Impact of β-myosin heavy chain isoform expression on cross-bridge cycling kinetics

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Rundell, Veronica L. M., Vlasios Manaves, Anne F. Martin, and Pieter P. de Tombe. Impact of β-myosin heavy chain isoform expression on cross-bridge cycling kinetics. Am J Physiol Heart Circ Physiol 288: H896–H903, 2005. First published October 7, 2004; doi:10.1152/ajpheart.00407.2004.—Myosin heavy chain (MHC) isoforms α and β have intrinsically different ATP hydrolysis activities (ATPase) and therefore cross-bridge cycling rates in solution. There is considerable evidence of altered MHC expression in rodent cardiac disease models; however, the effect of incremental β-MHC expression over a wide range on the rate of high-strain, isometric cross-bridge cycling is yet to be ascertained. We treated male rats with 6-propyl-2-thiouracil (PTU; 0.8 g/l in drinking water) for short intervals (6, 11, 16, and 21 days) to generate cardiac MHC patterns in transition from predominantly α-MHC to predominantly β-MHC. Steady-state calcium-dependent tension development and tension-dependent ATP consumption (tension cost; proportional to cross-bridge cycling) were measured in chemically permeabilized (skinned) right ventricular muscles at 20°C. To assess dynamic cross-bridge cycling kinetics, the rate of force redevelopment (k₀) was determined after rapid release-restretch of fully activated muscles. MHC isoform content in each experimental muscle was measured by SDS-PAGE and densitometry. α-MHC content decreased significantly and progressively with length of PTU treatment [68 ± 5%, 58 ± 4%, 37 ± 4%, and 27 ± 6% for 6, 11, 16, and 21 days, respectively; P < 0.001 (ANOVA)]. Tension cost decreased, linearly, with decreased α-MHC content [6.7 ± 0.4, 5.6 ± 0.5, 4.0 ± 0.4, and 3.9 ± 0.3 ATPase/tension for 6, 11, 16, and 21 days, respectively; P < 0.001 (ANOVA)]. Likewise, k₀ was significantly and progressively depressed with length of PTU treatment [11.1 ± 0.6, 9.1 ± 0.5, 8.2 ± 0.7, and 6.2 ± 0.3 s⁻¹] for 6, 11, 16, and 21 days, respectively; P < 0.05 (ANOVA)] Thus cross-bridge cycling, under high strain, for α-MHC is three times higher than for β-MHC. Furthermore, under isometric conditions, α-MHC and β-MHC cross bridges hydrolyze ATP independently of one another.

rate of force redevelopment; sodium dodecyl sulfate-polyacrylamide gel electrophoresis; mammalian myocardium

the speed with which muscle shortens is correlated to the intrinsic ATP hydrolysis activity of myosin (1). In vertebrate striated muscle there are several isoforms of myosin heavy chain (MHC); however, only two cardiac isoforms are expressed, α-MHC and β-MHC, which share 93% homology in the rat (26). Myofibrillar and myosin ATPase studies have reported that α-MHC has a higher ATPase activity than β-MHC; however, these measurements were made in low- or no-strain preparations (34). The maximal shortening velocity, which is correlated to the ATP hydrolysis rate, is significantly higher in fibers that contain a higher proportion of α-MHC (5, 36). Contractile power is increased in myocardial tissue containing high α-MHC as opposed to tissues with even a small amount of β-MHC (17, 18). Although the isometric tension generated by both isoforms is roughly identical (Refs. 16, 29, 41, but see Refs. 15 and 40), the cross-bridge cycling rate and amount of ATP consumed to generate a given level of tension development are significantly disparate, with α-MHC having an ATP hydrolysis rate three times that of β-MHC and a duty cycle that is nearly half (16, 40). This indicates that β-MHC is the more energetically economical isoform. Therefore, although MHC composition can affect cardiac contractility, it is unclear whether there is mechanical advantage in the expression of one isoform over the other.

