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ORIGINAL CONTRIBUTION

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Myocardial length-force relationship in end stage dilated cardiomyopathy and normal human myocardium: analysis of intact and skinned left ventricular trabeculae obtained during 11 heart transplantations

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Abstract The Frank-Starling-mechanism (FSM) was analyzed in isolated intact and skinned human left ventricular myocardium obtained from 11 heart transplantations (normal donor hearts (NDH), n = 8; dilated cardiomyopathy (DCM), n = 11). The new technique to utilize muscle strips from normal donor hearts which were actually implanted is described in detail.

Methods: I) In electrically stimulated left ventricular trabeculae (37 °C, oxygenated Krebs-Henseleit solution, supramaximal electrical stimulation, frequency 1 Hz) force development was analyzed as a function of muscle length (NDH = 8; DCM = 11). II) In an additional series left ventricular myocardium was demembranized ("skinned") by Triton-X-100. At different sarcomere lengths and calcium concentrations corresponding to pCa values of 4.3, 5.5, and 8.0 force development was measured (DCM = 11; NDH = 9).

Results: I) Developed force increased up to an optimum as a function of muscle length in intact NDH- and DCM-myocardium. However, the relative increment of developed force after any length step was smaller in DCM than in NDH. Near "Lmax" (muscle length associated with maximum developed force) passive resting tension was considerably elevated in DCM, indicating significantly incresed diastolic stiffness. II) In skinned left ventricular DCM- and NDH-myocardium developed force depended on sarcomere length with an optimum near 2.2 μm. However, a reduction of activator calcium concentration from pCa 4.3 to pCa 5.5 produces a smaller percent decline in force at short sarcomere lengths in DCM than it does in NDH.

Conclusion: the present study shows that except for diastolic stiffness and a smaller relative force increment after any length step in DCM the Frank Starling mechanism is still present in isolated human left ventricular DCM- as in NDH-myocardium. The current study does not allow to decide whether in skinned myocardium the smaller percent decline in force after reduction of activator calcium concentrations in DCM is caused by an increased calcium sensitivity at short sarcomere lengths or decreased sensitivity at long sarcomere lengths.

Key words Frank-Starling-mechanism – heart failure – normal donor hearts – dilated cardiomyopathy – heart transplantation – skinned fibres

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Introduction

Conflicting results are reported regarding the Frank-Starling mechanism (FSM) in failing and normal human myocardium. Schwinger et al. (30, 31) and Böhm et al. (9) hypothesized that the Frank Starling mechanism (FSM) was absent in isolated failing human myocardium. These results seemed to be supported by large animal experiments: using an experimental model based on pacing induced heart failure an exhaustion of the FSM had been described in dog myocardium (20). However, recent experiments presented by Holubarsch et al. (17) in a large and carefully conducted study on isolated human muscle strips and on isolated whole human hearts could not reproduce the experimental data obtained by Schwinger (30, 31) and Böhm (9). Holubarsch et al. had to conclude that the FSM is well preserved in failing human myocardium (17). As similar preparations and a similar experimental model was used in all groups the reason for these extremely divergent results is not clear. Thus further studies are required in order to decide whether the FSM is present or not in failing human myocardium.

For that reason the present study attempts to reinvestigate the Frank-Starling mechanism using exactly defined experimental conditions. While Holubarsch et al. had to use 2,3-Butanedione Monoxime (BDM) and Insulin for myocardial preservation to allow the 7 hour long distance transport of the myocardium from Oeynhausen to Freiburg (17) these substances were not used in the present study. Furthermore, in contrast to any other group and any other study presented before in the present study only normal myocardium of donor hearts, which were implanted later one, was used for experiments. The technique is described in detail.

Methods

Patients, inclusion criteria and muscle specimen

Left ventricular human myocardium of failing (dilated cardiomyopathy, DCM) and normal donor hearts (NDH) was obtained during 11 heart transplantations. Preoperative angiographic examination was carried out in all patients showing no evidence of significant coronary artery stenosis or heart valve disease. Presence of inflammatory processes was excluded by pre-operative myocardial biopsy. When accepted for heart transplantation all patients were classified according to the New York Heart Association as NYHA class III or IV. All of them had at least one period of myocardial decompensation requiring intensive care treatment. In some patients the intraaortic balloon pump had been implanted. None of the patients included in this study received preoperative support with assist devices.

