Increase in tension-dependent ATP consumption induced by cardiac troponin T mutation

Murali Chandra, Matthew L. Tschirgi, and Jil C. Tardiff. Increase in tension-dependent ATP consumption induced by cardiac troponin T mutation. Am J Physiol Heart Circ Physiol 289: H2112–H2119, 2005—First published July 1, 2005; doi:10.1152/ajpheart.00571.2005.—How different mutations in cardiac troponin T (cTnT) lead to distinct secondary downstream cellular remodeling in familial hypertrophic cardiomyopathy (FHC) remains elusive. To explore the molecular basis for the distinct impact of different mutations in cTnT on cardiac myocytes, we studied mechanical activity of detergent-skinned muscle fibers bundles from different lines of transgenic (TG) mouse hearts that express wild-type cTnT (WTGT), R92W cTnT, R92L cTnT, and Delta-160 cTnT (deletion of amino acid 160). The amount of mutant cTnT is ~50% of the total myocellular cTnT in both R92W and R92L TG mouse hearts and ~35% in Delta-160 TG mouse hearts. Myofilament Ca2+ sensitivity was enhanced in all mutant cTnT TG cardiac muscle fibers. Compared with the WTGT fibers, Ca2+ sensitivity increased significantly at short sarcomere length (SL) of 1.9 µm (P < 0.001) in R92W TG fibers by 2.2-fold, in R92L by 2.0-fold, and in Delta-160 by 1.3-fold. At long SL of 2.3 µm, Ca2+ sensitivity increased significantly (P < 0.01) in a similar manner (R92W, 2.5-fold; R92L, 1.9-fold; Delta-160, 1.3-fold). Ca2+-activated maximal tension remained unaltered in all TG muscle fibers. However, tension-dependent ATP consumption increased significantly in Delta-160 TG muscle fibers at both short SL (23%, P < 0.005) and long SL (37%, P < 0.0001), suggesting a mutation-induced change in crossbridge detachment rate constant. Chronic stresses on relative cellular ATP level in cardiac myocytes may cause a strain on energy-dependent Ca2+ homeostatic mechanisms. This may result in pathological remodeling that we observed in Delta-160 TG cardiac myocytes where the ratio of sarco(endo)plasmic reticulum Ca2+-ATPase 2/phospholamban decreased significantly. Our results suggest that different types of stresses imposed on cardiac myocytes would trigger distinct cellular signaling, which leads to remodeling that may be unique to some mutants.

Address for reprint requests and other correspondence: M. Chandra, 205 Wegner Hall, Dept. of VCAPP, Washington State Univ., Pullman, WA 99164 (e-mail: murali@vetmed.wsu.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
In the present study, we have compared steady-state mechanical activity of detergent-treated cardiac fiber bundles from TG mice that express two substitution mutants of mouse cTnT (R92L, R92W) and a mouse mutant cTnT in which Glu 160 has been deleted (Delta-160). Codon 92 in human cTnT (hcTnT) represents a hot spot for mutations in hcTnT because 3 of the 9 mutations have been found at this site (39). Each of these mutations (R92L, R92W, and R92Q) is lethal in humans (27, 28, 39). Because both R92 and E160 are conserved among all known sequences of troponin T, it is likely that they represent a structurally/or functionally important regions in cTnT. Both R92 and E160 lie within the region of cTnT (1–193) that is considered to be important for establishing the size of the regulated functional unit [tropomyosin (Tm)1- troponin (Tn)1-actin7] in the cardiac sarcomere (31) and for remodeling that may be unique to some mutants.

