Correlation Between Myofilament Response to Ca\textsuperscript{2+} and Altered Dynamics of Contraction and Relaxation in Transgenic Cardiac Cells That Express \(\beta\)-Tropomyosin


Abstract—We compared the dynamics of the contraction and relaxation of single myocytes isolated from nontransgenic (NTG) mouse hearts and from transgenic (TG-\(\beta\)-Tm) mouse hearts that overexpress the skeletal isoform of tropomyosin (Tm). Compared with NTG controls, TG-\(\beta\)-Tm myocytes showed significantly reduced maximal rates of contraction and relaxation with no change in the extent of shortening. This result indicated that the depression in contraction dynamics determined in TG-\(\beta\)-Tm isolated hearts is intrinsic to the cells. To further investigate the effect of Tm isoform switching on myofilament activity and regulation, we measured myofilament force and ATPase rate as functions of pCa (\(-\log\) of [Ca\textsuperscript{2+}]). Compared with controls, force generated by myofilaments from TG-\(\beta\)-Tm hearts and myofibrillar ATPase activity were both more sensitive to Ca\textsuperscript{2+}. However, the shift in pCa\textsubscript{0} (half-maximally activating pCa) caused by changing sarcomere length from 1.8 to 2.4 \(\mu\)m was not significantly different between NTG and TG-\(\beta\)-Tm fiber preparations. To test directly whether isoform switching affected the economy of contraction, force versus ATPase rate relationships were measured in detergent-extracted fiber bundles. In both NTG and TG-\(\beta\)-Tm preparations, force and ATPase rate were linear and identically correlated, which indicated that crossbridge turnover was unaffected by Tm isoform switching. However, detergent extracted fibers from TG-\(\beta\)-Tm demonstrated significantly less maximum tension and ATPase activity than NTG controls. Our results provide the first evidence that the Tm isoform population modulates the dynamics of contraction and relaxation of single myocytes by a mechanism that does not alter the rate-limiting step of crossbridge detachment. Our results also indicate that differences in sarcomere-length dependence of activation between cardiac and skeletal muscle are not likely due to differences in the isoform population of Tm. (Circ Res. 1999;84:745-751.)

Key Words: transgenic mice ■ myocyte ■ tropomyosin ■ sarcomere ■ thin filament

Activation of cardiac muscle myofilaments involves complex steric, allosteric, and cooperative mechanisms in which tropomyosin (Tm) plays a central role (see Solaro and Rarick\textsuperscript{1} and Tobacman\textsuperscript{2}). According to the steric model of myofilament activation, Ca\textsuperscript{2+} binding to troponin C (TnC) causes Tm to move to a different position on the thin filament, which exposes actin sites for myosin-head binding.\textsuperscript{3,4} Allosteric activation suggests that Tm may affect myofilament activity by a change in the reaction of actin with myosin heads and that Ca\textsuperscript{2+} binding to TnC is insufficient to fully activate the thin filament. In the allosteric model of myofilament activation, Ca\textsuperscript{2+} plays a role as a cofactor that influences the equilibrium between “off” and “on” states of Tm.\textsuperscript{1,2,5} End-to-end interactions between contiguous Tm molecules are also important in cooperative interaction of the thin filament.\textsuperscript{6,7}

An important and poorly understood function is how changes in Tm isoform population or covalent phosphorylation affect cardiac function. Moreover, the functional significance of a point mutation in \(\alpha\)-Tm (Asp175Asn), which is linked to familial hypertrophic cardiomyopathy, is unclear.\textsuperscript{8–10} All of these alterations involve changes in the charge of Tm, an important aspect of its reaction with other thin-filament proteins.\textsuperscript{11} Our hypothesis is that modification of electrostatic interactions between Tm and actin and between Tm and Tn affect myofilament activation and cardiac dynamics.

