Isometric tension development and its calcium sensitivity in skinned myocyte-sized preparations from different regions of the human heart

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Abstract

Objective: In this study we investigated whether differences exist or develop in patients with aortic or mitral valve disease in myofibrillar contractile function and contractile protein composition between subendo- and subepicardial human ventricular tissue. Isometric tension, its calcium sensitivity and contractile protein composition were studied in left ventricular subendo- and subepicardial and in atrial biopsies obtained during open heart surgery from 24 patients with mitral or aortic valve disease. Methods: Isometric tension was measured in mechanically isolated skinned myocyte-sized preparations at different free calcium concentrations at 15°C. Protein composition was analysed by one-dimensional gel electrophoresis. A comparison was made between the results of subendo- and subepicardial ventricular tissue within each New York Heart Association class and within the different hemodynamically overloaded groups. Results: Maximal isometric tension was significantly lower in atrial than in ventricular preparations. The concentration of calcium required for half-maximal activation was significantly higher in atrial than in ventricular preparations. Within the ventricle no differences were found in contractile protein composition, isometric tension and its calcium sensitivity between subendo- and subepicardial tissue when all patients were treated as one group or when patients were subdivided according to severity of heart disease or hemodynamic overload. Conclusions: In this group of patients with ventricular volume or pressure overload no regional differences exist or develop during cardiac disease in left ventricular myofibrillar protein composition and force production. Maximal isometric tension and its calcium sensitivity are smaller in atrial than in ventricular preparations. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Human heart; Contractility; Cardiac diseases; Ventricle; Atrium

1. Introduction

In the heart considerable differences are found in blood flow [1], metabolic demand [2,3] and electrophysiological properties [4–6] between, or within the subendo- and subepicardial ventricular regions. These differences, observed under normal circumstances, may be altered or even amplified during hypertrophy and heart failure and after myocardial infarction (see e.g. [6–9]).

However, it is unknown if regional differences are also present in the contractile protein composition of the human ventricular myocardium. Since contractile protein isoform composition is closely related to myofibrillar contractility, regional differences in contractile protein expression might result in different mechanical properties of subendo- and subepicardial ventricular myocardium.

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The expression of protein isoforms of the contractile apparatus changes under certain pathological conditions [10]. These changes may be adaptations to new functional demands of the heart, but also may be maladaptive. For instance, with cardiac overload and subsequent myocardial hypertrophy an improved economy of contraction has been observed in rats due to a shift from the fast α-myosin heavy chain isoform (α-MHC) to the slow β-MHC isoform [11,12]. In the adult human heart the β-MHC predominates in the ventricles [13]. Hence a similar shift in response to cardiac overload does not occur, but changes in other contractile proteins may alter the contractile performance of hypertrophied and failing human myocardium [14–16]. In some patients with cardiomyopathy the atrial myosin light chain 1 has been found in ventricular tissue [16], while reexpression of fetal troponin T isoforms has been found by others [14,17,18]. If transmural differences exist in contractile protein composition and function, altered conditions during cardiac disease (e.g. hemodynamic overload) may even accentuate these differences in contractile protein composition and function between subendo- and subepicardial ventricular layers.

In the present study isometric tension and its calcium sensitivity and contractile protein composition were determined in small subendo- and subepicardial ventricular biopsies obtained during open heart surgery from patients, with mitral or aortic valve disease, undergoing valve replacement or repair surgery. Furthermore, to examine whether differences develop with heart failure, a comparison was made between tension characteristics of patients from different New York Heart Association (NYHA) Classes, ranging from mild (class I) to severe heart disease (class IV). This group consisted of patients with no left ventricular overload (mitral stenosis), pressure overload (aortic stenosis) or volume overload (mitral and aortic insufficiency).

Tension characteristics of subendo- and subepicardial tissue were studied using mechanically isolated skinned myocyte-sized preparations. These preparations have the advantage that isometric tension and its calcium sensitivity can be measured without interference of extracellular matrix components. Furthermore, by removing all membranous structures (sarcoplasmic reticulum, mitochondria), myofibrillar contractile properties could be measured under standardized conditions (i.e. composition of the intracellular medium, sarcomere length) without disturbing factors present in the intact heart (i.e. hormonal factors, variable calcium concentrations). This is essential for an unambiguous determination of the relation between contractile properties and the contractile protein composition. Since only a small part of the biopsy was needed to isolate preparations for mechanical studies, sufficient tissue remained for protein analysis by SDS-gel electrophoresis.

In human studies, atrial tissue is often used [19] because it becomes available during cardiac surgery on a regular basis. Therefore, a comparison was also made of isometric tension, its calcium sensitivity and myofibrillar protein composition between atrial and ventricular tissue.

2. Methods

2.1. Biopsies

Small biopsies of about 3 mm³ were taken during open heart surgery from the free left ventricular wall, after cold cardioplegic arrest, from 24 patients with mitral or aortic valve disease (insufficiency or stenosis) (NYHA, class I to IV). Eleven subendocardial (five male, six female) and nine subepicardial (four male, five female) biopsies were taken from the left ventricle. Four biopsies were obtained from the right atrium (~10 mm³, four female). Seven patients also suffered from coronary artery disease. Two patients had diabetes mellitus. The clinical data of the patients are given in Table 1. This study was approved by the Ethical Committee of the Academic Hospital of the Free University. The patients gave informed consent and the investigation conforms with the principles outlined in the Declaration of Helsinki [Cardiovase Res 1997; 35:2–3].

