Diminished Post-Rest Potentiation of Contractile Force in Human Dilated Cardiomyopathy

Functional Evidence for Alterations in Intracellular Ca^{2+} Handling

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Abstract

Post-rest contractile behavior of isolated myocardium indicates the capacity of the sarcoplasmic reticulum (SR) to store and release Ca^{2+}. We investigated post-rest behavior in isolated muscle strips from nonfailing (NF) and end-stage failing (dilated cardiomyopathy [DCM]) human hearts. At a basal stimulation frequency of 1 Hz, contractile parameters of the first twitch after increasing rest intervals (2–240 s) were evaluated. In NF ($n = 9$), steady state twitch tension was 13.7 ± 1.8 mN/mm^2. With increasing rest intervals, post-rest twitch tension continuously increased to maximally 29.9 ± 4.1 mN/mm^2 after 120s ($P < 0.05$) and to 26.7 ± 4.5 mN after 240 s rest. In DCM ($n = 22$), basal twitch tension was 10.0 ± 1.5 mN/mm^2 and increased to maximally 13.6 ± 2.2 mN/mm^2 after 20 s rest ($P < 0.05$). With longer rest intervals, however, post-rest twitch tension continuously declined (rest decay) to 4.7 ± 1.0 mN/mm^2 at 240 s ($P < 0.05$). The rest-dependent changes in twitch tension were associated with parallel changes in intracellular Ca^{2+} transients in NF and DCM (aequorin method). The relation between rest-induced changes in twitch tension and aequorin light emission was similar in NF and DCM, indicating preserved Ca^{2+}-responsiveness of the myofilaments. Ryanodine (1 μM) completely abolished post-rest potentiation. Increasing basal stimulation frequency (2 Hz) augmented post-rest potentiation, but did not prevent rest decay after longer rest intervals in DCM. The altered post-rest behavior in failing human myocardium indicates disturbed intracellular Ca^{2+} handling involving altered function of the SR. (*J. Clin. Invest.* 1996. 98:764–776.) Key words: myocardial function • excitation-contraction-coupling • sarcoplasmic reticulum • aequorin • calcium handling

Introduction

Short periods of rest increase force of contraction of the first beat upon restimulation in isolated myocardium of most mammals (1) which was suggested to result from an increased amount of activator Ca^{2+} released from the sarcoplasmic reticulum (2, 3). Therefore, the influence of rest periods on post-rest isometric force is considered to be an index for the amount of Ca^{2+} released from the sarcoplasmic reticulum (SR). In animal species in which SR Ca^{2+} release is of minor relevance for contraction, such as the frog, post-rest potentiation is absent (4). However, as SR function becomes more important for excitation-contraction coupling processes, the potentiation of the first isometric twitch after a rest period gets more pronounced. In rat myocardium, having a strongly developed sarcoplasmic reticulum, post-rest potentiation is very pronounced even after long rest periods (3, 5).

In the human heart, SR function is considered to be of major importance for excitation-contraction coupling processes. According to Fabiato (6), it is believed that only a relatively small amount of Ca^{2+} enters the cell through the voltage-dependent sarcolemmal Ca^{2+} channels during depolarization. This "trigger" Ca^{2+} induces the release of a larger amount of Ca^{2+} from the SR which in turn is responsible for activation of the contractile proteins, and thus, contraction. Relaxation occurs as Ca^{2+} dissociates from the contractile apparatus and is sequestered into the SR by SR Ca^{2+} pumps or extruded from the cell mainly via the sarcolemmal Na^+/Ca^{2+} exchanger. Abnormalities in contractile function may result from altered intracellular Ca^{2+} handling or altered Ca^{2+} responsiveness of the myofilaments. Gwathmey et al. (7) and Morgan et al. (8) were the first to demonstrate prolonged intracellular Ca^{2+} transients and isometric twitches in hypertrophic and failing human myocardium. Disturbed excitation-contraction coupling and intracellular Ca^{2+} handling has been suggested from myothermal measurements, showing reduced Ca^{2+} cycling in association with reduced force generation in failing dilated cardiomyopathy (9). Accordingly, FURA-2 measurements showed reduced systolic Ca^{2+} transients in isolated myocytes from failing human hearts (10). In addition, the inverse force-frequency relation in failing human myocardium (11, 12) has been related to reduced systolic Ca^{2+} transients (13). In contrast, Gwathmey et al. (14) described an inverse force-frequency relation, but a positive aequorin light-frequency relation in failing human myocardium. The authors concluded that abnormalities of contractile function in failing human myocardium are not due to decreased availability of intracellular Ca^{2+}, but more likely reflect differences in myofibrillar Ca^{2+} responsiveness.

The recent finding of a close correlation between altered force-frequency behavior and reduced SR Ca^{2+} ATPase pro-

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1. *Abbreviations used in this paper:* SR, sarcoplasmic reticulum; TPT, time to peak tension.

tein levels suggests that SR Ca^{2+} handling may determine contractile function (15). Although there are contradictory results (16, 17), several studies demonstrated a reduction of SR Ca^{2+} ATPase gene expression, protein levels, and in vitro Ca^{2+} accumulation into the SR in failing hearts (13, 18–21). In addition, activity and expression of mRNA- and protein level of the sarcolemmal Na^+/Ca^{2+}-exchanger are increased in failing myocardium (22, 23). However, the relevance of these alterations for intracellular Ca^{2+} handling and contractile function are unclear.

Post-rest potentiation of force of contraction was suggested to reflect the amount of Ca^{2+} accumulated within and released from the SR (2, 3). Therefore, reduced SR Ca^{2+} accumulation and release capacity in association with increased activity of the Na^+/Ca^{2+}-exchanger in the failing human heart may result in disturbed post-rest behavior. Accordingly, the present study was performed to test the hypothesis that (1) post-rest potentiation of force of contraction is present in the nonfailing human heart and depends on Ca^{2+} released from the sarcoplasmic reticulum, and (2), post-rest potentiation of force of contraction and intracellular Ca^{2+} transients are diminished in the failing human myocardium.

Methods

Myocardial tissue. Experiments were performed in isolated, electrically driven left ventricular trabeculae from human myocardium. The preparations were obtained from a total of 12 nonfailing hearts which could not be transplanted for technical reasons and from 31 end-stage failing hearts due to idiopathic dilated cardiomyopathy at the time of heart transplantation. The age in the control group was 39±8 yr, 5 of the donors were female and 7 were male. All donors had normal left ventricular function. Coronary artery disease or valvular disease had been excluded in all patients with heart failure before transplantation. Heart failure patient's data, hemodynamic parameters and premedications are shown in Table I.

Table I. Patients' Data and Hemodynamic Parameters

Patients	Sex	Age	EF	PCW	CI	Previous medications
		yr	*%*	*mmHg*	*liter/min/m²*	
H.R.	F	48	20	30	2.1	Diu,ACEI, Dig, CaA, Nit
H.R.	M	48	20	24	1.8	Diu, ACEI, Dig, CaA, Cat
N.M.	M	29	15	29	2.1	Diu, ACEI, Dig
K.B.	F	27	22	29	1.8	Diu, ACEI, Cat
H.S.	M	28	26	19	2.7	Diu, ACEI
K.E.	M	46	16	28	2.2	Diu, ACEI, Dig, Cat
S.E.	M	55	15	26	1.0	Diu, ACEI, Dig, Nit, Cat, PDEI
C.A.	M	32	16	25	2.3	Diu, ACEI, Nit, Cat
J.H.	M	57	30	24	1.5	Diu, ACEI, Dig, Nit, Cat
H.U.	F	50	39	37	1.6	Diu, ACEI, Dig, Nit, Cat, PDEI
L.N.	F	38	25	37	2.9	Diu, ACEI, Dig, CaA, PDEI
M.J.	M	16	24	30	3.7	Diu, ACEI, Cat, PDEI
K.W.	M	49	31	26	3.3	Diu, ACEI, Dig, Nit, Cat
A.K.	F	51	13	40	2.5	Diu, ACEI, Nit, Dig, PDEI
K.H.	M	48	20	27	2.2	Diu, ACEI, Dig, Amiodarone, Cat, PDEI
K.S.	F	17	21	13	3.0	Nit, Amiodarone
L.H.	M	39	22	33	2.4	Diu, ACEI, Dig, Cat
A.E.	M	33	20	30	2.5	Diu, ACEI, Dig, Nit
K.W.	M	61	22	29	2.4	Diu, Dig
F.H.	M	55	26	17	1.5	Diu, ACEI, Dig,
L.G.	M	65	20	22	1.9	Diu, ACEI, Dig,
J.S.	M	66	11	—	1.3	Diu, ACEI, Dig,
L.H.	M	61	14	26	—	Diu, Dig, Nit, CaA,
S.E.	F	33	39	—	1.6	Diu, Dig, PDEI
W.H.	M	33	—	28	2.1	ACEI, Dig, Sotalol,
D.M.	M	25	25	9	1.3	Diu, ACEI, Dig, Amiodaron
S.H.	M	56	11	24	—	Diu, ACEI, Dig
L.P.	M	48	30	—	1.7	Diu, ACEI, Nit, Propafenone
B.H.	M	63	31	20	2.1	Diu, ACEI, Dig, Amiodarone
B.H.	M	78	17	—	—	Diu, ACEI, Dig, Nit, Metoprolol
W.P.	M	4	—	20	—	Diu, ACEI, Dig, Nit, Nitroprussid
mean±SEM		42.5±3.2	22±1	26±1	2.1±0.1	

M, male; F, female; EF, ejection fraction; PCW, pulmonary capillary wedge pressure; CI, cardiac index; Diu, diuretics; ACEI, ACE inhibitor; Dig, digitoxin; CaA, calciuantagonist; Cat, catecholamines; PDEI, phosphodiesterase inhibitor.

