Rate Dependence of $[Na^+]_i$ and Contractility in Nonfailing and Failing Human Myocardium

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Background—In the failing human heart, altered Ca$^{2+}$ homeostasis causes contractile dysfunction. Because Ca$^{2+}$ and Na$^+$ homeostasis are intimately linked through the Na$^+/Ca^{2+}$ exchanger, we compared the regulation of [Na$^+$], in nonfailing (NF) and failing human myocardium.

Methods and Results—[Na$^+$] was measured in SBFI-loaded muscle strips. At slow pacing rates (0.25 Hz, 37°C), isometric force was similar in NF (n=6) and failing (n=12) myocardium (6.4±1.2 versus 7.2±1.9 mN/mm$^2$), but [Na$^+$], and diastolic force were greater in failing (22.1±2.6 mmol/L and 15.6±3.2 mN/mm$^2$) than in NF (15.9±3.1 mmol/L and 3.50±0.55 mN/mm$^2$; $P<0.05$) myocardium. In NF hearts, increasing stimulation rates resulted in a parallel increase in force and [Na$^+$], without changes in diastolic tension. At 2.0 Hz, force increased to 136±17% of the basal value ($P<0.05$), and [Na$^+$], to 20.5±4.2 mmol/L ($P<0.05$). In contrast, in failing myocardium, force declined to 45±3%, whereas [Na$^+$], increased to 27.4±3.2 mmol/L (both $P<0.05$), in association with significant elevations in diastolic tension. [Na$^+$], was higher in failing than in NF myocardium at every stimulation rate. [Na$^+$], predicted in myocytes from Na$^+/K^+$-contraction relations was 8.0 mmol/L in NF (n=9) and 12.1 mmol/L in failing (n=57; $P<0.05$) myocardium at 0.25 Hz. Reverse-mode Na$^+/Ca^{2+}$ exchange induced significant Ca$^{2+}$ influx in failing but not NF myocytes, compatible with higher [Na$^+$], in failing myocytes.

Conclusions—Na$^+$, homeostasis is altered in failing human myocardium. At slow heart rates, the higher [Na$^+$], in failing myocardium appears to enhance Ca$^{2+}$ influx through Na$^+/Ca^{2+}$ exchange and maintain sarcoplasmic reticulum Ca$^{2+}$ load and force development. At faster rates, failing myocytes with high [Na$^+$], cannot further increase sarcoplasmic reticulum Ca$^{2+}$ load and are prone to diastolic Ca$^{2+}$ overload. (Circulation. 2002;106:447-453.)

Key Words: heart failure  contractility  sodium  calcium  diastole

A normal regulation of myocyte Ca$^{2+}$ with reduced systolic and increased diastolic [Ca$^{2+}$] underlies contractile dysfunction of the failing human heart.1,2 Both a reduced activity and expression of sarcoplasmic/endoplasmic reticulum Ca$^{2+}$-ATPase 2a (SERCA2a)1,3 and an increased expression of the Na$^+/Ca^{2+}$ exchanger (NCX)4 were related to disturbed intracellular Ca$^{2+}$ handling. An important aspect of the contractile defects of the failing heart is that they become more pronounced at faster beating rates. Whereas [Ca$^{2+}$], transients and twitch force increase with higher stimulation rates in nonfailing human myocardium (positive force-frequency relation), this frequency potentiation is blunted or reversed (negative) in end-stage failing myocardium.1,5 The negative force-frequency relation is directly related to altered Ca$^{2+}$ handling of the myocytes1,6 and to reduced SERCA2a protein expression.7

However, intracellular Ca$^{2+}$ handling is not only regulated by the expression of Ca$^{2+}$ handling proteins but is also intimately linked to intracellular Na$^+$ homeostasis via the activity and transport direction of NCX (for review, see Blaustein and Lederer). At negative membrane potentials and normal [Na$^+$], and [Ca$^{2+}$], the NCX functions in the (forward) Na$^-$ in/Ca$^{2+}$-out mode and reduces diastolic Ca$^{2+}$ levels by extruding Ca$^{2+}$ from the SR.12,13 Higher [Na$^+$], limits forward-mode and favors reverse-mode NCX14 and may contribute to disturbed Ca$^{2+}$ handling in the failing heart through its effect on NCX.