After excision, the biopsies were immediately immersed in a cold relaxing solution described below, and transferred from the operation room to the laboratory in about 10 min. The biopsies were divided into two parts. One part (~2 mg) was used for mechanical isolation of cardiac preparations of cellular size. The remaining part was directly frozen in liquid nitrogen to minimize degradation of the contractile proteins, freeze dried, and stored at −80°C. The freeze dryer (Edwards, Sussex, UK) operated at −70°C. The bottle which contained the tissue sample was kept at −30°C during the initial phase of the drying process. When sufficient samples were collected, protein analysis of the cardiac tissue was performed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), silver staining and laser scanning densitometry.

2.2. Mechanical isolation and experimental set-up

Myocyte-sized preparations were mechanically isolated as described previously [20,21]. Briefly, the tissue was put into a small glass cylindrical beaker of about 0.1 ml in volume containing relaxing solution and was mechanically disrupted with a rough glass piston within 5 to 10 s using a tissue homogenizer (Tamson, Zoetermeer, Netherlands) set at low speed (1000 r.p.m.). A suspension of small clumps of myocytes, myocyte-sized preparations, and cell fragments was obtained and kept in relaxing solution at 0°C for 10–24 h. Single myocyte-sized preparations were selected for mechanical measurements on the basis of size (100–150 μm long×15–30 μm diameter) and uniformity of the striation pattern. The preparation was attached with silicone adhesive (Dow Corning, MI, USA) to thin stain-
Table 1
Clinical characteristics of the patients

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¹ Coronary artery disease.
² Diabetes mellitus.

Abbreviations: M, male; F, female; epi, subepicardium; endo, subendocardium; A, atrium; S, stenosis; I, insufficiency; NYHA, New York Heart Association Classes I to IV; LV-mass, left ventricular mass normalized on body surface, and AoV-Gr, pressure gradient across aortic or mitral valve obtained from two-dimensional and Doppler echocardiography.

Medication: Ca²⁺ AG = calcium antagonist, β = beta-blocker, D = diuretics, n = nitrates, ACE = ACE-inhibitor, AC = anticoagulant, DX = digoxin, Ins = insulin.

less steel needles (tip about 15 µm) while viewed by an inverted microscope (Axiovert 40, Zeiss, Oberkochen, Germany). One needle was attached to a force transducer (SensoNor, Horten, Norway) and the other to a piezoelectric motor (Physike Instrumente, Waldbrunn, Germany) both connected to joystick-controlled micromanipulators. A thin carbon fibre of about 15 mm in length was glued to the force transducer element to obtain an adequate signal-to-noise ratio. The average maximal force amounted to 22 µN. The peak-to-peak noise level corresponded to ~1 µN. The output of the force transducer was filtered at 10 Hz. The force transducer as well as the extension for the piezoelectric motor were mounted at an angle of 75° with respect to the horizontal microscope stage to reduce the influence of surface tension on the output of the force transducer. The total compliance of force transducer and piezoelectric motor amounted to 0.054 µm/µN. After curing for 50 min the preparation was immersed for 45 s in a relaxing solution containing 0.3% Triton X-100 to remove all membranous structures. After the Triton treatment the preparation was transferred from the mounting area to a small temperature controlled well (volume 80 µl), which contained relaxing solution. From there the preparation could be transferred to a similar temperature controlled well containing activating solution. During attachment and subsequent force measurements the cardiac preparations were viewed at 320 × magnification. Images were captured by means of a CCD video camera (Phillips, Eindhoven, Netherlands) and stored on a personal computer. Average sarcomere length was determined by means of a spatial Fourier transform as described previously [21,22] and adjusted to approximately 2.2 µm in relaxing solution. Fig. 1 shows a Triton-skinned atrial preparation from a patient with mitral stenosis when viewed in the mounting area (A), in a well containing relaxing solution (pCa, i.e. −10 log[Ca²⁺], 9) (B) and in a well containing activating solution (pCa 4.5) (C). In Fig. 2 representative power spectra obtained with the spatial Fourier transform are shown, which were calculated from most of the image of the preparation between the attachments in relaxing (A) and activating (B) solution. Average sarcomere length of this preparation amounted to 2.21 and 2.15 µm in the relaxing and activating solution, respectively. The width of the power spectra is a measure of sarcomere non-uniformity within the preparation. The mean value of sarcomere length of all myocyte-sized preparations at rest amounted to 2.18±0.01 µm. As seen in Fig. 1C the image of the skinned preparation inside the well lost some of its
definition during activation. Sarcomere length determined in 20 preparations showed a decrease in sarcomere length of 0.07±0.01 μm, i.e., about 3% of the length of the preparation between the attachments. The diameters of the preparation were measured microscopically, in two perpendicular directions, at 320× magnification. The width of a myocyte-sized preparation was measured by means of the calibration image of the CCD camera. Its depth was determined by focusing on the lower and upper surface of the preparation and measuring the displacement of the objective. Cross-sectional area was calculated assuming an elliptical cross-section [23].