The objective of the present study was to determine the unique molecular basis for mutations in cTnT that induce different pathogenic responses in cardiac myocytes. All of the three lines of TG mouse heart fibers show significant increases in Ca2+ sensitivity and a prolongation of the relaxation phase in TG cardiac myocytes (13). However, the phenotype of Delta-160 TG mouse myocytes is distinctly different from those of R92W and R92L TG mouse hearts (8, 9). To provide new insight into the mechanism by which Delta-160 mutation elicits distinct alterations in myocytes, we determined the effect of cTnT mutations (Delta-160, R92L, and R92W) on the Ca2+-activated tension, ATPase activity, and tension-dependent ATP consumption in detergent-skinned muscle fiber bundles. An interesting observation in this study is that the tension-dependent ATP hydrolysis increased only in muscle fibers from Delta-160 TG mouse hearts. Our results suggest that different stresses imposed on cardiac myocytes would trigger distinct cellular signaling, which leads to cardiac myocyte remodeling that may be unique to some mutants.

METHODS

TG mice. hcTnT mutations were mapped to the mouse cTnT gene (36), and mouse cTnT mutants were expressed in the mouse heart as described below. A 2,996-bp rat α-MHC promoter (36) was used to drive the cardiac-specific expression of four different lines of TG mice expressing the wild-type mouse cTnT (WT), the R92L mutation in cTnT (R92L), the R92W mutation in cTnT (R92W), and the deletion of E160 in cTnT (Delta-160), as described before (36). To estimate the level of expression in the heart, cTnT in all TG constructs was tagged at the NH2 terminus with an 11-amino acid human c-myc epitope (36). Muscle fiber bundles from WTTG mouse hearts were used as the controls.

Isolation of detergent-skinned cardiac muscle fiber bundles from TG mouse hearts. All experiments were carried out according to the guidelines laid down by the Washington State University Institutional Animal Care and Use Committee. Left ventricular papillary muscle fiber bundles from freshly dissected mouse hearts were used for detergent-skinned muscle fiber experiments. Mice were anesthetized with pentobarbital sodium (50 mg/kg body wt), and the hearts were rapidly excised and placed into ice-cold high relaxing (HR) solution containing (in mM) 20 MOPS, pH 7.0, 53 KCl, 10 EGTA, 6.81 MgCl2, 5.35 Na2ATP, and 0.5 dithiothreitol (DTT). The total ionic strength of HR was 150 mM. A cocktail of protease inhibitors containing (in μM) 10 leupeptin, 1 pepstatin, and 100 PMSF was included in the buffer. Papillary muscles were dissected into thin bundles approximately 150–200 μm in width and 1.5–2.0 mm in length. Chemical skinning of the muscle fiber bundles was accomplished by bathing the fibers overnight in HR solution containing 1% Triton X-100.

Simultaneous measurement of steady-state isometric force and ATPase activity in detergent-skinned muscle fiber bundles from TG mouse hearts. For simultaneous measurement of force and ATPase activity, a system described by de Tombe and Stienen (7) and Stienen et al. (35) was used. The procedure for measuring force and ATPase activity was as described previously (5, 7). The composition of activating and relaxing solutions was based on a computer program (10). Relaxation buffer (pCa 9.0) contained (in mM) 100 N,N-bis(2-hydroxymethyl)-2-amino ethanesulfonic acid (BES), pH 7.0, 21.2 potassium propionate, 5.85 Na2ATP, 7.11 MgCl2, 20 EGTA, as well as (in μM) 200 diadenosine pentaphosphate (Ap5A) and 10 oligomycin. The composition of the activation buffer (pCa 4.3) was (in mM) 100 BES, pH 7.0, 1.55 potassium propionate, 5.97 Na2ATP, 6.59 MgCl2, 20 CaEGTA, as well as (in mM) 200 A2P5 and 10 oligomycin. A cocktail of inhibitors, containing (in μM) 10 leupeptin, 1 pepstatin, and 10 PMSF, was included in all the buffers. To determine the pCa-tension relation (pCa = −log of molar free Ca2+ concentration) and pCa-ATPase relations, muscle fiber bundles were sequentially bathed in solutions with pCa values ranging from 9.0 to 4.3.