The study protocol was reviewed and approved by the Ethical Committee of the University Clinics of Freiburg.

Muscle strip preparation. Immediately after explantation, the left ventricle was carefully opened and a thin layer of the subendocardial muscle wall was excised from the myocardium. Special attention was given to avoid damage of the fine trabecular network at the inner surface of the ventricle. The excised myocardium was stored in a special cardioplegic solution which was oxygenated with carbogen (95% O_2, 5% CO_2) and transported to the laboratory at room temperature. The solution contained (in mM): Na^+ 152, K^+ 3.6, Cl^- 135, HCO_3^- 25, Mg^{2+} 0.6, $H_2PO_4^-$ 1.3, SO_4^{2-} 0.6, Ca^{2+} 2.5, glucose 11.2 and 2,3-butanedione-monoxime 30. 10 IU/l Insulin were added. This special cardioplegic solution was shown to protect the myocardium during transportation and from cutting injury at the time of dissection (24). Its effects on the myocardium were fully reversible after washout (24). There was no influence of BDM on post-rest behavior ($n = 4$ muscle strip preparations from 2 hearts; data not shown). Small trabeculae were dissected with the help of a stereo-microscope and specially designed dissection chambers. All preparation steps were carried out in the cardioprotective solution. The trabeculae were then mounted horizontally in a cylindrical glass cuvette between miniature clamps and connected to an isometric force transducer (OPT1L, Scientific Instruments, Heidelberg, Germany) and superfused with a carbogen-bubbled modified Krebs-Henseleit solution of the composition given above, with the exception that 2,3-butanedione monoxime was omitted. The pH of the bathing solution was 7.4, all experiments were performed at a physiological temperature of 37°C. Muscle preparations were electrically stimulated by field stimulation or punctate stimulation via platinum electrodes (aequorin measurements). Stimulation voltage was set 20% above threshold. The means of stimulation did not influence post-rest behavior. After initially prestretching the muscle strips with 1 mN, they were allowed to equilibrate for 30 to 60 min at a stimulation frequency of 1 Hz. Thereafter, the muscles were gradually stretched along their length-tension curve in 0.05-mm steps until maximum isometric tension was reached (l_{max}).

Aequorin measurements. Intracellular Ca^{2+} transients were recorded using the Ca^{2+}-regulated bioluminescent photoprotein aequorin. 1 mg of lyophilized aequorin powder was dissolved in 700 μl of solution of the following composition (in mM): 154 NaCl, 5.4 KCl, 1.0 $MgCl_2$, 12 Hepes, and 0.1 EDTA, pH 7.4. Simultaneous measurement of aequorin light signals and isometric force was performed using a specially designed setup (Scientific Instruments, Heidelberg, Germany) as described previously (13). Briefly, the muscle preparation was placed in a horizontally mounted glass cuvette (internal diameter, 5 mm, length 15 mm) superfused with temperature-adjusted (37°C), oxygenated Tyrode's solution and attached to an isometric force transducer through the open ends of the glass cuvette. As for the contraction experiments, muscles were prestretched to l_{max}. By the time of complete mechanical stabilization of the muscle strips, electrical stimulation was discontinued for 5 min, and the flow of the superfusate was temporarily stopped. Aequorin was macroinjected into the quiescent muscle according to the method described by Kihara and Morgan (25) and is similar to the protocol of Urthaler et al. (26), who reported excellent beat-to-beat measurements of Ca^{2+} and tension in ferret cardiac trabeculae after macroinjection. Macroinjection was achieved by using a fine-tipped glass micropipette (external diameter 7–8 μm). 1–2 μl of dissolved aequorin was injected just beneath the endocardium of the muscle preparation by air pressure under microscopic control. The rate of injection was slow and required 30–60 s. It was not necessary to reduce the extracellular Ca^{2+} concentration in the Tyrode's solution before macroinjection. After macroinjection, superfusion and electrical stimulation was resumed after 1 min. The aequorin light signal was detected using a photomultiplier tube (XP 2802; Philipps) which was vertically mounted with its cathode just above the glass cuvette containing the aequorin-loaded muscle. To increase the optical efficiency of the system, an ellipsoidal mirror was placed beneath the glass cuvette reflecting photons to the photomultiplier tube. Light emissions (in mV photomultiplier output) and force signals were recorded simultaneously on-line on a personal computer and an oscilloscope with signal-averaging function (Nicolet PRO 10C; Nicolet Instrument Corporation, Madison, WI) as well as on a chart strip recorder (WR 3310, Graphtec, Ettlingen, Germany) for original registration. Aequorin that had not been taken up by the myocytes was completely discharged within 10–20 min by the high Ca^{2+} concentration in the Tyrode's solution. During this time period, aequorin light signals stabilized with respect to systolic and diastolic light emission. The experimental protocol was started at the time when the aequorin light signals were completely stable. Muscles strip preparations in which isometric twitch tension declined by more than 10% during the loading procedure were discarded from the evaluation.

Experimental protocol. After complete mechanical stabilization of the muscle at a basal stimulation frequency of 1 Hz, the experimental protocol was started. For post-rest contractions, electrical stimulation of the muscle strips was switched off at steady state conditions for increasing intervals (2, 5, 10, 20, 30, 40, 50, 60, 120, and 240 s). Upon restimulation, the first beat was evaluated for post-rest changes in isometric twitch tension (mN/mm^2), maximum rate of tension rise ($+dT/dt_{max}$), maximum rate of tension decline ($-dT/dt_{max}$) time to peak tension (TPT) and time to 50% and 95% relaxation. Twitch tension (mN/mm^2) is defined as the active tension developed during the isometric twitch. It is the amplitude of the twitch signal between peak systolic tension and diastolic tension at the end of the stimulus interval. After each rest interval, the muscle preparation was stimulated at

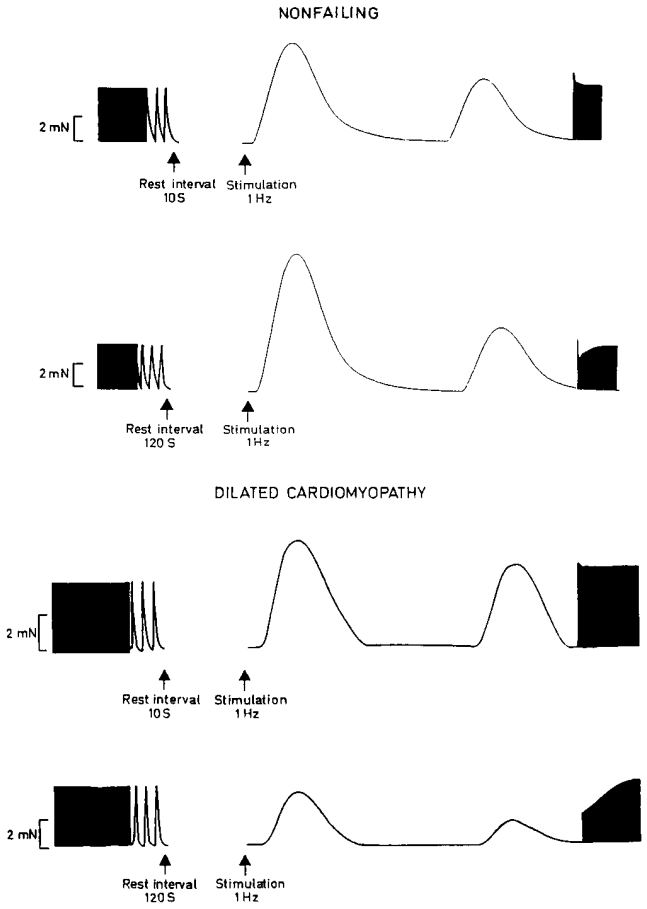

Figure 1. Influence of rest intervals on post-rest contraction. Original recordings of post-rest behavior in a muscle strip preparation from a nonfailing heart (*top*) and an end-stage failing heart (*bottom*). Rest intervals were 10 s and 120 s. Basal stimulation frequency was 1 Hz.