Ventricular MHC isoforms are expressed differentially across species and also during development within the same species. Likewise, it is well documented that there are changes in cardiac MHC isoform population in many mammals with developmental stage, aging, thyroid state, and cardiac disease (19, 27, 36, 38). Species-related differences in MHC isoform content are attributed to heart rate differentials that exist between small and large mammals. Small mammals may have developed high heart rates to maintain sufficient arterial diastolic pressure to allow for coronary perfusion with the constraint that the heart and arteries be of minimum size to maximize efficiency (8, 42). In normal adult large mammals (pigs, cats, dogs, and humans) ventricular β-MHC content is high, whereas in smaller mammals (mouse and rat) α-MHC is the predominant isoform. The high levels of α-MHC content in small mammals are considered adaptation because the faster ATP hydrolysis rate of α-MHC facilitates the extremely rapid rates of cardiac contraction and relaxation present in these animals (16). As a result, the use of rodent models to study cardiac disease is confounded first by investigating cardiac tissue, which predominantly contains the faster α-MHC, unlike human tissue, and second by the increased expression of β-MHC that naturally occurs as a consequence of cardiac insult.

There is considerable evidence of altered MHC expression in rodent models of cardiac disease (14, 23, 28, 32, 38); however, the effect of incremental β-MHC expression over a wide range on the rate of cross-bridge cycling is yet to be ascertained. Recently, van der Velden and colleagues (41) demonstrated a significant, and linear, decrease in cross-bridge cycling rate in skinned fibers prepared from young and old guinea pigs. Guinea pigs, however, predominantly express the β-MHC isoform, even in juvenile states, such that the range of α-MHC content was small in that study, only between 5% and

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30%. Thus the question remains as to whether or not crossbridge cycling rate is a linear function over a wide range of MHC isoform expression. 6-Propyl-2-thiouracil (PTU) interferes with the production of thyroid hormone (T3) by blocking iodination of thyroglobulin and also by blocking the coupling of diiodothyronine and monoiodothyronine (21). As a result, animals treated with PTU are rendered hypothyroid, silencing the α-MHC gene promoter and subsequent transcription and translation of the β-MHC gene (9, 26). As the turnover rate of myosin in the heart is relatively slow (5.4 days; Ref. 25), administration of the drug for short periods of time produces cardiac tissue that contains variable amounts of β-MHC. We have adapted the method of Laemmli (22), utilizing a low cross-linking acrylamide solution as previously described (12, 24). Modification of electrophoresis conditions enabled us to improve the resolution of separation between α-MHC and β-MHC not just in rat, but also in human, porcine, baboon, rabbit, guinea pig, and murine myocardial tissue. Using this improved method of gel electrophoresis allowed us to accurately determine MHC isoform content in the individual skinned muscles that were used for chemechanical experiments, thus allowing for a direct correlation between crossbridge cycling kinetics and MHC isoform content.

METHODS

Animals were treated and housed in accordance with the Animal Care and Use Committee guidelines at the University of Illinois at Chicago Biologic Resources Laboratory. Male Lewis Brown Norway-F1 rats (Harlan Laboratories) were treated with PTU (0.8 g/l in drinking water) for short intervals (6, 11, 16, and 21 days) to generate cardiac MHC patterns in transition from α-MHC to β-MHC expression. Treatment with PTU in this manner leads to a rapid reduction in circulating T3 levels in the rat, as we showed previously (5). Animals were anesthetized by intraperitoneal injection of pentobarbital sodium (200 mg/kg), and hearts were rapidly excised. Body and heart weights were obtained at the time of death. Right ventricular (RV) trabeculae (muscles) were dissected free and incubated overnight at 4°C in low-calcium solution containing 1% Triton X-100 to chemically remove all cellular membranes, which contained (in mM) 20 EGTA, 10 creatine phosphate, 100 N,N-bis-(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES), 5.93 ATP, 6.6 magnesium chloride, and 20.7 potassium propionate, at pH 7.0. Once permeabilized, muscles were attached to hooks mounted on a force transducer (AE 801 Sensonor) and a motor arm (model 403, Cambridge Scientific) in the experimental apparatus by aluminum T clips, as previously described (4). The sarcomere length (SL) in each muscle was set to 2.3 μm by laser diffraction. Briefly, a 10-mW HeNe laser was focused onto the muscle (~300-μm spot size), and the resulting diffraction pattern was projected onto a calibrated screen. Muscles were stretched by micromotopulators to set SL to 2.3 μm. SL was checked at regular intervals throughout the experiment and readjusted as required. In general, SL was stable after a first test contraction at full activation. Muscles in which a stable SL was not observed were discarded. The muscle dimensions were measured under a light microscope at ×40 power. Thickness of the preparation was measured at ×40 power with a 45° mirror positioned adjacent to the fiber. Dimensions were measured at three positions along the length of the muscle, and cross-sectional area was calculated from the average dimensions, assuming an elliptical shape. Muscles were exposed to a range of calcium solutions obtained by proportional mixing of activating and relaxing solutions, and the force generated and ATP consumed were measured simultaneously during contraction as previously described (4).

