Skinned Fibers of Human Atrium and Ventricle: Myosin Isoenzymes and Contractility

Ingo Morano, Holger Arndt, Christine Gärtner, and Johann Caspar Rüegg

Different myosin isoenzymes of pig and human atrium and ventricle were characterized by two approaches: pyrophosphate polyacrylamide gel electrophoresis (PP-PAGE) and analysis of the myosin P light chains by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). We further investigated the relation between atrial and ventricular myosin isoenzymes of human and pig and of rat ventricle and the maximum (unloaded) shortening velocity ($V_{\text{max}}$) and the Ca$^{2+}$ sensitivity of chemically skinned fibers of the same species. The myosin isoenzymes of both human and pig atrium comigrated in the PP-PAGE with rat V1 isomyosin, whereas the ventricle of human and pig comigrated with rat V2. In both human and pig ventricle, a myosin P light chain polymorphism exists (two phosphorylatable P light chains with the same molecular weight but different isoelectric points). In contrast, we found no P light chain polymorphism in the atrium of human and pig and in the ventricle of rat. A correlation exists between $V_{\text{max}}$, Ca$^{2+}$ sensitivity, and atrium- and ventricle-specific myosin isoenzymes of human and pig. $V_{\text{max}}$ was determined by the slack-test method. Plots of $A_1$ versus $A_t$ of atrial and ventricular skinned fibers were well fitted by a single straight line up to $A_1 = 15\%$ and $A_t = 13\%$, respectively. $V_{\text{max}}$ of skinned ventricular fibers was lower than $V_{\text{max}}$ of skinned atrial fibers in both human and pig. Ca$^{2+}$ sensitivity of skinned fibers of ventricle, however, was higher than Ca$^{2+}$ sensitivity of atrial skinned fibers in both human and pig.


ATPase activity and maximal shortening velocity ($V_{\text{max}}$) of skeletal and cardiac muscle depend on the contractile protein myosin and its isoenzymatic variety,1,4 which are, therefore, a major determinant of contractility.

The cardiac myosin molecule consists of two heavy chains with a molecular weight of 200 kDa each, two 28-kDa light chains (LC-1), and two 18-kDa light chains (LC-2). The human atrium6-7 and the rat ventricle2,3 can adapt to different work loads by changing their myosin heavy chains. Furthermore, ventricle-specific myosin light chains appear in the hypertrophied human atrium6 and atrium-specific myosin light chains in the hypertrophied human ventricle.2,6,11 The 18-kDa myosin light chain (LC-2) can be phosphorylated and dephosphorylated by a light chain kinase that is Ca$^{2+}$ and calmodulin dependent (MLCK) and light chain phosphatase, respectively.12,13 The cardiac LC-2 has, therefore, been designated as the P-light chain (P-LC) or regulatory light chain. We previously demonstrated that the phosphorylation level of the P-LC influenced the Ca$^{2+}$ sensitivity of skinned ventricular fiber bundles: MLCK-induced increased myosin P-LC phosphorylation sensitized and dephosphorylation desensitized chemically skinned cardiac fibers for Ca$^{2+}$.14,15 This observation could be verified by others16 and was also found in the skeletal muscle of rabbits.17 Because little is known about the different variations of the myosin P-LC in the cardiac muscle, we investigated the myosin P-LC of atrium and ventricle of human and pig and of the ventricle of the rat using a high-resolution two-dimensional gel electrophoretic system.

The different myosin isoenzymatic forms of the rat are generally analyzed by pyrophosphate polyacrylamide gel electrophoresis.2,4 We could characterize atrial and ventricular myosin isoforms of human and pig by the same method using rat isoenzymes as "carrier." A strong correlation exists between the myosin isoenzymatic forms and $V_{\text{max}}$ of the ventricle of rats7,8 and rabbits.19,20 We investigated whether $V_{\text{max}}$ correlated with atrium- and ventricle-specific myosin isoforms in pig and human. In addition, we studied whether the Ca$^{2+}$ sensitivity of chemically skinned cardiac fibers varied with different isoenzymes in pig and human. This approach seems to be reasonable because the modulation of the Ca$^{2+}$ sensitivity of cardiac myofibrils seems to be a basic physiological mechanism (for review see Rüegg21).