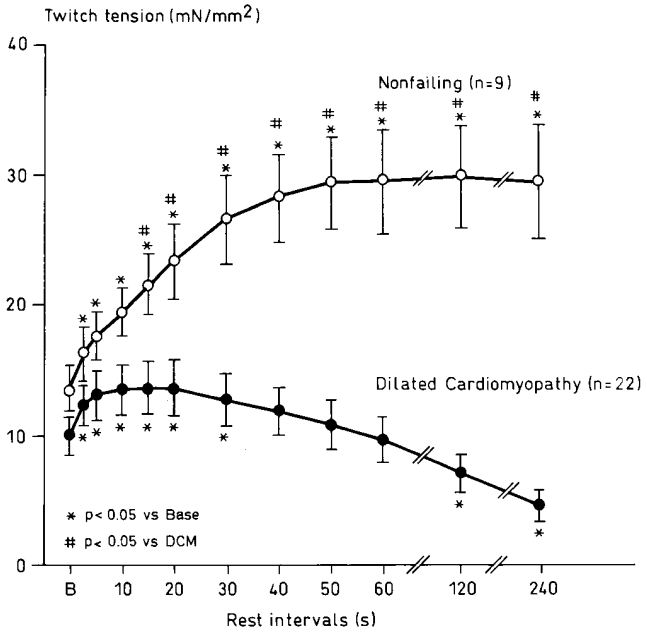

Figure 2. Average post-rest isometric twitch tension as a function of increasing rest intervals in nonfailing myocardium (O) and end-stage failing dilated cardiomyopathy (●). In nonfailing myocardium, post-rest tension was significantly increased at all rest intervals as compared to the basal value (B). In the failing myocardium, post-rest tension was significantly increased after rest periods of 2–30 s, and significantly reduced after a rest period of 120 and 240 s. Post-rest tension was significantly higher in nonfailing myocardium at rest intervals > 10 s.

the basal stimulation frequency for 5–10 min until complete mechanical stabilization, before the next rest interval was imposed. Further experiments were performed after preincubating muscle strips with ryanodine (1 μM) for 30 min at 1.0 Hz.

In additional experiments, intracellular Ca^{2+} transients were recorded simultaneously using the photoprotein aequorin. Aequorin light emission, reflecting intracellular Ca^{2+} transients, was related to isometric twitch tension at the different rest intervals. Aequorin light emission (mV amplifier output) is defined as the amplitude of the aequorin light signal between the peak systolic light emission and the diastolic light value at the end of the stimulus interval.

In addition, transsarcolemmal Ca^{2+} influx was altered by means of increasing or decreasing basal stimulation frequency to 2.0 or 0.5 Hz. Post-rest behavior of nonfailing and end-stage failing myocardium was investigated over the whole rest interval range at the higher or lower basal stimulation frequency. Furthermore, force-frequency- and post-rest behavior was determined in the same hearts. For force-frequency experiments, stimulation rate was increased in 0.5 Hz steps from 0.5 Hz to 3.0 Hz. Twitch tension was recorded at each stimulation rate at steady state. Then, stimulation rate was reduced back to 1.0 Hz, and post-rest behavior was characterized.

Cross-sectional area of the muscle strip preparations for normalization of force values was determined as the ratio of blotted muscle weight to muscle length. The trabeculae were blotted dry for 30 s with 10 g. The specific weight of the muscle was assumed as 1. Average cross-sectional area of the muscle strips was 0.26±0.03 mm^2 in the control group and 0.29±0.02 mm^2 in the heart failure group (difference not significant).

Materials. Aequorin was purchased in lyophilized form from J.R. Blinks, Friday Harbor Photoproteins (Friday Harbor, WA). Ryanodine was purchased from Sigma Chemicals Ltd. (Deisenhofen, F.R.G.).

Statistical analysis. Average values are given as means±SEM. Comparison within one group of myocardium was performed using the paired *t* test followed by Bonferroni-Holm correction. Comparison between different groups was performed by unpaired *t* test or analysis of variance followed by Newman-Keuls procedure, when appropriate. Correlations were examined by linear regression analysis. Analysis of covariance was used to assess the significance of differences between slopes of regression lines. A P value < 0.05 was considered significant.

Results

Post-rest behavior of nonfailing and failing human myocardium. Isometric twitch tension increases with increasing rest periods

Table II. Force and Time Parameters of Post-Rest Contractions at 1 Hz

Rest interval	Twitch tension	TT	TPT	RT_{50}	RT_{95}	+dT/dt	−dT/dt
s	*mN/mm²*	*ms*	*ms*	*ms*	*ms*	*mN/s/mm²*	*nN/s/mm²*
Nonfailing ($n = 9$)							
Base	13.7±1.8	501±42	165±7	116±6	334±43	138.2±18.1	108.6±15.6
5	17.4±1.9*	565±70	174±7	129±9*	390±68	169.1±14.6*	128.8±17.8*
10	19.6±2.1*	571±72	173±9	131±9*	398±70	204.4±24.8*	137.6±15.8*
30	26.6±3.4*	562±67	179±10	130±9*	393±63	281.7±46.5*	178.8±29.0*
120	29.9±4.1*	518±45	170±6	110±6	335±43	313.1±61.5*	200.3±41.1*
240	26.7±4.5*	465±43	169±10	107±4	327±44	282.5±65.9*	202.9±49.0*
Dilated Cardiomyopathy ($n = 22$)							
Base	10.0±1.5	499±16	186±5	117±4	311±12	95.1±15.0	80.3±11.1
5	13.1±2.0*	519±16*	194±7	128±5*	329±14*	112.2±17.2*	90.8±11.2*
10	13.3±2.0*	523±19	199±6*	130±6*	333±17	112.7±16.6*	91.4±10.7*
30	12.9±2.1*	526±21	199±8*	132±7*	339±20	102.0±15.7	83.9±10.2
120	7.1±1.5*	505±20	184±7	126±5	324±16	57.8±12.6*	43.4±8.0*
240	4.7±1.0*	476±24	179±7	127±6	319±16	40.5±11.1*	25.3±5.1*

TT, total twitch time; TPT, time to peak tension; RT_{50}, time to 50% relaxation; RT_{95}, time to 95% relaxation; +dT/dt, maximal tension rise; −dT/dt, maximal tension decline. *$P < 0.05$ vs. Base.

in non-failing myocardium (Fig. 1, *top*). As can be seen, the amplitude of the first post-rest beat was increased from 4.5 to 6.8 mN after 10 s rest interval, and from 4.3 to 11.9 mN after 120 s rest interval. In a muscle strip from an end-stage failing heart (Fig. 1, *bottom*), twitch amplitude increased after 10 s rest interval (from 3.9 to 5.9 mN) but decreased after the 120 s rest interval (from 3.8 to 3.5 mN).

Fig. 2 shows the relation between twitch tension of the first beat after restimulation and the duration of the rest interval in muscle strips from 9 nonfailing and 22 end-stage failing hearts. At a basal stimulation frequency of 1 Hz, rest intervals were stepwise increased from 2 to 240 s. In nonfailing myocardium, isometric twitch tension of the first post-rest beat continuously increased with increasing rest periods. Post-rest potentiation of tension was maximum at a rest interval of 120 s (increase from 13.7±1.8 mN/mm^2 to 29.9±4.1 mN/mm^2, $P < 0.05$) and then remained stable at 240 s rest interval. In contrast, in end-stage failing myocardium, twitch tension of the first post-rest beat increased slightly at short rest intervals (from 10.0±1.5 mN/mm^2 to 13.6±2.2 mN/mm^2 at 20 s rest interval; $P < 0.05$), and then continuously declined at longer rest intervals to 4.7± 1.0 mN/mm^2 at 240 s rest interval ($P < 0.05$ vs. the steady state value at 1 Hz). At rest intervals longer than 10 s, post-rest twitch tension was significantly higher in nonfailing as compared to failing myocardium. The rundown of steady state isometric twitch tension over the whole experiment was not significantly different between the two groups: from 13.7±1.8 to 11.1±1.9 mN/mm^2 in the nonfailing and from 10.0±1.5 to 7.8±1.3 mN/mm^2 in the failing myocardium. Interval-dependent changes in twitch tension, maximum rate of tension rise and fall and time parameters of the isometric twitch are given in Table II.

Influence of patient's age, ejection fraction and drug treatment on post-rest response in failing myocardium. To test for the influence of age, patients have been divided into three groups: age < 35 yr ($n = 7$), 35–60 yr ($n = 7$) and > 60 yr ($n = 8$).