Although substantial information on the in vitro properties of Tm exists, only recently has it become possible to alter specifically the isoform population of Tm in heart muscle with a transgenic (TG) approach.\textsuperscript{12,13} In an initial series of experiments, it was found that the exchange of \(\alpha\)-Tm isoform...
with \(\beta\)-Tm in the myofilament lattice influenced dynamics of relaxation in work-performing hearts\(^{12}\) and increased submaximal thin-filament activation through \(Ca^{2+}\) and strong crossbridges in skinned preparations.\(^{13}\) In the present experiments, we used intact single cells isolated from TG mouse hearts (TG-\(\beta\)-Tm) and nontransgenic mouse hearts (NTG) to show that the changes observed in the dynamics of contraction in whole-heart preparations are intrinsic to the cells. To understand the mechanism of these changes, we measured the ATPase rate and tension that was generated by the myofilaments. Our results provide the first evidence that the Tm isoform population modulates the dynamics of contraction and relaxation of intact single myocytes.

### Materials and Methods

#### TG Animals

TG mice (FVBN strain) that overexpressed \(\beta\)-Tm were generated as described by Muthuchamy et al.\(^{12}\) TG mice that overexpressed \(\alpha\)-Tm were generated through essentially the same method as TG-\(\beta\)-Tm mice except that the coding sequence for the mouse \(\alpha\)-Tm cDNA was used instead of \(\beta\)-Tm cDNA. Expression of the transgene was driven by the murine \(\alpha\)-myosin heavy chain promoter, which restricts expression to the cardiac compartment. The mice demonstrated no gross phenotypic abnormalities, no evidence of neonatal mortality, and no histological evidence of abnormalities or hypertrophy.\(^{12}\)

#### Myocyte Isolation

Myocytes were isolated as described by Wolska and Solaro.\(^{14}\) The animals were heparinized (5000 U/kg body wt) and after 30 minutes, anesthetized with pentobarbital sodium (50 mg/kg body wt IP). The hearts were quickly removed and put into cold, nominally Ca\(^{2+}\)-free control solution of the following composition (in mmol/L): NaCl 133.5, KCl 4, MgSO\(_4\) 1.2, HEPES 1.2, and glucose 11. The pH of the solution was adjusted with NaOH to 7.4. The ascending aorta was cannulated, and the hearts were perfused by the Langendorf method at 37°C at a perfusion pressure of 60 cm of H\(_2\)O. After 10 minutes, the hearts were removed and placed into a dish with the aid of 1.0% Triton X-100. Protein concentration was measured to one fourth of its original volume because of the small amount of protein obtained from myofilament preparation. The reaction continued at 30°C for 10 minutes in a solution of the following composition (in mmol/L): Mg\(^{2+}\) 1, MOPS 20, KCl 79.5, MgATP\(^{2-}\) 5, EGTA 1, and pCa (\(-\log[Ca]\)) with values that ranged from 8.0 to 4.875 at pH 7.0. The reaction was stopped with trichloroacetic acid and inorganic phosphate that was determined had been released as described by Carter and Karl.\(^{17}\) The ionic composition of all solutions was computed with an iterative computer program.\(^{18}\)

