

Original Article

Abnormal frequency-dependent responses represent the pathophysiologic signature of contractile failure in human myocardium

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Received 2 April 2003; received in revised form 22 July 2003; accepted 3 September 2003

Abstract

Background. – The normal increase in isometric developed force (DF) with faster pacing rates, known as the positive force–frequency response/relationship (FFR), is altered in failing myocardium, as shown by its negative response to increased pacing. The objective of this study was to determine if increasing Ca^{2+} influx with L-type Ca^{2+} channel (L-CaCh) agonists: BayK 8644 (BayK) and FPL 64176 (FPL) or increased extracellular Ca^{2+} could increase contractility and normalize the FFR in failing myocardium.

Methods. – Isometric DF was measured in right ventricular trabeculae from failing ($n = 28$) and non-failing ($n = 12$) human hearts at various stimulation frequencies (0.5–2.5 Hz) before and after bath application of BayK (250 nM), FPL (100 nM), or high Ca^{2+} (7.0 mM). Post-rest (PR) experiments were also conducted on several trabeculae.

Results. – In trabeculae from failing hearts, the DF decreased with an increase in pacing. Addition of L-CaCh agonists increased DF to similar levels in trabeculae from both failing and non-failing hearts at slow pacing rates, but did not alter the negative FFR in the failing group. During increased rest intervals, the amount of PR potentiation was diminished in trabeculae from failing hearts as compared to the non-failing preparations.

Conclusion. – This study demonstrates that the abnormal FFR observed in trabeculae from failing hearts is a reliable physiologic signature of the cardiomyopathic state even when DF, at slow stimulation frequencies, is relatively high. These studies further demonstrate that the impaired FFR is not due to an inability to further increase contractility. Rather, our findings suggest that the abnormal FFR and blunted PR potentiation alike are a reflection of an altered functional balance between Ca^{2+} re-uptake and Ca^{2+} extrusion.

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Keywords: Heart failure; Calcium; Contractility; Myocardial contraction; Sarcoplasmic reticulum

1. Introduction

Along with the Frank–Starling mechanism and responses to neurohumoral stimulation (e.g. catecholamines), the ability to increase myocardial contractility in response to increased stimulation frequency represents a key mechanism for cardiac adaptation to acute increases in workload [1]. Indeed, increased contractile force upon faster pacing rates (i.e. a positive force–frequency response/relationship (FFR)) is observed

in virtually all normal mammalian hearts. Conversely, a blunted, absent, or negative FFR is increasingly recognized as a signature abnormality among failing hearts from humans [2,3] and animal models alike [4–7]. Beyond its relevance as a marker of diseased myocardium, an abnormal FFR contributes to impaired cardiac contractile reserve and abnormal exercise tolerance among individuals with heart failure.

Numerous studies have investigated the possible mechanisms underlying this abnormal FFR in failing human hearts, and most studies have implicated defects in cellular Ca^{2+} handling, and particularly depressed sarcoplasmic reticulum (SR) uptake [8] coupled with an increase in SR leak [9], as

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contributors to the abnormal FFRs observed in failing hearts [10–18]. According to this perspective, the normal FFR involves increased Ca^{2+} entry into the cell via the L-type Ca^{2+} channel (L-CaCh) with a subsequent increase in contraction as more of this increased Ca^{2+} influx is sequestered into the SR. In this context, one possible reason for abnormal FFRs is that the depressed and leaky SR might already be maximally loaded in myocardium from failing hearts. In this scheme, any subsequent increase in pacing frequency and Ca^{2+} entry cannot further increase the Ca^{2+} load of SR and force generation. Alternatively, inhibitory effect of high intracellular Ca^{2+} on excitation–contraction coupling or increased Ca^{2+} buffering via mitochondria could also limit further SR Ca^{2+} loading and contribute to a blunted/negative FFR. Another possibility is that alterations in the balance between Ca^{2+} uptake by the SR Ca^{2+} ATPase (SERCA) and Ca^{2+} extrusion by the Na^{2+} – Ca^{2+} exchanger (NCX) in failing hearts contribute to the abnormal FFR. According to this hypothesis, in the setting of reduced SERCA uptake capacity, if increased Ca^{2+} entry during higher stimulation frequencies is associated with equivalent or even greater increases in Ca^{2+} extrusion by the NCX, a flat or negative FFR would result. Indeed, several investigators have reported correlations between reduced SERCA protein abundance and/or increased NCX abundance, and the negative FFRs observed in failing human hearts [10–12,17].

The general goal of this research was to further investigate the mechanisms underlying the negative FFR found in failing human hearts. Our specific objective was to determine whether an inability to further augment contractility explains the abnormal FFR. In order to accomplish this, we studied the frequency dependence of isometric contractions in human ventricular trabeculae from failing and non-failing hearts in the presence and absence of the L-CaCh agonists: BayK 8644 (BayK) and FPL 64176 (FPL), and increased extracellular Ca^{2+} . To explore the possibility that alterations in the balance between Ca^{2+} uptake by SERCA and Ca^{2+} extrusion by the NCX is an important determinant of responses to increased stimulation frequency, we also examined contractile responses after increases in the rest interval between contractions (post-rest (PR) behavior) in trabeculae from failing and non-failing hearts.

2. Materials and methods

2.1. Tissue procurement

Human myocardium was obtained from patients with end-stage heart failure undergoing cardiac transplantation (failing) or from donor hearts deemed unsuitable for transplantation (non-failing). Clinical information concerning patient demographics, etiology of heart failure, clinical history, and medications was obtained for all heart failure patients and controls. Prior to explantation in the cardiac surgical suite, cold, 4:1 blood cardioplegia was administered via an aortic

catheter in an antegrade fashion as previously described [19]. After explantation, the hearts were promptly transported to the laboratory in ice-cold Krebs–Henseleit buffer (KHB) containing (mmol/l): glucose (12.5), KCl (5.4), lactic acid (1.0), MgSO_4 (1.2), NaCl (130.0), NaH_2PO_4 (1.2), NaHCO_3 (25.0), and Na-pyruvate (2.0); pH 7.4. Upon arrival to the laboratory, the heart was weighed and rinsed in fresh, cold KHB. The apical septum and right ventricular free wall were removed for muscle strip experiments, and the tissue was placed in room temperature KHB solution containing 20 mmol/l 2,3-butanedione monoxime (BDM) and 0.25 mmol/l CaCl_2 .

