitivity at 1°C. 23,28 The RCC in nonfailing muscle is larger after 120 s versus 10 s of rest. In the failing muscle, the RCC amplitude is also lower than the steady-state twitch force, but in contrast to the nonfailing muscle, both twitch force and RCC amplitude are smaller after the long rest interval. These results are consistent with a gradual increase in SR Ca2+ load during rest in the nonfailing heart but a decrease in the failing heart.

Figure 2 summarizes average data from experiments similar to that shown in Figure 1. Nonfailing myocardium (n=7) showed a significant and progressive potentiation of twitch force after increasing rest intervals. At up to 240 s, postrest twitches were significantly larger (increase by 121±44%) than steady-state twitches at 1 Hz and were also significantly different from failing myocardium at 60 s and longer rest intervals. In failing human heart muscles (n=30), rest potentiation of twitch force increased significantly up to a rest interval of 10 s (by 45±11%) and then declined continuously with longer rest intervals. After 240 s, postrest twitch force was significantly smaller than the steady-state twitch at 1 Hz (decrease by 25±9%).

Figure 2 also shows the results from the postrest RCCs in nonfailing and failing human myocardium. With longer rest intervals, there was a continuous increase in the average RCC amplitudes in nonfailing muscles (P<0.05 at rest intervals >60 s; increase at 240 s was by 69±53%) that was also significantly different from the failing ventricle at 60 s and longer rest intervals. Therefore, the increase in postrest twitch amplitude in nonfailing myocardium is paralleled by an increase in SR Ca2+ content (although the percentage increase in twitch force was greater than for RCC). In contrast, in failing myocardium, no significant change in RCC ampli-
tudes (ie, SR Ca\textsuperscript{2+} content) could be observed after short rest intervals (5 to 30 s), but RCC amplitude significantly declined at longer rest intervals (>30 s; decreased at 240 s by 53\%±9\%). No differences existed in rest-dependent changes in postrest twitch force or RCCs between dilative or ischemic cardiomyopathy.

Figure 1. Postrest twitches and RCCs from isolated muscle strips from a nonfailing (A) and an end-stage failing (B) human heart. Isometric tension traces above temperature traces. Stimulation frequency is 1 Hz. After a rest interval of 10 s (left), postrest twitch amplitude was measured. Note that the faster recording speed was used during RCCs, rest intervals, and the few twitches before and after rest. After complete equilibration of twitch force, a second rest of 10 s was followed by a RCC. Postrest twitches and RCCs were also measured after rest intervals of 120 s. Note that RCC amplitudes were measured when force reached a maximum during the cooling contracture, but before the rewarming spike (attributed to increased Ca\textsuperscript{2+} sensitivity of myofilaments on rewarming).

Effects of Stimulation Frequency on Twitch Force and SR Ca\textsuperscript{2+} Content in Human Ventricle

Figure 3 shows the effects of increasing stimulation frequencies on isometric twitch force and RCC amplitudes in nonfailing (n=7) and end-stage failing (n=26) human myo-

Figure 2. Rest-dependent changes in the amplitudes of twitch force (left) and RCCs (right) in human myocardium. All values are normalized to the values obtained at steady-state conditions (1 s rest). Mean values for 7 muscle strips from 4 nonfailing (open symbols) and 30 muscle strips from 26 end-stage failing (filled symbols) human hearts. *Significantly different (P<0.05) to steady-state values; #significant difference (P<0.05) between nonfailing and failing myocardium.

