Divergent effects of $\alpha$- and $\beta$-myosin heavy chain isoforms on the N terminus of rat cardiac troponin T

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Divergent effects of $\alpha$- and $\beta$-myosin heavy chain (MHC) isoforms on contractile behavior arise mainly because of their impact on thin filament cooperativity. The N terminus of cardiac troponin T (cTnT) also modulates thin filament cooperativity. Our hypothesis is that the impact of the N terminus of cTnT on thin filament activation is modulated by a shift from $\alpha$- to $\beta$-MHC isoform. We engineered two recombinant proteins by deleting residues 1–43 and 44–73 in rat cTnT (RcTnT): RcTnT$_{1–43}$ and RcTnT$_{44–73}$, respectively. Dynamic and steady-state contractile parameters were measured at sarcomere length of 2.5 $\mu$m after reconstituting proteins into detergent-skinned muscle fibers from normal ($\alpha$-MHC) and propylthiouracil-treated ($\beta$-MHC) rat hearts. $\alpha$-MHC attenuated Ca$^{2+}$-activated maximal tension ($\sim$46%) in RcTnT$_{1–43}$ fibers. In contrast, $\beta$-MHC decreased tension only by 19% in RcTnT$_{1–43}$ fibers. Both $\alpha$- and $\beta$-MHC did not affect tension in RcTnT$_{44–73}$ fibers. The instantaneous muscle fiber stiffness measurements corroborated the divergent impact of $\alpha$- and $\beta$-MHC on tension in RcTnT$_{1–43}$ fibers, $p$Ca$_{50}$ (log of [Ca$^{2+}$]$_{1/2}$ required for half-maximal activation) decreased significantly by 0.13 pCa units in $\alpha$-MHC + RcTnT$_{1–43}$ fibers but remained unaltered in $\beta$-MHC + RcTnT$_{1–43}$ fibers, demonstrating that $\beta$-MHC counteracted the attenuating effect of RcTnT$_{1–43}$ on myofilament Ca$^{2+}$ sensitivity. $\beta$-MHC did not alter the sudden stretch–mediated recruitment of new cross-bridges ($E_R$) in RcTnT$_{1–43}$ fibers, but $\alpha$-MHC attenuated $E_R$ by 36% in RcTnT$_{1–43}$ fibers. The divergent impact of $\alpha$- and $\beta$-MHC on how the N terminus of cTnT modulates contractile dynamics has implications for heart disease; alterations in cTnT and MHC are known to occur via changes in isoform expression or mutations.

INTRODUCTION

We recently demonstrated the existence of two distinct regions in the N terminus of mouse cardiac troponin T (cTnT) that have diverse roles in mediating cardiac contractile activation (Mamidi et al., 2013a). For example, using normal adult mouse cardiac muscle fibers ($\alpha$-myosin heavy chain [MHC]), we demonstrated that the region 1–44 in mouse cTnT modulated the recruitment of force-bearing cross-bridges (XBs). Our data also showed that the cardiac-specific region 45–74 in mouse cTnT slowed XB recruitment mechanisms by augmenting thin filament cooperative processes. cTnT-induced slowing of XB recruitment mechanisms may be important for linking the dynamics of cardiac contraction to the heart rate (Mamidi et al., 2013a).

Apart from cTnT, XB also have a major effect on cardiac thin filaments through cooperative mechanisms, a process in which the strongly bound XBs promote the recruitment of other strong XBs by affecting the movement of tropomyosin (Tm) on the actin filament (Gordon et al., 2000; Fitzsimons et al., 2001; Moss et al., 2004; Fitzsimons and Moss, 2007). Because of their major effects on thin filament activation, differences in the expression of XB isoforms are associated with tissue-specific functions. For example, fast skeletal muscle type expresses a fast XB isoform ($\alpha$-MHC), and slow skeletal muscle type expresses a slow XB isoform ($\beta$-MHC). Moreover, the hearts of smaller mammals, with rapid heart rates, primarily express a faster cycling $\alpha$-MHC isoform; larger mammals, with slower heart rates, primarily express a slower cycling $\beta$-MHC isoform. Because of their significant differences in XB dwell times (shorter for $\alpha$-MHC isoform vs. longer for $\beta$-MHC isoform) in the force-producing state, fast and slow XB isoforms are expected to have divergent effects on cooperative processes in the thin filament (Ford et al., 2012).

Whether it is cTnT or a given MHC isoform, cooperative effects modulated by these proteins do not work in isolation. Therefore, the effects of both cTnT andXBs on thin filament cooperativity takes on a new significance when we consider the cTnT–XB interplay effects on cardiac contractile dynamics (Chandra et al., 2007). Collectively, these arguments lead us to hypothesize that the modulating effects of the N terminus of cTnT on contractile dynamics is affected differently by $\alpha$- and $\beta$-MHC isoforms. Our hypothesis has significant

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Abbreviations used in this paper: cTnT, cardiac troponin T; MHC, myosin heavy chain; ML, muscle length; NLRD, nonlinear recruitment distortion; PTU, propylthiouracil; RcTnT, rat cTnT; RU, regulatory unit; SL, sarcomere length; Tm, tropomyosin; Tn, troponin; XB, cross-bridge.

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implications for understanding how contractile dynamics are tuned by species-specific variation in the expression of fast versus slow MHC isoforms in small versus large animals. In this regard, it is worth noting that hearts of both small and large animals contain the cTnT isoform that has the cardiac-specific 1–44 and 45–74 regions—albeit with minor changes—but express MHC isoforms that possess vastly differing kinetic rates.