The advantage of chemically skinned fibers is the absence of membranes and the accessibility of the myofibrils for exogenous Ca$^{2+}$-containing solutions, ATP, drugs, and proteins.

Materials and Methods

Biological Material

We used the right atrium and left ventricle of patients with coronary heart disease because these cells were not hypertrophied.22 Samples were taken during surgery from the local university hospital and were transported in cardioplegic solution (4°C) to our laboratory.
Right atrium and ventricle of pigs were obtained from the local slaughterhouse. Rat ventricles were excised from 4- or 20-week-old rats of the Wistar-Kyoto (WKY) strain.

Biochemical Analysis

Pyrophosphate polyacrylamide gel electrophoresis (PP-PAGE). PP-PAGE was carried out as described. Whole tissues or skinned fibers were crushed in liquid nitrogen and extracted for 20 minutes at 4°C with 3.4 μl/mg muscle of a modified Guba-Straub solution (0.3 M KCl, 0.1 M NaPO₄, 1 mM MgCl₂, 10 mM Na₄P₂O₇, 10 mM EDTA, 1% Na₂ [wt/vol], 1% β-mercaptoethanol, pH 6.5). The extract was centrifuged at 20,000 rpm for 20 minutes, and the supernatant was mixed 1:1 with glycerol and stored at −20°C. Electrophoresis was performed in 4% polyacrylamide gels using a Pharmacia GE-4 apparatus, which allows circulation of the upper and lower chamber buffer (20 mM Na₄P₂O₇, 1 mM EDTA, 10% glycerol, 0.1% β-mercaptoethanol, pH 8.5). The temperature was maintained at 1°C. The electrophoresis was run for 20 hours at 84 V (constant). Gels were stained for 30 minutes in 0.025% Coomassie blue R-250, 10% methanol, and 7% acetic acid, and destained in 7% acetic acid.

Two-dimensional gel electrophoresis (2D-PAGE). The first dimension (isoelectric focusing) was carried out as described. In short, isoelectric focusing was performed overnight in gels composed of 6.8% acrylamide, 1.7% Triton X-100, 9 M urea, and 20% ampholine (pH 4.5–5.4, Pharmacia, Sweden) at 400 V. The second dimension (SDS-PAGE) was run as described.

To demonstrate P light chain polymorphism, chemically skinned fibers were phosphorylated for 15 minutes at room temperature at pH 6.7 in a solution containing ATP (10 mM), Ca²⁺ (pCa 5.45), calmodulin (4 μM), and MLCK (100 μM) as described and immediately denatured with ice-cold 15% TCA to exclude dephosphorylation by endogenous light chain phosphatase that survived the skinning procedure. We had to phosphorylate the myosin P-LEC especially for human skinned fibers because human cardiac tissue was transported in cardioplegic solution prior to the skinning procedure. The myosin P-LEC of human skinned cardiac fibers was, therefore, mainly dephosphorylated (not shown). If cardiac tissue is chemically skinned without previous incubation in cardioplegic solution, therefore, mainly dephosphorylated (not shown). If cardiac tissue is chemically skinned in a solution containing 50% glycerol, 20 mM imidazole, 10 mM NaN₃, 5 mM ATP, 5 mM MgCl₂, 4 mM EGTA, 2 mM DTE, pH 7.0, at 4°C for 1 hour and then in the same solution including 1% Triton X-100 at 4°C for 24 hours. The fibers were stored at −20°C in the first solution without detergent.