#### Force and ATPase Activity Measurements of Skinned Fiber Bundles

Measurements of the pCa-force relation at different sarcomere lengths were performed on fiber bundles prepared as follows: Adult mice were anesthetized with pentobarbital sodium (50 mg/kg body wt IP), and hearts were quickly removed and put into cold high-relaxing solution of the following composition (in mmol/L): KCl 53, EGTA 10, MOPS 20, free Mg\(^{2+}\) 1, MgATP\(^{2-}\) 5, creatine phosphate 12, and 10 IU/mL creatine phosphokinase. The pH of the solution was adjusted to 7.0 with KOH. The ionic strength of all solutions was 150 mmol/L. Papillary muscles from the left ventricle were dissected, and small fiber bundles \(\approx 150\) to 200 \(\mu\)m in width and 4 to 5 \(\mu\)m in length were prepared. Fiber bundles were mounted between a micromanipulator and a force transducer with cellulose-acetate glue. Fibers were skinned for 30 minutes in high-relaxing solution that contained 1% Triton X-100. A resting sarcomere length of 1.8, 2.0, and 2.4 \(\mu\)m was established from laser diffraction patterns.\(^{19}\) Isometric tension was recorded on a chart recorder. After skimming, the fibers were initially washed in high-relaxing solution and then sequentially bathed in low-relaxing solution followed by solutions of varying pCa values (pCa range from 8.0 to 4.5). Compared with high-relaxing solution, low-relaxing solution contained 0.1 mmol/L EGTA. All solutions also contained the protease inhibitors pepstatin A (2.5 \(\mu\)mol/L), leupeptin (1 \(\mu\)mol/L), and PMSF (50 \(\mu\)mol/L). In a separate series of experiments, we simultaneously measured ATPase activity and steady-state force.\(^{20,21}\) Detergent extracted fiber bundles were prepared from left papillary muscle as described above and placed in a standard relaxing solution to which 1% vol/vol Triton X-100 was added. The composition of this standard relaxing solution was as follows (in mmol/L): Na\(_2\)ATP 7.3, MgCl\(_2\) 10.6, EGTA 20, creatine phosphate 10, and BES 100. The pH of the solution was adjusted to 7.1 with KOH. The ionic strength was adjusted to 200 mmol/L with KCl. The preparations were left in this solution for 2 hours for complete extraction of membranes.

The skinned muscle was attached to a displacement generator (model 300B, Cambridge Technology) on one end and to a force transducer element (AE 801, SensoNor) on the other end with aluminum T clips. The natural frequency of the force transducer was \(\approx 2\) kHz. The muscle preparation was transferred manually between several baths to expose the muscle to the various solutions that were used in the present study (Table). The bath that was used for the ATPase assay had quartz windows to allow transmission of near-UV light (340 nm) for the measurement of NADH absorbance (see below). The volume of this bath was 30 \(\mu\)L, and the bath was continuously stirred by motor-driven vibration of a membrane positioned at the bottom of the bath. The baths were filled in aluminum blocks (anodized) and mounted on top of an aluminum base through which water was circulated to allow temperature control.
control of all solutions (20±1°C). The force and displacement generator position (ie, muscle length) signals were filtered at 1 kHz (corner frequency, slope −12 dB/oct). The NADH absorbance signal was filtered at 1 Hz (corner frequency, slope −12 dB/oct). The data were recorded on a chart recorder and sampled through an A/D converter on computer disk (Macintosh 7600, Apple). Samples were collected at a rate of 5 per second for 5 minutes. Sarcomere length of the preparation was measured in relaxing solution by means of a He-Ne laser and set at 2.3 μm.

The ATPase activity of the skinned muscle was measured online by means of an enzyme-coupled assay as described in detail previously.20 Formation of ADP by the muscle was stoichiometrically coupled first to the synthesis of pyruvate and ATP from phosphoenolpyruvate, a reaction that is catalyzed by the enzyme pyruvate kinase, and, subsequent to synthesis of lactate, a reaction that is catalyzed by the enzyme lactate dehydrogenase and during which NADH is oxidized to NAD⁺. The breakdown of NADH was determined photometrically. The ratio of light intensity at 340 nm, which is sensitive to the NADH concentration in the bath, and the light intensity at 410 nm, which serves as a reference signal, was obtained by means of an analog divider. After each recording in the assay bath, the NADH absorbance signal was calibrated by multiple injections of 0.25 nmol of ADP (0.025 μL of 10 mmol/L ADP solution) with a stepper motor-controlled calibration pipette (World Precision Instruments). With this method, the SE of the first time point was expressed as the percent of total Tm. Figure 3, compared with NTG myocytes, TG-β-Tm cells were expressed as the percent of total Tm.