Additional support for our hypothesis comes from the following critical observations: (a) the functional impact of disease-related mutations in the N terminus of cTnT is influenced by the type of MHC isoform (Tscharig et al., 2006; Rice et al., 2010; Ford et al., 2012); (b) the composition of MHC isoform is a key determinant of cardiac contractile activation (Stelzer et al., 2007); (c) small changes in the level of MHC isoform induce large changes in cardiac function both at the myofilament level (Fitzsimons et al., 1998; Rundell et al., 2005; Tscharig et al., 2006) and at the whole heart level (Tardiff et al., 2000; Korte et al., 2005; Rice et al., 2010); and (d) mutations in sarcomeric proteins that cause human heart failure are often accompanied by a near-complete shift to β-MHC (Miyata et al., 2000; Reiser et al., 2001).

To test our hypothesis, we deleted two specific regions (regions 1–43 and 44–73) in the N terminus of rat cTnT (RcTnT) to generate RcTnT1–43 and RcTnT44–73 proteins, respectively. Steady-state and dynamic contractile measurements were made in cardiac muscle fibers (containing either α- or β-MHC isoforms) reconstituted with RcTnT1–43 and RcTnT44–73 proteins. Novel findings from our study demonstrate that α- or β-MHC isoforms have divergent effects on how the N terminus of cTnT modulates contractile function. As demonstrated by the decrease in Ca2+-activated maximal tension and myofilament Ca2+ sensitivity, the thin filament activation was significantly attenuated by RcTnT1–43 fibers in the presence of α-MHC. In contrast, the attenuating effect of RcTnT1–43 was counteracted by β-MHC. The sudden stretch-mediated recruitment of new force-bearing XBs (Eβ) was attenuated in α-MHC + RcTnT1–43 fibers but not in β-MHC + RcTnT1–43 fibers. We discuss these data in terms of the differential synergy between α/β-MHC isoforms and the N terminus of cTnT.

MATERIALS AND METHODS

Ethical approval and animal treatment protocols

Muscle bundles were collected from the left ventricles of young adult male Sprague-Dawley rats. Rats used in this study were split into two groups: one group consisted of normal rats that predominantly expressed α-MHC, and the other group consisted of propylthiouracil (PTU)-treated rats (see below) that predominantly expressed β-MHC in their left ventricles. Rats were carefully handled to minimize the pain and suffering, according to the established guidelines of the National Academy of Sciences: Guide for the Care and Use of Laboratory animals.
then purified by ion-exchange chromatography on a DEAE-fast sepharose column (GE Healthcare). c-myc RcTnT and RcTnT44–73 were eluted with a 0–0.4 M NaCl gradient. The procedure used for the purification of RcTnT1–43A was similar to that of c-myc RcTnT, except that a CM sepharose column (GE Healthcare) was used for ion-exchange chromatography. RcTnT was purified as described previously (Chandra et al., 2007). RcTnC protein was purified as described previously (Mamidi et al., 2013a). Samples from eluted fractions were pooled and dialyzed extensively against deionized water containing 15 mM β-mercaptoethanol, lyophilized, and stored at −80°C.

Reconstitution of recombinant rat cardiac Tn subunits into detergent-skinned rat cardiac muscle fibers

Reconstitution was performed as described previously (Chandra et al., 2007). c-myc-tagged WT RcTnT (RcTnTWT) was used as a control so that the incorporation of the exogenously added WT Tn subunits could be assessed by the differential gel migration pattern of c-myc RcTnT. It has been shown that the use of cTnT with an 11-amino acid c-myc epitope at the N terminus does not affect cardiac function (Tardiff et al., 1998; Montgomery et al., 2001). The replacement of endogenous Tn was performed using an extraction solution containing RcTnT (c-myc RcTnT; RcTnT44–73A or RcTnT1–43A) (0.9 mg/ml, wt/vol) and RcTnI (1.0 mg/ml, wt/vol) that were first dissolved in buffer 1, which contained 50 mM Tris-HCl, pH 8.0, 6 M urea, 1.0 M KCl, 10 mM DTT, and a cocktail of protease inhibitors. High salt and urea in the extraction solution were removed by successive dialysis against the following buffers: 50 mM Tris-HCl, pH 8.0, 4 M urea, 0.7 M KCl, 0.2 mM PMSF, 2 mM benzamidine-HCl, 1 mM DTT, and 0.01% NaN₃ (buffer 2), followed by 50 mM Tris-HCl, pH 8.0, 2 M urea, 0.5 M KCl, 0.2 mM PMSF, 2 mM benzamidine-HCl, 1 mM DTT, and 0.01% NaN₃ (buffer 3), and finally dialyzed against an extraction buffer containing 50 mM BES, pH 7.0 at 20°C, 200 mM KCl, 10 mM BDM, 6.27 mM MgCl₂, 5 mM EGTA, 0.2 mM PMSF, 2 mM benzamidine-HCl, 1 mM DTT, and 0.01% NaN₃ (buffer 4). After the final dialysis, 6.15 mM MgATP²⁻ and a cocktail of protease inhibitors were added to the supernatant containing RcTnT and RcTnI. Undissolved protein was removed by spinning the protein solution at 3,000 rpm for 15 min. Detergent-skinned fibers were then treated with the extraction solution containing RcTnT and RcTnI for ~3 h at room temperature (20°C), with constant stirring. RcTnT–RcTnI-treated muscle fibers were then washed three times using buffer 4 for 10 min at room temperature. To complete the reconstitution procedure, RcTnT–RcTnI treated fibers were incubated with RcTnC (3.0 mg/ml, wt/vol) overnight at 4°C.