Mechanical Experiments

Skinning procedure. Fiber bundles of the right atrium and left ventricle of all species studied were chemically skinned in a solution containing 50% glycerol, 20 mM imidazole, 10 mM NaN₃, 5 mM ATP, 5 mM MgCl₂, 4 mM EGTA, 2 mM DTE, pH 7.0, at 4°C for 1 hour and then in the same solution including 1% Triton X-100 at 4°C for 24 hours. The fibers were stored at −20°C in the first solution without detergent.

Isometric tension registration. The chemically skinned fibers were mounted isometrically and connected to a force transducer (AME 801, SensoNor, Horten, Norway). Fibers were slightly stretched in relaxation solution (sarcomere length about 2 μm). The relaxation solution contained ATP 10 mM, MgCl₂ 12.5 mM, EGTA 5 mM, imidazole 20 mM, NaN₃ 5 mM, phosphocreatine 10 mM, and creatine kinase (Boehringer, Mannheim, FRG) 380 U/ml, pH 6.7.

The contraction solution had the same composition as the relaxation solution except that EGTA was substituted with 5 mM Ca-EGTA. The desired Ca²⁺ concentration was obtained by mixing the relaxation and contraction solutions in the appropriate proportions. The actual Ca²⁺ concentrations were calculated by a computer program similar to that reported by Fabiato and Fabiato.

Maximal (unloaded) shortening velocity (Vₘax). Vₘax was obtained at maximal Ca²⁺ activation (pCa 4.3) by the slack-test method as described. This procedure was used since shortening is directly measured under zero applied load. Skinned fibers (3–3.5 mm long, 0.15 mm thick) were first released in relaxation solution and then prestretched to the extent that resting tension was just “threshold” (sarcomere length, 1.9–2.0 μm). The fibers were then activated at maximal Ca²⁺ concentration, and quick releases of different amplitudes were applied to the fibers within 4–6 msec at isometric tension plateau. The release amplitudes introduced to one end of a fiber varied within 6% to 15% of the initial muscle length (ΔL/ΔL %). All release amplitudes abolished tension completely. After the onset of tension recovery, the fiber was restretched to the initial muscle length (see inset Figure 5A). Percent of initial fiber length (ΔL) was plotted versus slack-time (time lag between the onset of a release and the onset of tension recovery Δt [msec]). Straight lines were then fitted by the least-squares method. The slope of the regression line provided the measure of Vₘax. Experiments were carried out at 21°C.

Statistical Analysis

Results were expressed as mean ± SEM. The data of the normalized tension-pCa relations were fitted to the Hill equation:

\[ y = \frac{[Ca^{2+}]^n}{EC_{50} + [Ca^{2+}]} \]

where \( y \) is the fractional force, \( EC_{50} \) is the Ca²⁺ concentration half-maximal activation, \( n \) is the Hill coefficient that is an index of cooperativity.

\( V_{max} \) was determined by calculating the regression line using the least-squares method.

Results

Biochemical Analysis

Pyrophosphate polyacrylamide gel electrophoresis (PP-PAGE). We tried to characterize atrium-specific and ventricle-specific myosin isoenzymes of human...
and pig using PP-PAGE (Figures 1A and 1B). While a comigration of myosin crude extracts of pig atrium and ventricle yielded two well-separated bands, no separation of human atrium- and ventricle-specific myosins could be achieved.

However, when human myosin crude extracts of atrium and ventricle were comigrated together with myosin crude extract of rat ventricle containing the three isomyosins of rat ventricle, a clear separation of atrium- and ventricle-specific myosins of humans and, of course, of pigs, could be observed. In these comigration experiments with the rat ventricular myosin isoenzymes as carrier, human as well as pig atrial and ventricular myosin could be characterized: human and pig atrial myosin comigrated with rat V2, whereas human and pig ventricular myosin comigrated with rat V3. The ventricular myosin crude extract of a 20-week-old female rat was used as a carrier.