Technique of NDH muscle fibre preparation

The donor hearts were transported in 4 °C cold cardioplegic solution (Bretschneider's HTK-solution (composition in mM/I: NaCl 15.0; KCl 9.0; MgCl 2.6 H_2O 4.0; potassiumhydrogen-2 ketoglutarat 1.0; Histidin. HCl. H_2O 180.0; Tryptophan 2.0; Mannit 30.0; $CaCl_2$ 0.015; osmolarity: 310 mosmol/l), exposition period prior to implantation: 15 – 200 min). On arrival the NDH was prepared on a separate sterile table. The vena cava superior was ligated – except those cases with a bicaval, bipulmonary implantation technique – and the right atrium was incised from the vena cava inferior upward towards the right atrial appendage. A cuff of donor right atrium was formed to match the right atrial cuff of the recipient. Subsequently the orifices of the pulmonary veins were connected by excision of a left atrial "flap". This way a left atrial cuff was formed. Through this orifice, the mitral valve was directly visible. After carefully opening the valve with the thumb, left ventricular trabeculae could be exposed by careful digital impression of the free left ventricular wall. This way the free left ventricular wall could be directly inspected behind the orifice of the mitral valve. Now left ventricular trabeculae became visible and optimal trabeculae could be selected and excised under completely controlled conditions avoiding lesions of the mitral valve's subvalvular apparatus (Fig. 1). Having applied this technique in more than 150 heart transplanations during 5 years, we never observed a surgical complication related to the excision procedure.

Technique of DCM muscle fibre preparation

Left ventricular muscle fibres of DCM-hearts were excised within 12 minutes after onset of explantation and transported in the same 4 °C cold cardioplegic solution as the donor hearts. None of the muscle fibres was in contact with 2,3-Butanedione monoxime (BDM).

Due to the surgical implantation technique (bicaval, bipulmonary anastomosis) no tissue was available in 2 donor hearts. In a third donor heart the intact muscle fibre measurements were not possible for technical reasons.

All patients included in the study had previously agreed that myocardial tissue obtained from their explanted hearts was used for detailed scientific analysis and that prior to implantation small muscle strips were

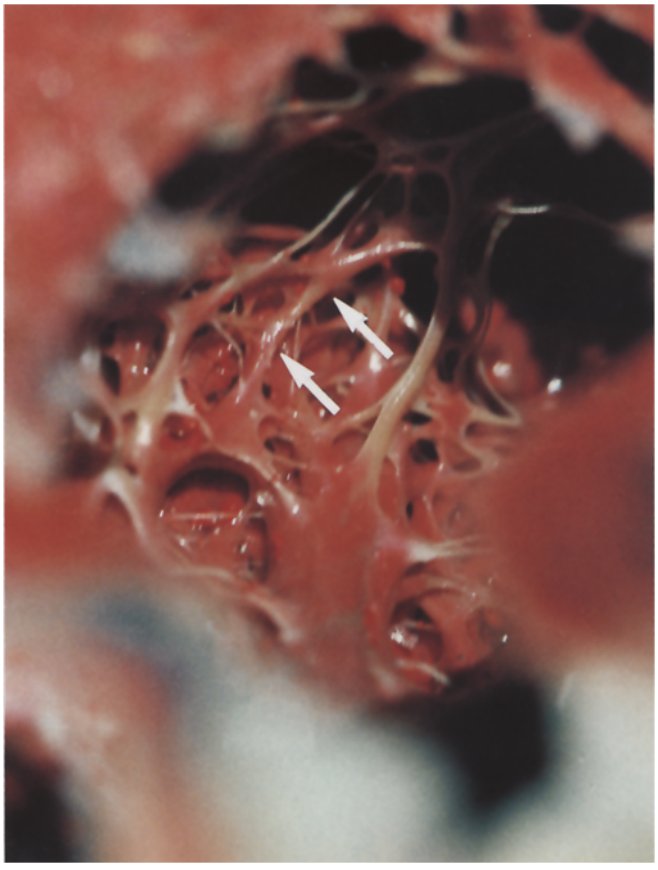

Fig. 1 Intraoperative view of left ventricular trabeculae of a donor heart. Adequate left ventricular trabeculae can be excized with ease through the mitral valve without affecting the subvalvular apparatus. Arrowheads are placed at the two points on a suitable trabeculum to indicate just where it would be cut. Distance between the trabeculum markers: 6 mm.

excised from every the donor heart for scientific purposes. The study was reviewed and approved by the Committee of Medical Ethics in Human Research of the University of Heidelberg.