2.2. Multicellular muscle preparation and mounting

Right ventricular tissue was completely immersed in supplemented KHB solution at room temperature while continuously being bubbled with a 95% O_2 –5% CO_2 gas mixture. Using a stereo dissecting microscope, thin, non-branching, and free-running trabeculae from the right ventricular free wall were carefully removed ($n = 40$, from 16 hearts) with a small cube of wall tissue on either side to facilitate attachment in the bath [20–22]. Average dimensions: 0.31 ± 0.02 mm wide, 0.25 ± 0.02 mm thick, and 2.48 ± 0.14 mm long. Only trabeculae that have a maximal thickness of 0.50 mm were used.

The preparations were then mounted in a slackened position, in a custom-designed muscle chamber (Scientific Instruments, Heidelberg, Germany) between a platinum/iridium wires with a basket-shaped extension of a force transducer at one end and a hook connected to a microdisplacement device at the other [20,21]. Following mounting of the trabeculae, the muscle chamber was completely sealed off to avoid contamination and evaporation of solution. The solution in the muscle bath was stirred continuously using a miniature Teflon-coated stirring bar, as a humidified 95% O_2 –5% CO_2 gas mixture was continuously pumped into the chamber. The supplemented KHB was replaced by BDM-free KHB containing 0.5 mmol/l CaCl_2 in three continuous steps, which involve replacing one-half of the solution at a time to ensure that the muscle remains under the surface of the solution. After 5 min, the KHB solution was exchanged in order to increase the Ca^{2+} concentration from 0.5 to 1.0 mmol/l. After five more minutes, the KHB solution was again exchanged for a 1.75 mmol/l CaCl_2 KHB solution. All solutions were kept at 37.0 °C, equilibrated by bubbling with a 95% O_2 –5% CO_2 gas mixture, and maintained at pH 7.4. In addition, the muscle chamber was electronically maintained at a temperature of 37.0 °C. The trabeculae were continuously stimulated at 0.5 Hz, by 3.0 ms asymmetric pulses with an energy 20% above threshold (typically 3–10 V). After initiating stimulation in solution containing 1.75 mmol/l Ca^{2+} , the trabeculae were left for 45 min at a very low preload to equilibrate.

Subsequently, the trabeculae were released to a “slack” length (the length at which no developed force (DF) occurs), in order to balance the force transducer. Slowly, the trabecu-

lae were stretched to a length (L_0) where an active DF can first be identified. The muscle length at L_0 was noted on the micrometer, and the muscle was further stretched to a length (L_{\max}) where maximal isometric force development occurred. The final length was set at 80% of the difference between L_{\max} and L_0 ($80\%(L_{\max} - L_0)$). At this length, the trabeculae exhibits stable isometric force measurements. In preliminary experiments, we observed unstable performance, possibly caused by overstretching, when studies were performed at lengths near L_{\max} . The trabeculae were paced at 0.5 Hz at $80\%(L_{\max} - L_0)$ for 30 min before the experiment was initiated. Muscle length was not re-adjusted during the rest of the experiment, unless otherwise noted. At this point, trabeculae were randomly chosen to undergo either force–frequency or PR experiments.

2.3. Contractile parameters experimental design

Once stabilized (at 0.5 Hz), steady-state (determined when DF varied <5% over a 3-min time period) basal contractile parameters were recorded in trabeculae from failing and non-failing hearts. After basal experiments were conducted, solutions containing either 0.25 $\mu\text{mol/l}$ BayK, 0.10 $\mu\text{mol/l}$ FPL, or 7.0 mmol/l Ca^{2+} were randomly added to the muscle chambers. These concentrations were chosen because they produced maximal force development without causing spontaneous contractions, which are signatures of SR Ca^{2+} overload [8]. After equilibration for 30 min in these solutions, steady-state twitches were recorded in order to compare contractile parameters to those under basal conditions.

2.4. Force–frequency experimental design

Once stabilized (at 0.5 Hz), trabeculae from failing and non-failing hearts underwent control force–frequency experiments. Steady-state twitches were recorded at 0.5, 1.0, 1.5, 2.0, and 2.5 Hz. After control measurements, solutions were randomly exchanged with those containing either 0.25 $\mu\text{mol/l}$ BayK or 0.10 $\mu\text{mol/l}$ FPL. Muscles were allowed to equilibrate for 30 min under these conditions. Again, these concentrations were chosen because they elicited maximal force development without causing spontaneous contractions. Steady-state twitches were recorded at the same stimulation frequencies used in control experiments.

2.5. Post-rest experimental design

Once stabilized (at 0.5 Hz), control PR experiments were conducted in trabeculae from failing and non-failing hearts. After the muscle has reached a steady state, a pre-rest twitch was recorded. Immediately after, the stimulator was turned off and the muscle was allowed to rest for a time interval of 5 s followed by resumption of pacing at 0.5 Hz. This procedure was repeated with subsequent rest intervals of 15, 30, 60, and 120 s. The analysis of these experiments consisted of comparing the first PR twitch to the pre-rest steady-state twitch.

2.6. Statistical analysis

Results are presented as mean \pm S.E.M. Statistical significance of basal contractile parameters was determined by two-tailed paired *t*-tests. Differences were considered significant if the probability of chance occurrence was <0.05. For force–frequency experiments, force data were analyzed using a mixed-model ANOVA for repeated measures. Differences between groups and frequencies were considered significant if the probability of chance occurrence was <0.05 using two-tailed tests. Due to non-normality, the data were transformed to normalized ranks prior to analysis. Between-group and within-group pair-wise comparisons of frequency means used a Bonferroni adjustment to maintain an experiment-wise type I error of 0.05 or less. For PR experiments, the dependent variable, isometric force of contraction was treated as a continuous variable and was calculated as the change from the immediately preceding force of contraction prior to the rest interval. The experimental design was a “within-subject” design measuring each strip at five different rest intervals for each treatment. Prior to analysis, all data were tested for normality. The data were analyzed using a mixed-model ANOVA for repeated measures followed by multiple comparisons to detect significant individual mean differences using the Bonferroni adjustment to maintain an experiment-wise type I error of 0.05 or less.