Decreased expression, isoform shifts, and altered function of Na$^+/K^+$-ATPase have been demonstrated in failing human myocardium15,16 and should produce increased [Na$^+$]. However, despite its potential major impact on contractile function, there are no data available on the regulation of [Na$^+$], in human cardiac muscle.

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The hypothesis of the present study is that $[\text{Na}^+]_{o}$ homeostasis is altered in the failing human heart. The main findings are that $[\text{Na}^+]_{o}$ is significantly elevated and contributes to preserved contractile function at slow stimulation rates but impairs force-frequency behavior and diastolic function at higher stimulation rates in failing myocardium.

**Methods**

**Human Myocardium**
Experiments were performed in muscle strips or myocytes from 12 nonfailing and 29 end-stage failing (New York Heart Association class IV) human hearts. The mean ages in the control and heart failure groups were 46±5 and 55±6 years, respectively. Control subjects had no history of heart disease and had normal left ventricular function. Mean ejection fraction of the failing hearts was 23±4%. The causes of heart failure were ischemic (n=14) and dilated (n=15) cardiomyopathies. The local ethics committees approved the study protocol.

**Fluorescence Measurements**
Muscles were dissected and prepared as described previously. To measure $[\text{Na}^+]_{o}$, muscles (cross-sectional area 0.39±0.08 mm$^2$) were incubated for 2 hours with SBFI-AM (35.5 μmol/L) at room temperature similar to previously described protocol. Muscles were then mounted in a cylindrical glass cuvette, connected to an isometric force transducer, and superfused with a modified Krebs-Henseleit solution (1.8 mmol/L Ca$^{2+}$, 95% O$_2$/5% CO$_2$). Muscles were stimulated at 1 Hz and stretched until maximum isometric developed force was reached.

Fluorescence was recorded and analyzed as described previously. Excitation light (100-W mercury lamp, Ushio) was passed alternatively through 340- and 380-nm band-pass filters at 125 Hz and focused on the muscle strip. The $[\text{Na}^+]_{o}$-dependent SBFI fluorescence was directed through a 510-nm band-pass filter and collected by a photomultiplier (Scientific Instruments). After subtraction of autofluorescence, the emitted signals at 340 and 380 nm were divided to obtain the 340/380 ratio. Fluorescence from the muscle strips declined by ~15% per hour without affecting the ratio or the calculated $[\text{Na}^+]_{o}$. The 340/380 ratios were calibrated by an in vivo procedure as described previously. To determine SBFI distribution in the myocytes, 50 μg/mL saponin was used to selectively permeabilize the sarcolemma, followed by 1% Triton X-100 to permeabilize all other cell compartments.

**Experimental Protocol**
Twitch force and $[\text{Na}^+]_{o}$ were measured at increasing stimulation rates from 0.25 Hz (basal rate) to 1, 2, and 3 Hz. To directly assess functional consequences of elevated $[\text{Na}^+]_{o}$, additional experiments were performed in muscles from failing hearts in the presence of the Na$^+/K^+$-ATPase inhibitor ouabain (0.03 μmol/L). The effects of increasing stimulation rates or rest intervals (2 to 240 seconds; postrest contractions) on twitch and diastolic tension were analyzed.