Figure 3. Frequency-dependent changes in twitch force amplitude (left) and RCCs (right) in 7 muscle strips from 4 nonfailing and 26 muscle strips from 26 end-stage failing human hearts. Average twitch amplitude at 1 Hz was 8.4±1.3 mN/mm\textsuperscript{2} in nonfailing and 8.3±0.7 mN/mm\textsuperscript{2} in failing myocardium (no significant difference). RCC amplitude at 1 Hz was 4.3±0.8 mN/mm\textsuperscript{2} in nonfailing and 4.0±0.7 mN/mm\textsuperscript{2} in failing myocardium (no significant difference). Percentage of changes of twitch force (left) and RCCs (right) from the basal value at 0.25 Hz. *Significantly different (P<0.05) from steady-state values at 0.25 Hz, #significant difference (P<0.05) between nonfailing and failing myocardium.
cardium. Average values are given as the percentage of the basal value at 0.25 Hz. In nonfailing myocardium, isometric twitch force increased continuously with higher stimulation frequencies (positive force-frequency relation). At 3 Hz, twitch force had increased by 71±17% (P<0.05). The positive force-frequency relation was accompanied by a parallel increase in RCC amplitude by maximally 247±55% at 3 Hz (P<0.05). Note that the percentage increase in RCCs was much greater than that of twitch amplitude. In contrast, in failing myocardium, isometric twitch force either did not change at moderate frequency or declined at higher stimulation rates (negative force-frequency relation). At 3 Hz, twitch force declined by 29±7% as compared with 0.25 Hz (P<0.05). RCC amplitude increased only slightly, albeit significantly in failing myocardium (by 36±14% at 3 Hz). There was a significant difference in twitch force and RCC amplitudes between nonfailing and failing myocardium at stimulation rates higher than 1 Hz.

RCCs could be completely abolished at all stimulation frequencies by preequilibration of muscles with either 1 μmol/L ryanodine (n=4) or 20 mmol/L caffeine (n=4, not shown). These data confirm that RCCs depend directly on SR Ca2+ content. Figure 3 includes the results from the experiments with ryanodine in failing myocardium. Ryanodine completely abolished RCCs at each stimulation frequency, which confirmed that RCCs rely on a ryanodine-sensitive Ca2+ compartment. In contrast, isometric contractions were not completely suppressed by ryanodine. At 1 Hz, the remaining isometric twitch force (presumably supported by Ca2+ influx) was ≈50% of the preryanodine value. No differences were observed in frequency-dependent changes in twitch force or RCCs between dilatative or ischemic cardiomyopathy.

Paired RCCs and Ca2+ Reuptake Into the SR in Human Ventricle

Paired RCCs were used to assess the relative competition among Ca2+ transport systems during relaxation. Figure 4 shows an example of paired RCCs in a nonfailing human muscle at 2 Hz. When RCC1 reaches a plateau, the muscle strip is rapidly rewarmed, and immediately after relaxation is complete, RCC2 is activated. RCC2 reflects Ca2+ that had been taken up by the SR during rewarming of RCC1. RCC2 is slightly smaller than RCC1 because of competitive Ca2+ elimination by Na+/Ca2+ exchange (which leaves less Ca2+ available for RCC2).

Figure 5 shows the influence of the stimulation frequency on RCC2/RCC1 in human nonfailing and failing myocardium. In nonfailing myocardium, RCC2/RCC1 continuously increased from 37±4% to 74±7% as the stimulation rate is increased from 0.25 to 3 Hz (P<0.05). The implication is that as the frequency increases, the SR Ca2+-ATPase transports a larger fraction of the Ca2+ that was released during RCC1 (versus the Na+/Ca2+ exchange). A simple explanation for this effect could be that as the frequency increases, the gradual increase in [Na+]i limits the ability of the Na+/Ca2+ exchange to compete with the SR Ca2+-ATPase. In addition, increasing frequency also accelerates [Ca2+]i decline because of an increased rate of SR Ca2+ transport possibly caused by Ca2+/calmodulin-dependent protein kinase II (CaMK-II). Thus, the SR Ca2+-ATPase becomes increasingly dominant over the Na+/Ca2+ exchange system in transporting Ca2+ from the cytosol at higher frequencies.