The ATPase activity of the muscle fiber bundle was measured by an enzyme-coupled assay as described previously (5, 7, 35). For ATPase measurements, the activation buffer included 0.9 mM NADH, 5 mM NaN3, 10 mM phosphoenolpyruvate, 4 mg/ml pyruvate kinase (500 U/mg), and 0.24 mg/ml lactate dehydrogenase (870 U/mg). ATPase activity in the detergent-skinned muscle fiber bundles was measured as follows: ATP regeneration from ADP was coupled to the breakdown of phosphoenolpyruvate to pyruvate and ATP catalyzed by pyruvate kinase, which was linked to the synthesis of lactate catalyzed by lactate dehydrogenase. The breakdown of NADH, which is proportional to the amount of ATP consumed, was measured online by UV absorbance at 340 nm. The ratio of light intensity at 340 nm (sensitive to NADH concentration) to the light intensity at 410 nm (reference signal) was obtained by means of an analog divider. After each recording, the UV absorbance signal of NADH was calibrated by multiple rapid injections of 0.25 nmol of ADP into the bathing solution, with a motor-controlled calibration pipette.

Gel electrophoresis and Western blot analysis of myofibrillar protein preparations from TG mouse hearts. Protein samples from TG mouse hearts were prepared, and 30 μg of protein/lane was run on 12.5% SDS-polyacrylamide gels, as described previously (4). The protein concentration was determined by using a Bio-Rad detergent-compatible (DC) protein assay kit. Proteins were transferred onto nitrocellulose for Western blot analysis using an anti-mouse primary antibody against the c-myc tag, as described previously (36).

Data analysis. All data are expressed as means ± SE. Data from the normalized pCa-force and pCa-MgATPase activity measurements were fitted to the Hill equation by using a nonlinear least-square regression procedure to obtain the pCa50 (pCa required for half-maximal activation) and the Hill coefficient (n). pCa values were converted to Ca2+ concentration ([Ca2+]i) to calculate the fold increase in Ca2+ sensitivity of TG muscle fibers containing mutant cTnT. Statistical differences were analyzed by an unpaired t-test, with each TG mutant fiber individually paired with WTTG fiber.

RESULTS

Generation of TG mice. Codon 92 (R92) in hcTnT represents a hot spot for mutations in hcTnT because three different mutations have been found at this site (40). Each of these mutations (R92Q, R92L, and R92W) is lethal in humans (39). Because R92 is conserved among all known sequences of troponin T, it is likely that R92 represents a structurally/or functionally important regions in cTnT. Different lines of TG
A length (SL) was adjusted to 1.9 m. pCa50 and Hill coefficient (n) values derived from the nonlinear fits are given in METHODS. A: gel probed with antibody against human c-myc epitope. B: gel probed with antibody against cTnT. C: gel probed with antibody against cTnT.

Fig. 1. Western blot analysis of proteins from cardiac muscle preparations from different lines of transgenic (TG) mouse hearts that express wild-type mouse cardiac troponin (cTnT) (WTTG), R92W mouse cTnT mutant, R92L mouse cTnT mutant, and Delta-160 mouse cTnT mutant (deletion of amino acid 160). A: gel probed with antibody against human c-myc epitope. B: gel probed with antibody against cTnT. C: gel probed with antibody against cTnT. NTG, muscle protein preparations from non-TG mouse hearts.

Western blot analysis of proteins from mouse heart muscle preparations. Western blot analysis of myofibrillar protein preparations from various TG mouse hearts is shown in Fig. 1. As previously demonstrated (36), the total amount of cardiac muscle cTnT remained unaltered when WT cTnT transgene was overexpressed. This feature is common to other TG animals that are used in this study. All cTnT constructs were tagged with the c-myc epitope that enabled us to quantitate the amount of exogenous protein expressed in the TG mouse heart.