**Experiments in Isolated Myocytes**
Left ventricular myocytes were isolated from human hearts by techniques described previously. All experiments were performed at 37°C in Tyrode’s solution (1 mmol/L Ca$^{2+}$). Contractions (video-edge detection) were induced at 0.25 or 0.5 Hz through voltage-clamp microelectrodes or with field stimulation. Standard suction pipette techniques were used either to record action potentials (current clamp) or to control membrane potential. Ca$^{2+}$ transients were measured by inclusion of 50 μmol/L Fluo-3 in the pipette solutions or by incubation of the cells with Fluo-3-AM. Ca$^{2+}$ influx via L-type Ca$^{2+}$ channels versus reverse-mode NCX was determined with a 2-step protocol. After 5 conditioning steps, the membrane potential was stepped to 10 mV for 1 second and then either maintained at this potential (at which the L-type Ca$^{2+}$ current is largest) or stepped up to 100 mV (at which Ca$^{2+}$ current is small and Ca$^{2+}$ influx via reverse-mode NCX is large).

**Results**

**Loading, Compartmentation, and Calibration of SBFI**
Autofluorescence at 340 and 380 nm was low before SBFI loading (Figure 1A). After incubation with SBFI-AM, fluorescence increased by a factor of 4 to 6. Selective permeabilization of the sarcolemmal membrane with saponin (50 μg/mL) led to a further reduction of SBFI fluorescence by ~20%. The remaining fluorescence was similar to the original autofluorescence. These data show that the largest SBFI fraction was within the cytoplasm.

To calibrate SBFI signals, the sarcolemmal Na$^+$ permeability was increased with monensin and gramicidin in 27 muscles. Under these conditions, $[\text{Na}^+]_{m}$ and $[\text{Na}^+]_{o}$ should eventually come into equilibrium. A stepwise reduction in $[\text{Na}^+]_{o}$ from 140 to 28 and 0 mmol/L resulted in a pronounced decline in the 340/380 fluorescence ratio because of an increase in the 380-nm fluorescence with only minor changes in 340-nm fluorescence.
The resulting calibration curve (Figure 2C) was used to convert the 340/380 ratios to [Na⁺].

**Influence of Stimulation Rate on [Na⁺] and Force**

Figure 3 shows the effects of increasing stimulation rates on [Na⁺], and force of contraction in a muscle strip from a failing heart. Whereas [Na⁺] was lowest at 0.25 Hz and increased with higher stimulation rates, isometric force declined, and diastolic tension increased at higher pacing rates. Similar experiments were performed in 6 nonfailing and 12 failing muscles (Figure 4; Table). Developed force at 0.25 Hz was not significantly different between nonfailing and failing muscles (6.4 ± 1.9 mN/mm², respectively). However, [Na⁺], and diastolic tension were higher in failing (22.1 ± 2.6 mmol/L and 15.6 ± 3.2 mN/mm²) than in nonfailing (15.9 ± 3.1 mmol/L and 3.50 ± 0.55 mN/mm²; P<0.05) myocardium. In nonfailing myocardium, developed force increased to 136 ± 7% at 2.0 Hz, and to 127 ± 9% at 3.0 Hz (P<0.05 versus 0.25 Hz). [Na⁺] increased in parallel from 15.6 ± 3.2 mmol/L at 0.25 Hz to 20.5 ± 4.2 mmol/L at 2.0 Hz and to 22.5 ± 4.6 mmol/L at 3.0 Hz (both P<0.05 versus 0.25 Hz). There were no significant rate-dependent effects on diastolic tension in nonfailing muscles (Table).

In failing muscles, the force-frequency relationship was negative. With increasing frequencies, developed force decreased to 45 ± 3% at 2.0 Hz and to 30 ± 2% at 3.0 Hz (both P<0.05 versus 0.25 Hz). Diastolic tension increased significantly over the same frequency range (Table). [Na⁺], increased with higher frequency to 27.4 ± 3.2 mmol/L at 2.0 Hz and to 31.4 ± 3.7 mmol/L at 3.0 Hz (both P<0.05 versus 0.25 Hz). These experiments show that frequency-dependent increases in [Na⁺], were positively correlated with developed tension in nonfailing muscles, whereas there was an inverse relationship in failing muscles. There was a significant positive correlation between frequency-dependent increases in [Na⁺], and diastolic force only in failing muscles. [Na⁺],
was significantly higher in failing than in nonfailing myocardium at every stimulation rate.