Intact muscle fibre experiments

The experimental set-up was similar as described in previous studies on skinned and intact myocardium (33, 34, 35). After transport into the laboratory (10 min) small muscle fibres (diameter: 0.3 – 0.6 mm, length: about 4 mm) were prepared under binocular control in a 4 °C cold Krebs-Henseleit solution (composition (in mM): NaCl 119.0; $NaHCO_3$ 25.0; KCl 4.6; KH_2PO_4 1.2; $MgSO_4$ 1.2; $CaCl_2$ 1.3; Glucose 11.0; pH: 7.4; $pO_2 > 500$ mmHg). The small preparations were carefully mounted between force transducer and vibrator (33) in a 37 °C oxygenated Krebs-Henseleit-solution (KHS) and electrically stimulated (1 Hz, impuls amplitude: 10 % above threshold, impuls duration: 5 ms, mode: square wave) under steady state conditions. In intact muscle preparation the technique of optimal length adjustment (Lmax) was used as reference as described in the literature (17, 21, 24, 28, 30, 31, 34, 35). Lmax is defined as the length at which developed force (isometric twitch amplitude) is maximal.

In order to analyse the length-force relationship of isolated trabeculae the muscle length was reduced in a stepwise fashion starting at Lmax down to 80 % of Lmax. After any length step an equilibration period of 10 minutes was used before passive resting tension and developed force were mesasured as a function of muscle length. At the end of the experiment the muscle was stretched from Lmax up to 110 % of Lmax. This procedure was carried out at the end of the experiments in order to avoid damage to the preparation due to overstretching. The same parameters were measured as described above including passive resting tension. Measurements were carried out in 11 failing hearts (DCM) and 8 normal donor hearts (NDH).

Skinned muscle fibre experiments

The muscle specimen were prepared in a dissection chamber visually controlled through a ×10 binocular microscope. The fibres were mounted in a slightly stretched state using needles on small cork-plates and immersed in small volumes of the solutions neccessary for the chemical skinning procedure. The solutions for skinning, activation, and relaxation had the following composition:
1) skinning procedure: 50 % glycerol, 20 mM imidazol, 10 mM NaN_3, 5 mM ATP, 5 mM $MgCl_2$, 4 mM EGTA, 2 mM DTE at 4 °C and pH = 7.0 for 1 hour; the same solution including 1 % Triton X-100 for 6 hours. Afterwards, the fibres were washed and stored up to 8 weeks at −20 °C in the first solution without detergent until use for experiments. The final preparation of muscle fibres for mechanical measurements was carried out immediately before use by cutting and shortening the fibre bundels until preparations of about 6 × 0.3 mm or less were obtained.

Skinned fibre preparations were mounted between force transducer and vibrator in relaxation solution (bath temperature: 26 °C). Sarcomere length was simultaneously recorded by laser diffraction (Scientific Instruments, Heidelberg). Force development was measured using contraction solutions with a pCa of either 4.3 or 5.5 or relaxation solution.
2) Activation- and relaxation-solutions: after isometrically mounting the muscle preparations the fibres were

activated or relaxed by respective solutions of the following composition: relaxation solution: 10 mM ATP, 12.5 mM $MgCl_2$, 5 mM EGTA, 20 mM imidazol, 5 mM NaN_3, 10 mM phosphocreatine and 400 U/ml CPK. In the contraction solution EGTA was substituted by CaEGTA. No Calmodulin was added. The negative logarithm of the free calcium concentration (pCa) of the standard relaxation- and contraction solution were 8.0 and 4.3

Sarcomere length measurements

A 670 nm laser beam was fed through a focussing lense into the illumination system of the measurement device and focussed on the skinned muscle preparation. The laser diffration pattern was detected by a 46 element photodiode array. A multiplexer circuit allowed to observe the diffraction pattern on the screen of an oscilloscope. In addition an analog output allowed to read the distance between the center beam and the first order reflection. The time resolution of the sarcomere length measurement unit was 1 ms (Scientific Instruments, Heidelberg).

Using left ventricular myocardium from 11 DCM hearts and 9 NDH-hearts the sarcomere-length- force relationship was analyzed. In order to construct active force versus sarcomere length diagrams, the skinned fibre preparation was initially adjusted to a sarcomere length of 2.0 μm in relaxation solution. A contraction was initiated by immersing the preparation in activating solutions and maintained for about 40 s. Then relaxation solution was used (180 s), and the sarcomere length was adjusted to a new value in the relaxed state. This activation-relaxation cycle was repeated at different sarcomere lengths (2.0; 1.8; 1.6; 2.0; 2.2; 2.4 μm) at pCa 5.5, pCa 4.3 and in relaxation solution while passive force and active force amplitude were measured.