3. Results

3.1. Clinical characteristics

Clinical information concerning the patients supplying heart tissue is summarized in Table 1. Of the 11 failing hearts from heart transplant recipients, all were males and most were failing due to coronary artery disease and ischemic cardiomyopathy, and two were failing due to non-ischemic causes. Non-failing hearts were unsuitable for transplantation due to donor age over 60 years [3], coronary artery disease without infarction [1], or positive hepatitis serology [1]. There was no age difference between the failing and non-failing hearts, but the average ejection fraction among failing hearts was 12% compared with 58% from non-failing hearts.

3.2. Basal contractile parameters

Isometric steady-state developed twitch force of trabeculae from failing ($n = 28$) human hearts at 0.5 Hz was significantly higher compared to trabeculae from non-failing ($n = 12$) hearts (Table 2). Diastolic force was also significantly higher in trabeculae from failing hearts as compared to trabeculae from non-failing hearts. Significant differences were also found in the rate of maximal force rise ($+dF/dt$) and maximal force decline ($-dF/dt$) when comparing trabeculae from failing to non-failing hearts. Representative steady-state twitch tracings of trabeculae from failing and non-failing hearts at 0.5 Hz are shown in Fig. 1A.

Table 1
Clinical characteristics of patients

Group	Gender	Age (years)	Etiology	LVEF (%)	RAP (mmHg)	PA systolic (mmHg)	Medications
Failing (n = 11)	11 Male	58 ± 3	9 ischemic 2 non-ischemic	12 ± 1 *	8 ± 2	45 ± 4	Dob 6/11, Mil 10/11, ACEI 9/11 ARB 6/11, BB 3/11, Dig 10/11 Diu 10/11, Nit 8/11, Amio 6/11 CCB 2/11, Hyd 3/11
Non-failing (n = 5)	3 Male 2 Female	58 ± 5	N/A	58 ± 4			Dopa 3/5

LVEF, left ventricular ejection fraction; RAP, right atrial pressure; PA, pulmonary artery; Diu, diuretic; Dig, digoxin; ACEI, ACE inhibitor; Cou, coumadin; Nit, nitrate; ARB, angiotension receptor blocker; Amio, amiodarone; Mil, mitrinone; Dob, dobutamine; Dopa, dopamine; BB, beta-blocker; Hyd, hydralazine; CCB, Ca²⁺ channel blocker; numeric data expressed as mean ± S.E.M.; * $P \leq 0.05$ vs. non-failing group.

Table 2
Twitch parameters

	Freq (Hz)	DevF (mN/mm ²)	TPF (ms)	DiaF (mN/mm ²)	+dF/dt (mN/s/mm ²)	-dF/dt (mN/s/mm ²)	RT ₅₀ (ms)	RT ₉₀ (ms)
Non-failing (n = 12)	0.5	16.7 ± 1.6	235 ± 13.4	4.7 ± 0.4	129.0 ± 17.4	98.3 ± 11.7	153 ± 7.1	309 ± 13.7
Failing (n = 28)	0.5	31.0 ± 2.8***	229 ± 7.4	7.9 ± 0.9**	217.6 ± 11.8***	152.5 ± 9.7**	172 ± 6.5	345 ± 12.6
Non-failing (n = 7)	2.5	30.3 ± 4.6	151 ± 6.1	5.2 ± 0.4	345.6 ± 66.9	246.8 ± 51.4	98 ± 7.7	173 ± 10.7
Failing (n = 8)	2.5	13.5 ± 1.8*	166 ± 8.8	7.0 ± 1.0	133.4 ± 18.8*	86.3 ± 6.9*	109 ± 6.7	201 ± 7.9

Freq, stimulation frequency; DevF, developed force; TPF, time-to-peak force; DiaF, diastolic force; +dF/dt, maximal force rise; -dF/dt, maximal force decline; RT₅₀, time to 50% relaxation; RT₉₀, time to 90% relaxation; average values are given as mean ± SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

3.3. Force–frequency relationship

Representative twitch tracings and average responses to increasing stimulation frequency in trabeculae from failing ($n = 8$) and non-failing ($n = 7$) hearts are shown in Fig. 1B,C. Trabeculae from failing hearts showed a non-significant decrease in twitch force when stimulation frequency was increased. In contrast, trabeculae from non-failing hearts exhibited significant increases in twitch force, as stimulation frequency was increased. As a result of these differential rate responses, at pacing rates of 2.5 Hz, trabeculae from non-failing hearts had significantly higher isometric developed twitch force as compared to trabeculae from failing hearts (Table 2). Moreover, at 2.5 Hz, trabeculae from non-failing hearts also demonstrated faster rates of force generation and relaxation compared with trabeculae from failing hearts (Table 2).

3.4. Effects of L-type Ca²⁺ channel agonists and bath [Ca²⁺] on contractility

The effects of L-CaCh agonists on contractile parameters in human myocardium are summarized in Table 3 with representative raw data presented in Fig. 2. In trabeculae from non-failing hearts, application of BayK ($n = 3$) caused a significant increase in DF. In trabeculae from failing hearts, addition of either BayK ($n = 15$) or FPL ($n = 8$) also caused significant increases in DF. In trabeculae from both non-failing and failing hearts, L-CaCh agonists induced signifi-

cant increases in the +dF/dt. At slow stimulation frequencies, L-CaCh agonists induced marked prolongation of relaxation times in trabeculae from failing hearts and a secondary component of relaxation was often observed (Fig. 2). When bath [Ca²⁺] was increased as a non-pharmacological, alternate means of increasing contractility in trabeculae from failing hearts ($n = 5$), increases in DF and +dF/dt were similar to those observed with the L-CaCh agonists, however, prolongation of relaxation times did not occur (Table 3). These differences are due to fundamentally different effects of L-CaCh agonists and extracellular Ca²⁺ on action potential duration [23,24].