**Isolated Myocyte Studies**

The objective of these experiments was to predict the [Na⁺]i of nonfailing and failing human ventricular myocytes from an alternative physiological approach. The strategy was to vary [Na⁺]i with cell dialysis and measure the steady-state level of contraction, thereby defining a standard relationship between [Na⁺]i and contraction magnitude (Figure 5A). In both nonfailing (n=7 from 5 hearts) and failing (n=12 from 6 hearts) myocytes, shortening amplitude increased with higher [Na⁺] in the patch pipette (Figures 5A and 5B). Shortening amplitudes were similar in nonfailing and failing myocytes when the pipette [Na⁺] was 0 or 10 mmol/L. Contractions were also induced by field stimulation at 0.25 Hz in nondialyzed nonfailing (n=18) and failing (n=57) myocytes, so that each cell maintained its own [Na⁺]i. Shortening was slightly but significantly larger in failing versus nonfailing myocytes, similar to our observations in muscles and previous observations in myocytes studied at slow frequencies.30 [Na⁺]i predicted from these experiments (Figure 5B) was 8.0 mmol/L in nonfailing and 12.1 mmol/L in failing myocytes.

Higher [Na⁺], in failing myocytes should alter cellular Ca²⁺ handling via the NCX. This hypothesis was tested in nonfailing and failing myocytes by induction of Ca²⁺ influx via reverse-mode NCX with voltage steps to 100 mV (Figure 6, top). There was a significant rise in cytosolic Ca²⁺ only in failing myocytes, and the Ca²⁺ transient of the next beat was potentiated, which demonstrated that SR Ca²⁺ was increased (Figure 6). Similar results were obtained in perforated patch experiments.

**Effects of Ouabain on Force-Frequency and Postrest Contractile Behavior**

Failing muscles were exposed to ouabain to elevate [Na⁺], and to test the hypothesis that elevated [Na⁺], of failing myocardium enhances contractility at slow pacing rates but favors systolic and diastolic dysfunction at higher rates. At 0.5 Hz, ouabain increased twitch force in failing myocardium from 9.1±2.9 to 15.4±2.3 mN/mm² (P<0.05). However, on increases in stimulation rate (up to 3 Hz), developed force declined more steeply, and diastolic tension increased to a
greater extent (Figure 7A). In contrast, after increasing rest periods (basal frequency 1 Hz), postrest contractions were more potentiated with ouabain (Figure 7B). These findings further support the idea that in failing myocardium with depressed SR function, elevated [Na\(^+\)], promotes SR Ca\(^{2+}\) loading at long diastolic intervals.

**Discussion**

This is the first report on intracellular Na\(^+\) handling in the failing human heart. The results show that (1) developed force and cell shortening are similar or greater but [Na\(^+\)], is significantly higher in failing versus nonfailing myocardium at slow stimulation rates; (2) [Na\(^+\)], increases with stimulation rates in all muscles, but developed force increases in nonfailing and declines in failing myocardium; (3) higher [Na\(^+\)], in failing myocardium persists with increasing stimulation rate and is related to diastolic dysfunction; and (4) elevation of [Na\(^+\)], with cardiac glycosides in failing muscles increases developed force at slow stimulation rates but impairs contractile function at high frequencies.