Statistical analysis

In intact muscle fibres the values for resting tension, developed force and muscle length of 60 subsequent twitches (Fig. 2) were always averaged for calculation of absolute values. In skinned myocardium, the mean value of at least 3 muscle fibres from any patient (donor, recipient) was calculated for the force data under the different experimental conditions. The number (n) always represents number of patients and not number of muscle fibres. Data are expressed as mean ± SD. For linear regression analysis the least square method was used. In order to evaluate statistical significances between the various groups, one way statistical analysis of variance followed by the Student t-test was carried out. For statistical analysis the SAS-software was used. Only for on line data analysis special software was used (Scientific Instruments, Heidelberg). A value of $p < 0.05$ was considered to indicate a statistically significant difference.

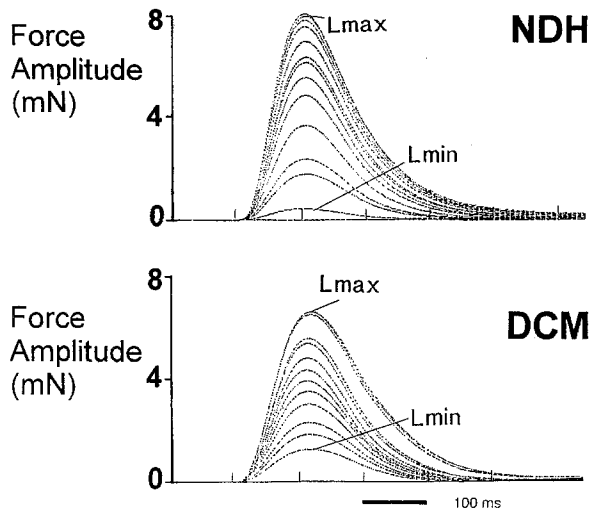

Fig. 2 Digitized recordings of the amplitudes of isometric twitches (developed force) as a function of muscle length in normal (upper taces) and failing myocardium (lower traces). At any length step ranging from minimal Length (= 60 % L/Lmax) up to Lmax 60 consecutive isometric twitches were averaged and superimposed (stimulation frequency: 1 Hz, sampling period: 1 min). Note, that there is a clear dependency of developed force on muscle length in NDH- and DCM-myocardium. The time to peak of force development is not affected by length changes in NDH- and DCM-myocardium.

Results

Force development and muscle length in intact normal and failing myocardium

In isolated intact, electrically stimulated human left ventricular trabeculae the developed force increased as a function of muscle length up to an optimal value in NDH – and DCM – myocardium as illustrated by the original recordings in Fig. 2. After any increase of passive muscle length there was an immediate fast response of the twitch amplitude followed by a more slowly developing further small increase of the twitch amplitude. A new steady state was observed about 5 min after induction of a length step. Fig. 3 shows passive resting tension and developed force as a function of muscle length. At L/Lmax = 0.8 developed force was 4.3 ± 2.1 mN in NDH (n = 8) and 3.8 ± 2.2 mN in DCM (n = 11). It increases up to a maximum in NDH (23.6 ± 3.1 mN/mm^2, n = 8) and DCM (17.6 ± 2.6 mN/

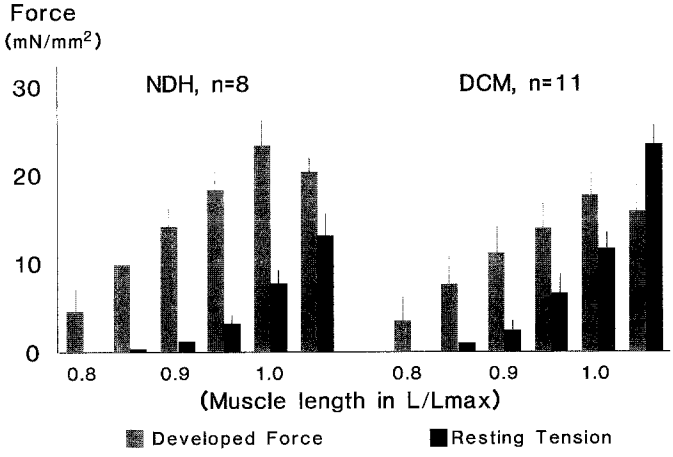

Fig. 3 Mechanical data in DCM- and NDH-myocardium. The developed force (= twitch amplitude) (mN/mm^2) and passive resting tension (mN/mm^2) are shown as a function of muscle length in normal (NDH, n = 8) and failing human myocardium (DCM, n = 11): black = passive resting tension; grey = developed force. Lmax is the passive resting length associated with maximum developed force. The muscle length is presented in L/Lmax. Total tension is not shown but can be calculated as sum of passive resting tension and developed force. Note that the passive resting tension increases considerably more in DCM-myocardium as a function of muscle length. Developed force depends on muscle length in NDH- and DCM-myocardium.

