Reduced Stretch-Induced Force Response in Failing Human Myocardium Caused by Impaired Na+-Contraction Coupling

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Background—Stretch elicits an immediate, followed by a delayed, inotropic response in various animal models and failing human myocardium. This study aimed to characterize functional differences in the stretch response between failing and nonfailing human myocardium.

Methods and Results—Experiments were performed in muscle tissue from 86 failing and 16 nonfailing human hearts. Muscles were stretched from 88% to 98% of optimal length. Resulting immediate (Frank-Starling mechanism [FSM]) and delayed (slow-force response [SFR]) increases in twitch force were assessed before and after blockade of nitric oxide synthase, phosphatidylinositol-3-kinase, or reverse-mode Na+/Ca2+ exchange. Stretch-induced changes in [Na+]i were measured using fluorescent indicator sodium-binding benzofuran isophthalate-AM. Nitric oxide synthase isoform expression was quantified by Western blot analysis. FSM was comparable between nonfailing (227±8%) and failing (222±9%) myocardium, whereas the additional increase during SFR (∼5 minutes) was larger in nonfailing myocardium (to 126±3% versus 119±2% of force of FSM, respectively; P<0.05). Basal [Na+]i and stretch-induced increase in [Na+]i were lower in nonfailing myocardium. Inhibition of the Na+/H+ exchange largely reduced the increase in [Na+]i and significantly blocked the SFR. In both groups, SFR was almost completely prevented by reverse-mode Na+/Ca2+-exchanger inhibition. Although neuronal and inducible nitric oxide synthase expression were significantly upregulated in failing myocardium, inhibition of nitric oxide synthase and phosphatidylinositol-3-kinase had no effect on FSM or SFR.

Conclusions—These data demonstrate a Na+-independent FSM and a Na+-dependent SFR in both nonfailing and failing human myocardium. The larger stretch-dependent increase in [Na+]i in failing myocardium was associated with a blunted functional response, indicating impaired Na+-contraction coupling in the failing human heart. (Circ Heart Fail. 2009;2:47-55.)

Key Words: contractility ■ heart failure ■ myocardial contraction ■ physiology

Mammalian cardiac muscle is characterized by a biphasic force response to stretch. In isolated myocardium, stretch induces an immediate increase in twitch force (Frank-Starling mechanism [FSM]), which is mediated by an increase in myofilament sensitivity for Ca2+. The exact underlying mechanism of this Ca2+-independent effect is still under discussion; however, an increase in longitudinal overlap as well as reduced radial distance between thin and thick filaments (lattice spacing) modulated by giant protein titin may be involved.1,2 This effect is followed by a slowly developing second phase in force increase (slow-force response [SFR]), which was first described by Parmley and Chuck3 in 1973. In contrast to the FSM, this delayed inotropic effect is associated with a parallel increase in intracellular Ca2+ transients4–6 and sarcoplasmic reticulum (SR) Ca2+ load7 of still undefined origin.

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Heart failure has been associated with reduced immediate stretch-dependent inotropy in dilatative cardiomyopathy,8 whereas broad experimental data on whole-heart preparations, trabeculae, and skinned fibers supports a maintained FSM in the failing human heart both in ventricular9–11 and atrial myocardium.12 The FSM response is attributed to an increase of myofilament calcium sensitivity; however, the underlying mechanisms are still controversial. In isolated rabbit hearts, the intramyocardial nitric oxide (NO) concentration changed in parallel to the cardiac cycle and was

Received May 27, 2008; accepted November 18, 2008.

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Circ Heart Fail is available at http://circheartfailure.ahajournals.org

DOI: 10.1161/CIRCHEARTFAILURE.108.794065

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increased by additional preload. Inhibition of constitutive NO release in turn limited the increase of cardiac output in response to an increased preload in isolated guinea-pig hearts, indicating a potential role of NO in the FSM.

