Functional Relevance of the Stretch-Dependent Slow Force Response in Failing Human Myocardium

Dirk von Lewinski, Burkhard Stumme, Florian Fialka, Claus Luers, Burkert Pieske

Abstract—Stretch induces immediate and delayed inotropic effects in mammalian myocardium via distinct mechanosensitive pathways, but these effects are poorly characterized in human cardiac muscle. We tested the effects of stretch on immediate and delayed force response in failing human myocardium. Experiments were performed in muscle strips from 52 failing human hearts (37°C, 1 Hz, bicarbonate buffer). Muscles were stretched from 88% of optimal length to 98% of optimal length. The resulting immediate and delayed (ie, slow force response [SFR]) increases in twitch force were assessed without and after blockade of the sarcoplasmic reticulum (SR; cyclopiazonic acid and ryanodine), stretch-activated ion channels (SACs; gadolinium, streptomycin), L-type Ca2+-channels (diltiazem), angiotensin II type-1 (AT1) receptors (candesartan), endothelin (ET) receptors (PD145065 or BQ123), Na+/H+ exchange (NHE1; HOE642), or reverse-mode Na+/Ca2+ exchange (NCX; KB-R7493). We also tested the effects of stretch on SR Ca2+ load (rapid cooling contractures [RCCs]) and intracellular pH (in BCECF-loaded trabeculae). Stretch induced an immediate (<10 beats), followed by a slow (5 to 10 minutes), force response. Twitch force increased to 232±6% of prestretch value during the immediate phase, followed by a further increase to 279±8% during the SFR. RCC amplitude significantly increased, but pH did not change during SFR. Inhibition of SACs, L-type Ca2+-channels, AT1 receptors, or ET receptors did not affect the stretch-dependent immediate or SFR. In contrast, the SFR was reduced by NHE1 inhibition and almost completely abolished by reverse-mode NCX inhibition or blockade of sarcoplasmic reticulum function. The data demonstrate the existence of a functionally relevant, SR-Ca2+-dependent SFR in failing human myocardium, which partly depends on NHE1 and reverse-mode NCX activation. (Circ Res. 2004;94:1392-1398.)

Key Words: stretch • human myocardium • contractile function • sodium • calcium

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), followed by a slowly developing second phase in force increase, which was first described by Parmley and Chuck1 in 1973. The immediate increase in force (phase 1) was related to an increased sensitivity of the myofilaments for Ca2+, whereas the slow force response (SFR) was associated with a parallel increase in intracellular Ca2+ transients.2,3

Recently, extensive work performed in isolated feline and rat heart muscle related the SFR to a stretch-dependent autocrine/paracrine release of angiotensin II (Ang II) and endothelin (ET)-1 with consecutive activation of the Na+/H+ exchanger (NHE1) and reverse-mode Na+/Ca2+ exchange in its reverse mode.4–6

In contrast to mammalian cardiac muscle, the existence of the immediate stretch induced inotropic response is controversial in failing human myocardium,7,8 and the SFR has never been described in the human heart to our knowledge. Failing human myocardium is characterized by elevated [Na+]i,9 prolonged action potentials and enhanced reverse-mode NCX function.10 These findings might even favor a putative [Na+]i-dependent and [Ca2+]i-dependent SFR in failing human myocardium.

Therefore, the aim of the present study was to identify and characterize a SFR in failing human myocardium. The main findings of the present study were that a functionally relevant SR-Ca2+-dependent SFR can be observed in human myocardium, which is related to stretch-dependent Na+/H+ exchange and reverse mode Na+/Ca2+ exchange activation.