Mechanical experiments. Calcium-dependent tension and tension-dependent ATP consumption were measured in skinned RV trabeculae at SL of 2.3 μm at 20°C. Additionally, to gain a measure of the dynamic turnover rate of cross bridges, the rate of force redevelopment (k redevelopment) was measured in high-calcium full-activating solution. In all preparations high-frequency stiffness was assessed by recording the strain elicited by rapid oscillation (500 Hz) of the preparation by 1% of the muscle length and sampled and analyzed by a dual-phase lock-in amplifier (Stanford Research Systems). ATP consumption was measured with an enzyme-coupled ultraviolet (UV) light absorbance assay (4, 7, 13). Briefly, ADP generated by the muscle was phosphorylated to ATP by the action of pyruvate kinase (PK; 0.5 mg/ml) with phosphoenolpyruvate (PEP; 10 mM) as substrate, causing the formation of pyruvate. Next, pyruvate was converted to lactate by the action of lactate dehydrogenase (LDH; 0.05 mg/ml) and the substrate NADH (~1 mM) to form NAD+. All enzymes and substrates were added at sufficient concentration such that the reactions proceeded rapidly and far from equilibrium to ensure an effectively stoichiometric reaction system. Because NADH absorbs UV light (340 nm) whereas NAD+ does not, the system allows for the determination of ATP consumption rate by measurement of NADH absorbance. Individual experimental examples are shown in Fig. 1A (NADH absorbance record at top and matched force trace at bottom). It is clear that NADH absorbance decreases immediately on incubation of the muscle in activating levels of calcium (50 μM) and stabilizes on its removal from the bath. A series of calibration steps is performed with every record by stepwise injection of 250 pmol of ADP. In the analysis of the raw record, the height of each step is measured and the average drop in absorbance is determined for each 250-pmol step of ADP. The slope of the NADH absorbance recorded during the contraction is then measured with this calibrated signal. As this calibration is done for each experimental contraction, we are able to accurately assess the concentration of NADH consumed in the ATP regeneration process and therefore also the ATP consumption because they are stoichiometrically linked. The calibrating steps also illustrate that during contraction, cross-bridge ADP production is the rate-limiting step in this measurement, not the enzyme cascade.

The experimental apparatus consisted of a cooled aluminum block in which three baths were placed. The preparation (muscle, force transducer, and motor) was mounted on a sliding arm such that it could be rapidly lifted and placed inside each of the three baths. The first bath contained relaxing solution, the second contained preactivating solution, and the third bath was the measuring chamber, which contained activating solution that could be titrated with relaxing solution to generate a range of calcium solutions to elicit variable levels of contraction. The fiber was moved sequentially from relaxing to preactivating baths and then to the measuring bath for determination of steady-state force development and ATP consumption. After steady-state force development and data acquisition, the muscle was returned to the relaxing bath until force returned to preactivation levels. At that time, it was placed in the preactivating bath to prepare for the next calcium incubation. In all, these fibers were exposed to 8–10 contractions during the course of an experiment. The final contraction was in saturating calcium, and the preparation was discarded if the developed tension was decreased by >10% from the first contraction. Activating solution contained (in mM) 20 Ca-EGTA, 1.55 potassium propionate, 6.59 magnesium chloride, 100 BES, 5 sodium azide, 1 DTT, 10 PEP, 0.01 leupeptin, 0.001 pepstatin, 0.01 oligomycin, 0.01 PMSF, and 0.01 AIPz. Relaxing solution was identical except it contained (in mM) 20 EGTA, 21.2 potassium propionate, and 7.11 MgCl2. Preactivating solution contained (in mM) 0.5 EGTA, 19.5 hexamethylenediamine-N,N,N',N'-tetraacetate (Fluka), and 21.8 potassium propionate. All solutions contained 0.5 mg/ml PK (386 U/mg) and 0.05 mg/ml LDH (880 U/mg) (Sigma, St. Louis, MO). All solutions had an ionic strength of 200 mM and 5 mM free ATP and 1 mM free magnesium as determined by the methods of Fabiato and Fabiato (10), assuming an apparent stability constant of the Ca2+-EGTA complex of 106.58 (4).
The final maximal activating contraction was accompanied by a rapid release-restretch protocol that allowed for measurement of the rate of force redevelopment (kₒ) maneuvers of the muscles illustrated in A. The top line represents the 6-day PTU treatment muscle, and the bottom line represents the 21-day PTU treatment muscle. The slope of the rate of tension rise following the rapid release-restretch of the muscle is fit by a single-exponential equation. It is apparent that the kₒ of the 21-day PTU treatment muscle is significantly slower than that of the 6-day PTU treatment muscle (6-day PTU: 11.06 s⁻¹; R = 0.99; 21-day PTU: 5.8 s⁻¹; R = 0.99). C: line fits of the kₒ data represented in B. The data have been normalized for illustration; however, the fit parameters were taken from the raw data trace. The top line indicates 6-day PTU treatment, and the bottom line indicates 21-day PTU treatment.