mm^2, n = 11). This corresponds to an increase by the factor 5.5 in NDH- and 4.6 in DCM-myocardium. Thus, the relative increment of developed force after any length step was smaller in DCM- than in NDH-myocardium (Fig. 3). Using the optimal length (Lmax) and the force developed at optimal length (Fmax) as reference, relative values could be calculated. Then a straight line relationship was approximated to all data pairs (F/Fmax); (L/Lmax) of the ascending limb of the Frank-Starling curve for NDH- and DCM-myocardium. The slope of these curves was significantly steeper in NDH- than in DCM-myocardium ($p < 0.0001$) confirming – in accordance with Fig. 3 – the larger relative increase of developed force after induction of muscle lengths changes in NDH.

At Lmax the time to peak was 175 ± 23 ms in NDH (n = 8) and 193 ± 20 ms in DCM (n = 11). The time required from peak force to 90 % relaxation was 240 ± 25 ms in NDH (n = 8) and 285 ± 31 ms in DCM (n = 11). None of these differences was statistically significant. Having reached Lmax, further streching of the muscle was associated with a reduction of the developed force (Fig. 3).

Passive resting tension increased with increasing muscle length in NDH- and DCM-trabeculae. However, this increase was more pronounced in DCM-myocardium (Fig. 3). If passive resting force at a given muscle length is expressed in % of the developed force measured at the same muscle length, passive force was 31.9 ± 4.2 % of the developed force at Lmax in NDH (n = 8), but 53.1 ± 7.4 % in DCM (n = 11, $p < 0.0001$). At a muscle length corresponding to 110 % Lmax the absolute values measured for passive resting tension were significantly higher in DCM- than in NDH-myocardium ($p < 0.0001$) and even higher than the twitch amplitude developed at this muscle length in DCM (Fig. 3).

Sarcomere length and force in normal and failing skinned human myocardium

In an additional series, left ventricular trabeculae excised during 11 heart transplantations were used to analyze the relationship between sarcomere length and developed force in skinned myocardium. Using supramaximal activation (pCa = 4.3), the developed force increased in NDH- and DCM-myocardium as a function of sarcomere length with an optimum near 2.2 μm (Fig. 4). Mean maximum force at supramaximal stimulation (pCa = 4.3) in NDH (39.6 ± 5.2 mN/mm^2, n = 9) and in DCM (36.8 ± 4.7 mN/mm^2, n = 11) did not differ. The sarcomere length associated with the maximum developed force was still 2.2 μm when submaximal calcium activation (pCa = 5.5) was used. However, at low sarcomere lengths and sub-

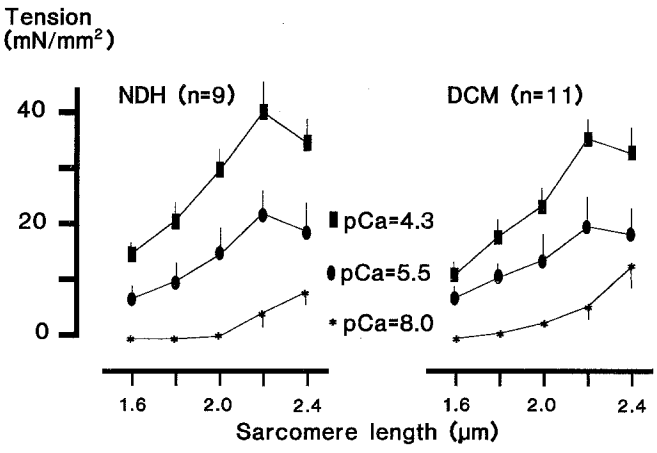

Fig. 4 Sarcomere Length-Force relationship in skinned myocardium. Developed force is shown as a function of sarcomere length in failing (DCM, n = 11) and normal human myocardium (NDH, n = 9) after stimulation with supramaximal calcium (pCa = 4.3), about half-maximal calcium (pCa = 5.5) and in relaxation solution. Note that developed force increases in NDH- and DCM-myocardium as a function of sarcomere length with a maximum force amplitude at a sarcomere length of about 2.2 μm in both types of preparation.