Methods

Human Myocardium

Experiments were performed in 114 isolated muscle strips from 52 end-stage failing hearts caused by dilated cardiomyopathy (n=27) and ischemic cardiomyopathy (n=25) on transplantation. The mean age of the patients was 53.6±2.1 years; 39 were male and 13 were female. The mean ejection fraction was 23.6±1.1%, cardiac index was 1.9±0.1 L/min per m², and pulmonary capillary wedge pressure was 21±1 mm Hg. Premedication consisted of angiotensin-converting enzyme (ACE) inhibitors or angiotensin II type-1 (AT1) blockers in 43, β-blockers in 40, cardiac glycosides in 33, and diuretics in 50 patients, respectively. The study protocol was

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approved by the local ethics committee and all patients gave informed consent.

Muscle Strip Preparation
Immediately after explantation, the heart was stored in ice-cold cardioplegic Tyrode solution containing (in mmol/L): Na⁺ 152, K⁺ 3.6, Cl⁻ 135, HCO₃⁻ 25, Mg²⁺ 0.6, H₂PO₄⁻ 1.3, SO₄²⁻ 0.6, Ca²⁺ 0.2, glucose 11.2, insulin 10 IU/L, and 2,3-butanedione-monoxime (BDM) 30, equilibrated with carbogen (95% O₂, 5% CO₂) to a pH of 7.4 and transported to the laboratory. This solution has been shown to protect the myocardium during transportation and from cutting injury at the time of dissection with full reversibility of the cardioplegic effects on washout. Small endocardial trabeculae ("muscle strips," cross-sectional area <0.5 mm²) were dissected with the help of a stereomicroscope. All preparation steps were performed in the cardioprotective solution, as previously described.⁹

Muscles strips were mounted in special chambers between miniature hooks, connected to an isometric force transducer (Scientific Instruments, Germany) and superfused with modified Tyrode solution of the composition given except that BDM was omitted and [Ca²⁺]₀ was increased stepwise to 2.5 mmol/L. Isometric twitches were evoked through electrical stimulation with a stimulation voltage 25% above threshold (pulse duration 5 ms) at the preload at which maximum steady-state twitch force was achieved (Lₘᵢₙ).³

Rapid Cooling Contractures
Rapid cooling contractures (RCCs) were elicited by a rapid decrease in the temperature of the muscle chamber from 37°C to 1°C by switching from a warm to a cold solution with solenoid pinch valves at the bath inlet as previously described.¹¹ The cold solution was maintained at −1°C by a cooling bath (RM20; Lauda, Lauda-Königshofen, Germany), which cools the solution and additionally surrounds the tubing that is connected to the chamber. During the cooling period, the muscle was not stimulated. Rapid cooling releases all the SR Ca²⁺ and inhibits Ca²⁺ transport, and the resulting cooling contracture is an index for SR Ca²⁺ content.¹¹

pH Measurements
Muscle strip preparations were loaded with the fluorescent intracellular pH (pHᵢ) indicator BCECF-AM as previously described,¹² mounted to a specially designed setup for simultaneous assessment of force and fluorescence (Scientific Instruments, Heidelberg, Germany), and illuminated by a 100-W mercury lamp (Ushio, Japan). The fluorescence intensities at each excitation wavelength were measured by a photomultiplier, and the fluorescence ratio F₄₉₅/F₄₄₀ was calculated. To minimize photobleaching, sampling intervals were selected during the protocol (20-second duration, every minute for 20 minutes during the protocol). At the end of each experiment, fluorescence emission was calibrated by the high-K⁺ nigericin method.¹² The calibration solution contained (mmol/L): KCl 140, MgCl₂ 1.2, HEPES 5.0, nigericin 0.01, and 2,3-BDM 30. Buffer pH was adjusted with KOH to 5 different values ranging from 6.8 to 7.6.