Gel electrophoresis. Cardiac α- and β-MHC isoforms were electrophoretically separated on 1.5-mm-thick, 14-cm-wide, and 16-cm-long gels with a Hoefer SE 600 system (Hoefer Scientific) and the Laemmli gel system (22) with minor modifications. The acrylamide solution used to make 6.5% resolving gels and 0.25% stacking gels contained 25% acrylamide and 0.05% bis-acrylamide (12). Acrylamide solutions were degassed for 10–15 min and polymerized by adding 10% ammonium persulfate and N,N′,N′,N′-tetramethylethylenediamine (Bio-Rad) to final concentrations of 0.125% (wt/vol) and 0.05% (vol/vol), respectively. Resolving gels were polymerized at room temperature and stored overnight at 4°C. Stacking gels were polymerized for at least 30 min. Lanes were formed with 1.5-mm-thick, 10-well combs. Samples were prepared by dilution with extraction buffer to 0.5 μg protein/μl and bromophenol blue was added to 45 mA/gel for 5–7 h for murine, human, baboon, rodent, and porcine samples. Frozen human atrial and ventricular tissues were the kind gift of Dr. R. J. Solaro. Rabbit and guinea pig samples could be best separated with electrophoresis for 20 min at 25 mA/gel, followed by 30 mA/gel for 14 h. Examples of MHC separation from these species with these electrophoretic conditions are illustrated in Fig. 2. Gels were cooled during the run by a circulating bath (Fisher Isotemp) set to 2°C to obtain a temperature of 1–2°C in the circulating bath and 4–6°C in the running buffer at the end of the run. Running buffer was stirred throughout electrophoresis with a magnetic stirring bar. At the completion of electrophoresis, gels were removed, stained with 0.03% Coomassie blue stain or Pierce Gel Code Blue stain for 2–16 h according to manufacturer’s instructions. Gels were scanned with a Bio-Rad GS 710 flatbed scanner and analyzed with Kodak 1D software.

Data analysis. The results of the mechanical experiments were fit to a modified Hill equation: F = F_max × [Ca²⁺]ⁿ/(EC₅₀ + [Ca²⁺]ⁿ), where F is peak steady-state force (skinned), F_max is the maximum saturated value F can attain; EC₅₀ is the concentration of Ca²⁺ ([Ca²⁺]) at which F is 50% of F_max, and n is the Hill coefficient and represents the slope of the force-[Ca²⁺] relation at EC₅₀. Each experimental trabecula was fit individually, these fit parameters were pooled, and the mean values are reported. Consumption of ATP was normalized to muscle volume and plotted vs. the force/cross-sectional area attained during each contraction. Linear regression was used to fit this relationship, the slope of each experimental line fit was pooled, and the mean value is reported as tension cost. The kₒ was calculated as a single-exponential fit of the initial rate of rise in tension following a rapid release-restretch maneuver as previously described (cf. Fig. 1, B and C, and Ref. 3). Individual fits were pooled to generate the mean value reported. Numbers (n) of trabeculae for each group studied were 10 (6 days), 11 (11 days), 9 (16 days), and 9 (21 days). These numbers reflect the total number of animals studied, as only one muscle per animal was used. Data were analyzed by ANOVA, and significance is reported at P < 0.05 with a commercially available statistical package (SPSS 10.0).
RESULTS

Characterization of model. Physical parameters of the animals used in the study are summarized in Table 1. The largest length of time animals were treated with PTU was 3 wk. There was no alteration in body weight, heart weight, or the heart weight-to-body weight ratio between groups. This indicates that there were no deleterious effects of PTU treatment on heart or body size in this short-term PTU treatment study.