The existence of the SFR has been demonstrated in various preparations of nonfailing animal hearts, in nonfailing human atrium, and in failing human ventricle. In contrast to the FSM, the SFR is Ca\(^{2+}\)-dependent, but the underlying mechanisms and pharmacology remain species-dependent and are still under discussion. For example, the SFR was related to a stretch-dependent autocrine/paracrine release of angiotensin-II and endothelin-1 in isolated canine and rat heart muscle. Although this could not be confirmed in other animal models and in failing human ventricular myocardium, the downstream signaling pathway seems to involve activation of the Na\(^+/H^+\) exchanger-1 (NHE1) and a secondary, Na\(^+/K^+\) dependent stimulation of the sarcolemmal Na\(^+/Ca^{2+}\) exchanger (NCX) in its reverse mode. However, pharmacological inhibition of NHE1 or rmNCX does not completely prevent the SFR and additional mechanisms may operate. For example, in isolated mouse cardiomyocytes phosphatidylinositol-3-kinase (PI3K) and NO derived from endothelial nitric oxide synthase (NOS) have been shown to mediate stretch-dependent increases in Ca\(^{2+}\) sparks and Ca\(^{2+}\) transients with a time course similar to the SFR. In addition, the relative importance of the SFR in nonfailing versus failing human heart has never been assessed. Therefore, we tested the effects of stretch on the FSM and SFR in nonfailing versus failing human myocardium.

The main findings of the present study are that the FSM is not altered in failing human myocardium, whereas it is shown for the first time that the SFR is significantly reduced due to impaired Na\(^+\)-contraction coupling.

**Methods**

**Human Myocardium**

Functional experiments were performed in 22 isolated muscle strips from 8 nonfailing hearts (not transplanted donor hearts) and in 170 muscle strips from 73 end-stage failing hearts due to dilatative cardiomyopathy in nonfailing and failing human myocardium. On stretch-dependence histograms were compared by Tukey post hoc test. A probability value of \(p < 0.05\) was used to declare the results significant.

**Statistical Analysis**

Data are expressed as mean±SEM. F test was used to compare mean values of nonfailing and failing (2-tailed). For the comparison of NO metabolite levels between nonfailing and failing (2-way ANOVA for repeated measurements, accounting for nonfailing/failing and drug effects (repeated factor). Individual mean values were compared by Tukey post hoc test. A probability value of \(p < 0.05\) was assumed to indicate a significant difference.

**Results**

Both nonfailing and failing human myocardium was characterized by a reproducible biphasic force response to stretch: an immediate first phase (FSM), followed by a delayed SFR. This is shown in an original tracing obtained in typical nonfailing (Figure 1A, upper panel) and failing (Figure 1A, lower panel) myocardium. On stretching the preparations from 88% to 98% of their optimal length, an immediate increase in twitch force was observed, followed to 88% of optimal length for 30 minutes before stretching them to 98% of optimal length.

**Sodium Measurements**

Na\(^+\) was assessed as described previously. Muscles were loaded with the fluorescent Na\(^+\) indicator SBFI-AM by 180 minutes incubation in Tyrode solution containing 35 \(\mu\)mol/L of the acetoxymethylester of the dye. Muscles were then mounted in a glass cuvette and connected to a force transducer. Isometric twitches were evoked through electric stimulation (0.2 Hz) in Tyrode solution at 30°C. SBFI was excited at 340 nm (F\(_{340}\)) and 380 nm (F\(_{380}\)), and the fluorescence emission was collected by a photomultiplier at 505 nm. The ratio F\(_{340}/F_{380}\) is a measure for [Na\(^+\)]. Photobleaching of the dye was minimized (1) by attenuating the intensity of the excitation light by means of a neutral density filter (1% transmittance) and (2) by limiting fluorescence recording to intervals of \(\sim 20\) s every 2 minutes. At the end of an experiment, the SBFI fluorescence ratio was converted to [Na\(^+\)], by a calibration procedure as reported previously.

**Western Blot**

Left ventricular tissue samples of 13 nonfailing and 15 failing human hearts were shock-frozen and lysed in Tris buffer and complete protease inhibitor cocktail (Roche Diagnostics). To optimize protein yield, lysis was maintained for 30 minutes on ice. Tissue homogenates were subjected to Western blotting (12% sodium dodecyl sulfate polyacrylamide gels) using monoclonal (neuronal and endothelial NOS) or polyclonal (inducible NOS) antibodies (brain derived). Chemiluminescent detection was done with SuperSignal West Pico Substrate (Pierce Biotechnology). Western blots were loaded with 20 \(\mu\)g (eNOS) or 40 \(\mu\)g (nNOS, iNOS) protein. Protein loading was normalized by coblotting GAPDH.