Drugs
To inhibit NHE1, HOE642 (Cariporide; Aventis Pharma, Frankfurt, Germany) was diluted from an aqueous stock solution (10 mmol/L). KB-R 7943 (Tocris, Bristol, UK) was added from a 10 mmol/L stock (50% DMSO, 50% water) to inhibit reverse-mode NCX. Candesartan (Candesartan; AstraZeneca, Möndal, Sweden) was dissolved in physiological saline and 2.5 vol% Na₂CO₃ (10 mmol/L stock), and used at 0.1 μmol/L to block AT₁ receptors. At this concentration, the inotropic effects of 10 μmol/L Ang II were completely prevented in rabbit trabeculae (n=7; data not shown); 0.3 μmol/L BD123 (Calbiochem, Bad Soden, Germany) was used to selectively block ET₄ receptors, and 10 μmol/L PD145065 (Sigma Chemicals) was used to block ET₄ receptors. Gadolinium (10 μmol/L, Sigma Chemicals) or streptomycin (70 μmol/L, Sigma Chemicals) were used to block stretch-activated channels (SACs), and diltiazem (5 μmol/L; Sigma Chemicals) was used to block l-type Ca²⁺ channels. Cyclopiazonic acid (CPA) (20 μmol/L; Sigma Chemicals) and ryanodine (1 μmol/L; Sigma Chemicals) were used to inhibit SR function. All other drugs and compounds were of best analytical grade available.

Statistical Analysis
Data are expressed as mean±SEM. Differences between basal values and values obtained after interventions were compared by 2-way ANOVA analysis. Statistical significance was taken as P<0.05.

An expanded Methods section is available in the online data supplement at http://circres.ahajournals.org.

Results
Failing human myocardium is characterized by a biphasic response to stretch: an immediate phase 1, followed by a delayed SFR. This is shown in an original tracing obtained in a typical muscle preparation (Figure 1A). On stretching the preparation from 88% to 98% of its optimal length, an immediate increase in twitch force could be observed, followed by a slowly (~10 minutes) developing further increase in twitch force. Both phases were reproducible: After unstretching the muscle back to 88% of its optimal length, a second stretch protocol revealed a comparable immediate force response and SFR.

Figure 1B summarizes average data from 75 experiments as outlined in Figure 1A. On stretch, force increased to

Figure 1. A, Influence of stretch on isometric twitch force in a muscle strip from a failing heart. At steady-state conditions, the muscle was stretched from 88% of its optimal length (Lₒ) to 98% of its optimal length (Lₘᵢₙ), resulting in an immediate (1st) and SFR. After mechanical stabilization, the muscle was unstretched to Lₒ, and the protocol was repeated. B, Relative increase in force during the immediate and the SFR. Average data from 75 muscle strips from 32 failing hearts. *P<0.05 vs prestretch values; #P<0.05 vs immediate phase. C, Reproducibility of the SFR. SFR-1 indicates slow force response during the first stretch protocol; SFR-2, slow force response during the second stretch protocol. Data from 12 stretch protocols as shown in Figure 1A. *P<0.05 vs force value at the end of the immediate phase (phase 1).

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Influence of Stretch on Twitch Kinetics During Phase 1 and the SFR

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<th>RT90</th>
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<th>+dT/dtmax</th>
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<td>−138±19†</td>
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Data from 17 stretch protocols.

RT50 indicates time to 50% relaxation (ms); RT90, time to 90% relaxation (ms); TPT, time to peak tension (ms); +dT/dtmax, maximum rate of tension increase (mN/s per mm²); −dT/dtmax, maximum rate of tension decline (mN/s per mm²).

*P<0.05 vs Lapse.
†P<0.05 vs phase 1.

232±6% of the prestretch force value during phase 1 and further to 279±8% during SFR. We also calculated the magnitude of the SFR related to force values at phase 1. Force further increased during SFR to 122±3% of phase 1 values (P<0.05; Figure 1C). After unstretching the preparations, the protocol was repeated. During the second stretch protocol, the immediate increase in force was to 233±20% of the unstretched value (NS versus first stretch protocol) and to 283±26% during SFR (or to 121±3% of twitch force values during phase 1; NS versus first stretch protocol; Figure 1C).

The effects of stretch on twitch kinetics are given in the Table. These experiments indicate the existence of a reproducible SFR in failing human myocardium, which contributes substantially (20% to 25%) to the total increase in twitch force during stretch.