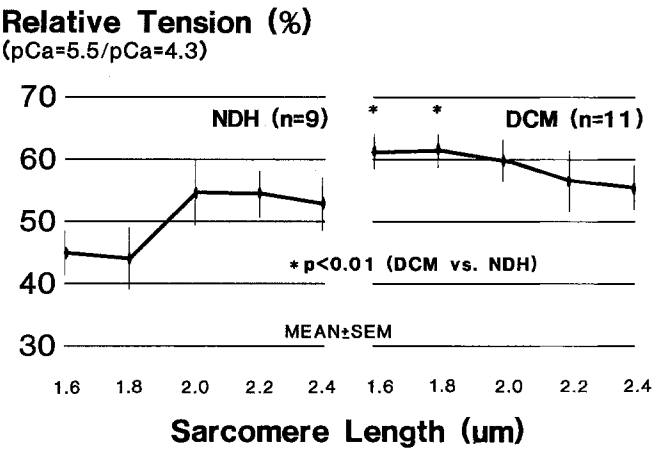

Fig. 5 Relative sarcomere-length force relationship in NDH and DCM myocardium. Using the maximum force amplitude (measured at pCa = 4.3) as reference (100 %), the force developed at a pCa = 5.5 could be expressed for any individual fibre as fraction of the maximum force. The respective data were calculated and are shown for DCM and NDH. Note, that in DCM significantly more force was developed in DCM at low sarcomere length when compared with NDH myocardium.

maximal activation skinned DCM trabeculae generated greater force relative to maximal activation than NDH did. For any individual muscle fibre force developed at submaximal stimulation (pCa = 5.5) was expressed in % of the force developed by the same fibre at pCa = 4.3. Fig. 5 shows the respective relative force values as a function of sarcomere length. At very short sarcomere lengths (1.6 and 1.8 μm) the ratio of force at low activation (pCa 5.5) to force at high activation (pCa 4.3) is significantly higher in DCM. In relaxation solution, passive resting force increases as a function of sarcomere length. At 2.4 μm sarcomere length passive resting force in relaxation solution was higher in DCM- than in NDH-myocardium ($p < 0.05$).

Discussion

A major strength of the current work is the technologic advance of utilizing human muscle strips from hearts which are actually implanted rather than utilization of those hearts rejected for several reasons by cardiac surgeons as usually done in such studies. The new and safe preparation technique may help to increase the availability of normal human myocardium.

The present study shows that in intact human myocardium the developed force depended on muscle length in normal and failing myocardium with an increased diastolic stiffness in failing myocardium. Despite the fact, that the Frank-Starling mechanism was basically present in intact DCM- and NDH-myocardium, the relative force increment after any length step was significantly larger in NDH (Fig. 3) indicating an important difference. In skinned myocardium maximum developed force was measured at the same sarcomere length (2.2 μm) in NDH- and DCM-myocardium. However, a reduction of activator calcium concentration from pCa 4.3 to pCa 5.5 produces a smaller percent decline in force production at short sarcomere lengths in DCM than it does in NDH. The present data are consistent with the hypothesis that although basically present in NDH- and DCM-myocardium the Frank Starling mechanism is not the same in both types of preparation.

Length-force relationship in intact DCM- and NDH-myocardium

The dependency of developed force on muscle length in DCM- and NDH-myocardium as described in the present study confirms observations on human (16, 17, 24, 28) and mammalian myocardium (3, 7, 14, 18, 19, 21, 25, 27). The mechanical data obtained in our study and those reported by Holubarsch et al. (17) are very similar strengthening the hypothesis of a preserved FSM in failing DCM myocardium. The reason, why the original data reported by Schwinger (30, 31) and Böhm (9) are so different is not clear. These authors stretched intact failing left ventricular muscle fibres from 2 mN up to 20 mN and observed a constant developed force of about 2 mN at any given passive resting force. When the same experiment was carried out in NDH myocardium these authors observed a small increase of developed force from 2 mN up to about 6 mN. This maximum value for developed force in NDH myocardium was measured at a muscle length that produced 20 mN passive resting force. According to their study near Lmax even in NDH-myocardium the preload (passive resting force) was by far higher than developed force (6 mN). As developed force remained constant at 2 mN at any preload in DCM-myocardium Schwinger et al. (30, 31) and Böhm et al. (9) concluded that the FSM was absent in intact failing myocardium.

Some of the differences may be related to methodological factors. In contrast to the present study and to that of Holubarsch (17), Schwinger (30, 31) and Böhm (9) did not analyze a length-force-relationship but presented a "resting tension-developed force" relationship. Thus the starting point of their length-force relationship is not clear making their results very difficult to interpret. The presence of a steep increase of diastolic stiffness in DCM-

myocardium beginning near Lmax (Fig. 3) may have further complicated their analysis.