2.3. Solutions

The relaxing solution used for the mechanical cell isolation was composed as follows (in mmol/l): MgCl₂ 1, KCl 100, ethylene glycol-bis(aminohyelodiehterytetraacetic acid (EGTA) 2, Na₂ATP 4, imidazole 10 (pH 7.0, adjusted with KOH). For the force measurements on skinned myocyte-sized preparations relaxing and activating solutions were used, which contained, respectively (in mmol/l): MgCl₂ 5.42 and 5.26, Na₂ATP 5.45 and 5.53, EGTA 7, imidazole 20, creatine phosphate 14.5 (pH 7.0, adjusted with KOH). The ionic strength of the solutions was adjusted to 180 mmol/l with KCl. The pCa values of the relaxing and activating solution were, respectively, 9 and 4.5. The composition of the solutions was calculated by means of a computer program similar to that described by Fabiato [24]. The calculated free Mg²⁺ and MgATP concentrations were 1 and 4 mmol/l, respectively. Solutions with intermediate free Ca²⁺ concentrations were obtained by appropriate mixing of the activating and relaxing solutions, using an apparent stability constant of the Ca–EGTA complex of 10⁻⁶.⁵ [24].

2.4. Force measurements

Isometric force was measured after the skinned preparation was transferred from the relaxing to the activating solution by laterally moving the stage of the inverted microscope. When steady force was reached the preparation was returned to the relaxing solution. After the first activation at saturating calcium concentration, sarcomere length was readjusted to ~2.2 μm, if necessary. The second measurement at pCa 4.5 was used to calculate maximal tension (i.e. force per cross-sectional area).
next three to four measurements were carried out at submaximal calcium concentrations and were followed by a control measurement at pCa 4.5. Tension was corrected for preparation deterioration by linear interpolation between maximal tension values. The intermediate results, obtained at higher pCa values, were normalized to the interpolated maximal tension values. Measurements were continued until a full tension–pCa curve was obtained or until the control tension was less than 80% of the first control measurement. The usual number of force measurements per skinned cardiac preparation amounted to 8, of which at least three were performed at maximal calcium concentration. On average tension decline, between the first and final maximal activation, was 3%. All tension measurements were performed at 15°C. This temperature was chosen to allow comparison with previous animal studies from which it also became apparent that sarcomere uniformity during maximal tension development was not well preserved at higher temperatures [20].

The skinned preparation, while kept in relaxing solution, was slackened from the initial sarcomere length of 2.2 μm by imposing a rapid shortening of ~20% of the cell length by means of a piezoelectric motor to record passive tension. The abrupt decrease in tension during this procedure equals the passive tension at 2.2 μm sarcomere length. Occasionally, when the motor was not in use, passive tension was obtained from the difference in tension found when the preparation was stretched from a resting sarcomere length of ~1.7 to 2.2 μm.

2.5. Protein analysis by SDS-PAGE

The freeze-dried samples (~100 μg dry weight) were used to determine the composition of the contractile proteins. SDS-PAGE was performed as described previously [25] using an acrylamide to bis-acrylamide ratio of 200:1 in the separating gel (12% total acrylamide, pH 9.3) and of 20:1 in the stacking gel (3.5% acrylamide, pH 6.8). A protein II xi cell was used (Bio-Rad, Hercules, CA, USA). The gel dimensions were: 20×16 cm×0.75 mm thickness. The stacking gel was 2.5 cm in length. Lane width was 3.5 mm. The samples were dissolved in a buffer, which contained (in mmol/l): Tris (pH 6.8) 62.5, dithiothreitol 15, phenylmethyl-sulfonyl fluoride 0.1, leupeptin 0.5 and 1% (w/v) SDS, 0.01% (w/v) bromophenol blue and 15% (v/v) glycerol. Comparable samples (5 μl ~1 μg dry weight) were loaded in each lane. The samples were run at constant current (24 mA) for a total of 5 h (~1800 Volt hours). Silver staining was performed as described by Giulian et al. [25]. Laser scanning densitometry was performed to detect differences in protein composition. Contractile protein bands were identified by Western immunoblotting using specific antibodies: α-myosin heavy chain antibody (mAb 249-5A4, diluted 1:50), β-myosin heavy chain antibody (mAb 169-1D5, 1:100), troponin T antibody (clone JLT-12, 1:200, Sigma), troponin I antibody (mAb 1691, 1:200, Chemicon), actin antibody (clone C4, 1:100, Boehringer Mannheim), myosin light chain antibody (clone MY-21, 1:200, Sigma) and the tropomyosin antibody (clone TM311, 1:400, Sigma). The antibodies against α- and β-myosin heavy chain were a kind gift from Dr. A.F.M. Moorman (Amsterdam, Netherlands). Molecular masses of the contractile proteins were determined using molecular mass standards (Bio-Rad, high range: 161-0303 and low range: 161-0304) run under identical conditions.

2.6. Data analysis

The tension–pCa relation was fit by a non-linear fit procedure [26] to a modified Hill equation:

\[ P(\text{Ca}^{2+})/P_o = [\text{Ca}^{2+}]^{n_{\text{Hill}}}/(K^{n_{\text{Hill}}} + [\text{Ca}^{2+}]^{n_{\text{Hill}}}) \]

where \( P \) is steady state tension, \( P_o \) denotes the steady isometric tension at saturating Ca\(^{2+}\) concentration. The Hill coefficient (\( n_{\text{Hill}} \)) is a measure of the steepness of the relationship, and \( K \) (or \( pK \)) represents the Ca\(^{2+}\) concentration at which tension = 0.5×\( P_o \), i.e. the mid-point of the relation. \( P_o \) was determined from the second maximal activation at pCa 4.5.