**Pharmacological Inhibition of Key Signaling Proteins**

\(N^\text{N}\)-nitro-l-arginine methyl ester (\(l\)-NAME) (NOS-inhibitor, Sigma Chemicals, Taufkirchen, Germany) was diluted from an aqueous stock solution (10 mmol/L). Wortmannin (Sigma Chemicals) was used to block the PI3K (0.1 \(\mu\)mol/L) and KB-R 7943 (Tocris, Ballwin) was added from a 10 mmol/L stock (50% dimethyl sulfoxide, 50% water) to inhibit reverse mode NCX. All other drugs and compounds were of best analytic grade available.
by a slowly (~5 minutes) developing further rise in twitch force. The increase in diastolic tension (normalized to maximum developed force during SFR) on stretch showed a tendency to be larger in failing compared with nonfailing myocardium (Δ diastolic tension/maximum deviation force, 0.34±0.03; failing, n=14) versus 0.28±0.08 (nonfailing, n=17; P=NS).

Figure 1B summarizes average data from 24 experiments in failing and 18 experiments in nonfailing human hearts as outlined in Figure 1A. On stretch, developed force increased to 227±8% (nonfailing) and 222±9% (failing) of the pre-stretch force value during FSM, respectively. Developed force further increased during SFR to 126±3% and 119±2% of FSM values in nonfailing and failing myocardium, respectively (P<0.05; Figure 1C). The time to complete development of SFR was similar in nonfailing and failing myocardium (343±31 s in nonfailing myocardium versus 308±34 s in failing myocardium; P=NS).

In general, nonfailing donor hearts are younger than the end stage failing hearts, and therefore differences in the SFR amplitude might depend on the age of the heart and not on the underlying disease. To test this, we compared donor hearts (31±4 years) with age matched failing hearts (34±4 years; n=22 muscle strips from 5 hearts) as shown in Figure 2A and correlated age and SFR amplitude in a larger sample of trabeculae from failing human hearts (Figure 2B). As can be seen from Figure 2A, the difference in SFR between nonfailing and failing was also present if only age-matched hearts were compared. In addition, SFR did not seem to depend on patient age in failing hearts (Figure 2B). We did also not observe gender differences regarding SFR amplitude (not shown).

To test the potential influence of the underlying cause of heart failure on SFR amplitude, we compared muscle strips from patients with dilative cardiomyopathy versus ischemic cardiomyopathy. As shown in Figure 2C, there was no significant difference between these groups. The data from this set of experiments suggest that subcellular alterations associated with heart failure, but not age or cause of cardiac disease, underlie the blunted SFR in failing human hearts.
To assess changes in Na\(^+\) homeostasis, we performed experiments with SBFI-incubated muscle strips to record intracellular Na\(^+\) directly. Basal [Na\(^+\)]\(_i\) was \(\approx 7\) mmol/L higher in failing myocardium than in nonfailing myocardium, which is comparable with recently published data of our group.\(^{28}\) After stretching the muscle strips [Na\(^+\)]\(_i\) increased during the SFR in both groups, but rate (nonfailing: 0.2 mmol L\(^{-1}\)min\(^{-1}\) versus failing: 0.54 mmol L\(^{-1}\)min\(^{-1}\)) and amplitude (\(\Delta[\text{Na}^+]\), nonfailing: 2\(\pm\)1 mmol/L versus failing: 5\(\pm\)1 mmol/L) of the increase were much more pronounced in failing myocardium. In sharp contrast SFR amplitude in failing myocardium was only \(\approx 50\%\) of that in nonfailing myocardium. NHE1-blocker HOE642 reduced the increase in [Na\(^+\)]\(_i\) in failing myocardium to the level seen in nonfailing myocardium and further decreased the SFR by another \(\approx 50\%\) (Figure 3).