**Relationship Between [Na\(^+\)], and Contractility in Normal Human Hearts**

[Na\(^+\)], has been measured in myocardial tissue of animal species, but only a few studies on Na\(^+\) handling have been reported in animal models of heart failure, and none with failing human tissue. We found that [Na\(^+\)], was significantly greater in failing versus nonfailing human myocardium at all stimulation rates. Increasing [Na\(^+\)], produces inotropic effects by shifting the reversal potential of the NCX to more negative values, which reduces Ca\(^{2+}\) efflux during diastole via forward-mode NCX and/or increases Ca\(^{2+}\) influx during depolarization via reverse-mode NCX. Therefore, high [Na\(^+\)], favors Ca\(^{2+}\) loading of the cytoplasm and the SR. A parallel increase in force and [Na\(^+\)], was observed in mammalian ventricular muscle and proposed as an important factor in the positive force-frequency relationship. Although a frequency-dependent increase in [Na\(^+\)], has also been shown by our group in rat heart, this was associated with a negative force-frequency relation. Therefore, frequency-dependent increases in [Na\(^+\)], are not sufficient for a positive force-frequency relation. Other factors, such as the capacity of the SR to load and release Ca\(^{2+}\), also appear to be involved. In small mammals, SR Ca\(^{2+}\) loading appears to be nearly maximum at slow pacing rates and does not increase further at higher frequencies even though [Na\(^+\)], increases. In large mammals and nonfailing human myocardium, the SR is not maximally loaded at slow heart rates. The rate-dependent increases in [Na\(^+\)], observed in the present study are likely to enhance SR Ca\(^{2+}\) loading and produce the increased force observed at higher stimulation rates in nonfailing human myocardium.

**Relationship Between [Na\(^+\)], and Contractility in Failing Human Hearts**

It is well established that SR Ca\(^{2+}\) uptake, load, and release can be abnormal in failing human myocardium and that reduced expression of SERCA2a, reduced phosphorylation of phospholamban, and increased expression of the NCX are involved in these abnormalities. Given the scope of changes in Ca\(^{2+}\) regulatory proteins in human heart failure, it is surprising that we and others find that peak systolic Ca\(^{2+}\) and the magnitude of contraction are similar in nonfailing and failing human myocardium at slow rates. Here we report for the first time that [Na\(^+\)], is significantly greater in failing versus nonfailing myocardium and suggest that this change helps maintain SR Ca\(^{2+}\) loading at slow heart rates. The higher level of [Na\(^+\)], in failing human myocytes, like that seen in animal models, will alter the thermodynamics of Ca\(^{2+}\) regulation via NCX to reduce forward-mode Ca\(^{2+}\) efflux and increase reverse-mode Ca\(^{2+}\) influx. In this regard, we observed (Figure 6) enhanced reverse-mode NCX-mediated Ca\(^{2+}\) influx during depolarization to 100 mV that enhanced SR Ca\(^{2+}\) loading in failing but not in nonfailing myocytes, consistent with our previous predictions. Our present findings suggest that reverse-mode Ca\(^{2+}\) entry and subsequent SR loading at slow frequencies is increased by the elevated [Na\(^+\)], in the failing human heart, consistent with predictions from the Bers laboratory.

Our results suggest that [Na\(^+\)],-dependent changes in NCX activity are the source of Ca\(^{2+}\) that allows the depressed SR of the failing myocyte to accumulate relatively normal amounts of Ca\(^{2+}\) without substantial elevations in diastolic Ca\(^{2+}\) at slow frequencies. This is only possible at long diastolic intervals with adequate time for Ca\(^{2+}\) reuptake in the presence of substantially reduced Ca\(^{2+}\) pump activity. As heart rates increase, it appears that the SR of failing myocytes cannot refill normally even though [Na\(^+\)], levels are still greater than normal. However, at these faster rates, the higher [Na\(^+\)], in the failing myocyte is associated with reduced systolic force and appears to be linked to diastolic Ca\(^{2+}\) overload and increased diastolic tension. Our hypothesis that high [Na\(^+\)], leads to enhanced Ca\(^{2+}\) loading of the poorly functioning SR of the failing heart at slow heart rates but also promotes rate-dependent diastolic dysfunction is supported by our observations with ouabain. Ouabain further increased [Na\(^+\)], increased force at slow rates, whereas at faster rates, both systolic depression and diastolic dysfunction were ex-
acerbated (Figure 7). Our finding that ouabain increased postrest potentiation⁶ is also consistent with enhanced [Na⁺]i-dependent SR Ca²⁺ loading during diastole.