3.5. Effects of L-type Ca²⁺ channel agonists on the force–frequency relationship

Force–frequency experiments were performed on trabeculae from failing hearts ($n = 8$) before and after application of the L-CaCh agonists BayK ($n = 4$) and FPL ($n = 4$). BayK and FPL did not alter the shape of the FFR, which remained slightly negative (Fig. 3). In trabeculae from non-failing hearts without BayK ($n = 7$), a positive FFR was observed. However, addition of BayK to trabeculae from non-failing hearts ($n = 3$) caused a flat FFR, similar to that observed in trabeculae from failing hearts.

3.6. Post-rest responses

To explore the possibility that alterations in the balance between Ca²⁺ uptake by SERCA and Ca²⁺ extrusion by the

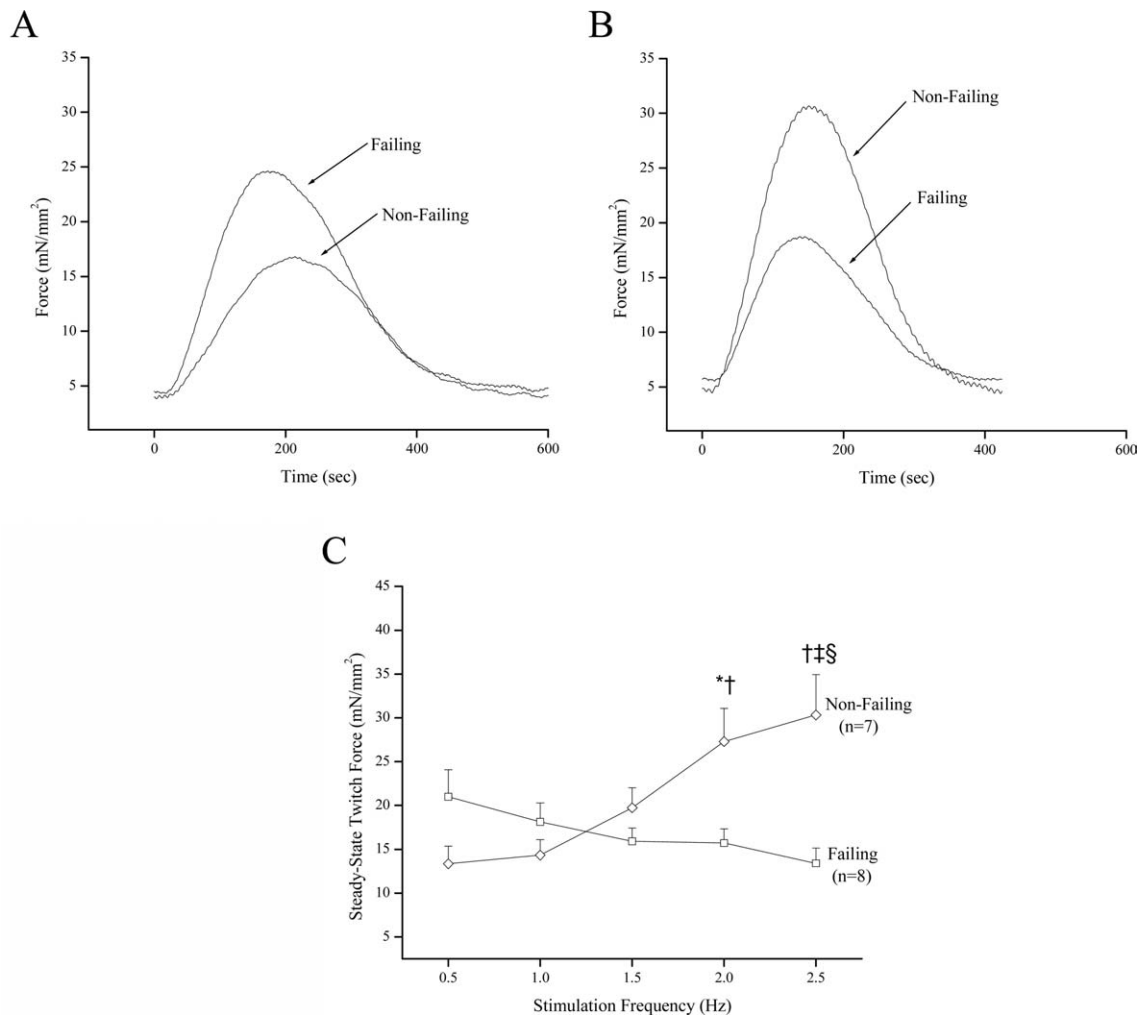


Fig. 1. Steady-state twitch tracings (1.75 mM Ca²⁺) of trabeculae from failing and non-failing human hearts at (A) 0.5 and (B) 2.5 Hz are represented. All tracings are raw, not averaged. (C) Average steady-state twitch force as a function of stimulation frequency in failing (□) and non-failing (◇) myocardium. * $P < 0.05$ when compared to within-group twitch force at 1.0 Hz; † $P < 0.01$ when compared to within-group twitch force at 0.5 Hz; ‡ $P < 0.01$ when compared to within-group twitch force at 1.0 Hz; § $P < 0.05$ when comparing between-groups twitch force.

NCX may be important determinants of responses to increased stimulation frequency, we studied contractile responses during stepwise increases in the rest interval between contractions [25]. Representative tracings of pre-rest steady-state twitches followed by the first twitch immediately after 5- and 120-s rest intervals are illustrated in Fig. 4A, and the average changes from pre-rest steady-state values at each rest interval tested are shown in Fig. 4B. Trabeculae from non-failing hearts ($n = 5$) exhibited PR potentiation after each rest interval, and the amount of potentiation increased as the rest interval was incremented to 120 s. Trabeculae from failing hearts ($n = 20$) had a significantly less PR potentiation and, after longer rest intervals, a time-dependent decrease in twitch force (rest decay) was observed.