### Contribution of Na\(^+\)/Ca\(^{2+}\) Exchange to Immediate and Delayed Inotropy

Stretch-dependent NHE1 activation was suggested to increase [Na\(^+\)]\(_i\) with resulting enhanced Ca\(^{2+}\) influx via reverse-mode NCX. We tested the effects of reverse-mode NCX inhibition on the FSM and the SFR. The results are summarized in Figure 4. KB-R7943 did not affect the FSM in both groups (nonfailing: 212\(\pm\)16\% before and 228\(\pm\)14\% after incubation; failing: 253\(\pm\)9\% and 250\(\pm\)11\%, respectively; Figure 4A). However, reverse-mode NCX blockade largely reduced the SFR both in failing and nonfailing. Twitch force increased to 132\(\pm\)7\% before and to 109\(\pm\)2\% in the presence of KB-R7943 in nonfailing myocardium (Figure 4B, left) and to 119\(\pm\)3\% before and to 106\(\pm\)3\% in the presence of KB-R7943 in failing myocardium (Figure 4B, right). The degree of inhibition, however, was not different between the 2 groups indicating that reverse-mode NCX activation underlies the SFR in failing and nonfailing myocardium. Furthermore, time to SFR\(_{\text{max}}\) was decreased in both groups after treatment with KB-R7943 correlating with the decrease in SFR amplitude (lower panel).

We further assessed signaling pathways potentially involved in the SFR (and altered in heart failure). NO was implicated in stretch-dependent effects on immediate force increase and intracellular Ca\(^{2+}\) handling.\(^{25,29}\) Furthermore, we found that nNOS and iNOS were significantly upregulated in failing human myocardium compared with nonfailing controls (Figure 5A). Therefore, we tested the effects of the unselective NOS inhibitor L-NAME on the stretch-induced force response. Preincubation with L-NAME did not affect FSM or SFR in human nonfailing or failing ventricle. FSM increased to 237\(\pm\)41\% before and to 245\(\pm\)45\% after incubation with the inhibitor in nonfailing myocardium (Figure 5B). SFR increased to 124\(\pm\)4\% and 117\(\pm\)3\% in the absence and presence of L-NAME in nonfailing and to 120\(\pm\)3\% and 120\(\pm\)3\%, respectively, in failing (Figure 5C). Time to the maximum of SFR was not altered in either group (Figure 5D).

Recently, we demonstrated that pharmacological PI3K activation results in positive inotropic effects of comparable time course and amplitude as seen during the SFR.\(^{30,31}\) In

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**Figure 2.** A, Increase in developed force during slow-force response, calculated as percent of immediate phase (FSM). Data for nonfailing (NF) represents the average of 18 muscle strips from 6 nonfailing donor hearts. Data for failing (F-young) represents 22 muscle strips from 5 failing hearts. B, Correlation of age (x axis) and slow-force response amplitude (y axis) in a total of 133 muscle strips of 52 failing human hearts. Mean values of SFRs were taken if \(\geq 1\) muscle strip per heart was used. C, Influence of the underlying etiology on the slow force response. Increase in developed force during SFR was calculated as percent of immediate phase (FSM). ICM indicates ischemic cardiomyopathy (n=40); DCM, dilative cardiomyopathy (n=28). *P<0.05 versus nonfailing hearts.
addition PI3K may be activated by endogenous peptides and potentially released during stretch. We therefore tested the effect of PI3K inhibition on the SFR in human myocardium. Preincubation with wortmannin did neither reduce the stretch induced inotropy in 7 human trabeculae from 3 nonfailing hearts nor in 6 trabeculae from 4 failing hearts (Figure 6). FSM increased to 233±10% before wortmannin and to 256±14% after incubation with the inhibitor in nonfailing myocardium and to 213±24% and 248±24% in failing myocardium (Figure 6, differences not significant). SFR was not altered in nonfailing (123±3% before wortmannin and 126±4% in the presence of the inhibitor) or in F (116±2% before wortmannin and 116±2% with the inhibitor; Figure 6B). Time to the maximum of SFR was not altered in either group as shown in Figure 6C.