### Solutions Used for Simultaneous Force and ATPase Activity Measurements

Three bathing solutions were used: a relaxing solution, a preactivating solution with low calcium-buffering capacity, and an activating solution. The composition of these solutions is shown in the Table. In addition, all solutions contained 0.9 mmol/L NADH, 100 mmol/L BES, 5 mmol/L Na-azide; 10 mmol/L phosphoenolpyruvate; 4 mg/mL pyruvate kinase (500 U/mg); 0.24 mg/mL lactate dehydrogenase (870 U/mg); 10 μmol/L oligomycin B; 0.2 mmol/L ATP; 100 μmol/L leupeptin. Ionic strength was set at 200 mmol/L by potassium propionate. The pH was adjusted to 7.1; 20°C. Free Mg²⁺ and MgATP concentrations were calculated at 1 and 5 mmol/L, respectively.

### Experimental Protocol

During a series of measurements, the muscle was incubated in the relaxing solution for 3 minutes, in the preactivating solution for 3 minutes, in the activating solution for ~1 minute, and then returned to the relaxing solution. Before the first activation-relaxation cycle, sarcomere length in the preparation, as measured in relaxing solution, was adjusted to 2.3 μm. Then, after an initial activation at a saturating calcium concentration (pCa 4.3), sarcomere length was readjusted to 2.3 μm. It was found that after this readjustment, resting sarcomere length remained stable throughout the experiment. Next, a second activation at the saturating Ca²⁺ was performed, which served as a first force and ATP consumption rate measurement. The next 5 to 6 contractures were performed at a range of intermediate Ca²⁺ concentrations that also included the relaxing solution (pCa 9). These measurements were then followed by a final control contracture at saturating Ca²⁺.

### Gel Electrophoresis

SDS-PAGE was performed according to the method of Laemmli.23 Proteins from isolated myofibrils or cells were separated on 12.5% SDS gels. To quantify expression of β-Tm, the gels were scanned by densitometric analysis (Personal Densitometer SI, Molecular Dynamics). Myofilament proteins were identified by comigration with known standards.

### Data Computation and Statistical Analysis

All results were presented as mean±SE. Data were analyzed with Inplot software (GraphPad Software) and fitted to a sigmoidal curve of variable slope to derive the pCa₅₀ (half-maximally activating pCa) and Hill coefficient. The significance of differences between the means was evaluated by the Student t test. A value of P≤0.05 was the criterion for significance.

### Results

Figure 1 shows the SDS-PAGE analysis of cardiac myofibrils (A) and isolated cells (B) from NTG and TG-β-Tm mouse hearts. The level of expression of β-Tm in line 28 used for skinned fiber and ATPase experiments was 64.9±2.8% (n=4) and in line 10, the level of expression was 61.0±3.2% (n=3) for experiments with isolated single cells. The β-Tm was expressed as the percent of total Tm.

Figure 2 illustrates the results of experiments in which we compared the extent of shortening and the kinetics of twitch contraction and relaxation in single myocytes isolated from the NTG and TG-β-Tm mouse hearts. As summarized in Figure 3, compared with NTG myocytes, TG-β-Tm cells showed no change in the extent of shortening. We also found no significant changes in cell length between NTG and TG-β-Tm hearts. However, a significantly reduced maximum rate of contraction and relaxation existed. To determine the mechanisms of this decreased rate of relaxation, we measured isometric force and ATPase rates of myofilaments prepared from NTG and TG-β-Tm mouse hearts. Results presented in

### Ionic Composition of the Solutions

<table>
<thead>
<tr>
<th></th>
<th>MgCl₂</th>
<th>NaATP</th>
<th>EGTA</th>
<th>HDTA</th>
<th>CaEGTA</th>
<th>KProp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relaxing solution</td>
<td>8.37</td>
<td>5.80</td>
<td>20</td>
<td>...</td>
<td>...</td>
<td>42.5</td>
</tr>
<tr>
<td>Preactivating solution</td>
<td>7.78</td>
<td>5.80</td>
<td>0.5</td>
<td>19.5</td>
<td>...</td>
<td>43.6</td>
</tr>
<tr>
<td>Activating solution</td>
<td>7.63</td>
<td>5.87</td>
<td>...</td>
<td>...</td>
<td>20</td>
<td>43.6</td>
</tr>
</tbody>
</table>