4. Discussion

The first major finding of this study is that trabeculae from failing human hearts produced relatively high isometric

forces at slow stimulation frequencies compared to non-failing hearts. However, trabeculae from failing hearts exhibited a negative or flat FFR, while trabeculae from non-failing hearts exhibited a significant positive FFR. These findings suggest that defects in frequency-dependent contractile responses, rather than reduced basal or peak DF, represent the pathophysiologic signature of the failing human heart. The next major finding is that the negative FFR in trabeculae from failing hearts is not due to an inability to further increase contractility. In failing preparations, L-CaCh agonists or higher bath Ca²⁺ increased basal (0.5 Hz) force generation, consistent with increased SR Ca²⁺ load and release but did not ameliorate the negative FFR. In non-failing preparations, the L-CaCh agonist BayK induced even greater increments in basal force production, but maximal DF in the presence of such agonists was equivalent in trabeculae from non-failing and failing hearts. These findings suggest that the concentration of L-CaCh agonists employed caused similar and possibly maximal levels of SR Ca²⁺ loading in non-failing and failing preparations. Finally, these experiments show that

Table 3
Twitch parameters before and after bath application of L-CaCh agonists

	Freq (Hz)	DF (mN/mm ²)	TPF (ms)	DF (mN/mm ²)	+dF/dt (mN/s/mm ²)	-dF/dt (mN/s/mm ²)	RT ₅₀ (ms)	RT ₉₀ (ms)
Non-failing (<i>n</i> = 3)	0.5	17.8 ± 3.7	218 ± 9.8	3.6 ± 0.3	132.3 ± 34.1	102.8 ± 25.6	158 ± 11.2	301 ± 18.1
BayK (paired)	0.5	49.8 ± 5.7**	253 ± 16.7	2.1 ± 1.0	316.5 ± 47.7*	242.3 ± 20.9*	185 ± 16.8	334 ± 29.4
Failing (<i>n</i> = 15)	0.5	34.4 ± 4.6	240 ± 10.4	8.2 ± 1.3	236.2 ± 19.1	163.1 ± 15.0	180 ± 10.1	362 ± 15.5
BayK (paired)	0.5	53.9 ± 7.1***	239 ± 8.7	9.4 ± 1.3	401.5 ± 21.5***	174.1 ± 15.7	280 ± 26.7***	542 ± 22.4***
Failing (<i>n</i> = 8)	0.5	27.4 ± 3.1	204 ± 10.2	7.8 ± 1.7	209.7 ± 12.0	140.5 ± 15.7	160 ± 8.1	312 ± 28.4
FPL (paired)	0.5	45.3 ± 7.6*	222 ± 8.5**	7.5 ± 2.0	255.3 ± 10.4**	148.3 ± 12.3	227 ± 12.6**	515 ± 22.5**
Failing (<i>n</i> = 5)	0.5	26.7 ± 4.1	237 ± 16.9	7.4 ± 1.4	181.7 ± 26.2	144.3 ± 21.3	167 ± 14.7	344 ± 23.4
High Ca ²⁺ (paired)	0.5	49.4 ± 9.6*	234 ± 21.9	9.4 ± 2.2	282.9 ± 22.3**	178.2 ± 22.6	159 ± 9.5	314 ± 25.5
Non-failing (<i>n</i> = 3)	2.5	19.8 ± 3.3	160 ± 5.2	4.2 ± 0.2	191.5 ± 36.6	130.2 ± 28.2	112 ± 9.1	194 ± 9.8
BayK (paired)	2.5	39.6 ± 2.3**	167 ± 4.0	4.1 ± 1.5	359.5 ± 32.7**	258.7 ± 19.8**	127 ± 1.3	199 ± 2.0
Failing (<i>n</i> = 4)	2.5	11.8 ± 1.8	176 ± 9.2	6.2 ± 0.5	123.1 ± 28.4	86.1 ± 10.7	106 ± 9.5	197 ± 9.7
BayK (paired)	2.5	17.7 ± 2.6**	177 ± 13.5	7.9 ± 0.9	172.7 ± 39.7*	139.0 ± 25.5**	128 ± 8.9	209 ± 11.5

Freq, stimulation frequency; TPF, time-to-peak force; DiaF, diastolic force; +dF/dt, maximal force rise; -dF/dt, maximal force decline; RT₅₀, time to 50% relaxation; RT₉₀, time to 90% relaxation; average values are given as mean ± S.E.M. * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

trabeculae from failing human hearts with a negative FFR also exhibit significantly smaller PR potentiation than trabeculae from non-failing hearts. These results suggest that the functional balance between Ca²⁺ re-uptake and Ca²⁺ extrusion may be the pivotal determinant of frequency-dependent contractile responses in human myocardium. In this regard, our results in intact trabeculae are consistent with our recent investigation examining competition between SERCA and NCX activity in single myocytes from non-failing and failing human hearts [8].

4.1. Basal contractile responses

Many researchers, past and present, have tried to distinguish between human non-failing and failing myocardium by comparing their basal twitch characteristics [2,14,26]. In our studies, DF at slow stimulation frequencies was significantly greater in trabeculae from failing hearts than in those from non-failing hearts. One implication of the greater basal tension observed in failing myocardium and the subsequent responses to increased stimulation frequency is that force production at a single slow stimulation rate is not a reliable assay for identifying failing myocardium. Rather, dynamic assays, such as responses to increased stimulation frequency or adrenergic agonists, may better distinguish failing from non-failing myocardium. Another interpretation of our findings is that increased force production observed at slow stimulation rates in trabeculae from failing hearts is “inappropriate” and that this phenomenon is a reflection of pathologic regulation of contractility. If we make the assumption that twitch amplitude is proportional to [Ca]_i transient amplitude and that [Ca]_i transient amplitude mainly reflects Ca²⁺ released from the SR, then high steady-state force generation at slow stimulation frequency represents a pathologic inability

of the cell to reduce SR Ca²⁺ load when appropriate. Supporting this notion of “supraoptimal” Ca²⁺ load in trabeculae from failing hearts are their relatively small increments in force generation with maneuvers that should markedly increase SR Ca²⁺ load including L-CaCh agonists and increases in bath Ca²⁺ [27].