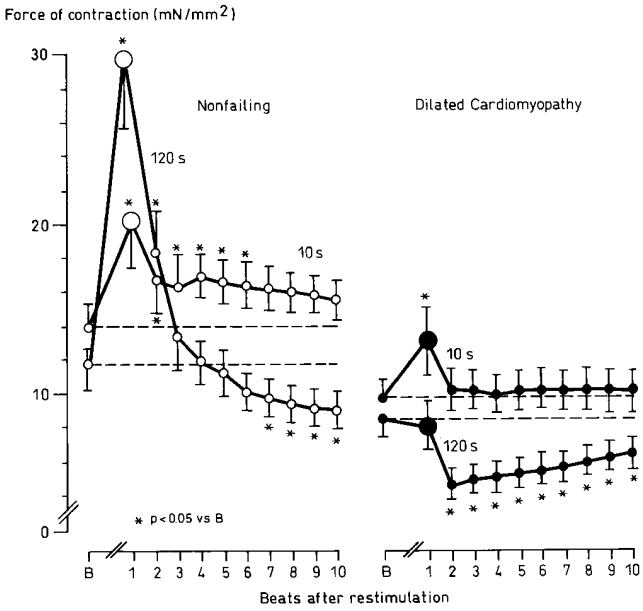

Figure 3. Staircase of contraction of the first 10 beats after restimulation. (*Left*, nonfailing myocardium; *right*, failing myocardium). In nonfailing myocardium, twitch tension was significantly increased at beat 1 after restimulation after 10 s rest and then continuously declined, reaching pre-rest levels after 6 beats. After 120 s rest, twitch tension was significantly increased at beat 1 and 2, but then declined below pre-rest tension values. Pre-rest tension levels were reached after 16 beats. In failing myocardium, twitch tension was potentiated after 10 s rest and was no longer significantly different from pre-rest tension at beat 2. After 120 s rest, no potentiation occurred at beat 1, and beat 2 was significantly below steady state tension. Twitch tension then gradually increased and reached pre-rest tension at beat 24.

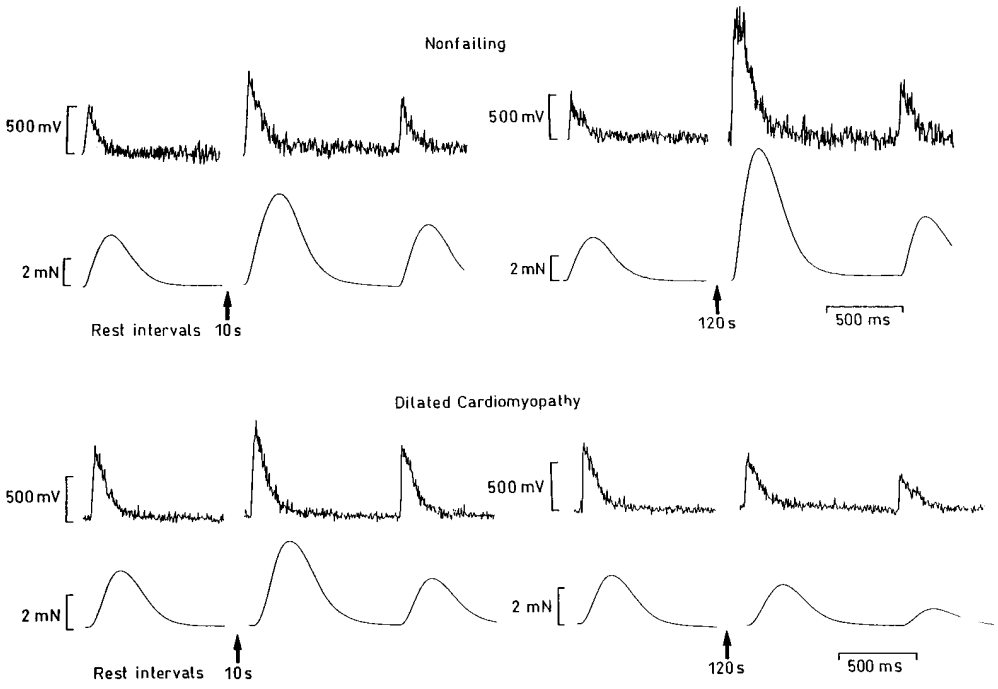

Figure 4. Original tracings of single aequorin light signals and the corresponding isometric twitch. Signals are not averaged. Upper panel, nonfailing myocardium; lower panel, failing myocardium. Steady state pre-rest signals and the first and second post-rest signal are shown for rest intervals of 10 and 120 s.

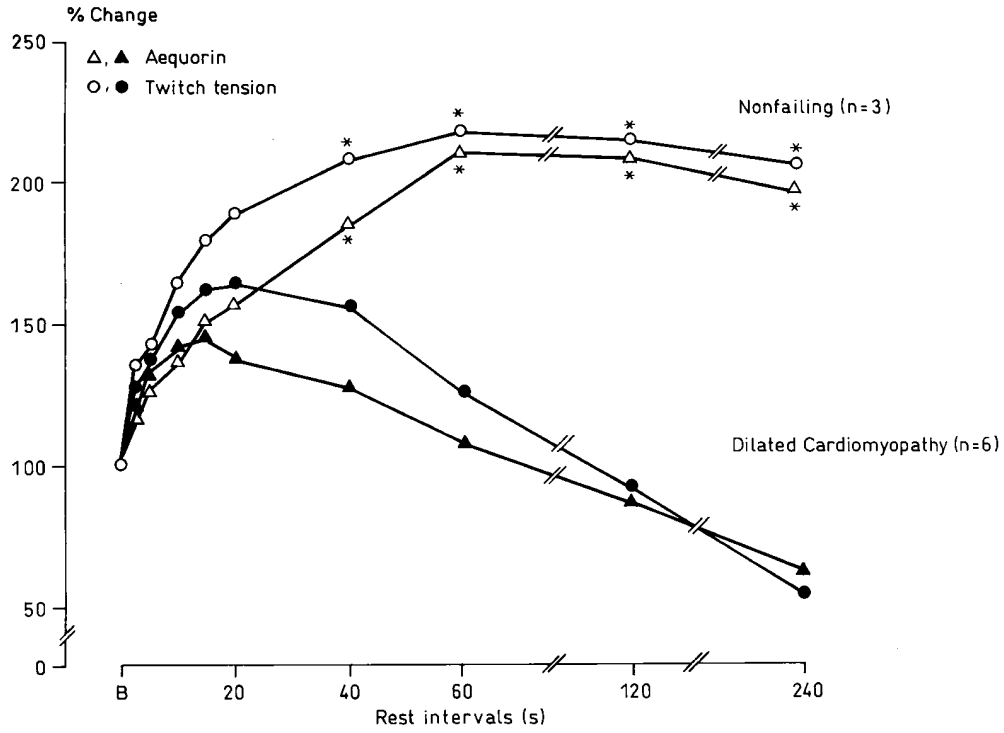

Figure 5. Average post-rest change in aequorin light emission and isometric twitch tension after increasing rest intervals in three muscle strips from three nonfailing hearts and in six muscle strips from six failing hearts (% change of basal value (*B*)). In nonfailing myocardium, post-rest twitch tension was significantly higher than base at all rest intervals and post-rest aequorin light emission at rest intervals between 5 and 60 s. In failing myocardium, both twitch tension and aequorin light were significantly higher than base at rest intervals between 2 and 40 s and significantly lower than base at a rest interval of 240 s. Post-rest aequorin light and twitch tension were significantly higher in nonfailing as compared to failing myocardium between 40 and 240 s rest interval.

Mean age in the three groups was 25±4, 49±2, and 63±2 yr ($P < 0.05$). Mean post-rest twitch tension after a 120 s rest interval was 76.4±10.2% (range: 44–114%), 91.7±13.4 (range: 50–154%) and 92.9±18.1% (range: 25–171%) of the steady state value, respectively. Analysis of variance showed no significant difference in post-rest behavior between the three age groups. The influence of ejection fraction as a measure of cardiac function on post-rest behavior was tested by dividing patients into three groups: EF < 20% ($n = 8$), 21–29% ($n = 9$) and > 29% ($n = 5$). Mean ejection fractions in the three groups were 15.0±1.0, 24.1±0.4 and 34.0±2.0% ($P < 0.05$). Mean post-rest twitch tension after a 120 s rest interval was 91.9±17.5% (range: 27–171%), 77.6±12.8% (range: 25–154%), and 96.8±8.2% (range: 69–120%) of the basal value, respectively. There was no significant difference between the three groups. In addition, analysis of variance was performed for the effect of age on post-rest behavior in failing myocardium using ejection fraction as covariant. Again, no effect of age or ejection fraction on the data could be detected.