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). 2D-PAGE of skinned fibers of atrium and ventricle was used to characterize the variation of the myosin P-LC. Two phosphorylatable P-LC exist in the ventricle of human and pig (P-LC polymorphism) (Figures 2A and 2B). In the atrium of human and pig (Figures 3A and 3B) and in the ventricle of the rat (Figure 3C), no P-LC polymorphism could be observed; only one phosphorylatable light chain was present.

Mechanical Experiments

Ca²⁺ sensitivity and cooperativity of force generation. We investigated the tension-pCa relation of human and pig atrium and ventricle and of the ventricle of 4-week-old rats. Ca²⁺ sensitivity was expressed as pCa⁰, i.e., the Ca²⁺ concentration (expressed as negative decadic logarithm of the Ca²⁺ concentration-pCa) at which half-maximal activation occurs. In both human and pig, the Ca²⁺ sensitivity of the ventricle (pCa⁰ = 5.67 for human; pCa⁰ = 5.53 for pig) was higher than that of the atrium (pCa⁰ = 5.5 for human; pCa⁰ = 5.4 for pig) (Figures 4A and 4B, Table 1). Skinned fibers of the rat ventricle showed a lower Ca²⁺ sensitivity (pCa⁰ = 5.2) than human and pig ventricular fibers (Figure 4C, Table 1).

The cooperativity factors (Hill coefficient “n”) of the tension-pCa relations of atrial and ventricular skinned fibers of human and pig were quite similar: we found cooperativity factors of 3.0 and 2.7 for human ventricle and atrium and cooperativity factors of 2.5 for pig atrium and ventricle (Figures 4A and 4B, Table 1). Again, the cooperativity factor of the ventricle of the rat was very distinct from that of human and pig ventricle: we calculated a Hill coefficient of 4.0 for the rat (Figure 4C, Table 1).

Maximal (unloaded) shortening velocity. The relation between slack-times (time lag between the onset of a release and the onset of tension recovery Δt/ms) and different release amplitudes (expressed as percent of the initial fiber length) of skinned cardiac fibers is biphasic containing an initial phase of steady high-velocity shortening and a subsequent phase of low-velocity shortening (Figures 5A and 5B). We calculated V⁰ from the initial high-velocity phase. Generally, the high-velocity phase of ventricular (Figure 5B) and atrial (Figure 5A) skinned fibers can well be fitted by a single straight line between release amplitudes of 6–13% and 8–15%, respectively, in our system. After initial visual inspection, plots, of Δt(%) versus Δt(ms) were fitted by the least-squares method, and
the slope of the regression line was used to calculate $V_{\text{max}}$.

In both human and pig, atrial skinned fibers showed a higher $V_{\text{TO}}$ than skinned ventricular fibers (Figures 6A and 6B): we found mean unloaded shortening velocities of 2.63 muscle length/sec and 4.0 muscle length/sec for atrial fibers of human and pig, respectively, and 1.65 muscle length/sec and 2.5 muscle length/sec for ventricular fibers of human and pig, respectively (Figures 6A and 6B, Table 1).

**Discussion**

Although the contractile proteins of the human heart have been the matter of many investigations, only few comparative data are available concerning the contractility of different cardiac cell types. Here we report the results obtained for maximal (unloaded) shortening velocity ($V_{\text{TO}}$) and the Ca$^{2+}$ sensitivity of chemically skinned atrial and ventricular fibers of the human heart with no sign of cardiac hypertrophy. We further compared these data with other species, namely pig and rat, because they are commonly used as animal models for physiological and biochemical studies.