Single classification analysis of variance (ANOVA) with unequal sample sizes was performed to determine the inter-patient and inter-preparation variation [27]. In the analysis of the inter-patient variation, the tension, \( pK \) and \( n_{\text{Hill}} \) values obtained from the individual preparations of each patient were averaged. Prior to the single classification ANOVA, the variances of the data from all patients were tested on equality because homogeneity of variances is an important prerequisite for the single classification ANOVA. Linear regression and multiple regression analysis were performed using the program spss [28].

Values are given as means±S.E.M. of \( n \) experiments. Differences were tested by means of Student’s \( t \)-test at a 0.05, 0.01 and 0.001 level of significance (\( P<0.05, P<0.01, P<0.001 \)).

3. Results

Suitable myocardial preparations were obtained from almost all biopsies taken. Only in two patients, with previous pericarditis and pericardial adhesions, the mechanical isolation failed (data not shown). From all other biopsies both protein composition and isometric tension development were determined. Isometric tension and its calcium sensitivity were determined in a total of 82 skinned preparations (37 subendocardial, 33 subepicardial and 12 atrial) derived from 24 patients.

Mean length of the preparations between the attachments was 78±3 μm. The mean cross-sectional dimensions, measured in two perpendicular directions, amounted
to 26±1 and 22±1 μm. No significant differences were found in the cross-sectional areas from preparations isolated from subendo- and subepicardial ventricular myocardium and from the right atrium (Fig. 3A).

3.1. Tension measurements

Recordings obtained during a contraction–relaxation cycle at three successive measurements at saturating [Ca$^{2+}$] (pCa 4.5), at submaximal [Ca$^{2+}$] (pCa 5.7) and again at saturating [Ca$^{2+}$] are shown in Fig. 4. In this case a maximum force of about 50 μN was attained during maximal activation, while maximum force reached at pCa 5.7 was considerably less. Maximal isometric tension was measured in skinned preparations from subendo- and subepicardial ventricular tissue and from atrial tissue. No significant difference was found in the average isometric tension at saturating calcium concentration between subendo- and subepicardial preparations (respectively, 55±6 and 48±5 kN/m²). However, the average maximal tension in atrial cells (27±4 kN/m²) was significantly lower than in ventricular preparations (Fig. 3A) ($P<0.05$). Passive tensions determined in relaxing solution (pCa 9.0) at 2.2 μm sarcomere length were very similar in subendocardial, subepicardial and atrial preparations and amounted to 3.5±0.7, 3.7±0.6 and 2.3±0.4 kN/m², respectively.

3.2. Calcium sensitivity

The Ca$^{2+}$ sensitivity of the contractile apparatus of human left ventricular subendocardial, subepicardial and atrial myocardium was determined by exposing the skinned preparations to different calcium concentrations and measuring the isometric tension. The data from each preparation were fitted to the Hill equation. The mean Hill parameters for subendocardial ($n=37$), subepicardial ($n=33$) and atrial ($n=12$) tissue were obtained by averaging the Hill parameters of all individual preparations. The mean tension–pCa relationships are shown in Fig. 5. The steepness of the relations ($n_{Hill}$), which amounted to 3.75±0.29, 3.69±0.16 and 3.52±0.20 in subendocardial, subepicardial and atrial skinned preparations, respectively, did not differ significantly. The mid-point of the Ca$^{2+}$ sensitivity curves (pK) did not differ significantly between subendo- and subepicardial preparations (pK = 5.59±0.01 and 5.55±0.02, respectively). However, the tension–pCa curve for atrial preparations (pK = 5.44±0.01) was shifted significantly to the right by 0.13 pCa units (or 0.94 μM) compared to ventricular tissue indicating that atrial cells are less sensitive to calcium ($P<0.01$).
Fig. 6. Tension–pCa relations from patient 2 and from all ventricular skinned preparations. The tension–pCa relations of preparations from patient 2 (NYHA class IV) and from all subendo- and subepicardial ventricular biopsies (without preparations from patient 2) were fitted to a modified Hill equation. Values are given ± S.E.M. The calcium sensitivity of isometric tension in skinned preparations from patient 2 (p\(K = 5.75 \pm 0.03\)) was significantly higher compared to ventricular preparations from all other patients (p\(K = 5.56 \pm 0.01\)) (\(P < 0.001\)).

An overview of the results is given in Table 2. The number of patients in NYHA class I, II, III and IV amounted to 3, 7, 9 and 1, respectively. The relatively low frequency of patients in NYHA class IV is an intrinsic property of this group of patients. Dimensions of the skinned preparations did not differ significantly between the NYHA classes and between subendocardial and subepicardial tissue from NYHA class II and III. No differences were found in tension measurements between subendo- and subepicardial preparations from NYHA classes II and III and between the different NYHA classes. Average isometric tension in the NYHA class IV patient (no. 2, Table 1) was very low (12 ± 2 kN/m², four myocytes) in comparison with the results from the other patients (\(P < 0.05\)). In addition, calcium sensitivity of tension in this patient (p\(K = 5.75 \pm 0.03\), \(n = 4\)) was significantly (\(P < 0.001\)) higher than that of all other patients (p\(K = 5.56 \pm 0.01\), \(n = 66\)). This difference is illustrated in Fig. 6, in which the tension–pCa

3.3. Severity of heart disease

The patients included in this study ranged from NYHA class I (mild cardiac disease) to severe heart failure (NYHA class IV). This allowed us to investigate whether the severity of the disease correlated with changes in contractile protein composition and/or in isometric tension and its calcium sensitivity. Regional differences were studied by comparing the results of subendocardial and subepicardial ventricular tissue within each NYHA class.