In failing myocardium, RCC2/RCC1 is relatively constant over the entire frequency range of 1 to 3 Hz, with 58±2%. This may mean that the frequency-dependent increase in SR Ca2+-ATPase activity is blunted or that the SR is already at its maximal load at a low stimulation rate before frequency is increased (note the limited rise of RCCs in Figure 3), thus SR cannot take up more Ca2+ or compete better against Na+/Ca2+ exchange.

Refractoriness of E-C coupling

Figures 2 and 3 show how twitch and SR Ca2+ load change with increasing stimulation frequencies or rest intervals. The
ratio of twitch/RCC normalizes the twitch amplitude for changes in SR Ca\(^{2+}\) load and thus provides an index of E-C coupling status. Assuming that the isometric twitch reflects the amount of Ca\(^{2+}\) released from the SR, the twitch/RCC ratio is analogous to fractional SR Ca\(^{2+}\) release at a twitch and allows us to consider how E-C coupling changes as function of frequency and rest interval. Twitch/RCC cannot be directly translated to a percentage of SR Ca\(^{2+}\) release because of nonlinearities of the force-[Ca\(^{2+}\)] relationship, kinetic constraints, and the effect of cooling on myofilament Ca\(^{2+}\) sensitivity. Nevertheless, it is a useful semiquantitative measure of how E-C coupling changes in a muscle with respect to frequency and rest interval.

Figure 6A shows that the twitch/RCC ratio progressively declines with increasing stimulation frequency in both failing and nonfailing myocardium (down to 50\% to 60\% at 3 Hz). This is indicative of increased refractoriness of E-C coupling at higher frequencies (ie, twitches are depressed with respect to SR Ca\(^{2+}\) load). However, the twitch/RCC ratio increases in both failing and nonfailing myocardium with longer rest intervals (Figure 6B) and may reflect the recovery of E-C coupling from refractoriness after a twitch. Overall, the recovery of E-C coupling can be described by 2 simple exponential time constants (\(\tau_{\text{fast}}\) and \(\tau_{\text{slow}}\); Figure 6C). The fast time constant (\(\tau_{\text{fast}} = 267 \text{ to } 353 \text{ ms}\)) may largely depend on action potential duration and recovery of sarcolemmal ion channels from inactivation and was slightly larger in the failing heart. The slow recovery (\(\tau_{\text{slow}} \approx 15 \text{ s}\)) was the same between failing and nonfailing myocardium and may reflect the slow phase of recovery of the ryanodine receptor from an inactivated or adapted state. The semiquantitative nature of this index precludes meaningful conclusions in regard to the relative amplitudes of twitch/RCC values (which were not statistically different).

Sample Size of Nonfailing Human Myocardium

In the present study, nonfailing human tissue availability was limited. To assure that these nonfailing muscles from the present study were representative, we compared both postrest and force-frequency relations with larger groups of nonfailing muscles from our previous studies. These studies included 16 muscles from 14 nonfailing hearts (for force-frequency protocols) and 21 muscles from 12 nonfailing hearts (for postrest protocols). The results were almost identical to the nonfailing data in Figures 2 and 3 and did not significantly differ at any frequency or rest interval. For force-frequency relation in nonfailing myocardium, the maximal force in both data sets occurred at 2.5 Hz (and was 179\% to 191\% present data; 191\% previous data). For postrest twitches, the maximum rest potentiation occurred at 120 s of rest in both sets (and was 225\% previous data). This indicates that the relatively small number of 7 nonfailing muscles from 4 hearts in the present study is representative of nonfailing human myocardium.

Discussion

The present study shows that (1) postrest potentiation of twitch force is associated with increased SR Ca\(^{2+}\) load in nonfailing human myocardium, whereas rest-decay of force and SR Ca\(^{2+}\) load occur in failing myocardium at long rest intervals; and (2) the positive force-frequency relation in nonfailing human myocardium is associated with a large increase in SR Ca\(^{2+}\) load, whereas the inverse force-frequency relation in failing myocardium is associated with blunted SR Ca\(^{2+}\) loading at higher stimulation rates.