Fibers reconstituted with RcTnT1–43A + RcTnI + RcTnC are referred to as “RcTnT1–43A + RcTnI + RcTnC” and those reconstituted with RcTnT44–73A + RcTnI + RcTnC are referred to as “RcTnT44–73A + RcTnI + RcTnC.” Fibers reconstituted with c-myc RcTnT + RcTnI + RcTnC are referred to as “RcTnTWT + RcTnI + RcTnC” and were used as controls. Reconstituted fibers were denatured and solubilized in 2% SDS solution (10 µl/fiber) for SDS-PAGE (Mamidi et al., 2012). SDS-solubilized fibers were mixed with an equal volume of protein loading dye that contained 125 mM Tris-HCl, pH 6.8, 20% glycerol, 2% SDS, 0.01% bromophenol blue, and 50 mM β-mercaptoethanol. Equal quantities of protein were separated on an 8% SDS gel and stained with Bio-Safe Coomassie blue G-250 (Bio-Rad Laboratories).

Proteins separated on the SDS gel were transferred onto a PVDF membrane for Western blot analysis using a Trans-Blot Turbo Transfer System (Bio-Rad Laboratories). The incorporation of c-myc RcTnT, RcTnT1–43A, or RcTnT44–73A was assessed using a monoclonal anti-TnT primary antibody (clone JLT-12; Sigma-Aldrich), followed by a horseradish peroxidase–labeled anti–mouse secondary antibody (GE Healthcare). Densitometric analysis of Western blots was performed using the ImageJ software (acquired from National Institutes of Health at: http://rsweb.nih.gov/ij/) (Mamidi et al., 2013b).

Measurement of steady-state isometric tension and ATPase activity

Isometric steady-state tension and ATPase activity were measured as described previously (Chandra et al., 2007, 2009). In brief, T-shaped aluminum clips were used to attach the muscle fiber between a motor arm (322C; Aurora Scientific Inc.) and a force transducer (AE 801; Sensor One Technologies Corp.). The sarcomere length (SL) of the muscle fibers was set to 2.3 µm under relaxing conditions as described previously (Chandra et al., 2007, 2009). After two cycles of maximal activation and relaxation, the SL was readjusted if necessary. The muscle fiber was then immersed in a constantly stirred chamber containing Ca²⁺ solutions, the concentration of which ranged from pCa 4.3 to 9.0 (pCa = −log of [Ca²⁺]₀). The concentration of various pCa solutions was calculated using methods described previously (Fabiato and Fabiato, 1979). The composition of the maximal Ca²⁺ activation solution (pCa 4.3) was (mM): 31 potassium propionate, 5.95 Na₂ATP, 6.61 MgCl₂, 10 EGTA, 10.11 CaCl₂, 50 BES, 5 sodium azide, and 10 phosphoenol pyruvate (PEP). The composition of the relaxing solution (pCa 9.0) was (mM): 51.14 potassium propionate, 5.83 Na₂ATP, 6.87 MgCl₂, 10 EGTA, 0.024 CaCl₂, 50 BES, 5 Na₂S, and 10 PEP. The activation and relaxing solutions also contained 0.5 mg/ml pyruvate kinase (500 U/mg), 0.05 mg/ml lactate dehydrogenase (870 U/mg), 20 µM diadenosine pentaphosphate, 10 µM oligomycin, and a cocktail of protease inhibitors. The pH of the Ca²⁺ solutions was adjusted to 7.0 using KOH, and the ionic strength was 180 mM. Ca²⁺-activated tension was digitally recorded on a computer. All measurements were made at 20°C.

Steady-state isometric ATPase activity was measured according to a protocol described previously (de Tombe and Stienen, 1995; Kirk et al., 2009; Rodgers et al., 2009; Gollapudi et al., 2012). In brief, near-UV light (340 nm) was projected through the muscle chamber, and then split (50:50) via a beam splitter and detected at 340 nm (sensitive to changes in NADH) and at 400 nm (insensitive to changes in NADH). ATPase activity was measured as follows: ATP regeneration from ADP was coupled to the breakdown of PEP to pyruvate and ATP catalyzed by pyruvate kinase, which was linked to the synthesis of lactate catalyzed by lactate dehydrogenase. The breakdown of NADH during the synthesis of lactate was proportional to the ATP consumption and was measured by changes in UV absorbance at 340 nm. The signal for NADH was calibrated by multiple injections of 250 pmol of ADP.

Measurement of muscle fiber mechano-dynamics

Once the force developed by the muscle fiber reached steady state (pCa 4.3), step-like length changes were applied (Ford et al., 2010). In brief, the motor arm was commanded to change the muscle length (ML) in a step-like pattern; the changes in ML were on the order of ±0.5, 1.0, 1.5, and 2.0% of the initial ML. The ML was initially increased by 0.5% ML (stretched) and held at the increased length for 5 s, and then the motor arm was commanded to rapidly return (released) to the initial ML. The same protocol was repeated for the remaining step-like length changes of 1.0, 1.5, and 2.0% ML. A nonlinear recruitment distortion (NLRD) model (Ford et al., 2010) was fitted to the elicited force responses to estimate the following model parameters: b, the rate by which new Xbs are recruited into the force-bearing state because of a change in ML; c, the rate by which the stiffness of distorted Xbs dissipate; E₀, the magnitude of an instantaneous increase in muscle fiber stiffness caused by a sudden change in ML; and Eᵦ, the magnitude of an increase in steady-state force caused by the sudden stretch–mediated recruitment of additional Xbs.
Measurement of rate of tension redevelopment (\(k_0\))

The measurement of \(k_0\) (pCa 4.3) was performed according to a modified protocol described originally by Brenner and Eisenberg (1986). Upon attaining a steady-state isometric force, the motor arm was commanded to slacken the muscle fiber by 10% of the ML using a high-speed length-control device (Aurora Scientific Inc.). After a brief shortening period of 25 ms, the motor arm was commanded to rapidly (within 0.5 ms) swing past the original set point by a 10% stretch. The stretch was applied to ensure that any remaining XB's were mechanically detached and the residual force was not more than \(\sim 10\%\) of the initial steady-state isometric force. \(k_0\) was determined by fitting the following mono-exponential equation to the force redevelopment:

\[
F = (F_m - F_\infty) \left(1 - e^{-k_0 t}\right) + F_\infty,
\]

where \(F\) is the force at time \(t\), \(F_m\) is the steady-state isometric force, and \(F_\infty\) is the residual force from which the redevelopment of force occurs.