MHC isoform expression. The utility of PTU treatment for increasing the level of cardiac α- and β-MHC expression has been well characterized (34). To assess the relative cardiac MHC isoform content we subjected all muscles to SDS-PAGE, as described in METHODS. The method we describe for separating cardiac α- and β-MHC is a modification of the Laemmli gel system that allows separation of the MHC isoforms in 6 h and permits gel staining by Coomassie blue. We did not find that the addition of glycerol improved the protein separation (35). After staining, gels were optically scanned with a densitometer and relative protein content was determined with analytical software (Kodak ID). The relative amounts of the two cardiac MHC isoforms could be quantified readily because all samples had at least 1.0 mm of separation from peak to peak in the densitometric scans. An example of the separation is shown in Fig. 3A. Rats treated with PTU had a significant and progressive decrease in α-MHC myocardial protein content with length of PTU treatment (68 ± 5%, 58 ± 4%, 37 ± 4%, and 27 ± 6% for 6, 11, 16, and 21 days of PTU, respectively; P < 0.001 by ANOVA), as illustrated in Fig. 3B. There was no indication that total MHC content was affected by PTU treatment.

Mechanical impact of hypothyroidism in RV skinned fibers. Fibers were subjected to a series of calcium-activated contractions, and the data were fit to a modified Hill equation as described in METHODS. The mean Hill fit parameters from each individual fiber experiment are reported in Table 2. Figure 4A illustrates the mean data collected in the 6- and 21-day PTU groups. As can be seen, maximal calcium-activated myofilamentary force increased significantly with the length of PTU treatment (102 ± 4%, 122 ± 7%, 216 ± 6%, and 223 ± 10% for 6, 11, 16, and 21 days of PTU, respectively; P < 0.001 by ANOVA). The Hill coefficients (n) also increased with PTU treatment (1.7 ± 0.1, 1.8 ± 0.1, 2.0 ± 0.1, and 2.0 ± 0.1 for 6, 11, 16, and 21 days of PTU, respectively; P < 0.001 by ANOVA). The mean maximum shortening velocity (vmax) decreased with PTU treatment (49 ± 3%, 30 ± 3%, 26 ± 3%, and 22 ± 3% for 6, 11, 16, and 21 days of PTU, respectively; P < 0.001 by ANOVA). The peak isometric force and length-tension relationship are depicted in Fig. 4B. The mean peak force was unchanged at 6 days of PTU treatment (100 ± 5%), but then decreased significantly with increasing PTU treatment (84 ± 7%, 62 ± 7%, and 58 ± 7% for 11, 16, and 21 days of PTU treatment, respectively; P < 0.001 by ANOVA). The mean length of the fiber at peak force was increased significantly with increasing PTU treatment (103 ± 3%, 113 ± 3%, and 117 ± 3% for 6, 11, and 16 days of PTU treatment, respectively; P < 0.001 by ANOVA).

Table 1. Physical parameters of PTU model

<table>
<thead>
<tr>
<th>Time on PTU</th>
<th>HW, g</th>
<th>BW, kg</th>
<th>HW/BW, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Days</td>
<td>0.81±0.05</td>
<td>0.231±0.02</td>
<td>3.6±0.16</td>
</tr>
<tr>
<td>11 Days</td>
<td>0.78±0.05</td>
<td>0.225±0.01</td>
<td>3.4±0.06</td>
</tr>
<tr>
<td>16 Days</td>
<td>0.91±0.05</td>
<td>0.257±0.01</td>
<td>3.6±0.15</td>
</tr>
<tr>
<td>21 Days</td>
<td>0.80±0.05</td>
<td>0.235±0.01</td>
<td>3.4±0.17</td>
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</table>

Values are means ± SE for n = 10 (6 days), 11 (11 days), 9 (16 days), and 9 (21 days) muscles (1 per animal). PTU, 6-propyl-2-thiouracil; HW, heart weight; BW, body weight. Statistical significance was measured by ANOVA.
ment tension development was not significantly different between groups, indicating that PTU treatment did not have any deleterious effects on the force-generating capability of the contractile proteins. Furthermore, there were no changes in myofilament calcium sensitivity as indexed by the $EC_{50}$, which force is half-maximal (EC 50) between groups with increasing length of PTU treatment. Therefore, PTU treatment did not have any impact on the contractile filament’s sensitivity to calcium. Additionally, there were no differences in $n_H$ between groups with increased length of PTU treatment, indicating that there was no change in the cooperativity of thin filament activation. Thus PTU treatment had no deleterious effects on myocardial mechanics in this short-term (maximum 21 days) PTU treatment model.