These findings indicate that reduced SR Ca\(^{2+}\) loading and the subsequent decreased SR Ca\(^{2+}\) release may be the dominant alteration that underlies disturbed frequency potentiation and postrest potentiation of contractile force in the failing human heart.

Postrest Behavior

During rest, Ca\(^{2+}\) is removed from the cytosol into the SR by Ca\(^{2+}\) pumps and across the sarcolemma mainly by the
Na+/Ca2+ exchanger.37–39 There is a finite rate of Ca2+ leak from the SR (eg, as Ca2+ sparks or otherwise). This released Ca2+ can either be taken back up by the SR (in which case SR Ca2+ load does not decrease) or it can be partly removed from the cytosol by Na+/Ca2+ exchange (in which case SR Ca2+ content declines). In the absence of SR Ca2+ depletion, rest potentiation of twitches appears to be the norm. Moreover, rest potentiation of twitches is not due to increased Ca2+ current and occurs even with constant SR Ca2+ content.33,34 This has been interpreted as a recovery of the E-C coupling mechanism from a refractory state (eg, adaptation or inactivation), with increased fractional SR Ca2+ release.33–36

The present results indicate that nonfailing human myocardium shows an increase in postrest twitch force with increasing rest intervals (by 121%) together with a rest-dependent increase in RCCs (by 69%). Thus, progressive SR Ca2+ loading during rest and recovery of SR Ca2+ release from refractoriness may contribute almost equally to the pronounced postrest potentiation of isometric force in nonfailing human myocardium observed in this study and in a previous study.7

In failing human myocardium, the initial postrest potentiation of twitches after short rest intervals is almost identical to nonfailing myocardium (Figure 2) and may again represent recovery of the Ca2+-release processes in the presence of unchanged SR Ca2+ content (because RCCs did not change significantly). However, after rest intervals of 30 s and longer, the decline of SR Ca2+ content (on the basis of RCCs) limits the postrest twitch amplitude, and with longer rest periods, the decline in twitch force parallels the decline in RCCs. For instance, if complete recovery of E-C coupling at 240 s increased fractional Ca2+ release to 150% of control, but the SR Ca2+ content was reduced by 50%, the resultant twitch would be 75% of control (as observed in Figure 2).

Rat ventricle shows rest potentiation similar to nonfailing human ventricle, whereas rabbit ventricle shows rest decay similar to failing human myocardium.1,33,34 Rabbit myocardium has a stronger Na+/-Ca2+ exchange, weaker SR Ca2+ pump, and lower resting [Na+], than rat myocardium.35,39 These 3 factors in rabbit and possibly failing human heart would bias the competition for cytosolic Ca2+ that leaks from the SR in favor of Ca2+ extrusion from the cell during rest (and hence rest decay of SR Ca2+ content). When rabbit ventricular myocytes are rested in Na+-free, Ca2+-free solution to block Na+/-Ca2+ exchange, the rest-induced loss of SR Ca2+ is completely blocked, which results in rest potentiation nearly identical to rat ventricular myocytes.34

For the postrest experiments, a basal stimulation rate of 1 Hz has been used for several reasons. First, it is a physiological human heart rate and it is a stimulation rate used in many studies that investigate the contractile behavior of isolated human cardiac tissue. In addition, it is a stimulation rate in which damage to the muscle caused by chronic core hypoxia, accumulation of inorganic phosphate, or limited access of glucose to the core of the muscle is minimized. Most importantly, we have tested the influence of the preconditioning stimulation rate on postrest contractile behavior in nonfailing and failing human cardiac muscles, and the results showed that a high heart rate (ie, 2 Hz versus 0.5 or 1 Hz) increases the amplitude of rest potentiation after low rest intervals but does not affect the pathological rest decay after longer rest intervals in failing human myocardium.7 Therefore, we feel that the use of 1 Hz of preconditioning stimulation is a reasonable stimulation rate in our experimental setup.