Data analysis

Contractile and mechano-dynamic parameters were analyzed using a two-way ANOVA. One factor in this analysis was the RcTnT variant (c-myc-RcTnT, RcTnT1-43\(\beta\), or RcTnT14-73\(\beta\)), and the other was the MHC isoform (\(\alpha\)- or \(\beta\)-MHC). After analysis by two-way ANOVA, post-hoc tests (planned multiple pairwise comparisons) were made using uncorrected Fisher's LSD method. Comparisons were made to determine the effects of RcTnT variants and MHC isoforms on various contractile and mechano-dynamic parameters. The Hill equation was fitted to normalized pCa-tension data to estimate \(pCa_{50}\) ( \(- \log \cdot [Ca^{2+}]_{ext}\) required for half-maximal activation) and the Hill coefficient (\(n_H\)). Values are reported as mean \(\pm\) SEM. The criterion for statistical significance was set at \(P < 0.05\).

Online supplemental material

The online supplemental material describes the development of the NLRD model, which was used to estimate the dynamic contractile parameters (Ford et al., 2010). It also contains characteristic features of the force responses of muscle fibers to step-like length perturbations. The online supplemental material is available at http://www.jgp.org/cgi/content/full/jgp.201310971/DC1.

RESULTS

Rationale for the generation of RcTnT\(1-43\(\beta\) and RcTnT\(44-73\(\beta\) deletion mutants

Recently, we identified two distinct functional regions (1-44 and 45-74) in the N terminus of mouse cTnT; the interplay between these two regions and the overlapping ends of contiguous Tm dimers were shown to affect thin filament activation and myofilament cooperativity (Mamidi et al., 2013b). Based on the sequence comparison shown in Fig. 1, we identified the corresponding regions in RcTnT: (a) a highly acidic peptide region comprising 1-43 amino acids, which shares only 51% sequence similarity with rat fast skeletal TnT (RfsTnT); and (b) a region comprising 44-73 amino acids, which is absent in RfsTnT. Residues 1-43 and 44-73 were deleted in RcTnT to generate RcTnT\(1-43\(\beta\) and RcTnT\(44-73\(\beta\) respectively. Deletion of these two distinct regions (RcTnT\(1-43\(\beta\) and RcTnT\(44-73\(\beta\)) amounted to a net removal of 22 and 6 acidic residues, respectively.

To understand how \(\alpha\)- and \(\beta\)-MHC isoforms affect the interplay between the N terminus of cTnT and the overlapping ends of Tm, we reconstituted these mutants into detergent-skinned rat cardiac muscle fibers from normal rats (\(\alpha\)-MHC) and PTU-treated rats (\(\beta\)-MHC).

Shift from \(\alpha\)- to \(\beta\)-MHC in PTU-treated rat hearts

PTU treatment has been shown to induce a shift from \(\alpha\)- to \(\beta\)-MHC isoform, without any significant changes in the expression of other myofilament proteins (Metzger et al., 1999; Herron et al., 2001; Ford et al., 2012). PTU-induced hypothyroidism silences the \(\alpha\)-MHC promoter and activates the \(\beta\)-MHC promoter, resulting in the myocardial expression of \(\beta\)-MHC (Chizzonite and Zak, 1984). As shown in Fig. 2 A, PTU treatment of rats induced a near-complete shift to \(\beta\)-MHC isoform in the left ventricles.

Western blot analysis of reconstituted rat cardiac muscle fibers

The c-myc–tagged WT RcTnT (RcTnT\(WT\)) was used as the control. In our reconstitution protocol, the exogenously added Tn complex consisted of RcTnT (RcTnT\(WT\), RcTnT\(1-43\(\beta\), or RcTnT\(44-73\(\beta\)), RcTnI, and RcTnC proteins. We have shown previously that the endogenous Tn complex is replaced when a vast excess of exogenously added cTnT competes with the endogenous cTnT (Chandra et al., 1999). Therefore, the level of RcTnT incorporated may be used as an index of the amount of endogenous Tn replaced. The differential migration

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<tr>
<td>RfsTnT</td>
<td>MSDETEQVEEEQQEVEQEEAPEPEEVEQEEKPR</td>
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Figure 1. Rationale for the generation of RcTnT\(1-43\(\beta\) and RcTnT\(44-73\(\beta\) deletion mutants. Sequence comparison of amino acids at the N-terminal ends of RcTnT and RfsTnT revealed two distinct regions in the N-terminal end region of RcTnT: (1) a highly negatively charged region comprising 1-43 amino acids in RcTnT that corresponds to 1-41 amino acids in RfsTnT; and (2) a region comprising 44-73 amino acids in RfsTnT that is missing in RcTnT. To understand how the functions of these two regions are modulated by \(\alpha\)- and \(\beta\)-MHC isoforms, we deleted these regions in RcTnT to generate RcTnT\(1-43\(\beta\) and RcTnT\(44-73\(\beta\) deletion mutants. ; identical amino acids; ., conservative substitution; empty space, nonconservative substitution; –, deletion introduced to maximize sequence similarities.

416 Divergent effects of MHC isoforms on cTnT function

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incorporation of exogenously added RcTnT proteins in PTU-treated rat fibers (β-MHC) was similar to that observed in the normal rat fibers.