To test for the influence of premedication on post-rest behavior, patients without ACE-inhibitors ($n = 3$), without digitalis ($n = 4$), with catecholamines ($n = 4$) or catecholamines and phosphodiesterase inhibitors ($n = 4$) before transplantation have been identified from Table I. No significant differences in post-rest behavior between these groups could be detected. According to Harding et al. (27), patients were divided by the daily diuretic dose prescribed before transplantation: furosemide (mg) 0–40 ($n = 4$), 80 ($n = 4$), 120 ($n = 5$), and

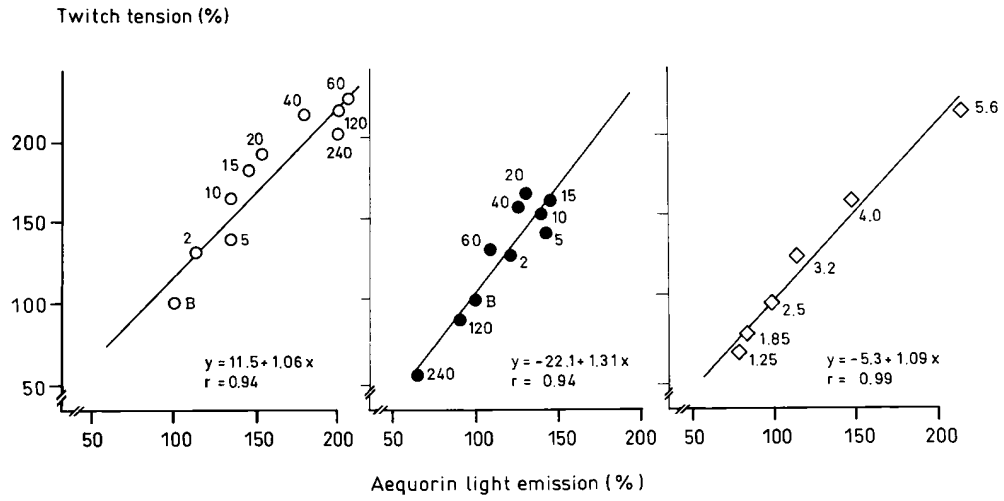

Figure 6. Change of post-rest peak twitch tension (in%; ordinate) plotted versus peak aequorin luminescence (in%; abscissa) at increasing rest intervals in nonfailing (*left*; $n = 3$) and end-stage failing (*middle*; $n = 6$) myocardium. Numbers denote rest intervals. For comparison, the plot of peak twitch tension versus peak aequorin luminescence for increasing concentrations of extracellular Ca^{2+} (1.25 to 5.6 mM; % change from the value at 1.25 mM) is given on the right for nonfailing myocardium ($n = 6$). There was no significant difference between the slopes of the regression lines of the three groups.

250–500 (n = 6). In three cases, the actual dose of diuretics could not be obtained from the records. No correlation between the dose of diuretics and post-rest behavior could be detected.

Staircase of contraction following restimulation. In nonfailing myocardium, average isometric twitch tension continuously declined after the potentiated beat after 10 s rest and was statistically no longer different from pre-rest twitch tension after 6 beats (Fig. 3, *left*). After 120 s rest, isometric twitch tension steeply declined after the potentiated beat and fell below pre-rest values. Twitch tension slowly recovered and was no longer statistically different from pre-rest values after 16 beats. In failing myocardium (Fig. 3, *right*), average post-rest twitch tension declined to pre-rest values at the second beat after 10 s rest. After 120 s, twitch tension of the second beat after restimulation fell below the first beat and then slowly recovered during the following 24 beats. During the rest intervals, there was no significant change in diastolic tension in both types of myocardium.

Intracellular Ca^{2+} handling. Fig. 4 shows typical original recordings of the last beat before the rest interval and the first and second beat after restimulation. Aequorin light signals (in mV) and the corresponding isometric twitches (in mN) from a muscle strip from a nonfailing heart (upper panel) and an end-stage failing heart (lower panel) are shown. Rest intervals were 10 and 120 s. In the preparation from a nonfailing heart, aequorin light emission and the corresponding isometric twitch increased after 10 s and even more pronounced after 120 s rest interval. In the preparation from the end-stage failing heart, aequorin light emission and the corresponding isometric twitch slightly increased upon restimulation after 10 s rest, but decreased after 120 s rest. The parallel changes in aequorin light emission and isometric twitch tension could be seen over the whole range of rest periods (Fig. 5). In nonfailing myocardium (n = 3), isometric twitch tension and aequorin light emission increased to maximally 217±23% and 207±57%, respectively, after a rest interval of 60 s ($P < 0.05$) and remained stable at longer rest intervals. In failing myocardium (n = 6), isometric twitch tension increased to maximally 166±8% after a rest interval of 20s ($P < 0.05$) and aequorin light emission to 145±11% after a rest interval of 15 s ($P < 0.05$), and then continuously declined to 55±14% and 64±10%, respectively, after a rest interval of 240 s ($P < 0.05$ vs. pre-rest values). Poten-

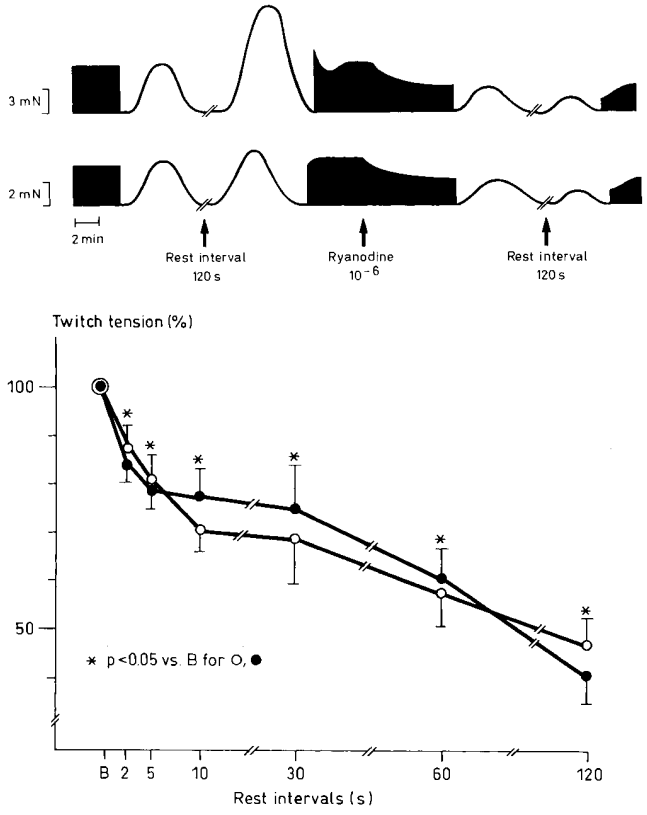

Figure 7. Effect of ryanodine on post-rest behavior. (*Top*) Original tracings in a muscle strip preparation from a nonfailing (*above*) and an end-stage failing heart (*below*). Post-rest contractile behavior (120 s) was tested before and after addition of ryanodine (1 μM). (*Bottom*) Average post-rest behavior (% change) in 4 muscle strip preparations from 3 nonfailing hearts (O) and 13 muscle strip preparations from 10 end-stage failing hearts (●) after ryanodine. Note that ryanodine converted rest potentiation to rest decay.

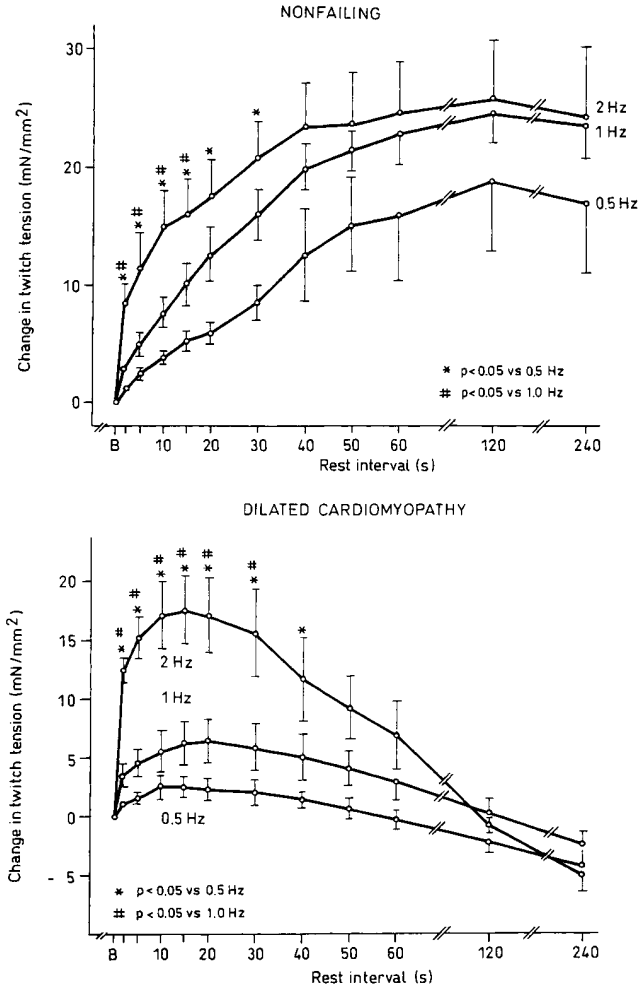

Figure 8. Influence of basal stimulation frequency on post-rest contraction in muscle strip preparations from nonfailing hearts (*top*) and from end-stage failing hearts (*bottom*). Basal stimulation frequency was 0.5, 1.0, or 2.0 Hz. The change in post-rest twitch tension is given in mN/mm^2. At 2 Hz, post-rest potentiation was significantly augmented as compared to 0.5 Hz at rest intervals between 2 s and 30 s for nonfailing and 2 s and 40 s for failing myocardium.