The SFR in human myocardium is Ca²⁺-dependent. This can be seen from the rapid cooling results shown in Figure 2 (left). In these experiments, the SFR was accompanied by a consistent and significant increase in the amplitude of the rapid cooling contractions to 124±6% and 109±3%, respectively (both P<0.05). RCCs during phase 1 were not significantly different from RCCs in the unstretched muscle, although there was a tendency toward an increase in RCC amplitude, which might be attributable to an increase in myofilament Ca²⁺ sensitivity. The SFR also depends on SR Ca²⁺ uptake and release in failing human myocardium: Blocking SR function with CPA (20 μmol/L) and ryanodine (1 μmol/L) did not affect the immediate increase in force (relative to basal values) on stretch, but almost completely prevented the SFR (delayed increase in force was to 117±3% before and to 102±2% in the presence of CPA and ryanodine; Figure 2, right). These data indicate that the SFR is associated with increased SR Ca²⁺ load and that SR function is a prerequisite for the SFR.

Because nitric oxide (NO) was implicated in stretch-dependent effects on intracellular Ca²⁺ handling, we tested the effects of the NO-synthase inhibitor L-NAME on the SFR. Preincubation with L-NAME (500 μmol/L) did not affect the SFR in 8 human trabeculae from 4 hearts. SFR increased to 123±4% before L-NAME and 121±3% after incubation with the inhibitor (NS; data not shown).

Influence of Stretch on Stretch-Activated Ion Channels and L-Type Ca²⁺ Channels

To test whether SACs are involved in the SFR, muscle strips were preincubated with the SAC inhibitors gadolinium (10 μmol/L) or streptomycin (70 μmol/L) for 25 minutes after a first stretch protocol (control). Neither intervention affected the SFR (Figure 3, left for streptomycin). Twitch force increased to 120±3% before and to 121±3% in the presence of gadolinium and to 122±6% before and to 126±6% in the presence of streptomycin. In addition, SAC blockade did not affect basal contractility or the immediate response to stretch. Similar experiments were performed with diltiazem (5 μmol/L—equivalent to IC₅₀ values in failing human myocardium). Developed force increased during the SFR to 119±3% before administration of diltiazem and to 118±3% in the presence of the L-type Ca²⁺ channel blocker (Figure 3, right).

Contribution of Na⁺/H⁺ Exchange, pHᵢ, and Na⁺/Ca²⁺ Exchange to Delayed Functional Responses to Stretch

In a further set of experiments, we assessed the contribution of stretch-dependent NHE1 activation to the SFR. For this purpose, muscle strips were incubated with the selective NHE1 inhibitor HOE642 (3 μmol/L) for 25 minutes after an initial stretch protocol. The results are shown in Figure 4A.
Before NHE1-inhibition, twitch force increased to 123±3% of the value at the end of phase 1. After incubating the preparations with HOE642, the SFR was significantly reduced to 116±2 (P<0.05 versus value before HOE642). HOE642 did not affect the immediate increase in twitch force during phase 1 (to 205±14% and 220±18%, respectively).

Stretch-dependent NHE1 activation may mediate functional effects via intracellular alkalinization, followed by enhanced Ca2+ responsiveness of the myofilaments. We directly assessed pH, during stretch in 8 muscle strip preparations with the fluorescent pH indicator BCECF. Figure 4B shows an original recording of a typical experiment. The upper tracing depicts the fluorescence signals, the lower tracing the force recordings. During stretch, a characteristic immediate force response and SFR are observed without any detectable changes in the fluorescent signal. At the end of the experiment, a typical calibration experiment is performed. That allows conversion of ratiometric values to pH. Although force increased to 120±3% of phase 1 values during the SFR, pH was 7.24±0.05 before and 7.22±0.04 at the plateau phase of the SFR (n=8; NS). These experiments indicate that intracellular alkalinization does not occur during stretch in physiological HCO3−-buffered bath solution. This is supported by unchanged twitch time parameters during SFR as shown in the Table.