Divergent effects of α- and β-MHC isoforms on Ca2+-activated maximal tension and ATPase activity in RcTnT1–43 or RcTnT44–73-reconstituted rat cardiac muscle fibers

Ca2+-activated maximal tension was measured at pCa 4.3. Two-way ANOVA revealed a significant interaction effect (P < 0.001), demonstrating a divergent impact of α- and β-MHC isoforms on how RcTnT1–43 and RcTnT44–73 modulated Ca2+-activated maximal tension. To determine the factor that was responsible for the significant interaction effect, we performed subsequent post-hoc tests using multiple pairwise comparisons (uncorrected Fischer’s LSD). Post-hoc tests revealed that α- and β-MHC isoforms had differential impact on maximal tension in RcTnT1–43 fibers but not in RcTnT44–73 fibers. Ca2+-activated maximal tension decreased by ~46% in α-MHC + RcTnT1–43 fibers (Fig. 3 A) and by only 18% in β-MHC + RcTnT1–43 fibers (Fig. 3 B). Thus, β-MHC significantly counteracted the attenuating effect of RcTnT1–43 on maximal tension. On the other hand, incorporation of exogenously added RcTnT proteins in PTU-treated rat fibers (β-MHC) was similar to that observed in the normal rat fibers.

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hand, both α- and β-MHC isoforms did not alter Ca\textsuperscript{2+}-activated maximal tension in RcTnT\textsubscript{44-73}\textsubscript{A} fibers (Fig. 3, A and B).

Ca\textsuperscript{2+}-activated maximal ATPase activity was measured in reconstituted fibers at pCa 4.3. Two-way ANOVA revealed a significant interaction effect (P < 0.01), demonstrating a divergent impact of α- and β-MHC isoforms on how RcTnT\textsubscript{1-43} or RcTnT\textsubscript{44-73} affected Ca\textsuperscript{2+}-activated maximal ATPase activity. Post-hoc multiple comparisons revealed that a shift from α- to β-MHC had a differential impact on ATPase activity. In the presence of α-MHC, ATPase values (in pmol \times mm\textsuperscript{-3} \times s\textsuperscript{-1}) were $174.89 \pm 4.87$, $118.23 \pm 4.31$, and $162.43 \pm 5.70$ for RcTnT\textsubscript{WT}, RcTnT\textsubscript{1-43}, and RcTnT\textsubscript{44-73} fibers, respectively. However, in the presence of β-MHC, ATPase values were $98.84 \pm 6.71$, $73.38 \pm 3.00$, and $93.27 \pm 4.09$ for RcTnT\textsubscript{WT}, RcTnT\textsubscript{1-43}, and RcTnT\textsubscript{44-73} fibers, respectively. When compared with the control fibers, RcTnT\textsubscript{1-43} decreased ATPase activity by ~32% in the presence of α-MHC and by ~26% in the presence of β-MHC. In the presence of α- or β-MHC, RcTnT\textsubscript{44-73} did not alter maximal ATPase activity when compared with the respective control fibers. Therefore, Ca\textsuperscript{2+}-activated maximal ATPase activity showed trends that were similar to Ca\textsuperscript{2+}-activated maximal tension.

Divergent effects of α- and β-MHC isoforms on the magnitude of an instantaneous increase in muscle fiber stiffness (E\textsubscript{0}) in RcTnT\textsubscript{1-43}\textsubscript{A} or RcTnT\textsubscript{44-73}\textsubscript{A} reconstituted rat cardiac muscle fibers

To examine whether the effect of RcTnT\textsubscript{1-43} on maximal tension was caused by changes in the number of strongly bound XBs, we measured E\textsubscript{0} by imposing step-like length perturbations in constantly activated muscle fibers (Ford et al., 2010). The amplitude of the ML-induced instantaneous force (see \textsuperscript{F}\textsubscript{1} in Fig. 4 and Fig. S1) increases with the amount of stretch and with the level of Ca\textsuperscript{2+} activation because it is an approximate function of the number of force-bearing XBs. Therefore, a comparison of \textsuperscript{F}\textsubscript{1} in Fig. 4 (A and B) suggests that the number of strongly bound XBs is attenuated more in α-MHC + RcTnT\textsubscript{1-43} fibers. E\textsubscript{0} was estimated as described in Fig. 4 and Fig. S1. Two-way ANOVA revealed a significant interaction effect (P < 0.001), suggesting a divergent impact of α- and β-MHC isoforms on how RcTnT\textsubscript{1-43} or

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Effect of RcTnT\textsubscript{1-43} and RcTnT\textsubscript{44-73} on E\textsubscript{0} in the presence of α- or β-MHC isoform. E\textsubscript{0} was measured at pCa 4.3. Step-like length perturbation protocol was as described previously (Ford et al., 2010). \textsuperscript{F}\textsubscript{1}, \textsuperscript{F}\textsubscript{ss}, and \textsuperscript{F}\textsubscript{nss} are described in the supplemental text. (A) Force responses to a 2% stretch in ML of α-MHC fibers reconstituted with RcTnT\textsubscript{WT} and RcTnT\textsubscript{1-43}. (B) Force responses to a 2% stretch in ML of β-MHC fibers reconstituted with RcTnT\textsubscript{WT} and RcTnT\textsubscript{1-43}. Force traces shown in A and B are the averaged responses measured from at least 11 fibers. \textsuperscript{F}\textsubscript{1} decreases to a greater extent in RcTnT\textsubscript{1-43} + α-MHC fibers. E\textsubscript{0} (the slope of the relationship between \textsuperscript{F}\textsubscript{1} and change in ML) was estimated as described in the supplemental text. (C) E\textsubscript{0} in fibers containing α-MHC. (D) E\textsubscript{0} in fibers containing β-MHC. RcTnT\textsubscript{1-43} decreased E\textsubscript{0} by ~39% in α-MHC fibers, whereas RcTnT\textsubscript{1-43} decreased E\textsubscript{0} by only 19% in β-MHC fibers. Values are reported as mean ± SEM. ***, P < 0.001; **, P < 0.01.
RcTnT<sub>1–43</sub> altered E<sub>0</sub>. Post-hoc multiple comparisons revealed that α- and β-MHC isoforms differentially affected E<sub>0</sub> in RcTnT<sub>1–43</sub> fibers but not in RcTnT<sub>44–73</sub> fibers. E<sub>0</sub> decreased by ~39% in α-MHC + RcTnT<sub>1–43</sub> fibers (Fig. 4 C). On the other hand, E<sub>0</sub> decreased by only 19% in β-MHC + RcTnT<sub>1–43</sub> fibers (Fig. 4 D). Thus, our estimates of E<sub>0</sub> suggest that the attenuation of maximal tension in RcTnT<sub>1–43</sub>-reconstituted fibers is caused by a decrease in the number of force-bearing XBs. Furthermore, multiple comparisons also revealed that the E<sub>0</sub> of β-MHC + RcTnT<sub>1–43</sub> fibers is significantly higher when compared with that of α-MHC + RcTnT<sub>1–43</sub> fibers. As observed for maximal tension, both α- and β-MHC isoforms did not alter E<sub>0</sub> in RcTnT<sub>44–73</sub> fibers (Fig. 4, C and D).