The reason for the extremely high passive resting tension at Lmax reported by Schwinger (30, 31) and Böhm (9) is not clear. According to the cited authors even at Lmax developed force averaged only about 30 % of the resting force in NDH-myocardium (developed force: 6.2 mN/mm^2; resting force 20 mN/mm^2) and only 10 % in DCM-myocardium (developed force 2.4 mN/mm^2; passive resting force 20 mN/mm^2) (5, 6, 56). In contrast to these data – and confirming the results of Holubarsch et al. (17) – we measured that developed force at Lmax was always higher than passive resting force in NDH and DCM (with a factor of about 2.0 in NDH- and 1.5 in DCM-myocardium). The surgical aspects of tissue harvesting are not specified by Schwinger (30, 31). An increased basal tonus due to core hypoxia and other factors in their preparations would at least be a simple explanation for the differences. It seems likely, that the experimental conditions and the inclusion criteria of preparations were different in Schwinger's and our study (explantation protocol, short time interval between excision and onset of measurement, special protocol for myocardial protection without BDM, measurements on real donor heart myocardium).

Despite these differences, however, we agree with Schwinger, that the FSM is not the same in NDH- and DCM-myocardium in intact human trabeculae.

Length-force relationship in skinned DCM- and NDH-myocardium

The present study confirms the results of Holubarsch (17), that the FSM is present on sarcomere level in NDH- and DCM-myocardium. This in accordance with observations of ter Keurs et al. (32) who obtained similar SL-force relationships in normal myocardium as in trabeculae obtained from hypertrophied rat hearts, that had been treated with thyroid hormone or in which hypertrophy was induced by hypoxia. They concluded that the mechanism, responsible for SL-force relation is similar in normal and hypertrophied myocardium. This is consistent with findings of DelMonte et al. (10) who observed that the sarcomere lengths of isolated myocytes obtained from human myocardium did not differ as a function of the NYHA class and ranged between 1.82 and 1.84 μm at rest and 1.57 – 1.59 μm during systolic shortening.

The decrease of force development as a function of decreasing sarcomere length at a constant calcium concentration appears to be mainly attributable to a length dependent change in the sensitivity of the contractile proteins for calcium and to the variation of the overlap region of the contractile proteins at different sarcomere lengths (1, 3, 7, 14). Beside the fact that the sarcomere-length dependency of developed force was similar in NDH- and DCM-myocardium the maximum force produced by both types of preparations did not differ significantly at supramaximal activation. Thus the number of cross bridges available for force generation is not reduced in DCM.

Considering only the contractile apparatus the increase in diastolic stiffness in intact (Fig. 3) and skinned DCM-myocardium (Fig. 4) could theoretically indicate that higher sarcomere lengths and subsequently an increased degree of stretching is required in DCM to obtain Lmax. However, the skinned fibre experiments showing the force optimum at 2.2 μm sarcomere length in NDH and DCM do not support this hypothesis (Fig. 4). Thus the steep increase in passive resting force near optimal length in DCM-myocardium may be related to other factors including perimysial and endomysial fibrosis (29, 33).

Are activation processes involved in the FSM?

It was suggested that in mammalian myocardium aside from sarcomere length related factors length dependent alterations of the intracellular calcium metabolism may also contribute to the FSM (12, 13, 21, 27). Fabiato was the first to suggest that aside from the calcium-sensitivity on the contractile apparatus level other mechanisms influenced the FSM. He argued that the calcium induced calcium release from the sarcoplasmic reticulum was sarcomere-length dependent (11). Lopez observed an increase of metabolic activity at higher muscle lengths and related it to increased intracellular calcium concentrations (22). In several studies Allen and collegues provided evidence, that intracellular calcium levels were directly related to muscle length (2, 4 – 6). Nichols hypothezised that in cat papillary muscle diastolic calcium is directly modulated by muscle length (25).

The role of intracellular calcium metabolism for the maintenance of the FSM in human myocardium had not been analyzed in detail. However, it is known that severe disturbances of intracellular calcium metabolism are present in DCM myocardium (8, 15, 16, 23, 24, 28, 31, 35). Characteristical length dependent alterations of intracellular calcium metabolism were observed in DCM during afterloaded contractions (35). It is tempting to speculate that the reduced incremental force increase after any length step in DCM-myocardium (Fig. 3) is related to pathologically altered length dependent activation processes (calcium metabolism).