In summary, the results of this study indicate for the first time directly that during rest the SR in failing human myocardium loses Ca2+, whereas SR Ca2+ content from nonfailing human hearts increases. In addition, it seems that human myocardium exhibits a fast and a slow restitution phase of E-C coupling that is similar in nonfailing and failing human myocardium.

Why might the failing human myocardium lose Ca2+ during rest? One attractive if not unique explanation is consistent with biochemical data. Namely, the reduced SR Ca2+-ATPase activity and increased Na+/Ca2+-exchange activity in the failing (versus nonfailing) myocardium would make Ca2+, which leaks from the SR during rest more likely to be extruded via Na+/Ca2+ exchange. This would then result in the observed progressive Ca2+ depletion of the SR during rest and the postrest decay of twitch force in failing human myocardium.

**Force-Frequency Relation**

Increasing frequency can cause both a negative effect (by refractoriness of E-C coupling; Figure 6A) and a positive effect on contractility (by increased SR Ca2+ load; Figure 3).40 The balance of these factors will determine whether the force-frequency relation is positive, negative, or a biphasic combination of both.41

In nonfailing human myocardium, the positive force-frequency relation is accompanied by a large increase in SR Ca2+ content. Thus, in the nonfailing human myocardium, the positive effect of increased SR Ca2+ content more than compensates for the putative negative effect of refractoriness at higher stimulation frequencies. For example, RCCs increased to 350% at 3 Hz (Figure 3), although our index of fractional SR Ca2+ release decreased by ~50% (Figure 6A), which resulted in a twitch amplitude ~175% of control at 3 Hz (Figure 3). The increase in SR Ca2+ content is attributable to the increased Ca2+ influx into the cell at higher stimulation rates and reduced time and [Na+], gradient available for Ca2+ efflux per unit time and relies on the ability of the SR to take up more Ca2+. These effects may increase the amount of SR Ca2+ available for release, the trigger for release,42 and also the fractional release for a specific trigger.43

In the failing human ventricle, the increase in SR Ca2+ content at 1.5 Hz (to 136%, Figure 3) may be sufficient to offset the negative effect of refractoriness (79%, Figure 6A) so that twitch force is relatively unchanged (101%, Figure 3). As frequency is raised further, no additional increase in SR Ca2+ content is observed, thus allowing the negative effect of refractoriness to be unopposed. This explains the observation that twitch force declines markedly at higher frequencies, although SR Ca2+ content is slightly higher than at 0.25 Hz. It also clarifies our previous findings of decreasing Ca2+ transients with increasing frequency, which we had speculated might be due to decreased SR Ca2+ content.6

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SR Ca\(^{2+}\) Uptake Versus Ca\(^{2+}\) Extrusion via Na\(^{+}\)/Ca\(^{2+}\) Exchange

Paired RCC studies indicate that in nonfailing human myocardium at very low stimulation frequencies, a smaller fraction of Ca\(^{2+}\) may be removed from the cytosol by the SR Ca\(^{2+}\)-ATPase versus the Na\(^{+}\)/Ca\(^{2+}\) exchanger. However, with increasing stimulation frequency, SR Ca\(^{2+}\) uptake becomes the increasingly dominant mechanism for Ca\(^{2+}\) removal. This may result from a frequency-dependent activation of SR Ca\(^{2+}\)-ATPase activity, for example, by CaMK-II\(^{2+}\) in combination with a decrease of Ca\(^{2+}\)-dependent extrusion via Na\(^{+}\)/Ca\(^{2+}\) exchange as a consequence of frequency-dependent increased [Na\(^{+}\)].\(^{31}\)