Divergent effects of α- and β-MHC isoforms on myofilament Ca<sup>2+</sup> sensitivity and cooperativity in RcTnT<sub>1–43</sub>- or RcTnT<sub>44–73</sub>-reconstituted rat cardiac muscle fibers

Normalized tension values were plotted against a range of pCa to construct the pCa–tension relations (Fig. 5, A and B). The pCa–tension relation of α-MHC + RcTnT<sub>1–43</sub> was shifted to the right of α-MHC + RcTnT<sub>WT</sub> (Fig. 5 A), indicating a desensitization of myofilament Ca<sup>2+</sup> sensitivity (pCa<sub>50</sub>) in α-MHC + RcTnT<sub>1–43</sub> fibers. This rightward shift of the pCa–tension relation was ablated in β-MHC + RcTnT<sub>1–43</sub> fibers (indicated by arrows in Fig. 5 B), suggesting that β-MHC counteracted the desensitizing effect of RcTnT<sub>1–43</sub> on pCa<sub>50</sub>. Two-way ANOVA revealed a significant interaction effect (P < 0.05) on pCa<sub>50</sub>, suggesting that α- and β-MHC isoforms divergently affected the way RcTnT<sub>1–43</sub> and RcTnT<sub>44–73</sub> altered pCa<sub>50</sub> (Fig. 5, C and D). The main contributing factor for the significant interaction effect on pCa<sub>50</sub> was caused by the divergent effects of α- and β-MHC isoforms on pCa<sub>50</sub> in RcTnT<sub>1–43</sub> versus RcTnT<sub>44–73</sub> fibers.

Comparison of α-MHC + RcTnT<sub>1–43</sub> fibers (Fig. 5 C) with β-MHC + RcTnT<sub>1–43</sub> fibers (Fig. 5 D) shows that β-MHC negated the desensitizing effect of RcTnT<sub>1–43</sub> on pCa<sub>50</sub>. In contrast, RcTnT<sub>44–73</sub> increased pCa<sub>50</sub> in the presence of α- or β-MHC (Fig. 5, C and D).

The Hill equation was fitted to the pCa–tension relationships to estimate the Hill coefficient (n<sub>H</sub>). Two-way ANOVA revealed a significant interaction effect (P < 0.05), suggesting that n<sub>H</sub> was affected differentially in RcTnT<sub>1–43</sub> and RcTnT<sub>44–73</sub> fibers, regardless of the type of MHC isoform present. Post-hoc multiple comparisons showed that RcTnT<sub>1–43</sub> did not affect n<sub>H</sub>, but RcTnT<sub>44–73</sub> decreased n<sub>H</sub> in the presence of α- or β-MHC. In the presence of α-MHC, n<sub>H</sub> values were 3.62 ± 0.17, 4.10 ± 0.31, and 2.18 ± 0.10 for RcTnT<sub>WT</sub>, RcTnT<sub>1–43</sub>, and RcTnT<sub>44–73</sub> fibers, respectively. In the presence of β-MHC, n<sub>H</sub> values were 3.92 ± 0.34, 2.98 ± 0.15, and 2.21 ± 0.08 for RcTnT<sub>WT</sub>, RcTnT<sub>1–43</sub>, and RcTnT<sub>44–73</sub> fibers, respectively.

Divergent effects of α- and β-MHC isoforms on the magnitude of sudden stretch-mediated recruitment of new force-bearing XBs (E<sub>R</sub>) in RcTnT<sub>1–43</sub>- or RcTnT<sub>44–73</sub>-reconstituted rat cardiac muscle fibers

We measured E<sub>R</sub> to determine if the attenuation of thin filament activation in α-MHC + RcTnT<sub>1–43</sub> fibers was caused by a decrease in E<sub>R</sub>. Two-way ANOVA revealed a significant interaction effect (P < 0.05), suggesting a divergent impact of α- and β-MHC isoforms on how RcTnT<sub>1–43</sub> or RcTnT<sub>44–73</sub> altered E<sub>R</sub>. Post-hoc multiple comparisons showed that RcTnT<sub>1–43</sub> and RcTnT<sub>44–73</sub> did not alter E<sub>R</sub> in α-MHC fibers. In the presence of β-MHC, E<sub>R</sub> in α-MHC fibers decreased significantly (P < 0.05); however, in the presence of β-MHC, E<sub>R</sub> in α-MHC fibers decreased significantly (P < 0.05). These results suggest that β-MHC counteracts the desensitizing effect of RcTnT<sub>1–43</sub> on E<sub>R</sub> in α-MHC fibers, whereas α-MHC negates the desensitizing effect of RcTnT<sub>1–43</sub> on E<sub>R</sub> in β-MHC fibers.