Stretch-dependent NHE1 activation may also increase cytosolic and subsarcolemmal [Na+], with resulting enhanced Ca2+ influx via reverse mode of the NCX. We tested the effects of reverse-mode NCX inhibition with KB-R7943 on the SFR. The results are summarized in Figure 5. KB-R7943 largely prevented the SFR. Twitch force increased to 121±4% before and to 107±3% in the presence of KB-R7943. In additional experiments (n=3), KB-R7943 almost completely prevented the increase in RCC amplitudes during SFR (to 102±4%, NS). KB-R7943 did not affect the immediate phase of force increase on stretch (to 259±11% before and 252±19% in the presence of KB-R7943). These data indicate that reverse-mode NCX activation underlies the SFR and that NCX-mediated Ca influx during stretch is stored within the SR.

Influence of AT1 or ET Receptor Blockade on the SFR

An autocrine–paracrine release and action of Ang II and ET-1 underlies the SFR in rat and feline cardiac tissue.4 We tested the potential role of these peptides in mediating the SFR in isolated human myocardium. The selective AT1 receptor antagonist candesartan (0.1 μmol/L) did not affect the SFR (Figure 6, left). Likewise, neither the selective ETα receptor antagonist BQ123 (0.3 μmol/L) (Figure 6, middle) nor the mixed ETα and ETβ receptor antagonist PD145065 (10 μmol/L) (Figure 6, right) prevented or reduced the delayed stretch-dependent functional responses. Force increased to 120±3% before and to 120±2% in the presence of candesartan, to 120±2% before and to 120±2% in the presence of the BQ123, and to 118±5% before and 119±6% in the presence of PD14065.

Discussion

The results of the present study demonstrate that: (1) a functionally relevant SR-Ca2+-dependent SFR exists in failing human myocardium; (2) activation of NHE1 and reverse-mode NCX contribute to the delayed inotropic response to stretch; and (3) functional effects are independent from NO, changes in pH, or activation of SACs, i-type Ca2+ channels, or AT1/ET1AB receptors.
A biphasic response to stretch was initially demonstrated by Parmley and Chuck in isolated mammalian cardiac muscle. In more detailed analyses the initial response to stretch (Frank-Starling mechanism) was shown to be independent from increases in [Ca\(^{2+}\)], and was related to enhanced responsiveness of the myofilaments for Ca\(^{2+}\). In contrast, Allen et al and others demonstrated slowly increasing Ca\(^{2+}\) transients as the underlying mechanism for the delayed inotropic response to stretch, but the origins of elevated [Ca\(^{2+}\)] remained obscure. During the past years, the subcellular mechanism for the delayed inotropic response to stretch was further elucidated. Using feline myocardium, Cingolani et al reported a stretch-dependent autocrine/paracrine stimulation of angiotensin II and endothelin-1 receptors with subsequent activation of NHE1. The latter resulted in elevated [Na\(^{+}\)], with subsequent activation of the NCX in its reverse mode. The stretch-dependent release of angiotensin II and endothelin-1 from isolated myocardium was in accordance with previous work of Sadoshima and Izumo in isolated rat myocytes.

**Influence of Stretch on Twitch Force in Isolated Human Myocardium**

Direct functional responses to stretch are poorly characterized in isolated human cardiac muscle. The functional importance of the immediate response to stretch in failing human myocardium is controversial, and its existence was even denied in a recent publication. Furthermore, a biphasic response to stretch with a delayed second phase in force increase has never been reported for human cardiac muscle. Here we clearly demonstrate a stable and reproducible biphasic response to stretch, including the initial Frank-Starling-like response, in end-stage failing human myocardium. Prestretching muscle strips from 88% of optimal length to 98% of optimal length resulted in an overall ∼140% immediate increase in isometric twitch tension. This increase is comparable to previous work in nonfailing or failing human or mammalian tissue. In addition, there was a consistent delayed increase in twitch force in failing human myocardium. This second phase developed over a time course of 5 to 10 minutes after the stretch and contributed ∼20% to 25% to the total increase in twitch force. Therefore, the second phase in twitch force is of potential functional relevance in the failing human heart.