Concentrations are expressed in mmol/L. All solutions contained 0.9 mmol/L NADH, 100 mmol/L BES, 5 mmol/L Na-azide; 10 mmol/L phosphoenolpyruvate, 4 mg/mL pyruvate kinase (500 U/mg), 0.24 mg/mL lactate dehydrogenase (870 U/mg), 10 μmol/L oligomycin B, 0.2 mmol/L ATP, and 100 μmol/L leupeptin. Ionic strength was set at 200 mmol/L by potassium propionate (Kprop); pH 7.1; 20°C. Free Mg²⁺ and MgATP concentrations were calculated at 1 and 5 mmol/L, respectively.
Figure 4A indicate that compared with controls, force generated by myofilaments from TG hearts was more sensitive to Ca$^{2+}$. The pCa 50 was 5.65 ± 0.00 (n = 7 from 4 different hearts) for NTG preparations and 5.78 ± 0.00 (n = 9 from 6 different hearts) for TG-β-Tm preparations. Figure 4B shows results of experiments in which we measured Ca$^{2+}$-activated MgATPase activity of unloaded myofibrils. We found no significant difference in maximum ATPase activity between myofibrils prepared from NTG and TG-β-Tm mouse hearts. However, as in the case of force developed by skinned fiber bundles, ATPase activity of myofibrils from TG-β-Tm mouse hearts was more sensitive to Ca$^{2+}$ compared with NTG hearts. The pCa 50 of the ATPase activity was 5.97 ± 0.00 (n = 21 from 7 preparations) for NTG myofibrils and 6.15 ± 0.00 (n = 13 from 5 preparations) for TG-β-Tm. We also compared simultaneous measurements of isometric force and ATPase activity in skinned preparations prepared from NTG and TG-β-Tm mouse hearts at sarcomere length 2.3 μm. Figure 5 shows the relationship between average steady-state force and pCa in both groups of animals. Consistent with data presented in Figure 4A, the force of fiber bundles prepared from TG-β-Tm mouse hearts was more sensitive to Ca$^{2+}$. The pCa 50 of the isometric force was 5.95 ± 0.00 (n = 9 from 4 different hearts) in NTG and 5.11 ± 0.00 (n = 9 from 4 different hearts) in TG-β-Tm mouse hearts. Myofilaments from TG-β-Tm mouse hearts also showed a significant decrease in cooperativity compared with myofilaments from NTG hearts (Hill coefficient was 3.3 ± 0.1 in NTG and 2.2 ± 0.1 in TG-β-Tm hearts). Interestingly, TG-β-Tm mouse hearts also showed a significant decrease in maximum force (at pCa 4.30) when compared with NTG hearts. Maximum force was 47.7 ± 4.4 mN/mm$^2$ in NTG and 33.7 ± 4.6 mN/mm$^2$ in TG-β-Tm mouse hearts. Figure 6 shows the relation between the rate of ATP consumption by the skinned muscle preparation and the level of average steady-state force. Myofilaments from both groups demonstrated a rate of ATP consumption that was linearly correlated...
with steady-state force regardless of Tm isoform population. In addition, the slope of the tension-ATPase relationship was similar between groups such that the data clustered close to a common line. Therefore, we conclude that the economy of force maintenance was not affected by Tm isoform composition.

To examine whether the isoform population of Tm influences length-dependent activation of the myofilaments, we measured pCa-force relations at sarcomere lengths 1.8 and 2.4 μm. The shift in pCa50 caused by changing the sarcomere length was not significantly different between NTG and TG-β-Tm fiber preparations. The ΔpCa50 was 0.12±0.01 (n=5 from 3 different hearts) for NTG and 0.14±0.03 (n=7 from 5 different hearts) for TG-β-Tm preparations.

As a control experiment for β-Tm overexpression, we overexpressed native α-Tm and measured isometric force of myofilaments prepared from TG-α-Tm mouse hearts and their litter mates (Figure 7). The pCa-force relationship was identical in both groups. The pCa50 was 5.74±0.03 (n=7 from 4 different hearts) in NTG and 5.73±0.02 (n=6 from 3 different hearts) in TG-α-Tm mouse hearts. Hill coefficient was 3.55±0.12 in NTG and 3.33±0.22 in TG-α-Tm mouse hearts.