**Figure 5.** Effect of RcTnT<sub>1–43</sub> and RcTnT<sub>44–73</sub> on pCa<sub>50</sub> in the presence of α- or β-MHC isoform. Normalized tension values were plotted against a range of pCa to derive the pCa–tension relationships. (A) pCa–tension relationships in fibers containing α-MHC. (B) pCa–tension relationships in fibers containing β-MHC. Arrows in B indicate that a shift to β-MHC counteracts the desensitizing effect of RcTnT<sub>1–43</sub> on myofilament Ca<sup>2+</sup> sensitivity. Note that the curve for RcTnT<sub>1–43</sub> is shifted to the right in A, but the rightward shift is attenuated in B (see arrows). The Hill equation was fitted to the pCa–tension relationships to estimate pCa<sub>50</sub>. (C) Estimates of pCa<sub>50</sub> in fibers containing α-MHC. (D) Estimates of pCa<sub>50</sub> in fibers containing β-MHC. RcTnT<sub>1–43</sub> increased pCa<sub>50</sub> significantly in α-MHC fibers, an effect that was counteracted in β-MHC fibers. Number of determinations was at least seven for each group. Values are reported as mean ± SEM. ***P < 0.001.
comparisons revealed that α- and β-MHC isoforms had differential impact on $E_R$ in RcTnT$_{1-43}$ fibers but not in RcTnT$_{44-73}$ fibers. $E_R$ decreased significantly (~36%) in α-MHC + RcTnT$_{1-43}$ fibers (Fig. 6 A) but not in β-MHC + RcTnT$_{1-43}$ fibers (Fig. 6 B). Furthermore, multiple comparisons also revealed that the $E_R$ of α-MHC + RcTnT$_{1-43}$ fibers is significantly higher when compared with that of α-MHC + RcTnT$_{1-43}$ fibers. Thus, β-MHC counteracted the attenuating effect of RcTnT$_{1-43}$ on $E_R$. On the other hand, both α- and β-MHC isoforms did not alter $E_R$ in RcTnT$_{41-73}$ fibers (Fig. 6, A and B).

Effects of α- and β-MHC isoforms on the rates of XB recruitment and detachment dynamics in RcTnT$_{1-43}^{-}$ or RcTnT$_{44-73}^{-}$ reconstituted rat cardiac muscle fibers

The rate constant of XB recruitment ($b$) was measured by fitting the NLRD model to force responses elicited by step-like ML perturbations (see the supplemental text for details). Two-way ANOVA showed no significant interaction effect but revealed a significant main effect of MHC isoforms on $b$. Post-hoc multiple comparisons demonstrated that $b$ decreased in all groups of α-MHC–containing fibers (Table 1). This is not surprising because β-MHC has an intrinsic ability to slow $b$ (Chandra et al., 2007; Stelzer et al., 2007). Therefore, we sought to determine if the impact of α- and β-MHC on XB detachment kinetics had any effect on how RcTnT$_{1-43}$ and RcTnT$_{44-73}$ modulated contractile dynamics. The rate of XB distortion dynamics ($c$) was measured by fitting the NLRD model to force responses elicited by step-like ML perturbations (Ford et al., 2010). Two-way ANOVA showed no significant interaction effect but revealed a significant main effect of α- and β-MHC on $c$. Post-hoc multiple comparisons showed that $c$ decreased in all groups of β-MHC–containing fibers when compared with the corresponding groups of α-MHC–containing fibers. However, RcTnT$_{1-43}$ and RcTnT$_{44-73}$ did not alter $c$ in α- or β-MHC fibers (Table 1).

**DISCUSSION**

Conclusions drawn from our data have significant implications for the regulation of cardiac thin filament activation. The most important finding from our study is that α- and β-MHC isoforms have a divergent impact

**TABLE 1**

<table>
<thead>
<tr>
<th>Dynamic contractile parameters</th>
<th>RcTnT$_{WT}$</th>
<th>RcTnT$_{1-43}$</th>
<th>RcTnT$_{44-73}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>α-MHC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$b$ (s$^-1$)</td>
<td>18.14 ± 0.62</td>
<td>18.38 ± 1.10</td>
<td>17.46 ± 0.43</td>
</tr>
<tr>
<td>$k_r$ (s$^-1$)</td>
<td>7.52 ± 0.17</td>
<td>7.25 ± 0.31</td>
<td>6.79 ± 0.29</td>
</tr>
<tr>
<td>$c$ (s$^-1$)</td>
<td>21.41 ± 0.83</td>
<td>20.60 ± 1.53</td>
<td>20.14 ± 0.07</td>
</tr>
<tr>
<td><strong>β-MHC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$b$ (s$^-1$)</td>
<td>7.55 ± 0.54</td>
<td>7.84 ± 0.17</td>
<td>7.53 ± 0.22*</td>
</tr>
<tr>
<td>$k_r$ (s$^-1$)</td>
<td>4.15 ± 0.11</td>
<td>4.38 ± 0.08*</td>
<td>4.40 ± 0.11*</td>
</tr>
<tr>
<td>$c$ (s$^-1$)</td>
<td>10.72 ± 0.40</td>
<td>10.70 ± 0.35*</td>
<td>11.68 ± 0.30*</td>
</tr>
</tbody>
</table>

The SL of the muscle fiber was set to 2.3 µm. $b$ and $c$ were estimated as described previously (Ford et al., 2010). $k_r$ was estimated using a rapid slack/restretch protocol (Brenner and Eisenberg, 1986). Number of determinations was at least 11 for each group. Values are reported as mean ± SEM. *P < 0.001 when compared to the corresponding groups in α-MHC fibers.
on how the cardiac-specific N terminus of cTnT modulates cardiac thin filament activation. The magnitude of cardiac thin filament activation depends on how cooperative mechanisms are modulated by the actions of both cTnT and XBs. Thus, how the 1–44 and 45–74 regions of cTnT modulate thin filament activation and desensitize cardiac thin filaments to Ca\(^{2+}\) (Mamidi et al., 2013a) will not only depend on how these two regions by themselves exert their effects on the thin filament but also on how such actions are further modulated by the MHC isoform-mediated effects on the thin filament.