**Influence of Stretch on SR Function**

We have previously reported that the immediate response to stretch in nonfailing or failing human myocardium does not depend on increased intracellular Ca\(^{2+}\) transients. An increase in intracellular Ca\(^{2+}\) transients was identified as the mechanism for the delayed increase in stretch-dependent force in mammalian myocardium. However, the effect of stretch on sarcoplasmic reticulum Ca\(^{2+}\) content remains unknown. In the present study, we observed unchanged SR Ca\(^{2+}\) content during the initial inotropic response, but a significant increase in SR Ca\(^{2+}\) content during the SFR. In addition, blockade of SR function with CPA and ryanodine did not affect the immediate increase in force but completely prevented the SFR. This extends previous work in mammalian myocardium and demonstrates a SR-dependent and Ca\(^{2+}\)-dependent mechanism for the delayed inotropic response to stretch in the failing human heart. However, Kentish and Wrzosek reported SR-independent SFR in rat myocardium at room temperature and low [Ca\(^{2+}\)]. Besides differences in experimental conditions, this discrepancy could be based on a much higher SR dependence of the EC coupling process in rat compared with failing human myocardium. The presumably low-loaded SR in failing human myocardium may store extra Ca\(^{2+}\) that enters the cell during stretch, whereas stretch-activated Ca\(^{2+}\) influx may directly contribute to myofilament activation in rats.

Neuronal nitric oxide synthase (nNOS) has recently been shown to modulate basal contractility and SR Ca\(^{2+}\) handling in mice in vitro and in vivo. More specifically, ryanodine receptors have been shown to be directly regulated by NO-dependent nitrolyzation. In isolated rat cardiomyocytes, unselective inhibition of NO synthesis with L-NAME prevents the stretch-induced increase in calcium spark frequency and calcium transients, suggesting an important role of NO in stretch-induced contractile activation. The stretch-induced increase in calcium cycling was absent in cardiomyocytes isolated from eNOS knockout mice. Our experiments with L-NAME did not reduce the SFR in failing human myocardium. In line with that, exogenous NO may even decrease contractility in isolated trabeculae from failing and nonfailing human hearts. These data indicate that NO does not play a major role in mediating the SFR in the failing human heart.

**Stretch-Sensitive Ion Channels and L-Type Ca\(^{2+}\) Channels**

Sarcolemantal stretch-sensitive ion channels are potential candidates to mediate the increase in intracellular Ca\(^{2+}\) and the delayed inotropic response to stretch (for review, see Hu and Sachs). We used the trivalent lanthanide gadolinium for pharmacological blockade of cation SACs. Gadolinium suppresses stretch-induced transient depolarizations and premature beats and prevents stretch-mediated contractile dysfunction in guinea pig papillary muscle. However, pretreatment of muscle strip preparations from end-stage failing human hearts with gadolinium did not affect the...
stretch-induced immediate or delayed inotropic response. Because these experiments were performed in bicarbonate-buffered solutions,26 we confirmed the results in additional experiments with an alternative SAC blocker, streptomycin. As with gadolinium, streptomycin did not reduce the SFR. Although a specific SAC blocker is currently not available, we conclude from these experiments that the SFR does not depend on stretch-mediated SAC channel activation in the failing human heart. This conclusion is in accordance with previous reports on rat papillary muscle.27

To elucidate whether the initial or delayed inotropic response to stretch might be mediated by L-type Ca2+ currents,28 further experiments were performed in the presence of diltiazem. Diltiazem did not affect the relative increase in force during the initial or delayed inotropic response after stretch. These data suggest that in failing human myocardium, neither the immediate nor the delayed inotropic response to stretch depends on enhanced transsarcolemmal Ca2+ entry via L-type Ca2+ channels.