### Table 2

Tension and calcium sensitivity in subepicardial and subendocardial skinned preparations classified according to the severity of heart disease

<table>
<thead>
<tr>
<th></th>
<th>NYHA I</th>
<th>NYHA II</th>
<th>NYHA III</th>
<th>NYHA IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>(F) (kN/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subepicardium</td>
<td>−</td>
<td>55±8 (13)</td>
<td>52±6 (16)</td>
<td>12±2* (4)</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>47±10 (15)</td>
<td>63±11 (9)</td>
<td>58±12 (13)</td>
<td>−</td>
</tr>
<tr>
<td>(pK)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subepicardium</td>
<td>−</td>
<td>5.50±0.02 (13)</td>
<td>5.54±0.02 (16)</td>
<td>5.75±0.03* (4)</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>5.62±0.02 (15)</td>
<td>5.57±0.04 (9)</td>
<td>5.58±0.03 (13)</td>
<td>−</td>
</tr>
<tr>
<td>(nHill)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subepicardium</td>
<td>−</td>
<td>3.62±0.21 (13)</td>
<td>3.60±0.22 (16)</td>
<td>4.28±0.78 (4)</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>3.33±0.18 (15)</td>
<td>4.18±0.61 (9)</td>
<td>4.00±0.73 (13)</td>
<td>−</td>
</tr>
</tbody>
</table>

\(F\), maximal tension; \(pK\), mid-point and \(nHill\), steepness of the tension–pCa relationship. Values are given as mean ± S.E.M. The number of myocyte-sized preparations is given between brackets.

* and † indicate that the values of NYHA class IV are significantly different from the values of NYHA class II and III at a level of \(P < 0.01\) and \(P < 0.001\), respectively.
curve from four skinned preparations from this patient is compared to the mean tension–pCa curve from all other subendo- and subepicardial ventricular myocyte-sized preparations.

Patients were also classified, according to their hemodynamic overload, into: no left ventricular overload (mitral stenosis, n = 2), pressure overload (aortic stenosis, n = 7) and volume overload (mitral and aortic insufficiency, n = 11). Left ventricular mass, obtained by two-dimensional echocardiography, from the individual patients is shown in Table 1. The average values for the different hemodynamic overloaded groups are given in Table 3. Left ventricular mass normalized on body surface ranged from 90 to 327 g/m². The average value of the whole group of patients studied (189±17 g/m²; n = 17) was significantly larger than normal (~92 g/m²) [29]. However, in patients without left ventricular overload left ventricular mass was not increased. In patients with pressure or volume overload, i.e. with concentric and eccentric hypertrophy, respectively, left ventricular mass normalized on body surface was significantly increased compared to normal (Table 3). A small but significant positive correlation was found when cross-sectional area of the ventricular preparations was plotted against left ventricular mass normalized on body surface (Fig. 3B, correlation coefficient R = 0.38, P < 0.05). However, mean ventricular cell dimensions did not differ significantly between the different hemodynamic overloaded groups. The averaged results of tension measurements in subendo- and subepicardium in the different groups of hemodynamic overload are also shown in Table 3. Isometric tension and its calcium sensitivity did not significantly differ between subendo- and subepicardial skinned preparations or between the different hemodynamic overloaded groups. No significant correlations were found when plotting tension, the mid-point (pK) and steepness (nHill) of the tension–pCa relationship against left ventricular mass normalized on body surface.

3.4. Inter-patient and inter-preparation variation

Since no significant differences were found in the force characteristics of subendo- and subepicardial skinned preparations, in the patients from NYHA class I to III, single classification ANOVA was performed on all ventricular myocyte-sized preparations (19 patients, 66 preparations), to determine inter-patient and inter-preparation variation in the force measurements. In the comparison between patients the averaged values obtained from all preparations of each patient were used. The distribution of variance of the average tension between patients was tested on equality and found to be homogeneously distributed among patients. A significant added variance (44% of the total variation) was present in maximal tension between patients. The relative coefficient of variation (i.e. standard deviation expressed as a percentage of the mean) amounted to 39%. Little variation was found in the mid-point (pK) and steepness (nHill) of the tension–pCa relationship of skinned preparations between patients and within one patient and no added inter-patient variance was present in these parameters.