Discussion

The results of the present study demonstrate that (1) FSM and SFR exist in failing and in nonfailing human ventricular myocardium, (2) FSM is not altered whereas SFR is reduced in failing human myocardium, (3) functional effects are independent of NO or PI3K, (4) reverse-mode NCX contributes equally to the SFR in both groups, (5) basal [Na⁺]i, as well as the increase in [Na⁺]i in response to stretch are significantly higher in failing myocardium, but (6) elevated Na⁺ is not translated effectively into increases in contractility.

In vivo, increases in ventricular end-diastolic volume caused either by an increase in venous return or a rise in aortic resistance are followed by an increase in force of contraction. This mechanism allows the heart to maintain sufficient cardiac output even at elevated pre- and afterload and equalizes the output of the left and right ventricle. Both immediate and delayed stretch-induced inotropic effects might underlie this mechanism. The functional importance of the immediate response to stretch in failing human myocardium is controversial and its existence was even denied. The present data clearly demonstrate an immediate force response of unaltered amplitude in failing and nonfailing human myocardium and contrasts with the recently published hypothesis of altered FSM in failing myocardium due to down-regulated eNOS. In our hands, this immediate phase accounts for ~75% of total stretch-induced inotropy.

Delayed Response to Stretch (SFR)

In contrast to the FSM, Allen and Kurihara and others demonstrated slowly rising Ca²⁺ transients as the underlying mechanism for the SFR. Today, there is agreement on the downstream part of the subcellular mechanism accounting for ~2/3 of the delayed inotropic response, involving NHE1 stimulation and resulting elevation in [Na⁺], with subsequent stimulation of the NCX in its reverse mode. The only reported exception to this finding is human atrial myocardium showing a SFR of comparable amplitude but independent of NHE1 and NCX inhibition. In addition, stretch-dependent increases in Ca²⁺ sparks within the same time frame have been reported to result from activation of PI3K and NOS in rat myocytes.
nism that would be fast enough and could well be altered in failing myocardium are differences in the elastic properties of the preparation resulting in a more pronounced dip in (less fibrotic and more elastic) nonfailing myocardium.

Role of Na\(^+\) and Ca\(^{2+}\) Homeostasis

Positive inotropic effects are closely linked to intracellular Ca\(^{2+}\)-homeostasis and this is markedly influenced by the intracellular sodium homeostasis via the electrogenic NCX. NCX is upregulated in heart failure and contributes significantly to the contractile state of myocytes. Largely regulated by membrane voltage and Na\(^+\) and Ca\(^{2+}\) gradients, the exchanger works in the forward mode (Ca\(^{2+}\) out) or the reverse mode (Ca\(^{2+}\) in). The latter is involved in the SFR and blocked by reverse-mode inhibitors such as KB-R7943. There is broad evidence that [Na\(^+\)]\(_i\) increases after stretch\(^6,16,17\) and various models have shown a consecutive increase in [Ca\(^{2+}\)]\(_i\) and developed force.

We have shown previously in nonfailing rabbit myocardium that interventions increasing [Na\(^+\)]\(_i\) (eg, cardiac glycosides) result in elevated SFR amplitude whereas interventions decreasing [Na\(^+\)]\(_i\) (eg, exchange of extracellular sodium with Lithium) result in reduced SFR amplitude.\(^7\) We here report stretch-induced increases in Na\(^+\) for both nonfailing and failing myocardium with larger increase in [Na\(^+\)]\(_i\) on stretch in failing myocardium despite an already elevated basal [Na\(^+\)]\(_i\) level. This observation likely results from altered Na\(^+\) handling in heart failure. First, NHE1 activity is significantly increased in human heart failure compared with nonfailing myocardium.\(^3,6\) Because protein levels are not changed posttranslational mechanisms play a pivotal role and may include phosphorylation by Ca\(^{2+}\)/calmodulin-dependent kinase,
which is elevated in heart failure.\textsuperscript{37} In a heart failure animal model elevated NHE1-activity has been shown to be causal to increased $[\text{Na}^+]_i$ indicating functional relevance.\textsuperscript{38} Second, the $\text{Na}^+/\text{K}^+$-ATPase is downregulated by $\approx 42\%$\textsuperscript{39} resulting in reduced $\text{Na}^+$-elimination capacity. Recent data also demonstrate that phospholemman regulates $\text{Na}^+/\text{K}^+$-ATPase activity.\textsuperscript{40} In heart failure phospholemman expression is reduced, but intrinsic phospholemman activity is increased.\textsuperscript{41} Therefore, functional effects are difficult to predict but reduced $\text{Na}^+/\text{K}^+$-ATPase expression and activity might be enhanced by increased inhibition via phospholemman. Although pharmacological inhibition of the $\text{Na}^+/\text{K}^+$-ATPase has been shown to increase SFR in vitro\textsuperscript{7} premedication with digitalis did not affect the SFR.