We have previously demonstrated that the presence of the c-myc epitope at the NH2 terminus of mouse cTnT and the transgene (WT cTnT) expression had no undesired effect on mouse cardiac sarcomere structure or the intact heart function (5, 36). In our previous study, we demonstrated that expression of 67% or 92% of R92Q mouse cTnT in the mouse heart caused an increase in Ca2+ sensitivity, hypercontractility, diastolic dysfunction, and impaired relaxation, which are the hallmarks of FHC in humans (5, 26, 36). In the present study, we have extended our investigation of FHC with the study of three new lines of TG mice that overexpress R92W, R92L, and Delta-160 mutant cTnT in the mouse heart. R92W TG mouse hearts are ~14% smaller than controls; myocytes exhibit moderate to severe disarray and little fibrosis. R92L TG mouse hearts are ~10% larger than controls; myocytes exhibit mild to moderate myofibrillar disarray and no significant fibrosis. Delta-160 TG mouse hearts are slightly smaller than those of R92W TG mice. Myocytes from Delta-160 TG mouse hearts show moderate myofibrillar disarray, without any significant fibrosis (Refs. 8 and 9; Ertz-Berger BR, He H, Dowell C, Factor SM, Haim TE, Nunez S, Schwartz SD, Ingwall JS, and Tardiff JC, unpublished observations).

Ca2+ sensitivity of myofilament activation in detergent-skinned mouse cardiac muscle fiber bundles. Even though there are differences in phenotypes, many of the FHC mutations studied so far indicate that an increase in myofilament Ca2+ sensitivity is common to these mutations. Therefore, something other than myofilament Ca2+ sensitivity is altered in some mutations that may be responsible for distinct phenotypes. To determine the molecular basis for differences in phenotypes of R92W, R92L, and Delta-160 TG mouse hearts, we measured the effects of these mutations on myofilament Ca2+ sensitivity, Ca2+-activated maximal tension, ATPase activity and the tension-dependent ATP consumption in muscle fiber bundles from TG mouse hearts. Figure 2 demonstrates the impact of cTnT mutations on the length-dependent activation of muscle fiber bundles from WTTG, R92W, R92L, and Delta-160 TG mouse hearts. The relationship between the steady-state isometric tension and pCa were compared at both short (1.9 m) and long sacromere lengths (SL; 2.3 m), respectively. When pCa-tension relations of WTTG cardiac muscle fibers measured at short SL were compared with those of mutant TG muscle fibers, there was a significant (P < 0.0001) increase in Ca2+ sensitivity, indicated by a 2.2-fold increase in R92W, 2.0-fold increase in R92L, and 1.3-fold increase in Delta-160 TG muscle fibers. pCa-tension relations...
measured at long SL also suggested a significant (P < 0.0001) increase in Ca\(^{2+}\) sensitivity of all mutant TG cardiac muscle fibers. At SL of 2.3 \(\mu\)m, Ca\(^{2+}\) sensitivity increased by 2.5-fold in R92W, 2.0-fold in R92L, and 1.3-fold in Delta-160 TG muscle fibers. The magnitude in pCa\(_{50}\) shift (from 1.9- to 2.3-\(\mu\)m SL) produced by all TG cardiac muscle fibers was similar compared with that of WTTG fibers, which indicated that length-dependent changes in Ca\(^{2+}\) sensitivity were not altered in any of the TG mouse cardiac muscle preparations tested (Table 1).