Cross-bridge cycling kinetics are depressed in hypothyroid cardiac fibers. The two-state model of cross-bridge cycling predicts that the overall steady-state rate of the cross-bridge cycle is proportional to the overall flux of either cross-bridge attachment or detachment (2, 20). Force is determined by the fraction of attached cross bridges and the force per cross bridge. A two-state model predicts that the fraction of attached cross bridges is proportional to the first-order rate constant of attachment ($f$) divided by the sum of $f$ and the first-order rate constant of detachment ($g$), i.e., $f/(f + g)$. ATP consumption is proportional to the product of the number of attached cross bridges and $g$, i.e., $g \times f/(f + g)$. Tension cost is equal to the ATP consumption divided by force, which is therefore directly proportional to $g$ (4, 20). To assess the impact of PTU treatment on cross-bridge cycling kinetics, we determined tension cost in all groups. All measures of cross-bridge cycling kinetics were significantly and progressively depressed with continued

Table 2. Mean fit parameters from individual Hill fits to data

<table>
<thead>
<tr>
<th>Time on PTU</th>
<th>Tension, mN/mm²</th>
<th>Hill Coefficient</th>
<th>$EC_{50}$, µM Ca²⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Days</td>
<td>52.2±4.8</td>
<td>8.1±1.3</td>
<td>2.63±0.11</td>
</tr>
<tr>
<td>11 Days</td>
<td>45.8±1.6</td>
<td>7.7±0.6</td>
<td>2.59±0.08</td>
</tr>
<tr>
<td>16 Days</td>
<td>48.3±4.2</td>
<td>9.1±1.5</td>
<td>2.65±0.12</td>
</tr>
<tr>
<td>21 Days</td>
<td>47.5±5.4</td>
<td>9.6±1.2</td>
<td>2.63±0.09</td>
</tr>
</tbody>
</table>

Values are means ± SE. There were no significant differences in tension development, Hill coefficient, or $EC_{50}$ as determined from the Hill fits of data recorded as described in METHODS.
administration of PTU. The mean data are summarized in Table 3, and Fig. 4B illustrates the difference in tension cost measured in the 6- and 21-day PTU groups. Maximal ATP consumption rate was significantly and progressively reduced with increased length of PTU treatment \((P < 0.01)\). Likewise, tension cost, the measure of steady-state cross-bridge detachment, was also progressively depressed with length of PTU administration \((P < 0.01)\). Dynamic cross-bridge turnover, flux through the cross-bridge cycle \((f + g)\), can be assessed by the \(k_t\) following a rapid release restretch maneuver in saturating calcium aimed to disengage all attached cross bridges \((3)\). The subsequent rise in tension was fit with a single-exponential equation. The mean values for \(k_t\) are given in Table 3, and it is clear that \(k_t\) was significantly and progressively depressed with increased length of PTU treatment \((P < 0.001)\).

Cross-bridge cycling kinetics are depressed linearly with decreasing \(\alpha\)-MHC. Considering that PTU treatment results in decreasing \(\alpha\)-MHC content and that this subsequently reduces cross-bridge cycling rate, it is important to determine whether this depression is a linear function of isoform expression. Therefore, we plotted the relative \(\alpha\)-MHC content of each fiber vs. the measured tension cost and \(k_t\) values for the fiber. Figure 5 illustrates that, indeed, the relationships between \(\alpha\)-MHC content and tension cost and \(k_t\) are linear. Additionally, as this relationship is linear, and if we can assume that thick filaments will be composed of random arrangements of \(\alpha\)- and \(\beta\)-MHC homo- and heterodimers, at points between the extremes, this indicates that there is no effect of \(\beta\)-MHC cross bridges on those of \(\alpha\)-MHC. That is to say, the longer cycle time of \(\beta\)-MHC cross bridges does not slow down the cycle time of \(\alpha\)-MHC cross bridges to any measurable degree in isometrically contracting skinned fiber preparations.