4.2. Force–frequency responses

Our observation that trabeculae from non-failing human hearts enhance their force generation after an increase in stimulation frequency is consistent with many previous studies [2,3,11,12,14,15,26,28,29]. The normal positive FFR is thought to result from a frequency-dependent increase in Ca²⁺ entry that causes an increase in Ca²⁺ uptake by the SR, an increase in SR Ca²⁺ loading, and ultimately more SR Ca²⁺ release to more fully activate the myofilaments [11,15,29]. This positive staircase is commonly found in non-failing human myocardium and in normal myocardium from virtually all mammalian species [4,7,30–32]. The magnitude of the relative FFR is, however, very much dependent on the size of the animal. Where humans and dogs have a relative large frequency reserve, this is smaller in the rabbit and guinea pig, and even smaller (or absent) in rat and mouse. These interspecies differences suggest quantitative and qualitative differences in intracellular Ca²⁺ handling among animals [33], and makes extrapolation of findings in small animal models to humans, difficult.

In trabeculae from failing human hearts, the reductions in DF we observed during increased stimulation frequency are consistent with the negative or blunted FFRs reported in most previous human [2,11,12,14,15,18,26,29,34] and animal studies [4,7,32]. An important aspect of the present experiments is the demonstration that the impaired FFR in failing

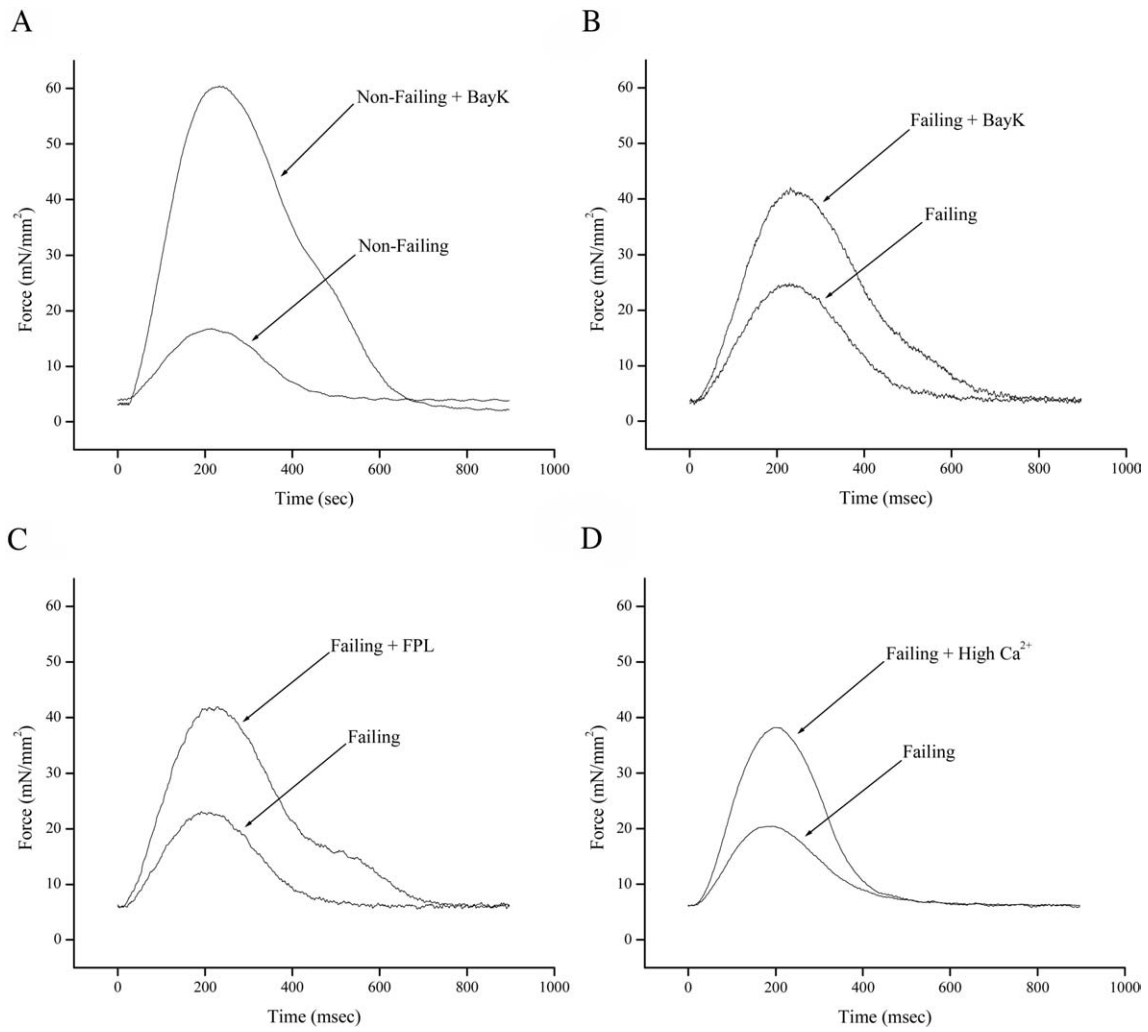


Fig. 2. Representative steady-state twitch tracings of trabeculae from non-failing and failing human hearts at 0.5 Hz stimulation frequency, and (1.75 mM Ca²⁺). (A) Non-failing before and after addition of BayK (0.25 μ M). (B) Failing before and after addition of BayK (0.25 μ M). (C) Failing before and after addition of FPL (0.10 μ M). (D) Failing before and after addition of high Ca²⁺ (7.0 mM). All tracings are raw, not averaged.