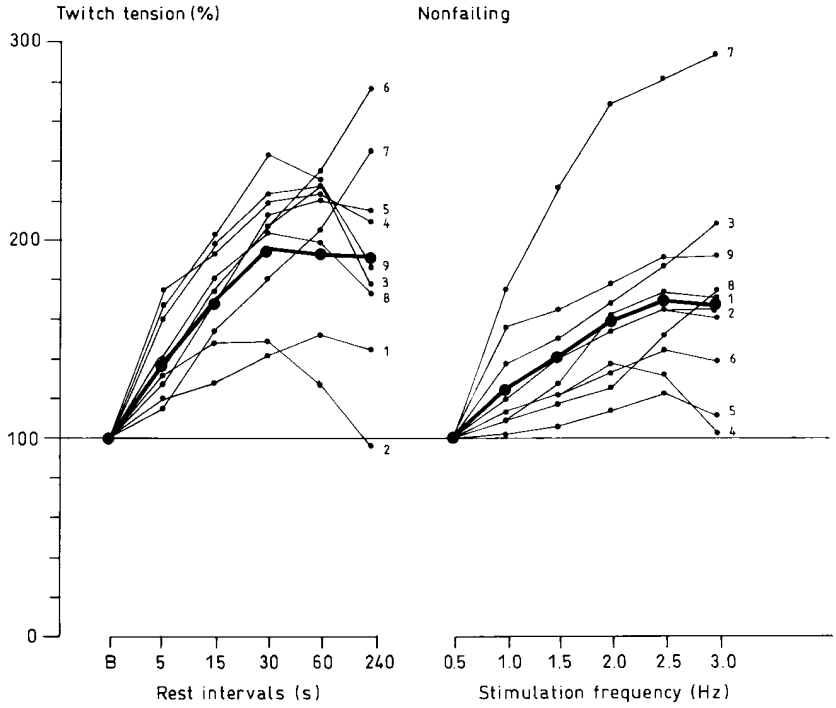

Figure 9. Post-rest and force-frequency behavior in human nonfailing myocardium ($n = 9$). % change in isometric twitch tension. Individual experiments are denoted by numbers. In six experiments, post-rest (*left*) and force-frequency (*right*) behavior was tested in the same muscle strip preparations. In three experiments, force-frequency and post-rest was tested in distinct left ventricular muscle strips from the same hearts. There was no significant relationship between post-rest and force-frequency behavior.

tiation of aequorin light signals and isometric twitch tension was significantly higher in nonfailing myocardium after rest intervals longer than 20 s. Therefore, post-rest behavior of contractile force is associated with parallel changes in the intracellular Ca^{2+} transients. To investigate whether rest induces alterations in the sensitivity of the myofilaments for Ca^{2+}, a plot of isometric tension versus peak aequorin light luminescence at each rest period was performed according to an approach described by Blinks (28) (Fig. 6). The resulting data were compared to the relation between peak isometric twitch tension and peak aequorin light emission after stepwise increasing the extracellular Ca^{2+}-concentration from 1.25 to 5.6 mM (Ca^{2+} reference curve; $n = 6$ muscle strip preparations from 6 nonfailing hearts). There was no statistically significant difference in the slopes of the post-rest curves between nonfailing and failing myocardium. The slopes of both curves did not differ significantly from the slope of the Ca^{2+} reference curve.

Influence of ryanodine on post-rest behavior. To test whether a functional sarcoplasmic reticulum is necessary for post-rest potentiation, the Ca^{2+} release channel of the sarcoplasmic reticulum was blocked with ryanodine (1 μM). At that concentration, ryanodine exerted its maximal negative inotropic effect, as assessed in concentration-response experiments in failing myocardium (0.01–10 μM; maximal decrease in force of contraction by 49.5±5.5%; $P < 0.05$, $n = 8$). Post-rest twitch tension increased after a rest interval of 120 s in the nonfailing and was unchanged in the failing myocardium (Fig. 7, *top*). After ryanodine, the twitch amplitude of the first post-rest beat was severely depressed after a rest interval of 120 s in both types of myocardium, and then slowly recovered to pre-rest steady state levels during the following beats. In nonfailing myocardium ($n = 4$), average post-rest twitch tension was maximum at a rest interval of 60 s (182±22% of basal value, $P < 0.05$). In failing myocardium ($n = 13$), average post-rest potentiation was maximum at a rest interval of 10 s (133±10% of basal value, $P < 0.05$). Ryanodine (1 μM) decreased steady state twitch tension by 46.7±6.7% ($P < 0.05$) in nonfailing and by 53.8±4.7% ($P < 0.05$) in end-stage failing myocardium. With ryanodine, post-rest potentiation of the isometric twitch was completely abolished in both types of myocardium and converted to rest decay (Fig. 7, *bottom*). Twitch tension was 58±7% of the basal value at a rest interval of 60 s in nonfailing myocardium ($P < 0.05$) and 76±7% at a rest interval of 10 s in failing myocardium ($P < 0.05$).

Influence of basal stimulation frequency on post-rest behavior. Since stimulation rate modulates transsarcolemmal Ca^{2+}-influx, we tested the influence of basal stimulation rate on post-rest behavior. In nonfailing myocardium, reducing basal stimulation frequency from 1.0 Hz to 0.5 Hz ($n = 5$) resulted in a decrease of isometric twitch tension by 1.1±1.2 mN/mm^2 (n.s.) and of diastolic tension by 0.2±0.7 mN/mm^2 (n.s.), whereas an increase to 2.0 Hz ($n = 6$) increased isometric twitch tension by 5.6±2.0 mN/mm^2 ($P < 0.05$) and of diastolic tension by 0.6±0.5 mN/mm^2 (n.s.). Post-rest potentiation of the isometric twitch was lowest at 0.5 Hz and highest at 2 Hz over the whole range of rest intervals (Fig. 8, *top*). Significant differences between these two groups occurred at rest intervals from 2–30 s. At 10 s rest interval, the first beat upon restimulation was potentiated by 4.1±1.1, 7.2±1.5 and 14.8±4.4 mN/mm^2 at 0.5, 1.0 and 2.0 Hz, respectively ($P < 0.05$ vs. pre-rest values). At 120 s, post-rest potentiation was 22.0±9.8, 27.2±4.9 and 27.6±7.0 mN/mm^2, respectively ($P < 0.05$ vs. pre-rest values).

In dilated cardiomyopathy (Fig. 8, *bottom*), neither isometric twitch tension nor diastolic tension were significantly influenced by changing stimulation frequency to 0.5 Hz (−1.2±0.8 mN/mm^2 and −0.2±0.4 mN/mm^2; $n = 10$) and to 2.0 Hz (−1.8±1.5 mN/mm^2 and +0.6±0.5 mN/mm^2, $n = 9$). Increasing basal stimulation frequency enhanced post-rest potentiation after