Several independent factors (i.e. medication, age, sex, coronary artery disease) could underlie the observed variation in maximal tension between patients. Linear regression analysis indicated that no significant relations were present between maximal isometric tension and the individual factors. Since the difference may be obscured by an interaction between the different factors multiple regression analysis was also performed in which NYHA class and type of valvular defect, which were already discussed.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Patients subdivided into three groups based on hemodynamic overload</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No overload (MS, n = 3)</td>
</tr>
<tr>
<td>LVMass (g/m²)</td>
<td>127±18</td>
</tr>
<tr>
<td>F (kN/m²)</td>
<td>Subepicardium 72±1 (2)</td>
</tr>
<tr>
<td>Subendocardium 61±16 (3)</td>
<td>59±8 (24)</td>
</tr>
<tr>
<td>pK</td>
<td>Subepicardium 5.43±0.06 (2)</td>
</tr>
<tr>
<td>Subendocardium 5.59±0.07 (3)</td>
<td>5.62±0.02 (24)</td>
</tr>
<tr>
<td>nHill</td>
<td>Subepicardium 4.19±0.36 (2)</td>
</tr>
<tr>
<td>Subendocardium 3.53±0.07 (3)</td>
<td>3.42±0.24 (24)</td>
</tr>
</tbody>
</table>

Abbreviations: MS, mitral stenosis; AS, aortic stenosis; AI, aortic insufficiency; ML, mitral insufficiency. LVMass, left ventricular mass normalized on body surface was obtained from two-dimensional echocardiography; n denotes the number of patients.

* LVMass per body surface is significantly higher than normal (92 g/m²) at P < 0.05.

F, maximal tension; pK, mid-point and nHill, steepness of the pCa–tension relationship.

Values are given as mean±S.E.M. The number of myocyte-sized preparations is given between brackets.
above, were included. Nevertheless, using multiple regression analysis no significant relationship was observed between maximal tension and the combined independent factors either. Linear and multiple regression analysis between the Hill parameters ($pK$ and $n_{Hill}$) and the independent factors mentioned also did not reveal significant relationships.

Another factor which may be involved in the variation between patients might be the quality of the preparations. However, little variability was found in the yield of suitable preparations between biopsies. Therefore, we suppose that the quality of the preparation is not an important factor in the comparison between patients.

### 3.5. Protein composition of the biopsies

In Fig. 7A a typical silver-stained 12% polyacrylamide gel of electrophoretically separated contractile proteins from subendocardial (lane 1), subepicardial (lane 2) and atrial tissue (lane 3) are shown. In the atrial tissue the fast $\alpha$-MHC and slow $\beta$-MHC could be separated by SDS-PAGE (Fig. 7B). Subsequent laser scanning densitometry revealed the presence of two peaks in atrial tissue (Fig. 7C). The fraction of $\alpha$-MHC ($\alpha/\alpha + \beta$) present in atrial tissue determined from the area underneath these two peaks, ranged from 0.49 to 0.67, with an average of $0.55 \pm 0.04$ (four biopsies). It can also be seen in Fig. 7 that

---

**Fig. 7.** (A) Silver stained gel of myocardial contractile proteins. 1 = subendocardium (patient 15), 2 = subepicardium (patient 12), 3 = atrium (patient 11). Protein bands include: MHC, myosin heavy chain; A, actin; TnT, troponin T; TM, tropomyosin; TnI, troponin I; VLC-1 and 2, ventricular light chain 1 and 2; ALC-1 and 2, atrial light chain 1 and 2. The two ventricular myosin light chain 2 isoforms cannot be separated by one-dimensional gel electrophoresis, because of equal molecular masses. (B) MHC ($\alpha$- and $\beta$-isoform) composition in atrial tissue (patient 11) and in subendocardial ventricular tissue (patient 3), and corresponding laser densitometric scans (C). AU, arbitrary units.
the β-MHC was the predominant isoform in ventricular tissue. In some ventricular biopsies (e.g. Fig. 7B) a small α-MHC band could be distinguished. Laser scanning densitometry indicated that the α-MHC band present in this ventricular sample comprised only 1.6% of the total MHC staining (Fig. 7C). Atrial and ventricular tissue also differed in their myosin light chain (MLC) composition. Atrial light chain 1 (ALC-1) had a slightly higher molecular mass than the ventricular light chain 1 (VLC-1). As found by Price et al. [30] atrial light chain 2 (ALC-2) comigrated with ventricular light chain-2 (VLC-2).

Careful visual inspection of all silver-stained SDS-PAGE gels (Fig. 7) and corresponding laser densitometric scans (Fig. 8) did not reveal apparent differences between the myofibrillar protein composition of subendo- and subepicardial biopsies from NYHA classes I to III with one exception. In the subendocardial biopsy from a patient with aortic stenosis (Table 1, no. 10, NYHA class I) an extra protein band was present at the level of troponin T (TnT), which may correspond to the fetal TnT4 (Fig. 9A, lane P10; lane P9 = control). Inspection of the tension measurements performed on this biopsy revealed an increased calcium sensitivity of tension (pK = 5.63 ± 0.03; P < 0.1). Furthermore, in the subepicardial tissue from patient 2, who showed significant alterations in maximal isometric tension and its calcium sensitivity (Table 1, NYHA class IV), an extra protein band was also observed at the level of TnT (Fig. 9A, lane P2; lane P1 = control). This protein band might correspond to the fetal TnT2 isoform. Laser densitometric scans corresponding to Fig. 9A are shown in Fig. 9B.

4. Discussion

In this study we investigated whether regional differences exist or develop during cardiac disease in contractile protein composition and myofibrillar function. Isometric tension and its calcium sensitivity were measured in left ventricular subendo- and subepicardial and in right atrial skinned myocyte-sized preparations from 24 patients with mitral or aortic valve disease. Contractile protein composition was analysed by one-dimensional gel electrophoresis.