Figure 3 illustrates the relationship between the Ca\(^{2+}\)-activated ATPase activity and the steady-state isometric tension in detergent-skinned fiber bundles from WTTG, R92W, R92L, and Delta-160 TG muscle fibers. Ca\(^{2+}\)-activated ATPase activity increased in proportion to the isometric tension development. When pCa-ATPase relations of WTGG cardiac muscle fibers measured at short SL were compared with those of mutant TG muscle fibers, there was a significant (P < 0.001) increase in Ca\(^{2+}\) sensitivity, indicated by a by 2.2-fold increase in R92W, 2.1-fold increase in R92L, and 1.3-fold increase in Delta-160 TG muscle fibers (Table 2). pCa-ATPase relations measured at long SL also suggested a significant (P < 0.001) increase in Ca\(^{2+}\) sensitivity of all mutant TG cardiac muscle fibers. At SL of 2.3 \(\mu\)m, Ca\(^{2+}\) sensitivity increased by 2.5-fold in R92W, 2.0-fold in R92L, and 1.3-fold in Delta-160 TG muscle fibers. Here again, the magnitude in pCa\(_{50}\) shift (from 1.9- to 2.3-\(\mu\)m SL) produced by all fibers was similar compared with that of WTGG muscle fibers, which indicated that length-dependent increase in Ca\(^{2+}\) sensitivity was not altered by R92W, R92L, and Delta-160 mutations in cTnT. There were no significant differences in Hill coefficient values between WTGG, R92W, and Delta-160 TG muscle fibers in both pCa-tension and pCa-ATPase relationships. However, the Hill coefficient values for R92L TG muscle fibers were significantly different from WTGG fibers in all measurements and at both short and long SL.

**Table 1. Normalized pCa-tension relationship in detergent-skinned TG mouse cardiac muscle fiber bundles**

<table>
<thead>
<tr>
<th>SL ((\mu)m)</th>
<th>WTTG</th>
<th>R92W</th>
<th>R92L</th>
<th>Delta-160</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCa(_{50}) values</td>
<td>5.76±0.01</td>
<td>6.11±0.02*</td>
<td>6.07±0.01*</td>
<td>5.89±0.02*</td>
</tr>
<tr>
<td>Hill coefficient (n) values</td>
<td>3.8±0.3</td>
<td>3.4±0.3</td>
<td>2.6±0.2*</td>
<td>3.3±0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Data from the normalized pCa-force measurements were fitted to the Hill equation by using a nonlinear least square regression procedure to derive the pCa\(_{50}\) and Hill coefficient (n) values. R92W, R92L, and Delta-160 mutations in cardiac muscle fiber bundles at both short and long sarcomere lengths (SL). Transgenic (TG) mice express wild-type cTnT (WTTG), the R92L mutation in cTnT (R92L), the R92W mutation in cTnT (R92W), or the deletion of E160 in cTnT (Delta-160), respectively. *P < 0.001.

Fig. 3. Normalized pCa-ATPase relationship in detergent-skinned cardiac muscle fibers. Experimental conditions are given in METHODS. The buffer included, in addition to the buffer conditions mentioned in Fig. 2 legend, 0.9 mM NADH, 10 mM phosphoenolpyruvate, 4 mg/ml pyruvate kinase (500 U/mg), 0.24 mg/ml lactate dehydrogenase (870 U/mg). ○, WTTG muscle fibers; ●, R92W TG muscle fibers; ▲, R92L TG muscle fibers; ▲, Delta-160 TG muscle fibers. A: pCa-ATPase relation at SL of 1.9 \(\mu\)m. B: pCa-ATPase relation at SL of 2.3 \(\mu\)m. No. of determinations is at least 9 for each case. Data points are means and the error bar represents SE. pCa\(_{50}\) and Hill coefficient (n) values derived from the nonlinear fits are listed in Table 2.
Impact of mutations in cTnT on tension-dependent ATP consumption. The slope of force-ATPase relations is widely accepted as an index of rate of cross-bridge detachment (3, 17). Therefore, we simultaneously measured steady-state isometric ATP consumption, and Ca\(^{2+}\) activation of muscle fibers from TG mice that overexpress R92L, R92W, and Delta-160 mutant cTnT in the heart.