Third, $\text{Na}^+/\text{Ca}^{2+}$ exchanger is upregulated in failing human hearts (for review, see reference 42) and thus, in part, accounts for the elevated basal $[\text{Na}^+]_i$ in heart failure due to its predominant forward mode. However, NCA function is shifted toward reverse mode as a result of increasing $[\text{Na}^+]_i$, and therefore represents a passive mechanism following NHE1 activation. Consistently, the relative effect of reverse-mode inhibition was comparable in nonfailing and failing myocardium. Fourth, tetrodotoxin-sensitive $\text{Na}^+$ current is elevated in heart failure mainly due to delayed inactivation of the $\text{Na}^+$ current resulting in elevated $[\text{Na}^+]_i$ levels and contributing to the prolongation of action potential duration in heart failure.\textsuperscript{43-44}

Reducing the increase in intracellular sodium to the level seen in nonfailing human myocardium by inhibiting the NHE1, SFR was even reduced to only a quarter of what we observed in nonfailing myocardium. This was despite the fact that basal $[\text{Na}^+]_i$ was markedly elevated in failing hearts compared with the control hearts, which additionally shifts NCX function toward more reverse mode.

Therefore, increases in $\text{Na}^+$ are translated into developed force differently between failing and nonfailing myocardium. This is in line with previous data\textsuperscript{45} that a given rise in $\text{Na}^+$ increases $\text{Ca}^{2+}$ transients and developed force in nonfailing myocardium but not in failing myocardium. Kayhan et al.\textsuperscript{12} demonstrated a stretch-induced increase in diastolic $\text{Ca}^{2+}$ using FURA-2 in failing human atrial myocardium whereas diastolic $\text{Ca}^{2+}$ levels were not altered in nonfailing atrial myocardium. This is consistent with the larger increase in diastolic tension in the failing group in the present study strengthening the evidence of stretch-induced diastolic $\text{Ca}^{2+}$-overload as a mechanism underlying the reduced SFR in failing myocardium and the impaired $\text{Na}^+$-contraction coupling.

Sarcoplasmic reticulum calcium ATPase expression and activity are reduced and SR storage capacity is altered due to higher leakage of the ryanodine receptors in heart failure, and therefore altered SR-function represents a potential mechanism for the impaired translation of elevated $\text{Na}^+$ into developed force and the reduced SFR. A $\text{Ca}^{2+}$-dependent mechanism like the SFR that is associated with an increase in SR-$\text{Ca}^{2+}$ load\textsuperscript{7,21} might therefore be altered. However, increased $\text{Ca}^{2+}$ load only seems to be secondary as Calaghan and White have shown that SFR amplitude was unchanged after blocking SR function using thapsigargin and ryanodine. In addition, altered translation of $[\text{Na}^+]_i$ into force was even present at increasing SR $\text{Ca}^{2+}$ load in failing human myocardium.\textsuperscript{45}