4.1. Tension measurements in general

During maximal activation only a small (≈3%) change in sarcomere length occurred, which mainly originated from the compliance of the mechanical parts of the set-up [21]. This indicates that the attachment procedure resulted in a firm connection of the cardiac preparations to the set-up. The mean maximal isometric tensions found in subendo- and subepicardial human skinned ventricular preparations are comparable with tension values found previously in skinned cardiac trabeculae from rat [31] and guinea-pig [32], as well as in cardiac myocytes from rat [33,34]. This suggests that the contractile properties are well preserved during the procedures followed for obtaining the small biopsies, tissue transportation and further experimentation.

Considerable variation in maximal isometric tension between and within patients was found. The calcium sensitivity parameters (pK and nHill) which were determined from normalized tension values, however, showed little variation. Part of the variation in maximum isometric tension is probably due to inaccuracy in the determination of cross-sectional area of the myocyte-sized preparations. However, an added component of variation was found between patients, which cannot be explained by inaccuracy in the determination of cross-sectional area. It is unlikely that the observed inter-patient variation in maximal isometric tension is due to factors such as medication, age, sex or need for coronary artery bypass, because the linear and multiple regression analysis performed did not reveal significant correlations. A factor which may be involved in variation between patients is the severity of heart disease. We did not observe a significant
Fig. 9. (A) Silver-stained gel of actin and troponin T present in myocardial tissue of patients 1, 2, 9 and 10 (respectively, P1, P2, P9 and P10). In patients 10 (NYHA class I) and 2 (NYHA class IV) an extra protein band (see arrow) was present at the level of troponin T, which was absent in all other biopsies (see e.g., patients 9 and 1). Accompanying laser densitometric scans are shown in (B). AU, arbitrary units.

difference in average maximal tension between patients from NYHA class I, II and III, but in one patient (NYHA class IV) a significant decrease in tension was observed.

4.2. Tension and protein composition in left ventricular tissue

Myofibrillar protein composition and contractility were determined in both subendo- and subepicardial tissue from patients in different stages of heart disease. The patients studied were subdivided into different groups. The first group of interest consisted of patients with mitral valve stenosis. This group served to answer the question whether regional differences were present in the absence of left ventricular overload. Furthermore, to address the question whether regional differences develop during cardiac disease, patients with mitral stenosis were compared with patients with left ventricular pressure or volume overload. Alternative ways to see whether regional differences develop are to distinguish between patients on the basis of the degree of hypertrophy, as reflected by left ventricular mass normalized on body surface, or on the basis of the NYHA class. Below the results will be discussed from these different perspectives.

The maximal power of the comparisons in tension, pK and nHill between subendocardial and subepicardial values was 36%. Therefore, caution should be exerted in the interpretation of our findings. The low statistical power is, in part, due to the small differences in the mean values of the mechanical parameters between subepicardial and subendocardial tissue. Given these small differences it can be estimated that in order to increase the power of the study to 80% about 200–300 measurements would be required.

4.3. Regional left ventricular differences without overload?

In patients with mitral valve stenosis no difference in tension and its calcium sensitivity were observed between subendo- and subepicardial preparations. Furthermore, it was found that subendo- and subepicardial tissue did not differ in their contractile protein composition. Kuro-o et al. [35] observed a transmural difference in the myosin heavy
in the subendocardial and subepicardial regions. However, this was not found by others [36]. In our study only trace amounts of α-MHC of less than 5% were detected, which were much smaller than the values given by Kuro-o et al. [35]. The reason for this discrepancy is unclear, but in our study no indications were obtained of regional left ventricular differences in contractile function and protein composition in the absence of hemodynamic overload.

4.4. Regional left ventricular differences with overload?

In animal studies the differences in growth response which occur with pressure and volume overload (i.e. concentric and eccentric hypertrophy) give rise to differences in the expression of contractile proteins [10,12]. These results contrast our findings, since no differences in isometric tension production, its calcium sensitivity and contractile protein composition were observed between patients with mitral valve stenosis, aortic valve stenosis (pressure overload) and mitral or aortic valve insufficiency (volume overload). Moreover, no differences in tension production, its calcium sensitivity and contractile protein composition were found between subendo- and subepicardial myocardium. This indicates that in the group of patients studied here no transmural differences develop with hemodynamic overload. Left ventricular mass normalized on body surface was not significantly increased in patients with mitral stenosis. On the other hand, a significant increase in left ventricular mass compared to normal was found in both volume and pressure overloaded hearts, which provides evidence for substantial left ventricular hypertrophy in these patients (Table 3). This increase in left ventricular mass was also discernible at the preparation level, because a significant positive correlation was observed between cross-sectional area of the skinned preparations and left ventricular mass normalized on body surface (Fig. 3B). Our results indicate that the magnitude of the growth response per se is not a determining factor for alterations in force characteristics, because plotting isometric tension and the Hill parameters (pK and nHill) as a function of left ventricular mass normalized on body surface did not reveal significant correlations. Hence, in our investigation on a group of 20 patients, hemodynamic overload appears not to affect myofibrillar protein composition and isometric tension development.