Previously, we have shown that increased NCX protein levels in the failing human heart are correlated with reduced diastolic dysfunction.³¹ These data are consistent with studies showing that overexpression of NCX by gene transfer³⁵ or transgenic mice¹² can increase forward-mode NCX and reduce diastolic Ca²⁺. However, these studies¹²,³⁵ also show that reverse-mode NCX can be increased when the NCX density is increased. Therefore, changes in the abundance of the NCX are not a sufficient predictor of changes in Ca²⁺ homeostasis. Our current results suggest that it is NCX energetics (elevated [Na⁺]i) rather than abundance that biases the NCX in failing myocytes more toward reverse-mode activity, and this maintains SR Ca²⁺ loading at slow heart rates but leads to diastolic Ca²⁺ overload at higher rates.

Potential Mechanisms for Higher [Na⁺]i in Failing Human Myocardium

The cause of increased [Na⁺]i, in failing human myocardium is not known. An increased Na⁺ influx secondary to an increase in a slowly inactivating component of I_Na could be involved.³⁶,³⁷ In addition, the increased expression and activity of the NCX may contribute.¹⁰,³⁸ as could an increased activity of the Na⁺/H⁺ exchanger.³⁹ However, the most likely cause of elevated [Na⁺]i in human heart failure is defective Na⁺ elimination (for review, see Bers³⁴) by Na⁺/K⁺-ATPase. Reduced expression and activity of Na⁺/K⁺-ATPase has been reported in failing human hearts,¹⁵,¹⁶ but there are only potential experiments in animals that directly relate expression or activity of the sodium pump to [Na⁺]i.³⁴ These studies support the idea that reduced capacity for Na⁺ elimination through decreased Na⁺/K⁺-ATPase in concert with increased Na⁺ influx underlies the increased [Na⁺]i, we observed in the failing heart.

Potential Technical Limitations of the Approaches

It is not clear why different absolute values for [Na⁺]i were obtained with SBFI and the bioassay techniques. However, these independent approaches led us to the same conclusion, i.e., that [Na⁺]i is increased in failing human myocardium. It is possible that SBFI overestimated [Na⁺]i, because diffusional equilibrium between bath and cell Na⁺ was not reached during the calibration procedure or because of differences in the proportion of nonmyocytes in failing and nonfailing preparations. Because muscles were of similar size and equilibration times were identical in all calibration experiments, these should have been fairly consistent errors that would put the absolute values in question but should not invalidate our conclusions. Likewise, the bioassay technique could overestimate or underestimate [Na⁺]i, because the relationships between [Na⁺]i and contraction were determined in dialyzed myocytes and the predictions were made in nondialyzed myocytes. If cell dialysis modifies the relationship between Na⁺ and contractility, the predicted values would be inaccurate, but again, the technical protocol was identical in nonfailing and failing cells.

Clinical Relevance of Altered [Na⁺]i in Human Heart Failure

High [Na⁺]i, is likely to be involved in a number of prominent features of heart failure. Elevated [Na⁺]i helps explain the increased sensitivity of the failing human heart to cardiac glycosides. Most importantly, elevated [Na⁺]i, affects basal and rate-dependent contractility. High [Na⁺]i increases Ca²⁺ influx via reverse-mode NCX and reduces Ca²⁺ efflux via forward-mode NCX.³³ These effects promote SR Ca²⁺ loading in normal myocardium but cause diastolic Ca²⁺ overload in failing hearts with defective SR Ca²⁺ transport capabilities. These findings also highlight the importance of heart rate control in patients with heart failure and/or cardiac glycoside therapy.

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