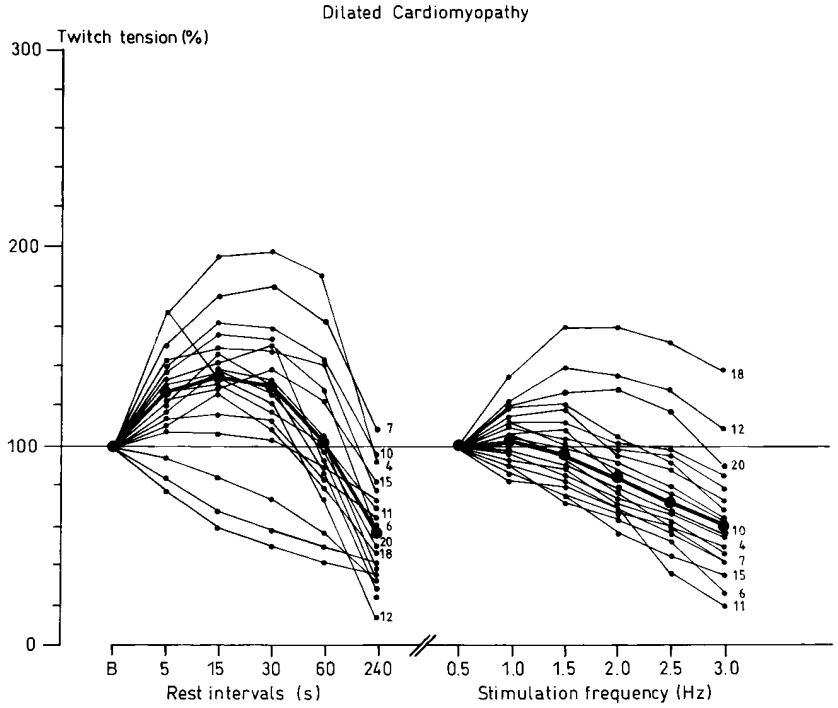

Figure 10. Post-rest and force-frequency behavior in human end-stage failing myocardium ($n = 20$). % change in isometric twitch tension. Individual experiments are denoted by numbers. In 14 experiments, post-rest (*left*) and force-frequency (*right*) behavior was tested in the same muscle strip preparations. In six experiments, force-frequency and post-rest was tested in distinct left ventricular muscle strips from the same hearts. There was no significant relationship between post-rest and force-frequency behavior.

short rest intervals (for 10 s rest interval: 2.6±1.0, 5.4±1.6, and 17.2±3.0 mN/mm^2 at 0.5, 1.0, and 2.0 Hz, respectively; $P < 0.05$ vs. pre-rest values). It did not prevent the blunted post-rest behavior of failing myocardium after longer rest intervals (−2.3±1.1, 0.1±1.2 and −0.9±2.3 mN/mm^2 at 120 s, n.s.). Post-rest contractile force at 2 Hz was significantly enhanced at rest intervals from 2–40 s as compared to 0.5 Hz.

Relation between force-frequency and post-rest behavior. Force-frequency and post-rest behavior were studied in muscle strip preparations from the same nonfailing or end-stage failing hearts (Fig. 9). In nonfailing myocardium, an increase in stimulation rate resulted in a stepwise increase in average force of contraction to maximal 168±17% of the basal value at 2.5 Hz ($n = 9$; $P < 0.05$). In the same myocardium, average post-rest potentiation at a basal stimulation frequency of 1.0 Hz continuously increased with increasing rest intervals to maximal 191±17% at a rest interval of 40s ($n = 9$; $P < 0.05$). In end-stage failing dilated cardiomyopathy, force-frequency behavior was inverse (decline of force of contraction to 80±15% of the basal value at 2.5 Hz; $n = 20$; $P < 0.05$). In the same myocardium, post-rest potentiation of isometric force was maximal at 15 s (increase to 138±21% of the basal value; $n = 20$, $P < 0.05$), but then continuously declined with longer rest intervals to 61±22% after 240 s rest ($P < 0.05$). However, no significant correlation between force-frequency behavior and post-rest behavior could be detected in nonfailing or failing myocardium.

Discussion

The results demonstrate a pronounced post-rest potentiation of intracellular Ca^{2+} transients and force of contraction in human nonfailing myocardium. In contrast, in end-stage failing human myocardium, post-rest potentiation of Ca^{2+} transients and contractile force was severely blunted and even transformed to rest decay at longer rest intervals. The data support previous findings that disturbed intracellular Ca^{2+} handling involving disturbed function of the SR may be the major pathological factor for altered contractile function in human dilated cardiomyopathy.

Altered intracellular Ca^{2+} handling associated with disturbed contractile function in failing human myocardium has been suggested from myothermal measurements (9) and from experiments using intracellular Ca^{2+} indicators such as FURA-2 (10) or Aequorin (7, 8, 13). However, some authors observed decreased peak systolic contractile force and Ca^{2+} amplitudes (10, 13), while others reported increased diastolic tension and delayed decline of intracellular Ca^{2+} transients (7, 8). Furthermore, Gwathmey et al. (14) suggested that decreased responsiveness of the myofilaments for Ca^{2+} rather than diminished cytosolic activator Ca^{2+} causes altered contractile function in the failing human myocardium. Therefore, the present study was performed to test the hypothesis that altered Ca^{2+} handling is associated with altered systolic activation in failing human dilated cardiomyopathy.

During depolarization, Ca^{2+} enters the myocytes through voltage-dependent Ca^{2+} channels and induces the release of a larger amount of Ca^{2+} from the SR (6). This activator Ca^{2+} binds to Troponin C, thereby inducing contraction. Contractile force depends on the amount of Ca^{2+} available on the level of Troponin C and the Ca^{2+} responsiveness of the contractile proteins. Relaxation is brought about by dissociation of Ca^{2+} from Troponin C and reuptake into the SR by phospholamban-regulated Ca^{2+} pumps. To maintain Ca^{2+} homeostasis, part of the cytosolic Ca^{2+} is extruded out of the cell. In mammalian cardiac myocytes, the Na^+/Ca^{2+} exchanger is the major pathway for transsarcolemmal Ca^{2+} efflux (29). SR Ca^{2+} pump proteins compete with other mechanisms for cytosolic Ca^{2+}, such as the Na^+/Ca^{2+} exchanger, the sarcolemmal Ca^{2+} pump proteins or the mitochondrial-cytosolic Ca^{2+} exchange. Their

relative contribution to Ca^{2+} elimination from the cytosol may influence post-rest behavior, as do properties of the SR Ca^{2+} release channels or leakage of Ca^{2+} from the SR. Therefore, post-rest behavior is multifactoriell and reflects intracellular Ca^{2+} handling.

It has been suggested that rest potentiation of force occurs in species with a highly developed SR, such as the rat, and is inverse (rest decay) in animals with sparse sarcoplasmic reticulum, such as the frog (3, 4). At long rest intervals, rest decay occurs in many mammalian species. Using rapid-cooling or caffeine-contractures, rest potentiation was attributed to SR Ca^{2+} loading during rest (30–32), while rest decay was associated with a gradual SR Ca^{2+} loss (33, 34). In the present study, post-rest potentiation of contractile force was pronounced in nonfailing human myocardium up to rest intervals of 240 s, but severely blunted and even altered to rest decay at rest intervals above 20 s in failing myocardium. Parallel changes occurred in rest-dependent intracellular Ca^{2+} transients. These results indicate that intracellular Ca^{2+} handling is severely altered in the failing human myocardium and determines altered systolic activation of the contractile proteins. The close correlation between aequorin light emission and isometric tension indicates that post-rest behavior of contractile force is not associated with changes in the Ca^{2+}-sensitivity of the myofilaments. This is supported by the finding that the relationship between changes in aequorin light emission and isometric force was not different after increasing rest intervals or increasing the extracellular Ca^{2+} concentration. The latter exerts a positive inotropic effect by increasing the intracellular Ca^{2+} concentration without changing the Ca^{2+}-sensitivity of the myofilaments (38). Furthermore, the relationship between changes in contractile force and aequorin light emission was similar in nonfailing and failing myocardium, which suggests similar sensitivity of the myofilaments for Ca^{2+} in nonfailing myocardium and dilated cardiomyopathy. Previous studies on Ca^{2+} sensitivity in failing human myocardium yielded controversial results (35–37). Taken together, it seems reasonable to assume that changes in post-rest contractile force primarily result from parallel changes in the amplitude of the intracellular Ca^{2+} transients. Although the parallel change in post-rest aequorin light emission and contractile force in human nonfailing and failing myocardium suggests a mechanistic relationship, it cannot be excluded that some intracellular compartmentalization of aequorin, e.g., to the mitochondria, had occurred and influenced the results. However, it is unlikely that intracellular Ca^{2+} sinks distinct from the SR contribute to Ca^{2+} cycling on a beat-to-beat basis. In addition to post-rest potentiation, the staircase of contraction after restimulation was different in failing compared to nonfailing myocardium (Fig. 3). The finding that after a pause of 10 s only the first twitch is potentiated upon restimulation in failing myocardium may indicate that the capacity of the SR to handle increased availability of Ca^{2+} is depressed.