As demonstrated by PP-PAGE both human and pig atrial and ventricular myosin isoenzymes comigrated with the rat V$_3$ and rat V$_2$ myosin isoenzymes, respectively. The different charges of human cardiac myosin isoenzymes correspond to different myosin heavy chains and light chains in atrium and ventricle.$^{7,10-24}$

Whereas pig atrium myosin can be separated from pig ventricular myosin by PP-PAGE, this was impossible with human extracts. Using rat ventricular isomyosin as carrier, however, atrium and ventricle isomyosins could also be separated and characterized by PP-PAGE. The reason for this phenomenon is unclear and needs further investigation. In the future, it will be interesting to study whether the variety of ventricular myosin in the hypertrophied human atrium can be identified by PP-PAGE and rat ventricle as carrier. In the ventricle of rabbit, guinea pig, cow,$^{32-33}$ chicken,$^{34}$ pig,$^{14}$ and human,$^{31}$ two forms exist of the P-LC with the same molecular weight but different isoelectric points.

In this study, the two phosphorylatable P-LC forms and their phosphorylated derivatives were studied using skinned fibers analyzed by 2D-PAGE. They were designated as LC-2, LC-2*, LC-2-P (the phosphorylated form of LC-2), and LC-2*P (the phosphorylated form of LC-2*), following the nomenclature of Price et al.$^{31}$ We observed two phosphorylatable P-LC in the ventricle but only one phosphorylatable P-LC in the atrium of human and pig even after preincubation of skinned fibers with MLCK. This observation is in agreement
with the concept of Westwood et al., who showed that a P-LC polymorphism exists in slow but not in fast muscle of mammalia as the atrium of human and pig showed a higher shortening velocity than the ventricle. In contrast, Price et al. observed two phosphorylatable P-LC in the human atrium. Furthermore, the same authors also demonstrated several forms of the myosin LC-1 in 2D-PAGE, which is quite surprising because LC-1 is not modified by phosphorylation and several forms of LC-2 (P-LC) have different molecular weights and different isoelectric points. No P-LC polymorphism exists in the rat ventricle either in young or in adult animals. We feel that this close correlation between shortening velocity and the phenomenon of a P-LC polymorphism in the striated muscle of mammalia might be important but further studies will have to prove a direct relation between these physiological and biochemical parameters. The phosphorylation level of LC-2 has no influence on the shortening velocity either of skinned skeletal and heart fibers or of intact skeletal muscles. In one report, however, a reduced $V_{\text{max}}$ was observed after increasing the P-LC phosphorylation of skeletal muscle. The P-LC phosphorylation influences the Ca$^{2+}$ sensitivity of skinned skeletal and cardiac fibers in that the higher the phosphorylation level the higher the Ca$^{2+}$ sensitivity. Ventricular skinned fibers of humans were more sensitive to Ca$^{2+}$ than skinned fibers of pig ventricles although human fibers were, in contrast to pig fibers, mainly dephosphorylated. This may be due to the fact that human cardiac tissue was transported in cardioplegic solution prior to the skinning procedure. Further, although the myosin P-LC of both human atrial and ventricular skinned fibers were mainly dephosphorylated (authors' unpublished results), ventricular fibers were more Ca$^{2+}$-sensitive than atrial fibers. Therefore, we conclude that the different Ca$^{2+}$ sensitivities of atrial and ventricular skinned fibers observed in different species may not be related to different myosin P-LC phosphorylation levels but to different myosin isoenzymes present in the heart muscle. A similar correlation was observed by Fabiato in the ventricle of rats. He found a higher Ca$^{2+}$ sensitivity of ventricular fibers of rats two days prepartum than in adult rats, and it is known that fetal rats have pure $V_{1}$ and that adult rats have high amounts of $V_{1}$. Arndt et al. reported that the Ca$^{2+}$ sensitivity of hypertrophied human atria was increased, which supports our finding of different Ca$^{2+}$ sensitivities of atrial and ventricular fibers since in the hypertrophied human atrium ventricle-specific myosin heavy chains and myosin light chains appear. In contrast, no change of Ca$^{2+}$ sensitivity of