DISCUSSION

There were no detrimental effects of increased \(\beta\)-MHC expression on the force-generating capability of skinned RV trabeculae in the rat. Also, the myofilament sensitivity for calcium as indexed by the EC\(_{50}\) was not altered with increased length of PTU treatment in this study. These data are in agreement with our previously published work \((6)\) and that of others \((11, 17)\) in the PTU-treated rat. In contrast, there are reports of decreased myofilament sensitivity for calcium in the mature guinea pig and PTU-treated rat, both of which had very high levels of \(\beta\)-MHC isoform expression \((29, 41)\). Van der Velden and colleagues \((41)\) report a shift in the EC\(_{50}\) of calcium between treatment groups of 0.80 \(\mu\)M, and Metzger and colleagues \((29)\) report an even greater shift in EC\(_{50}\) \((1.01 \mu\)M) after 6 wk of PTU treatment in the rat. The reasons behind the discrepancy are unclear, however.

The widespread use of rodent models of cardiac disease has prompted a reinvestigation of the effects of MHC isoform shifts in measures of cardiac contractility and energetics, where the confounding effect of MHC switching has a significant impact. As discussed above, \(\alpha\)-MHC is the predominant isoform expressed in rodent cardiac tissue, as opposed to \(\beta\)-MHC in human myocardium; however, during the course of disease development, regardless of etiology, \(\beta\)-MHC becomes more highly expressed. This is seen as an adaptive mechanism initially, in that \(\beta\)-MHC is the more economical ATPase and has at least the same, if not greater, force-generating capability as \(\alpha\)-MHC \((37, 40)\). However, the unloaded velocity of shortening of this isoform \((\beta\)-MHC) is considerably less, and recent evaluation of cells expressing only \(\beta\)-MHC indicated a considerable deficit not only in loaded shortening velocity but also in overall power output-generating capacity \((5, 17, 33)\). Thus increasing the \(\beta\)-MHC content in myocardium can have a deleterious effect on myocardial contractility. This was further

### Table 3. Mean measures of cross-bridge cycling kinetics

<table>
<thead>
<tr>
<th>Length of PTU</th>
<th>Max ATPase, pmol·s(^{-1})·mm(^{-3})</th>
<th>Tension Cost</th>
<th>(k_t), s(^{-1})</th>
<th>Stiffness/Tension</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Days</td>
<td>395±25*</td>
<td>6.7±0.4*</td>
<td>11.1±0.6*</td>
<td>1.7±0.2</td>
</tr>
<tr>
<td>11 Days</td>
<td>288±20*</td>
<td>5.7±0.5*</td>
<td>9.1±0.5*</td>
<td>1.4±0.2</td>
</tr>
<tr>
<td>16 Days</td>
<td>221±21*</td>
<td>4.0±0.4*</td>
<td>8.2±0.7*</td>
<td>1.2±0.3</td>
</tr>
<tr>
<td>21 Days</td>
<td>218±29*</td>
<td>3.9±0.3*</td>
<td>6.2±0.3*</td>
<td>2.0±0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Maximal ATP consumption and tension cost decreased significantly and progressively with increasing length of PTU treatment. The rate of force development \((k_t)\) decreased in the same fashion. *\(P < 0.001\) by ANOVA.
demonstrated by using a transgenic approach to express a small amount of β-MHC (12%), without alteration of thyroid state, in mouse myocardium. The resultant effect was a 25% decrease in myofibrillar ATPase activity and a 15% decrease in systolic function measured as developed pressure in a Langendorff-perfused heart preparation (39). Furthermore, recent studies in human cardiac tissue have demonstrated that α-MHC RNA is not only transcribed but also translated, comprising ~7% of the total MHC content in normal human myocardium, whereas there is virtually no α-MHC RNA and undetectable amounts of protein in failing myocardium (30, 31). Thus it seems that small perturbations in MHC isoform expression can lead to severe deleterious consequences in pump function of the working heart, despite perhaps little alteration in maximal myofilament force-generating capability.