Discussion

Results presented here provide the first evidence that the specific exchange of α-Tm with β-Tm (1) slows the kinetics of contraction and relaxation of single intact isolated myocytes, (2) reduces maximum tension developed by the myofilaments, (3) shifts pCa50 of the pCa-ATPase activity and pCa-force relation to lower Ca2+, and (4) does not alter length-dependent myofilament activation. Important questions that we address here are (1) What are the mechanisms responsible for the observed increase in Ca2+ sensitivity and decrease in maximum force and ATPase activity associated with replacing myofilament α-Tm with β-Tm and (2) What are the mechanisms responsible for depressed rates of contraction and relaxation in single myocytes from TG-β-Tm mouse hearts when compared with NTG?

A plausible mechanism for the increase in Ca2+ sensitivity of myofilaments from TG-β-Tm hearts is charge-dependent differences between the affinity of α-Tm and β-Tm for their
actin than wild type familial hypertrophic cardiomyopathy binds more weakly to the increased Ca\(^{2+}\) sensitivity of the myofilaments with no change in the maximum rate of force-generating crossbridges is additionally associated with an increase in pCa\(_{50}\), which agrees with our data from skinned fiber bundles.

Our results from studies of isolated cardiomyocytes are in agreement with results of previous studies that compared in vitro work-performing hearts of NTG and TG-\(\beta\)-Tm mice.\(^{12}\) Although cells isolated from TG-\(\beta\)-Tm mouse hearts showed the same extent of shortening as cells isolated from control mice, these cells demonstrated significant differences in the dynamics of contraction and relaxation. The decreased rate of relaxation of myocytes corresponds to decreased \(-dP/dt_{\text{max}}\) and the time to half relaxation in work-performing hearts. In cells isolated from TG-\(\beta\)-Tm mouse hearts, we also observed a small (36\%) but significant decrease in the maximum rate of contraction, despite no change in the maximum rate of contraction (\(dP/dt_{\text{max}}\)) in work-performing hearts. This difference in results obtained from studies with isolated myocytes and with work-performing hearts may be due to the different loads (essentially 0 for cells) against which these preparations contract. Moreover, our experiments were performed at room temperature, whereas experiments with work-performing hearts were performed at 35°C.

Our investigation of the length dependence of myofilament Ca\(^{2+}\) activation showed that myofilaments that contained predominantly \(\alpha\)-Tm and myofilaments that contained predominantly \(\beta\)-Tm demonstrated the same shift in pCa\(_{50}\) with changes in sarcomere length. We conclude from these results that differences in sarcomere length dependence of activation among different muscle types are not likely to be due to differences in the isoform population of Tm. The mechanism of length dependent activation is in dispute, but it is apparent that an important aspect of the mechanism is mediated by changes in interfilament spacing associated with changes in sarcomere length.\(^{28,30}\) The idea here is that the crossbridge reaction is promoted when the filaments are closer together and depressed when the filaments are farther apart. Thus, at a particular pCa value, force will rise or fall depending on interfilament spacing and give rise to shifts in the pCa-force relations. The mechanism appears to be modulated by fiber type variations in the activation state of the thin filament induced by the bound crossbridges. For example, recent results of McDonald et al\(^{31}\) that show differential effects of length on Ca\(^{2+}\) activation of slow versus fast skeletal muscle fibers were interpreted on the basis of a greater ability of strongly bound crossbridges to induce activation in the fast muscle fibers. Yet, it is apparent in our experiments that differences in cooperativity between \(\alpha\)-Tm and \(\beta\)-Tm that contained myofilament were not sufficient to account for the differences in length-dependent activation. Other factors that could play a role are differences in the TnI and TnT isoform population. Differences in TnC isoforms probably do not account for differences in length-dependent activation.\(^{32}\)