In addition to potential alterations of activation processes, the present study indicates that as compared to NDH myocardium sarcomere length dependent alterations of the responsiveness of the contractile apparatus for cal-

cium seem to be present in DCM. Fig. 5 illustrates that a reduction of activator calcium concentration from pCa 4.3 to pCa 5.5 produces a smaller percent decline in force production at short sarcomere length in DCM than it does in NDH. As claimed by Schwinger et al. (30, 31) this could indicate that DCM myocardium has a greater sensitivity to calcium at shorter sarcomere lengths than does NDH. However, this interpretation is not supported by the data in Fig. 4. The tension-length curves for DCM and NDH preparations at pCa 5.5 are virtually identical, especially at 1.6 and 1.8 μm sarcomere lengths. If there were an increased sensitivity to Ca in DCM at short sarcomere lengths the DCM fibres should have generated more tension than the NDH's. Since at pCa 4.3 the DCM tension-length curve falls consistently below the NDH curve the more obvious explanation for the higher pCa 5.5/pCa 4.3 ratios for DCM in Fig. 5 is that DCM produces less pCa 4.3 tension at all sarcomere lengths than does NDH. In the absence of the definitive experiment comparing the entire tension vs. pCa curves for DCM and NDH at 1.6 and 2.2 μm it is not possible to decide whether the higher DCM ratios in Fig. 5 are caused by an increased Ca sensitivity at short sarcomere lengths or decreased sensitivity at long sarcomere lengths.

Limitations

1) A new method for the analysis of muscle fibres obtained from donor hearts was described. The advantages of this method include a) the availability of normal donor myocardium and DCM-myocardium during the same heart transplantation, b) standardized treatment, explantation procedure and conservation of donor hearts, c) the possibility for simultaneous measurements of donor and recipient hearts that minimizes experimental bias and d) use of hearts that were in fact normal enough to be implanted in patients. However, it has to be considered, that "normal donor hearts" used during heart transplantations are obtained from patients that underwent the pathophysiology of brain death. Whenever the bicaval bipulmonary implantation technique is used, neither the right nor the left atrium is incised. Then no NDH-trabeculae are available for experiments.

2) Sarcomere length measurements were only performed in skinned, not in intact human myocardium. In the literature there is – at least to our knowledge – still no report regarding sarcomere lengths measurements in intact human left ventricular muscle strips. Konamura hypothesized (20) that myocytes of failing myocardium do not work near Lmax but at a length longer than Lmax. Although direct sarcomere length measurements in diseased mammalian myocardium (32) and isolated human myocytes obtained from failing hearts (10) do not seem to support his hypothesis it has to be stressed, that a simple extrapolation to the contractile performance of skinned muscle strips to intact muscle fibres is not possible.

3) Trabeculae which are utilized in the present study may not be reflective of the majority of myofibres of the majority of the free wall.

4) Increased diastolic stiffness may alter the ability of the DCM-ventricle to utilize the FSM, especially if it is working near optimal length. Thus a simple extrapolation of the present data to the behaviour of the whole heart under in vivo-conditions is not possible, as long as the in vivo sarcomere lengths of DCM- and NDH-myocardium are not known. According to Page (26) the physiological working range of mammalian cardiac muscle is restricted to sarcomere lengths between 1.6 μm and 2.3 μm, that were analyzed in the present study. Kentish et al. analyzed the sarcomere-length force relationship of six rat trabeculae before and after skinning measuring sarcomere length with a laser diffraction method (1.5 μm – 2.3 μm) (18, 19). Their result, that force production at SL = 1.6 μm was not zero but about 40 % of the force at 2.2 μm in skinned preparations is very similar to our results in human myocardium (Fig. 4). Confirming these data ter Keurs et al. (32) varied sarcomere length between 1.6 and 2.2 μm at different calcium concentrations and found about 30 % force at 1.6 μm SL as compared to 2.2 μm.

Summarizing, the present study shows that the Frank-Starling-mechanism is basically present in both, left ventricular DCM- and NDH-myocardium. However, although present, the FSM was different in DCM and NDH: in intact trabeculae the incremental increase of developed force after any length step was smaller in DCM- than in NDH-myocardium; in both, skinned and intact muscle fibres diastolic stiffness was higher in DCM; in skinned myocardium a reduction of activator calcium concentration from pCa 4.3 to pCa 5.5 produces a smaller percent decline in force at short sarcomere lengths in DCM than it does in NDH. Further studies have to show, whether muscle length dependent alterations of activation processes (e.g. intracellular calcium handling) may further explain these differences.

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