Role of Na+/H+ Exchange and Reverse-Mode Na+/Ca2+ Exchange

The NHE1 is an electroneutral cotransport system that regulates pHi by controlling transsarcolemmal transport of Na+ and H+. Its activation results in the outward transport of H+ ions for inward transport of Na+ ions in a 1:1 stoichiometry.29

In the present study, using physiological, bicarbonate-containing buffer, inhibition of NHE1 partly prevented the delayed inotropic response to stretch in failing human myocardium. This indicates that NHE1 acts as a sarcolemmal mechanosensor or mechanotransducer that directly or indirectly converts mechanical stress into functional responses in the human heart.

Inotropic effects after NHE1 activation could be related to intracellular alkalinization with enhanced myofilament responsiveness for Ca2+, or increases in [Na+]i, that thermodynamically favor the NCX to operate in its reverse mode.30 In bicarbonate-buffered solutions, using the fluorescent indicator BCECF, we did not detect any stretch-induced intracellular alkalinization. The physiological significance of alkalinization for inotropic responses after NHE1 activation has been recently challenged and was possibly related to the use of unphysiological, bicarbonate-free buffers in previous experimental work. In fact, Alvarez et al, using physiological CO2/HCO3−-buffered medium, demonstrated a stretch-induced NHE1-activation with consecutive increases in [Na+]i, [Ca2+]i, and twitch force, but without changes in pHi in rat trabeculae.5 We recently confirmed these observations in rabbit trabeculae.17

Major changes in pHi are also unlikely based on our findings that twitch kinetics were unchanged during the delayed phase in force increase (Table). The latter observation is typical for an inotropic intervention that depends on an increase in [Ca2+]i, whereas increased myofilament sensitivity is typically associated with a prolongation of twitch relaxation parameters. Therefore, we suggest that NHE1 activation mediates a part of the delayed inotropic response to stretch as a consequence of NHE1-dependent increase in [Na+]i, followed by the observed increase in [Ca2+]i.

The mechanism of stretch-dependent NHE1 activation remains to be clarified. Previous reports in feline and rat papillary muscle demonstrated autocrine/paracrine activation of AT1 and ET receptors as the initial step for NHE1 activation and the SFR.5 However, the SFR was not related to activation of AT1 or ET receptors in the present work. Therefore, in contrast to feline and rat myocardium, NHE1 activation seems independent from Ang II or ET-1 release on stretch in failing human myocardium. This is in accordance with previous work from Calaghan and White31 and our own reports17 in which AT1 receptor– and ET, receptor–independent SFR was observed in ferret and rabbit papillary muscle. Partial inhibition of the SFR with the mixed ET receptor antagonist PD145065 as reported in ferret papillary muscle31 was not observed in the present study in failing human myocardium. Therefore, species-dependent differences in activation pathways for the SFR may exist. Direct activation by mechanical signals or indirect activation through distinct mechanosensors, such as focal adhesions or cytoskeletal proteins, might contribute to NHE1 activation in the human heart.32

Although NHE1 inhibition reduced the delayed inotropic response to stretch by ~35%, blockade of the reverse-mode NCX with KB-R7943 almost completely prevented the SFR. This indicates that reverse-mode NCX is the major subcellular mediator for the SFR and might be activated through additional mechanisms distinct from NHE1. Consistent with our data, Pérez et al demonstrated that Ca2+ entry through reverse-mode NCX is the last step in a chain of events underlying the SFR after stretch in feline myocardium.6 Besides NHE1-activation, further mechanisms, such as action potential prolongation,33 increased open probability of Na+ channels, inhibition of the sodium pump,34 or direct effects of stretch on NCX may contribute to reverse-mode NCX activation and the SFR in human cardiac muscle.