Further evidence for this notion follows from the observation that the average maximal isometric tension, its calcium sensitivity and contractile protein composition remained the same up to NYHA class IV. In addition, it was found that, in NYHA class II and III no transmural differences in tension characteristics and protein composition were present or had developed. In one patient classified to NYHA class IV (Table 1, no. 2) an increase in calcium sensitivity of isometric tension and a decrease in maximal isometric tension was found. In the subepicardial tissue from this patient an extra protein band was observed at the level of the fetal TnT isoform 2. An additional protein band in the TnT region was also observed in the subendocardial tissue from another NYHA class I patient (Table 1, no. 10). Calcium sensitivity of isometric tension in this patient was rather high, which suggests that also in this case a shift in the TnT isoform composition coincides with a change in calcium sensitivity of tension. The shift in calcium sensitivity in the NYHA class IV patient was larger, which suggests that the change in calcium sensitivity originating from TnT is more profound or of a distinct nature in end-stage heart failure than with moderate heart failure. This conclusion is based on the data from one patient only, but it is supported by the increased calcium sensitivity in end-stage failing human hearts observed by Wolff et al. [15] and by Morano et al. [16]. Wolff et al. [15] suggested that the increased calcium sensitivity observed in failing myocardium could be due to a reduction of the β-adrenergically mediated phosphorylation of myofibrillar proteins such as troponin I (TnI) and protein C. Morano et al. [16] attributed the increased calcium sensitivity of tension to the expression of atrial essential myosin light chain (ALC-1) in ventricular tissue of failing hearts. The three alternative explanations (altered TnT expression, decreased β-adrenergic stimulation and expression of ALC-1 in ventricular tissue) for an altered calcium sensitivity during end-stage heart failure, discussed above, may all be true. Presumably, a combination of changes in isoform expression and level of phosphorylation is involved in the changes in myofibrillar contractile function during end-stage heart failure.

The decrease in maximal isometric tension observed in the skinned preparations from the NYHA class IV patient in our study is not in agreement with the results obtained by Wolff et al. [15] and Morano et al. [16]. They both found an unchanged maximal isometric tension in the failing myocardium compared to the controls. The maximal isometric tension values found by Wolff et al. [15] were lower compared to our values found in NYHA classes I to III, but similar to the tension values we found in NYHA class IV. These differences may be caused by both mechanistic and methodological factors such as the severity of heart disease (NYHA class III and IV), the cause of the disease (i.e. valvular heart disease versus idiopathic and ischemic dilated cardiomyopathy) and/or a difference in the isolation procedure, since myocytes were prepared from frozen biopsies in Wolff et al. [15]. A decline in maximal isometric tension, similar to what we have found, was reported recently after pressure overload in right ventricular myocytes from rat [22]. Interestingly, in this study, a reduced calcium sensitivity of isometric tension was observed, which is different from our findings and that of others discussed above on human tissue.
4.5. Tension and protein composition in atrial tissue

Since in many human studies atrial tissue is assumed to be representative of the whole heart, maximal isometric tension and its calcium sensitivity were also measured in skinned atrial preparations. Both maximal tension and calcium responsiveness were lower in atrial compared to ventricular preparations. Morano et al. [37] also found a lower calcium sensitivity in human atrial skinned fibers compared to fibers of the ventricle. This difference in calcium sensitivity between atrial and ventricular tissue might be due to a difference in myosin light chain (MLC) composition. The two ventricular myosin light chains, VLC-1 and VLC-2, have been found in atria from patients with moderate to severe heart failure [38,39], and ALC-1 is expressed in failing ventricles [16,40,41]. The observation that calcium sensitivity was increased in atria [39] and ventricles [16] from patients with cardiomyopathy (severe heart failure) therefore suggests that MLC composition may be involved in the difference in calcium responsiveness between atrium and ventricle.

In normal human atrial tissue the α-MHC versus β-MHC ratio equals 1 [42] and decreases with heart failure indicating that α-MHC is replaced by β-MHC [36,42]. Densitometric analysis of the atrial tissues which originated from patients in NYHA classes II and III revealed a mean α-to β-MHC ratio above 1 (mean 1.29±0.25). This suggests that in our group of patients similar changes in myosin heavy chain composition have not taken place.

Isometric tension was smaller in atrial than in ventricular preparations. This might be caused by a lower density of myofibrils in the atrial tissue. However, in view of the differences in contractile protein composition discussed above, an interaction between different contractile proteins (MHC isoforms, MLC isoforms and actin) in atrial and ventricular myocardium might be involved as well.

5. Concluding remark

In this study clear differences in myofibrillar contractile function and protein composition were observed between atrial and ventricular tissue, but not between subendo- and subepicardial ventricular myocardium. It appeared that no regional differences in contractile protein composition and myofibrillar function existed or developed in patients with valvular defects. An interesting implication of this finding is that small ventricular biopsies taken during cardiac surgery may be representative of the whole left ventricle. Contractile protein composition, tension and calcium sensitivity of tension remained unaltered up to NYHA class IV. This indicates that alterations in contractile proteins are not the main cause of the decrease in pump function observed in diseased hearts, but opens the possibility that (transmural) differences in contractile protein composition and myofibrillar contractile function in the left ventricle could be indices of end-stage heart failure.

Acknowledgements

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References


[33] Fabiato A, Fabiato F. Contraction induced by a calcium-triggered release of calcium from the sarcoplasmic reticulum of single skinned cardiac cells. J Physiol 1975;249:469–495.

[34] Strang KT, Moss RL. α1-Adrenergic receptor stimulation decreases maximum shortening velocity of skinned single ventricular myocytes from rats. Circ Res 1995;77:114–120.