An inordinate slowing of the maximum sliding filament velocity of α-MHC populations when small increments of β-MHC are present in the in vitro motility assay has been reported (15). The data of these experiments reflect a curvilinear MHC-velocity relationship in which the velocity of actin filaments slows increasingly as more β-MHC is added to the preparation. Harris and colleagues (15) surmised that the slower ATPase of β-MHC was causing a drag effect on the α-MHC cross bridges as a result of their longer duty cycle. However, those experiments measured unloaded cross-bridge cycling and also had little control regarding orientation of myosin heads in regard to actin filament position. Under isometric conditions we have determined that there is no effect of β-MHC cross bridges on α-MHC cross bridges, as evidenced by the linear shape of both the α-MHC vs. tension cost and α-MHC vs. \( k_r \) relationships. Furthermore, because we found no differences in the high-frequency stiffness vs. tension relationship (see Table 3 and Fig. 4C), we can assume that force per cross bridge was not altered in this model. Thus the differences in cross-bridge cycling kinetics we have observed are limited to the inherent kinetic properties of the MHC isoforms, and not their force-generating capacity. This result indicates that isometrically cycling cross bridges act independently, that is, each cross bridge hydrolyzing ATP at its intrinsic rate governed solely by its individual MHC isoform.

PTU had the effect of progressively lowering the myocardial content of α-MHC in otherwise healthy, young rodents. Concomitant with this decreased expression was a decrease of all cross-bridge cycling parameters measured, including total ATP consumption rate, tension cost, and \( k_r \). By extrapolation of the linear curve fit we were able to derive that there was a 2.5- to 3-fold reduction in cross-bridge cycling rate as indexed by tension cost and \( k_r \). The linear decrease in tension cost with increasing β-MHC content is in accordance with previously published work in the guinea pig (41). However, the difference in tension cost at nearly 100% β-MHC expression from that measured at 100% α-MHC expression that we report in the rat (3 times less at 100% β-MHC) is lower than that reported for the guinea pig (5 times less at 100% β-MHC). It may be that the discrepancy lies in the narrow range of MHC isoforms investigated in that study and the need to extrapolate those data to cover the range from 30% to 100% α-MHC content (41). We have determined here and previously (6) that the force-calcium relationship is unchanged with increasing expression of β-MHC. We assume that the force per cross bridge between conditions is unchanged, as supported by the finding that the relationship between high-frequency stiffness and force development was not affected by PTU treatment. As described above, tension cost is proportional to \( g \) and \( k_r \) is equal to \((f + g)\), and we find that both are decreasing by a factor of 3. If we then apply the two-state cross-bridge model of contraction described by Brenner (2), we are able to derive that not only \( g_{\text{app}} \), but also \( f_{\text{app}} \), decreases approximately three times in the transition from 100% α-MHC to 100% β-MHC. The \( f_{\text{app}} \) would necessarily be decreased by three times, to result in a proportional decrease in both tension cost and \( k_r \) while maintaining isometric tension development at the same level.

Limitations of study. We (5) and others (29) have shown that administration of PTU results in a dramatic decrease in the circulating levels of T3 and thyroxine, although these values were not measured in the present study. Furthermore, with respect to the myofilament proteins, it has been extensively reported that only MHC isoform expression is altered with PTU treatment (11, 17, 29). However, we did not investigate the possibility of other myofilament protein alteration, and, as such, it cannot be completely excluded. It is known that there is significant alteration of β-adrenergic receptor and calcium-handling protein expression in the PTU-treated rat; however, this study investigated skinned muscle, where there would be no difference in calcium delivery to or adrenergic regulation of the myofilaments. Hence, we do not anticipate that these factors affected our conclusions. Also, there was no measurement of SL during the course of the \( k_r \); however, it was examined before and after the maneuver, and the preparation was discarded if the SL deviated from its set point of 2.3 μm. Wolff and colleagues (43) examined the \( k_r \) in cardiac muscle under conditions of strict SL control and found that lack of SL control resulted in ~10% underestimation of \( k_r \). Hence, we cannot exclude that the \( k_r \) measurements were slightly underestimated. However, this would apply to all muscles in all groups, and therefore in our study this factor would not alter our main finding of an approximately three times slower \( k_r \) in β-MHC as opposed to α-MHC myocardium.

The experiments presented here confirm earlier work and expand the scope of our understanding of the impact of MHC isoform expression on cardiac energetics to include intermediate points in the transition from 100% α-MHC content to 100% β-MHC content. This enabled us to conclude accurately that there exists a linear relationship between cross-bridge cycling kinetics and MHC isoform expression.

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