Enhancement of myofilament Ca\(^{2+}\) sensitivity in R92W, R92L, and Delta-160 TG mouse cardiac muscle fiber bundles. It is now apparent that an enhancement of thin filament Ca\(^{2+}\) sensitivity is common to many of the mutations associated with FHC. This provides a molecular basis for hypercontractility that is commonly observed in human patients and in studies with TG mouse models. All three TG lines we tested in this study show a significant increase in Ca\(^{2+}\) sensitivity of cardiac thin filaments. The mechanism by which mutations in cTnT alter Ca\(^{2+}\) activation of thin filaments has been linked to either an inefficient binding of improperly folded mutant cTnT or a direct effect of mutation on the Tn complex or through mutant cTnT impact on actin-Tm. TG studies of R92Q, R92W, and R92L mutations suggest that the mutation-induced alterations in protein structure have no major impact on their ability to bind to thin filaments. Nearly 90% of R92Q, 50% of R92W, and R92L are stably incorporated into the thin filaments, and Ca\(^{2+}\)-activated maximal tension in all of these fibers is not altered. When expressed in substantial quantity (as in R92W, R92L, and Delta-160 TG mouse hearts), any improper folding of the mutant would likely result in myofilament structural disruptions, which were not observed in TG myofilaments that stably incorporated cTnT mutants.

Our previous work on another TG mouse model for FHC suggested that R92Q mutation in cTnT enhances Ca\(^{2+}\) sensitivity of thin filaments by decreasing the threshold for Ca\(^{2+}\) activation (5). This would imply that both R92W and R92L mutations may also work by altering cTnT-Tm interactions, which leads to a partial disinhibition of actin monomers underneath the Tn-Tm complex. The idea that R92W and R92L mutations may alter Tm-dependent function of cTnT is supported by Palm et al. (29), who used hcTnT peptides and cosedimentation assay to measure the binding between hcTnT\(_{170-170}\)-Tm and actin (29). Their study showed that mutations in this region (near codon 92) decreased hcTnT\(_{170-170}\) impact on Tm binding to actin. This weakening of Tm interaction with actin makes the Tn-Tm complex less inhibitory, which leads to greater force at submaximal [Ca\(^{2+}\)] when increasing number of cross bridges bind cooperatively to actin. However, none of the mutant TG muscle fibers showed any

---

### Table 3. Ca\(^{2+}\)-activated maximal tension and ATPase activity in detergent-skinned TG mouse cardiac fiber bundles

<table>
<thead>
<tr>
<th>SL ((\mu)m)</th>
<th>WTGG</th>
<th>R92W</th>
<th>R92L</th>
<th>Delta-160</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9 (\mu)m</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pCa(_{50}) values</td>
<td>27.8 ± 0.8</td>
<td>27.6 ± 1.8</td>
<td>26.6 ± 0.9</td>
<td>26.4 ± 1.4</td>
</tr>
<tr>
<td>Hill coefficient (n) values</td>
<td>264 ± 7</td>
<td>277 ± 17</td>
<td>261 ± 8</td>
<td>300 ± 10*</td>
</tr>
<tr>
<td>2.3 (\mu)m</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tension, mN/mm(^2)</td>
<td>43.8 ± 1.2</td>
<td>44.3 ± 2.6</td>
<td>46.3 ± 1.7</td>
<td>41.7 ± 1.3</td>
</tr>
<tr>
<td>ATPase, pmol/(\mu)L(^{-1})s(^{-1})</td>
<td>320 ± 8</td>
<td>334 ± 15</td>
<td>341 ± 10</td>
<td>371 ± 10*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Ca\(^{2+}\)-activated maximal tension and ATPase activity were measured in activation buffer (pCa 4.3, pH 7.0), and the buffer composition is given in Figs. 2 and 3 legends. Tension and ATPase activity were measured online as described previously (5, 7, 35). Number of determinations is 14 muscle fibers for WTGG, 9 muscle fibers for R92W, 10 muscle fibers for R92L, and 12 muscle fibers for Delta-160. Delta-160 mutation in cTnT decreases ATPase activity in cardiac muscle fiber bundles at both short and long SL. *P < 0.01.