Recently, Ezzaher et al. (39), using a rabbit model of heart failure, showed almost identical mechanical changes in post-rest behavior between nonfailing and failing rabbit myocardium. The authors speculated that altered function of the sarcoplasmic reticulum may be the underlying defect. In human myocardium, several authors reported a reduced Ca^{2+}-uptake capacity of the sarcoplasmic reticulum in failing dilated cardiomyopathy (13, 21, 40), which was suggested to result from a diminished expression of the SR Ca^{2+} ATPase on mRNA (18, 19) - and protein (15, 20) levels. Due to a reduced Ca^{2+} uptake capacity of the SR, less Ca^{2+} may be sequestered into the SR during a rest interval and more Ca^{2+} may be competitively removed from the cytosol by alternative Ca^{2+} eliminating mechanisms. In addition, a diminished Ca^{2+} storage capacity, alterations at the level of the SR Ca^{2+} release channels and/or a higher leakage of Ca^{2+} from the SR may contribute to altered Ca^{2+} handling and diminished post-rest potentiation in failing dilated cardiomyopathy. Altered storage capacity of the SR is unlikely to occur in dilated cardiomyopathy, since protein expression of the storage proteins calreticulin and calsequestrin is unchanged (20). Furthermore, no significant alteration in mRNA (41) or protein (20) levels of the SR Ca^{2+} release channel in failing dilated cardiomyopathy could be detected. Holmberg and Williams (42) did not find abnormal functional activity of single SR Ca^{2+} release channels under voltage clamp conditions from end-stage failing human hearts. However, D'Agnolo et al. (43) found an increased threshold for caffeine to release Ca^{2+} from the SR in idiopathic dilated cardiomyopathy and postulated an abnormal gating mechanism of the Ca^{2+} release channel. In addition, an increased efflux of Ca^{2+} from SR microsomes was recently described by Nimer et al. (44) in human dilated cardiomyopathy. Therefore, we cannot exclude that in addition to reduced SR Ca^{2+} uptake, altered release of Ca^{2+} from the SR might contribute to the blunted post-rest behavior in failing dilated cardiomyopathy. Furthermore, altered fractional release of Ca^{2+} from the SR (45–47) may contribute to the observed changes in post-rest behavior in failing myocardium.

To evaluate whether a functional SR is a prerequisite for post-rest potentiation in human myocardium, the influence of ryanodine on post-rest contractions was investigated. Ryanodine was shown to deplete SR Ca^{2+} stores (48) and has been found to abolish post-rest potentiation of contractile force in ferret (49), canine (50) and rat (5, 51) myocardium. This effect was attributed to a ryanodine-induced increased leak of Ca^{2+} from the SR by blocking the release channel in a subconducting open state (52). In the present study, ryanodine completely abolished post-rest potentiation in the nonfailing and end-stage failing myocardium. This indicates that in the human myocardium, post-rest potentiation critically depends on the function of the SR.

Besides altered SR Ca^{2+} uptake or release, altered function of other mechanisms for Ca^{2+} removal from the cytosol may contribute to the observed changes in post-rest behavior. Recently, an increase in mRNA levels, protein expression and activity of the Na^+/Ca^{2+} exchanger in human dilated cardiomyopathy was reported (22, 23). This may be of functional importance, since from recent work of Bers et al. (53) in isolated rabbit myocytes it has been estimated that Na^+/Ca^{2+} exchange contributes significantly to cytosolic Ca^{2+} elimination and relaxation. If both SR Ca^{2+} uptake and sarcolemmal Na^+/Ca^{2+} exchange were inhibited, relaxation was significantly prolonged, demonstrating their major importance for relaxation. In guinea pig myocytes, SR Ca^{2+} ATPase and Na^+/Ca^{2+} exchange produce about 97% of the relaxation (54). Functional data are lacking for human myocardium, but Sham et al. (55) recently described similar Ca^{2+}-activated Na^+/Ca^{2+} exchange activities in guinea-pig ventricular and human atrial myocytes. Therefore, it is reasonable to assume that other sarcolemmal Ca^{2+}-eliminating mechanisms, such as the Ca^{2+} pumps, are of minor relevance for relaxation. In addition, rest decay of contractile force in rabbit myocardium can be substantially slowed

by inhibition of the Na^+/Ca^{2+} exchanger (56). In consequence, less Ca^{2+} is extruded out of the cell, and more Ca^{2+} is available for reuptake to the SR. Therefore, we may hypothesize that in the failing human myocardium, both a decrease in SR Ca^{2+} pump capacity and an increase in Na^+/Ca^{2+} exchange activity contribute to rest decay instead of rest potentiation of contractile force. Since in the present study, significant changes in diastolic aequorin light emission or diastolic tension have not been observed even at long rest intervals, it is likely that Ca^{2+} ions which have not been accumulated within the SR have been eliminated from the cytosol by other mechanisms. In addition to sarcolemmal mechanisms, intracellular Ca^{2+} sinks such as mitochondria may contribute to cytosolic Ca^{2+} removal (57).

In this regard, it is important to note that in both types of myocardium, post-rest twitch tension progressively declined with longer rest intervals in the presence of ryanodine (rest decay). This ryanodine-induced transformation of rest potentiation to rest decay was also seen in isolated rabbit myocardium (58). In the latter study, the authors measured rest-dependent Ca^{2+} efflux to the extracellular space with the help of Ca^{2+} selective microelectrodes and found a ryanodine-induced increase in Ca^{2+} efflux with longer rest intervals. Hansford and Lakatta (59) demonstrated an increase in quin-2 fluorescence after addition of ryanodine to resting rat ventricular myocytes. From both experiments it was concluded that due to the action of ryanodine on the SR Ca^{2+} release channels, Ca^{2+} continuously leaks from the SR and is extruded via the Na^+/Ca^{2+} exchanger to the extracellular space, hence lost for post-rest potentiation. It seems reasonable to speculate that in the human myocardium, the pronounced rest decay after ryanodine is due to the same mechanism.

ACE inhibitor treatment has been reported to increase the number of β-adrenoceptors (60) and to improve intracellular Ca^{2+} handling (61) in the failing heart. Therefore, the influence of premedication on post-rest behavior was investigated. However, no significant differences in post-rest behavior was observed in relation to pretreatment of the patients. In addition, no significant correlation between age or ejection fraction of the patients on post-rest behavior could be detected.

Increasing stimulation frequencies result in an increase in time-averaged Ca^{2+} entry across the sarcolemmal membrane via L-type Ca channels (62, 63). An increased amount of Ca^{2+} within the cell increases Ca^{2+} loading of the SR. Accordingly, in the present study, post-rest potentiation was enhanced with higher basal stimulation frequencies in both types of myocardium. However, the rest intervals for maximum post-rest potentiation were not influenced by stimulation frequency, and rest decay at longer rest intervals in failing myocardium could not be prevented. Therefore, basal stimulation frequency does not determine the fundamental differences between nonfailing and failing human myocardium with respect to post rest behavior. In addition, these findings make it unlikely that diminished availability of intracellular Ca^{2+} determines rest decay in heart failure.

It was recently suggested that disturbed intracellular Ca^{2+} handling may be a major defect associated with the inverse force-frequency relationship in failing human myocardium (13). Since both force-frequency relation and post-rest behavior depend on intracellular Ca^{2+} handling, we tested force-frequency and post-rest behavior in the same muscle strips from nonfailing and end-stage failing hearts. In nonfailing myocardium, average force-frequency and post-rest behavior was positive, while an inverse force-frequency relation and rest decay was observed in failing myocardium. However, there was no direct relationship between frequency-behavior and post-rest behavior. This is in line with findings of Phillips et al. (64), who showed a dissociation of frequency-potentiation and post-extrasystolic potentiation of force in failing human myocardium. These findings may indicate that the contribution of the various intracellular Ca^{2+} handling mechanisms are different in frequency-dependent and rest-dependent modulation of contractile force. We may speculate that "fast" Ca^{2+} cycling systems, such as SR Ca^{2+} pumps predominantly determine force-frequency behavior, while "slower" factors such as Ca^{2+} leak from the SR and sarcolemmal and cytosolic Ca^{2+} transport systems become more relevant for post-rest contractile behavior. In this context, it is important to note that post-rest potentiation of contractile force did not depend on increased transsarcolemmal Ca^{2+} influx in ferret and rabbit myocardium (3, 65) upon restimulation. Beuckelmann et al. (66) and Nanasi et al. (67) described normal L-type Ca^{2+} currents in isolated human myocytes from end-stage failing hearts. However, recent evidence from patch-clamp experiments suggests diminished frequency-induced increases in L-type Ca^{2+} currents in failing human myocardium (63). Therefore, blunted force-frequency behavior of failing myocardium may depend more on altered inward Ca^{2+} currents than post-rest behavior.

In addition to intracellular Ca^{2+} handling, force-frequency behavior may be influenced by energetic factors such as oxygen supply to the core of the muscle, depletion of high energy phosphates and inorganic phosphate accumulation (68). It is unlikely that these factors influence post-rest behavior. Therefore, post-rest behavior of isolated heart muscle may even more directly than force-frequency behavior reflect intracellular Ca^{2+} handling independent of metabolic changes.

The present findings support the hypothesis that disturbed intracellular Ca^{2+} handling is a major pathological finding and determines altered contractile function in failing dilated cardiomyopathy. A decreased SR Ca^{2+} uptake and release capacity and an increased activity of the sarcolemmal Na^+/Ca^{2+} exchanger in the failing myocardium may shift intracellular Ca^{2+} cycling of the myocytes to transsarcolemmal Ca^{2+} exchange.

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