Our data show that although Ca\(^{2+}\) sensitivity in fiber bundles that contain \(\beta\)-Tm is increased, the maximum number of force-generating crossbridges is depressed, as reflected in the reduced tension at maximum free Ca\(^{2+}\). These results indicate that the Ca\(^{2+}\)-dependent transition to the “open” state of Tm is eased in fiber bundles that contain \(\beta\)-Tm, whereas neighbors on the thin filament. Switching from native \(\alpha\)-Tm to \(\beta\)-Tm, in which Ser\(^{229}\) →Glu, and His\(^{276}\) →Asn involves a -2 charge change.\(^{24}\) These charge changes are likely to affect Tm interactions with TnT and actin.\(^{11,25}\) The Tm-coiled coil possesses repeating motifs of charged and uncharged residues that react with actin and most likely TnT through a slower rate of force development in preparations from NTG and TG-\(\alpha\)-Tm, in which Ser\(^{229}\) →Glu, and His\(^{276}\) →Asn involves a -2 charge change.\(^{24}\) These charge changes are likely to affect Tm interactions with TnT and actin.\(^{11,25}\) The Tm-coiled coil possesses repeating motifs of charged and uncharged residues that react with actin and most likely TnT through mobile and accessible for binding to neighboring protein. This design of Tm provides versatile and flexible interactions with the thin filament and would be expected to be perturbed by charge changes. For example, Golitsina et al\(^{26}\) have shown that \(\alpha\)-Tm with a point mutation (Asp\(^{175}\)Asn) linked to familial hypertrophic cardiomyopathy binds more weakly to actin than wild type \(\alpha\)-Tm. Of course, the functional differences between the TG and NTG myofilaments reported could well be due to other amino acid substitutions that occur when switching from \(\beta\)-Tm to \(\alpha\)-Tm.

What are the mechanisms responsible for depressed rates of contraction and relaxation in single myocytes from TG-\(\beta\)-Tm mouse hearts when compared with NTG? This difference does not appear to arise from differences in the rate of crossbridge detachment, the limiting step of the crossbridge cycle. Our data obtained from the simultaneous determination of tension and ATPase rate indicate that tension is proportional to ATPase rate regardless of the Tm isoform in the myofilaments. A slower rate of relaxation could arise from the increased Ca\(^{2+}\) sensitivity of the myofilaments with no change in the rate of Ca\(^{2+}\) transport by the sarcoplasmic reticulum and Na-Ca exchanger.

Yet, according to the model of Campbell,\(^{27}\) the development of force is slowed by a cooperative action of force-generating crossbridges feeding back on activation. Because myofilaments that contain \(\beta\)-Tm are more sensitive to activation by strong crossbridges,\(^{13}\) the Campbell model predicts a slower rate of force development in preparations from TG-\(\beta\)-Tm hearts. The Campbell model also predicts that the cooperative feedback of force-generating crossbridges is more pronounced at low levels of Ca\(^{2+}\) activation, a condition that most likely exists during the basal activity of intact cells studied in this experiment. The increased cooperative action of force-generating crossbridges is additionally associated with an increase in pCa\(_{50}\), which agrees with our data from skinned fiber bundles.
the maximum level of thin-filament activation is depressed. At maximum levels of free Ca\(^{2+}\), we would expect TaC to be fully saturated; thus, Tm open state would depend on cross-bridge interaction with the thin filament. It is apparent that the ability of strongly bound force generating crossbridges to fully activate the thin filament is depressed in fiber bundles in which \(\beta\)-Tm has replaced \(\alpha\)-Tm.

We conclude from our results that TG mouse hearts with myofilaments having an increased population of the \(\beta\)-Tm isoform demonstrate altered dynamics due to changes that are intrinsic to the myocytes and are mainly caused by charge differences between \(\alpha\)-Tm and \(\beta\)-Tm. We think that changes in dynamics result from either increased sensitivity of myofilament force and ATPase to Ca\(^{2+}\) or altered feedback effects of force bearing crossbridges on activation. Our results may also have important implications for better understanding of the etiology of familial hypertrophic cardiomyopathy linked to point mutation of \(\alpha\)-Tm.

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References

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