### Table 1. Ca$^{2+}$ Sensitivity and Maximal (Unloaded) Shortening Velocity ($V_{\text{max}}$) of Atrial and Ventricular Chemically Skinned Fibers of Human, Pig, and Rat

<table>
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<tr>
<th></th>
<th>Human Atrium</th>
<th>Human Ventricle</th>
<th>Pig Atrium</th>
<th>Pig Ventricle</th>
<th>Rat Ventricle</th>
</tr>
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<tbody>
<tr>
<td>$V_{\text{max}}$</td>
<td>2.63 ± 0.4</td>
<td>1.65 ± 0.2</td>
<td>4.0 ± 0.4</td>
<td>2.5 ± 0.3</td>
<td>—</td>
</tr>
<tr>
<td>pCa$_{50}$</td>
<td>5.5</td>
<td>5.67</td>
<td>5.4</td>
<td>5.53</td>
<td>5.2</td>
</tr>
<tr>
<td>n</td>
<td>2.7</td>
<td>3.0</td>
<td>2.5</td>
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Ca$^{2+}$ sensitivity is expressed as pCa$_{50}$, the Ca$^{2+}$ concentration (expressed as pCa = −log free Ca$^{2+}$) at which half-maximal activation occurs. The degree of positive cooperativity is expressed by the Hill coefficient n. $V_{\text{max}}$ is expressed in muscle length/sec.

Number of fibers in parentheses. Values are mean ± SEM. pCa$_{50}$ and cooperativity factors were calculated from tension-pCa relation of whole data collected for muscle type studied. $V_{\text{max}}$ was calculated using regression line for every fiber separately. Regression coefficients for every fiber were between 0.92 and 0.999.
skinned ventricular fibers of rats and rabbits could be found as a function of myosin isoenzymes and cardiac hypertrophy, respectively. The different calcium sensitivity of (slow) ventricular and (fast) atrial skinned fibers seems to be in agreement with results from skeletal muscle: in the rat, slow-twitch fibers are more sensitive to Ca^{2+} than fast-twitch fibers.

The Ca^{2+} sensitivity of chemically skinned cardiac fibers was species-dependent. Thus, skinned ventricular fibers of 4-week-old rats (exclusively V) were less Ca^{2+} sensitive than ventricular fibers of human and pig.

In addition to the correlation between myosin isoenzymes and Ca^{2+} sensitivity, we observed a correlation between myosin isoenzymes and V_max: in both human and pig, the atrial fibers revealed a higher V_max than ventricular fibers. This observation corresponds to the higher myosin ATPase activity in the human atrium compared with the human ventricle. Thus, a transition of myosin isoenzymes in the human atrium to the ventricular type should decrease the shortening velocity. Indeed, it could be shown that hypertrophied human atria have a lower V_max than nonhypertrophied.

We calculated V_max from plots of Δl versus Δt at the initial high-velocity shortening phase. As we used fibers of 3–3.5 mm length, length steps up to 500 μm for atrial and 400 μm for ventricular skinned fibers could be applied to the preparations. Above these values, a phase of low-velocity shortening commenced, e.g., the relation Δl versus Δt becomes biphasic. Accordingly, length steps up to 400 μm were applied to fast- and slow-twitch skinned skeletal muscle fibers to evaluate V_max from the high-velocity shortening phase when activated at maximal Ca^{2+} concentration. Biphasic plots of Δl versus Δt appeared at submaximal Ca^{2+} concentration of both fast- and slow-twitch skinned skeletal muscle fibers or at maximal Ca^{2+} concentration after partial extraction of troponin-C.

To summarize, we observed that both Ca^{2+} sensitivity and V_max of chemically skinned human and pig cardiac fibers correlate with the myosin isoenzyme pattern. Future work will show whether it is possible
to causally relate the different Ca\(^{2+}\) sensitivities and shortening velocities to different crossbridge kinetics of myosin isoenzymes, as proposed by Brenner.\(^{43}\)

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