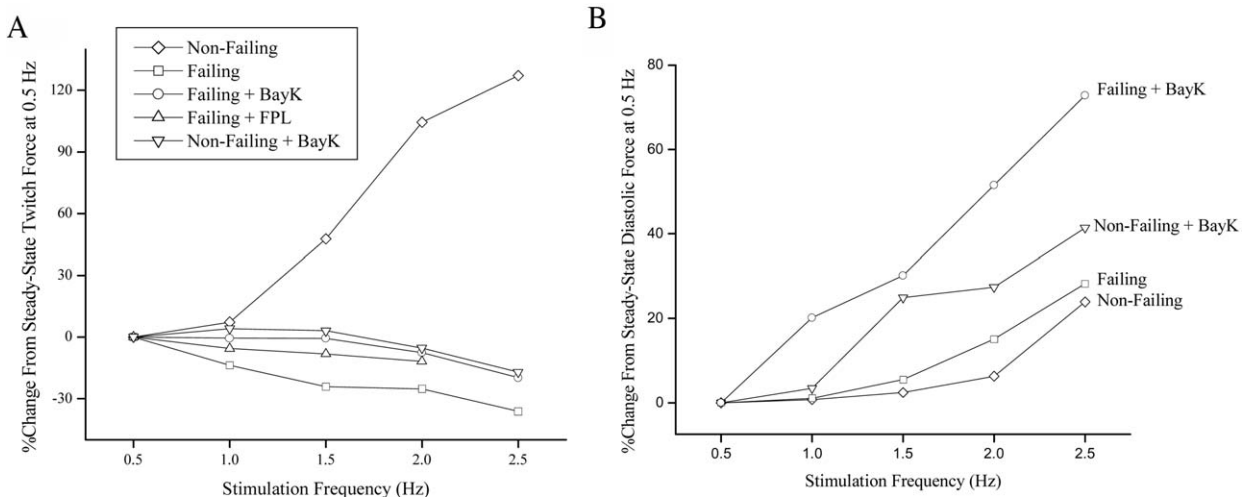


Fig. 3. (A) Average percent change from steady-state twitch force at 0.5 Hz as a function of stimulation frequency in non-failing myocardium (\diamond), failing myocardium (\square), failing + FPL (0.10 μ M) (\triangle), failing + BayK (0.25 μ M) (\circ), and non-failing + BayK (0.25 μ M) (∇). (B) Average percent change from steady-state diastolic force at 0.5 Hz as a function of stimulation frequency in paired non-failing myocardium (\diamond), failing myocardium (\square), failing + BayK (0.25 μ M) (\circ), and non-failing + BayK (0.25 μ M) (∇).

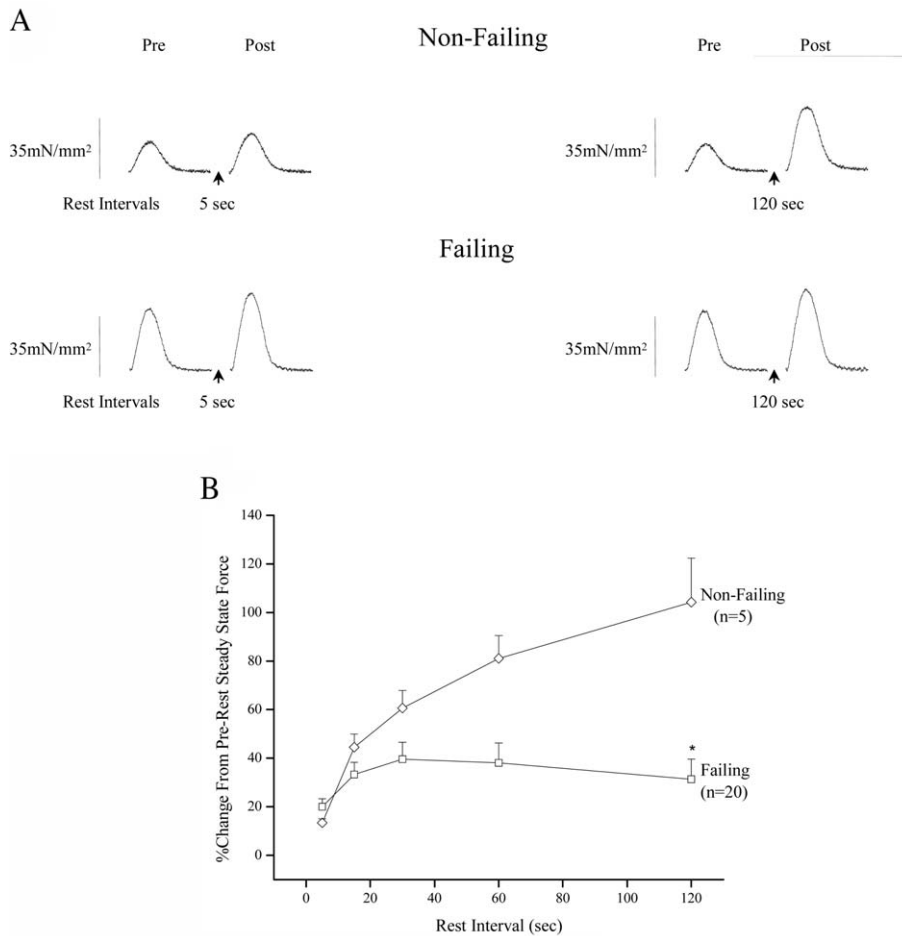


Fig. 4. (A) Representative twitch tracings of PR behavior in trabeculae from non-failing and failing human hearts. Rest intervals were 5 and 120 s. Basal stimulation frequency was 0.5 Hz. All tracings are raw, not averaged. (B) Average percent change of PR isometric twitch force from pre-rest steady-state isometric twitch force as a function of increasing rest intervals in non-failing myocardium (\diamond) and failing myocardium (\square). * $P < 0.05$ when compared between groups.

human myocardium is a consistent feature of cardiomyopathic phenotype even when basal DF is normal or supranormal. In fact, if the high force at 0.5 Hz in trabeculae from failing hearts is itself viewed as indicative of “inappropriate” Ca^{2+} regulation, the responses to increased pacing rates simply represent another manifestation of the same defect.