---

### Table 2. Normalized pCa-ATPase relationship in detergent-skinned TG mouse cardiac fiber bundles

<table>
<thead>
<tr>
<th>SL ((\mu)m)</th>
<th>WTGG</th>
<th>R92W</th>
<th>R92L</th>
<th>Delta-160</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9 (\mu)m</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pCa(_{50}) values</td>
<td>5.86 ± 0.01</td>
<td>6.21 ± 0.02*</td>
<td>6.19 ± 0.01*</td>
<td>5.99 ± 0.02*</td>
</tr>
<tr>
<td>Hill coefficient (n) values</td>
<td>4.9 ± 0.4</td>
<td>4.1 ± 0.2</td>
<td>3.0 ± 0.3*</td>
<td>4.5 ± 0.4</td>
</tr>
<tr>
<td>2.3 (\mu)m</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pCa(_{50}) values</td>
<td>5.96 ± 0.01</td>
<td>6.36 ± 0.02*</td>
<td>6.26 ± 0.02*</td>
<td>6.09 ± 0.02*</td>
</tr>
<tr>
<td>Hill coefficient (n) values</td>
<td>3.9 ± 0.3</td>
<td>3.2 ± 0.4</td>
<td>2.4 ± 0.2*</td>
<td>4.0 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. Data from the normalized pCa-ATPase measurements were fitted to the Hill equation by using a nonlinear least square regression procedure to derive the pCa\(_{50}\) and Hill coefficient (n) values. R92W, R92L, and Delta-160 mutations in cTnT increase Ca\(^{2+}\) sensitivity in cardiac muscle fiber bundles at both short and long SL. *P < 0.01.
increase in the Hill coefficient values (Table 1). On the other hand, R92L TG muscle fibers showed a significant decrease in the Hill coefficient values at both short and long SL. Hinkle and Tobacman (16) showed that the mutation-induced impact on the flexibility of TnT tail domain affected Tn binding Tm but had no significant effect on actin interaction with the Tn-Tm regulatory complex. Unlike the mutations at position 92 of cTnT, Delta-160 mutation did not impair the cTnT ability to stabilize Tm head-to-tail overlap (25, 26). Overall, these observations suggest that it is difficult to ascertain whether there is a straightforward link between mutation-induced increase in myofilament cooperativity and thin filament Ca\(^{2+}\) sensitivity.

Impact of mutation in cTnT on increase in tension-dependent ATP consumption: implications for altered cross-bridge cycling kinetics. Our observations with R92W, R92L, and Delta-160 TG mice demonstrate that these mice show different phenotypes despite the fact that all of the TG muscle fibers exhibited an enhancement in myofilament Ca\(^{2+}\) sensitivity. Delta-160 TG mice show a relatively more severe phenotype than R92L and R92W both at the TG mouse level and in humans. This is a classic example of two different mutations in cTnT with similar effect on myofilament Ca\(^{2+}\) sensitivity that lead to distinct phenotypes in TG cardiac myocytes and in humans. Because cTnT-related FHC is a disease that starts at the myofilament level, it is possible that these different mutations affect myocyte function in more than one way that is unique to each mutation. To test this hypothesis, we determined the effect of cTnT mutations (Delta-160, R92L, and R92W) on the tension-dependent ATP consumption in detergent-skinned TG muscle fiber bundles. An interesting observation is that the tension-dependent ATP hydrolysis increased only in Delta-160 TG muscle fibers at both short (24% increase) and long (37% increase) SLs, suggesting that cross-bridge detachment rate constant is altered by the Delta-160 mutation in cTnT. The change in the rate of ATP hydrolysis reflects a change in cross-bridge cycling kinetics (3). The increase in ATP consumption must be due to an increase in intrinsic rate of ATP hydrolysis because there was no significant change in maximal tension at both short and long SL.