Limitations of the Study

KB-R7943 was used as an inhibitor of the NCX reverse mode. However, KB-R7943 is probably not selective, because it might also affect K+, Na+, and Ca2+ channels and affects Ca2+ transients even in NCX knockout heart tubes.35 In addition, the apparent selectivity for outward versus inward NCX current is not well understood.36,37 Using bicarbonate- and phosphate-containing physiological buffer solutions, the concentration of free gadolinium may be lower than the concentration applied to the organ bath, because gadolinium may bind in complexes with bicarbonate or phosphate.26 Therefore, we confirmed the data obtained with gadolinium by use of streptomycin, an SAC blocker that is not affected by physiological buffers. For future studies, it would be desirable to use pharmacological compounds with high potency and selectivity for reverse-mode NCX or SAC inhibition, but such agents are currently not available. We used BDM for cardioprotection during transport, storage, and preparation of muscle strips. However, BDM has multiple subcellular actions and potential long-lasting effects of the compound despite full mechanical recovery after washout cannot be completely ruled out.
Nevertheless, we demonstrated for the first time a sustained SFR in failing human myocardium. This SFR is SR-dependent and Ca\(^{2+}\)-mediated and involves NHE1 and reverse-mode NCX activation. Because the SFR contributes 20\% to 25\% to the total increase in force on stretch, it could be of functional relevance in the volume overloaded failing human heart.

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References

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Methods

For immunohistochemistry, hearts from 4 wildtype (Wt) mice and 4 transgenic MHC-TGFcys\(^{33}\)ser (Tx) mice were frozen rapidly in liquid nitrogen and stored at -80°C. 5µm thick longitudinal cryosections were cut and fixed to coverslip coated with 50 mg/l poly-L-lysine. Rabbit polyclonal anti-Cx40 (Alpha Diagnostics Int. Inc., Sant Antonio, TX) and anti-Cx43 (Chemicon Int. Inc., Temecula CA) were used to detect connexins. At high concentrations, the anti-Cx40 antibody cross-reacted with Cx43, as was evident from immunostaining of gap junctions between ventricular myocytes. At the dilution used in this study (1:3000), anti-Cx40 showed clear immunostaining of gap junctions between endothelial cells in the ventricle, but not between ventricular myocytes. Connexin antibodies were used in combination with mouse monoclonal anti-desmin antibody (DAKO A/S, Denmark) to characterize myocyte identity and orientation.

Sections were permeabilized with 0.2% Triton-X100 in phosphate buffered saline (PBS) for 1 hour, incubated with 2% bovine serum albumin (BSA) in PBS for 30 minutes, and with the primary antibodies overnight. Subsequently, sections were incubated with 2% BSA for 30 minutes, and for 2 hours with a combination of the secondary antibodies goat-anti-rabbit FITC and donkey-anti-mouse Texas Red (Jackson Immunoresearch, Inc., West Grove, PA). In negative controls, the first antibodies were omitted. Tissue sections were viewed using a Leitz Laborlux S microscope equipped for fluorescence microscopy. Images were digitized using a Spot camera (Diagnostics Instruments, Inc.).
Results & Discussion

Figure 1. Connexin expression in Wt and Tx mice. All sections were immuno-labeled with anti-desmin (red color) and either anti-Cx40 or anti-Cx43 (green color). For both the LA and the RA, expression levels of both connexins between adjacent myocytes was comparable between Wt and Tx.

Both Cx40 and Cx43 were expressed atrial myocytes of Wt mice. Expression levels of remaining myocytes in Tx mice were comparable to that in Wt myocytes. Therefore, we do not expect an intrinsic alteration in connexin expression to be a causative factor in the conduction abnormalities which were observed in the Tx. However, in areas where myocytes are physically separated by fibrous tissue, electrical coupling does not occur. In the Tx LA and RA, fibrosis is strongly increased, which could lead to increased tortuosity of conduction pathways.