NO impacts on cardiomyocyte function both in a paracrine and autocrine manner, and all three NOS isoforms are expressed within the cardiomyocyte constitutively (NOS I and III) or after inflammatory induction (NOS II). The activity of the constitutive isoforms is compartmentalized to the sarcolemma (NOS III) and sarcoplasmic reticulum (NOS I), and promotes $\beta$-adrenergic responsiveness, the force-frequency response and maintenance of the cell’s redox state. In contrast, NO derived from the high output isoform NOS II is not restricted to a subcellular compartment, and via formation of peroxynitrite depresses contractility (for review, see references 29 and 46). Inhibition of constitutive NO synthesis markedly diminishes the preload-recruitable increase of cardiac output in isolated guinea-pig hearts.\textsuperscript{14} In line with that, unselective inhibition of NO synthesis with $\text{L-NAME}$ in isolated rat cardiomyocytes prevented the stretch-induced increase of calcium spark frequency and calcium transients, and in turn the stretch-induced increase in calcium cycling was absent in cardiomyocytes isolated from eNOS knockout mice.\textsuperscript{25} However, we clearly demonstrate that neither FSM
nor SFR are affected by NOS-inhibition in nonfailing human myocardium. Such negative finding has also been reported in rat papillary muscle and rat myocytes. In accordance with previous studies, we observed an upregulation of nNOS and iNOS protein expression in failing human myocardium but again, neither FSM nor SFR were affected by NOS inhibition. Although we cannot exclude that unselective NOS inhibition may mask opposing effects of single isoforms, our results rather argue against a major role of NO for stretch-induced force development in isolated human myocardium.

Besides its role in stretch-dependent stimulation of NO and Ca$^{2+}$, PI3K is critically involved in mechanotransduction of vascular smooth muscle cells in vitro as well as in vivo. Furthermore, PI3K mediates Ca$^{2+}$-dependent inotropy in human myocardium in response to insulin and IGF-1. This inotropic effect was of similar amplitude and time course compared with the SFR. However, inhibition of the PI3K using wortmannin in a concentration completely precluding IGF-1-mediated inotropy did not alter the SFR in failing or nonfailing human myocardium. Corresponding data have been obtained for rat myocytes and papillary muscles using the PI3K inhibitor LY294002.

Limitations of the Study

The SFR is known to be a Ca$^{2+}$-dependent mechanism and Ca$^{2+}$ homeostasis is altered in failing human myocardium. Therefore, direct intracellular Ca$^{2+}$ measurements in both groups would be helpful to draw a more complete picture. In addition, further pharmacological interventions would support the notion of an unaltered basal signal transduction. However, availability of nonfailing donor hearts is very limited, which forced us to concentrate on the key experiments in nonfailing myocardium.

In conclusion, we demonstrated for the first time a reduced SFR in failing human myocardium. This reduction is present, despite the fact that basal [Na$^+$], as well as the stretch-induced increase in [Na$^+$], is higher in failing myocardium partially compensating an impaired Na$^+$-contraction coupling in failing human myocardium.

Sources of Funding

This work was supported by Deutsche Forschungsgemeinschaft (PI-414/1, PI-414/2, and KFO 155 to B.P. and J.K.).

Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Increases in ventricular end-diastolic volume caused either by elevated preload (increase in venous return) or afterload (eg, pulmonary embolism or rise in aortic resistance) are followed by an increase in force of contraction. This mechanism allows the heart to maintain sufficient cardiac output even at elevated preload and afterload and synchronizes the output of the left and right ventricles. Both immediate and delayed stretch-induced inotropic effects, as seen in the multicellular preparations, may underlie this mechanism. The reduced slow force response in failing human myocardium would therefore translate into less inotropy after hemodynamic challenges in vivo, and the already failing heart would have even less capacity to compensate for elevated load than would the nonfailing heart. The understanding of the underlying signal transduction mechanisms of the stretch-induced inotropy and its alterations in disease might lead to therapeutic approaches supporting the (failing) heart to respond sufficiently to acute alterations in preload and afterload. In addition, the presented data describe an altered sodium-contraction coupling in failing human myocardium. Therefore, the failing human heart does not only suffer from an altered calcium handling but also from an even more proximally located impaired transduction. This can also impact other inotropics that increase intracellular sodium, such as treatment with cardiac glycosides.
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_Circ Heart Fail_. 2009;2:47-55
doi: 10.1161/CIRCHEARTFAILURE.108.794065

_Circulation: Heart Failure_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1941-3289. Online ISSN: 1941-3297

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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