Our data suggest that an alteration in cTnT is capable of modulating cross-bridge cycling kinetics. How Tn may enhance cross-bridge detachment rate is not well understood. Mutations in cTnT could alter cross-bridge cycling kinetics by altering the state of actin monomers so that the kinetics of myosin head binding to actin is altered. In the presence of TnT, Tm binds strongly to actin, and, therefore, TnT has the potential to impact actin (32, 38). There are some data suggesting

---

Table 4. The slope of tension-ATPase relationship (tension cost) in detergent-skinned TG mouse cardiac muscle fiber bundles

<table>
<thead>
<tr>
<th></th>
<th>WTTG</th>
<th>R92W</th>
<th>R92L</th>
<th>Delta-160</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope of tension-ATPase relationship</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SL 1.9(\mu)m</td>
<td>9.5±0.4</td>
<td>10.0±0.3</td>
<td>9.7±0.4</td>
<td>11.7±0.5*</td>
</tr>
<tr>
<td>SL 2.3(\mu)m</td>
<td>6.7±0.3</td>
<td>7.5±0.4</td>
<td>7.2±0.4</td>
<td>9.2±0.5†</td>
</tr>
</tbody>
</table>

Values are means±SE. ATPase measurements were made at different pCa values, and data were fitted using a linear regression analysis as described in Fig. 4 legend. The slopes of tension-ATPase relationships (in pmol-mM\(^{-1}\)-mm\(^{-1}\)-s\(^{-1}\)) are mean values calculated from linear fits (see Fig. 4) of 14 muscle fibers for WTTG, 9 muscle fibers for R92W, 10 muscle fibers for R92L, and 12 muscle fibers for Delta-160. Delta-160 TG muscle fiber bundles consume more ATP for a given amount of tension at both short and long SL. *\(P<0.005\), †\(P<0.0001\).

---
that TnT may interact directly with actin (6, 14). Whether this is an indirect effect of TnT on actin through Tm or a direct effect of TnT on actin remains to be explored.

A link between hypercontractility and cardiac dysfunction suggests that the hearts under stress may not be able to meet the increased energy demand (5, 26, 34). In the case of Delta-160 TG muscle fibers, even more stress is imposed on the cardiac myocytes because of an increase in the cost of force development. Chronic stress on relative cellular ATP pool in cardiac myocytes may cause a strain on energy-dependent Ca\(^{2+}\) homeostatic mechanisms. This may lead to a pathological cardiac response. When I79N TG mice were inotropically stimulated with isoproterenol, there was a significant increase in sudden death due to arrhythmia (17).

REFERENCES

Grants K08-HL-68619 – 04 and R01-HL-075619 – 02 (J. C. Tardiff). Heart Association (M. Chandra), and National Heart, Lung, and Blood Institute Scientist Development Award AHA 0335131-N from the National American Heart Association of the Northwest Affiliate (M. Chandra), Grant-in-AidAHA-0151180 from the American Heart Association, National Heart, Lung, and Blood Institute Grant R01-HL-075643– 02 (M. Chandra), Grant-in-AidAHA-0151180 from the American Heart Association, National Heart, Lung, and Blood Institute Grants K08-HL-68619 – 04 and R01-HL-075619 – 02 (J. C. Tardiff).

ACKNOWLEDGMENTS

We thank Drs. R. John Solaro and Pieter de Tombe for their support.

GRANTS

This work was supported in part by National Heart, Lung, and Blood Institute Grant R01-HL-075643–02 (M. Chandra), Grant-in-Aid AHA-0151180 from the American Heart Association of the Northwest Affiliate (M. Chandra), Scientist Development Award AHA 0335131-N from the National American Heart Association (M. Chandra), and National Heart, Lung, and Blood Institute Grants K08-HL-68619 – 04 and R01-HL-075619 – 02 (J. C. Tardiff).

REFERENCES