To determine whether the negative FFRs in failing myocardium was due to a general inability to increase contractility, we treated trabeculae with two different L-CaCh agonists, BayK and FPL, and increased extracellular Ca^{2+} . Each of these maneuvers should increase Ca^{2+} influx into the myocyte and should increase contractility via an increase in SR Ca^{2+} loading [24,35,36]. These approaches were chosen to increase SR Ca^{2+} load without directly triggering any downstream signaling pathways associated with PKA- or PKC-dependent agonists. Both trabeculae from non-failing and failing hearts had a significant increase in DF upon application of L-CaCh agonists or high bath Ca^{2+} , without a rise in diastolic force, suggesting that the increased Ca^{2+} was sequestered into the SR. This large increase in DF with no

change in diastolic force strongly suggests that the SR, in both trabeculae from failing and non-failing hearts, is not maximally loaded with Ca^{2+} under basal conditions (0.5 Hz, 1.75 mM Ca^{2+}). Interestingly, the maximum level of DF, reached by both non-failing and failing preparations after being treated with the agonists, was not significantly different. These results are consistent with the idea that L-CaCh agonists might have allowed the SR to become fully loaded with Ca^{2+} , and that the maximal SR Ca^{2+} load may be similar in failing and non-failing myocardium.

After treatment with BayK or FPL, trabeculae from failing hearts continued to exhibit negative FFRs. Given that BayK and FPL responses suggest that SR Ca^{2+} loading is not maximal in failing myocardium under our basal conditions, the persistent negative FFR after BayK implies that an alternative mechanism must be at work. These results differ from the findings of Reuter et al. [34], who observed that addition of BayK to failing human papillary muscle was able to restore the positive FFR, normally found in non-failing myocardium. This disparity likely reflects the fact that Reuter et

al. used a lower dose of the BayK and this resulted in less than maximal SR Ca^{2+} loading at slow pacing rates. In trabeculae from non-failing hearts, which previously exhibited a positive FFR, there was also a blunted response to an increase in stimulation frequency after BayK. These findings support our hypothesis that at slow pacing rates, our dose of BayK caused maximal SR Ca^{2+} loading. We conclude that the shape of the FFR (positive or negative) is substantially influenced by the state of SR loading at slow pacing rates. If SR Ca^{2+} loading is near maximal levels at slow pacing rates, then only flat or negative FFRs will be observed. If SR Ca^{2+} loading is low at slow pacing rates, then increased frequency can further enhance loading and produce positive FFRs. Although our results suggest that SR Ca^{2+} loading is not maximal at slow pacing rates in trabeculae from failing hearts, this does not preclude the possibility that the “inappropriately” high Ca^{2+} load, at slow stimulation frequencies, might contribute to the negative FFR.

4.3. Post-rest responses

Previous studies show that the shape of the PR (potentiation or decay) relationship is determined by the relative capacity of SERCA to load the SR with Ca^{2+} vs. the capacity of the NCX to extrude Ca^{2+} [16,25]. In our studies, we observed much greater PR potentiation in trabeculae from non-failing hearts than in trabeculae from failing hearts. In this context, the decreased PR potentiation observed in trabeculae from failing hearts likely reflects a functional decrease in SERCA activity relative to forward mode NCX activity during the rest interval, as we have recently shown in more biophysical studies involving single myocytes [8]. However, pinpointing the exact cause of such a functional imbalance is difficult with the techniques used in this study.

4.4. Limitations

There are a number of important limitations to this study. First, cardiac trabeculae were derived from patients with different heart failure etiologies, who varied somewhat in their medications and other factors. Nevertheless, the consistency of abnormal force–frequency, agonist-induced, and PR response patterns in trabeculae from failing hearts suggests that these are typical features of advanced human cardiomyopathy irrespective of underlying etiology. Another important distinction between the present study and previous work is the use of right ventricular trabeculae. Morphologically, right ventricular trabeculae are advantageous in that they are typically spared from involvement with myocardial infarction. Also, as they are very thin, they are less prone to core ischemia during *in vitro* experiments requiring increased metabolic demand. Moreover, because left ventricular failure is more prominent in most patients requiring cardiac transplantation, use of right ventricular trabeculae may provide an opportunity to examine a somewhat less severe degree of cardiomyopathy. Indeed, the basal DF observed in our failing

right ventricular myocardium is higher than that observed in most previous studies employing human left ventricular trabeculae [11,16]. Another concern relates to the possibility that pharmacologic L-CaCh agonists may have other effects on the myocardium [37]. By demonstrating that two alternative L-CaCh agonists, BayK and FPL, as well as an increase in bath Ca^{2+} , all increase basal contractile force in failing myocardium without correcting the abnormal force–frequency behavior, we have tried to address concerns about potential non-specific pharmacological effects.

5. Conclusions

In summary, the present studies demonstrate that the abnormal FFR observed in failing human myocardium is a reliable pathophysiologic signature of the cardiomyopathic state even when contractility at slow stimulation frequencies is not reduced. These studies further demonstrate that the impaired FFR is not due to a generalized inability to increase DF suggesting that failing myocytes can further increase their SR Ca^{2+} load. Rather, our findings are most consistent with the interpretation that abnormal FFRs and blunted PR potentiation alike are a reflection of an altered functional balance between key Ca^{2+} -handling proteins, namely SERCA and the NCX. Future studies are needed to define, which maneuvers for altering the functional balance between SERCA and the NCX can correct the abnormal FFRs. Treatments that normalize the FFR in failing hearts are likely to improve myocardial contractility reserve and exercise tolerance among patients with advanced cardiomyopathy.

Acknowledgements

The authors wish to express their gratitude to fellow laboratory members, the Cardiovascular Research Group, and the Temple University Hospital Cardiac Transplant Team for their assistance with these studies. This research was supported by grants from the National Institutes of Health, Bethesda, MD (HL33921 and HL61495 to S.R.H. and HL03560 and AG17022 to K.B.M.), from the Southeastern Pennsylvania Affiliate of the American Heart Association (0110063U to E.I.R.), and from the W.W. Smith Foundation (to K.B.M.).

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