In contrast, in failing human myocardium, Ca\(^{2+}\) removal by SR Ca\(^{2+}\)-ATPase is similar to that by Na\(^{+}\)/Ca\(^{2+}\) exchange and the relative contributions do not change with higher stimulation rates. This may indicate that the depression of Ca\(^{2+}\) extrusion via Na\(^{+}\)/Ca\(^{2+}\) exchange does not occur in the failing heart or that the frequency-dependent increase of SR Ca\(^{2+}\)-ATPase activity is blunted. Alternatively, if fractional SR Ca\(^{2+}\) release is less in failing myocardium,\(^{10}\) at higher frequencies, there would be more residual Ca\(^{2+}\) left in the SR. This may prevent increased net SR Ca\(^{2+}\) uptake at higher frequencies because of the steeper thermodynamic gradient the SR Ca\(^{2+}\) pump must face. In addition, it might be speculated that an elevated diastolic [Ca\(^{2+}\)] in the failing human heart\(^{4}\) could already have maximized Ca\(^{2+}\)-dependent activation of the SR Ca\(^{2+}\)-ATPase at the expense of reduced functional reserve of SR Ca\(^{2+}\) pumping with higher stimulation rates.

What Changes Occur in the Failing Human Ventricle

Altered force-frequency relation and postrest behavior of failing human myocardium have been difficult to fully understand, despite molecular data that indicate reduced SR Ca\(^{2+}\)-ATPase and increased Na\(^{+}\)/Ca\(^{2+}\) exchange. This is partly because parallel measurements of twitch force and SR Ca\(^{2+}\) content were unavailable. In nonfailing myocardium (with relatively high SR Ca\(^{2+}\)-ATPase and low Na\(^{+}\)/Ca\(^{2+}\)-exchange activity), SR Ca\(^{2+}\) content can increase greatly with frequency, such that it more than offsets the depressant effect of frequency-dependent refractoriness of E-C coupling. In failing myocardium (with lower SR Ca\(^{2+}\)-ATPase activity and stronger Ca\(^{2+}\) extrusion by Na\(^{+}\)/Ca\(^{2+}\) exchange activity), SR Ca\(^{2+}\) content can increase greatly with frequency, such that it more than offsets the depressant effect of frequency-dependent refractoriness of E-C coupling. In failing myocardium (with lower SR Ca\(^{2+}\)-ATPase activity and stronger Ca\(^{2+}\) extrusion by Na\(^{+}\)/Ca\(^{2+}\) exchange), the increase in SR Ca\(^{2+}\) content at high frequency is only \(\approx 10\%\) of that in nonfailing myocardium. This limited increase in SR Ca\(^{2+}\) content cannot compensate for the refractoriness that accumulates, and the result is a blunted or negative force-frequency relation.

The increased ratio of Na\(^{+}\)/Ca\(^{2+}\) exchange to SR Ca\(^{2+}\)-ATPase in failing human heart\(^{19}\) also readily explains why the rest potentiation observed in nonfailing hearts is abbreviated and gives way to rest decay at longer rests (even if the SR Ca\(^{2+}\) leak is unchanged). That is, a given resting leak of Ca\(^{2+}\) from the SR is more likely to be extruded from the cell in failing myocardium.

If limitation of SR Ca\(^{2+}\) load underlies the blunted force-frequency relation in failing human myocardium, therapeutic approaches guided toward increasing SR Ca\(^{2+}\) uptake may prove beneficial. Indeed, low concentrations of forskolin can partially normalize the blunted force-frequency response in failing myocardium.\(^{44}\) Moreover, knockout of the phospholamban gene in mouse hearts and adenovirus-mediated SR Ca\(^{2+}\)-ATPase overexpression in rat cardiac myocytes improved contractile function.\(^{35,46}\) Therefore, therapeutic interventions guided toward specifically increasing SR Ca\(^{2+}\) load may prove more beneficial than those that nonselectively increase [Ca\(^{2+}\)].

Acknowledgments

This work was supported by grants from the Zentrum für Klinische Forschung II der Universität Freiburg, the Deutsche Forschungsgemeinschaft (HA1233/3-3), the Boehringer Ingelheim Fonds, and the National Institutes of Health (HL-52478).

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* Circ Res. 1999;85:38-46 
doi: 10.1161/01.RES.85.1.38

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

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