A shift from \(\alpha\) to \(\beta\)-MHC counteracts the attenuating effect of RcTnT\(_{1–43}\) on thin filament activation

The functional significance of the N terminus of cTnT is demonstrated by the findings that deletions in the N terminus of cTnT attenuate thin filament activation by depressing both Ca\(^{2+}\)-activated maximal tension (Chandra et al., 1999; Communal et al., 2002; Sumandea et al., 2009; Mamidi et al., 2013a) and myofilament Ca\(^{2+}\) sensitivity (Mamidi et al., 2013a). Deletions in the N terminus of cTnT have been shown to alter Tn–Tm interactions (Chandra et al., 1999), as well as Tn–Tm interaction with actin (Mamidi et al., 2013a). In view of the observation that cardiac contractile dynamics are tuned by a significant interplay between Tn isoforms and MHC (Chandra et al., 2007), and that \(\alpha\) and \(\beta\)-MHC isoforms differentially modulate thin filament activation (Ford and Chandra, 2013), we sought to determine how the impact of deletions in the N terminus of cTnT is modified by a shift from \(\alpha\) to \(\beta\)-MHC background. An interesting observation in the present study is that the attenuating effect of RcTnT\(_{1–43}\) on Ca\(^{2+}\)-activated maximal tension—observed under an \(\alpha\)-MHC background (Mamidi et al., 2013a)—is suppressed by a shift to \(\beta\)-MHC background (Fig. 3, A and B). Specifically, RcTnT\(_{1–43}\) attenuates maximal tension by \(\sim 46\%\) under an \(\alpha\)-MHC background (Fig. 3 A), whereas a shift to \(\beta\)-MHC background attenuates maximal tension by only \(\sim 18\%\) (Fig. 3 B). Thus, our data demonstrate that RcTnT\(_{1–43}\) attenuates thin filament activation to a greater extent in the presence of \(\alpha\)-MHC.

How does the \(\beta\)-MHC isoform counteract the RcTnT\(_{1–43}\)-mediated attenuation of cardiac thin filament activation? The answer may lie in the innate property of \(\beta\)-MHC to slow XB cycling dynamics (Moss et al., 2004; Rundell et al., 2005; Chandra et al., 2007; Stelzer et al., 2007; Locher et al., 2009). XB cycling dynamics—as estimated by the rate of XB recruitment (\(b\)) and the rate of XB detachment (\(c\))—are significantly slower in fibers containing \(\beta\)-MHC (Table 1 and Fig. 2S). This slowing effect on XB cycling dynamics is independent of the deletion in cTnT because both \(b\) and \(c\) are unaffected by RcTnT\(_{1–43}\) (Table 1). The slower cycling \(\beta\)-MHC may amplify cooperative mechanisms in the thin filament because of its longer XB dwell time in the strongly bound state; such an effect on thin filament cooperativity is expected to cause an increase in the recruitment of additional force-bearing XB in RcTnT\(_{1–43}\)-reconstituted fibers (schematically depicted in Fig. S3). This notion is supported by our \(E_0\) estimates, which demonstrate that the number of force-bearing XB is significantly higher in RcTnT\(_{1–43}\ + \beta\)-MHC fibers when compared with RcTnT\(_{1–43}\ + \alpha\)-MHC fibers (Fig. 4, C and D). Furthermore, the ML-mediated increase in stiffness (\(E_0\)) is significantly higher in RcTnT\(_{1–43}\ + \beta\)-MHC fibers when compared with RcTnT\(_{1–43}\ + \alpha\)-MHC fibers (Fig. 6). Mechanisms other than changes in thin filament cooperativity may also be involved. Recent studies (Coffee Castro-Zena and Root, 2013) show that the myosin lever arm tends to attain the pre–power stroke orientation when bound closer to the Tn complex but assumes the post–power stroke orientation when bound away from the Tn complex, suggesting that Tn is responsible for the formation of myosin-binding target zones along the thin filament. Whether or not charge changes in cTnT (caused by deletions in the N terminus of cTnT in our study) may have influenced the XB formation requires further investigation. Collectively, our data demonstrate that \(\alpha\) and \(\beta\)-MHC isoforms divergently modulate the activity of RcTnT\(_{1–43}\) because of intrinsic differences in their kinetic properties.

A shift from \(\alpha\) to \(\beta\)-MHC ablates the RcTnT\(_{1–43}\)-mediated desensitization of myofilament Ca\(^{2+}\) sensitivity

Another novel finding in this study is that the attenuation of myofilament Ca\(^{2+}\) sensitivity—observed in \(\alpha\)-MHC + RcTnT\(_{1–43}\) fibers (Fig. 5, A and C)—is ablated in \(\beta\)-MHC + RcTnT\(_{1–43}\) fibers (Fig. 5, B and D). These observations clearly demonstrate that \(\alpha\) and \(\beta\)-MHC isoforms have a divergent impact on myofilament Ca\(^{2+}\) sensitivity. Based on our recent studies, the attenuation of myofilament Ca\(^{2+}\) sensitivity by RcTnT\(_{1–43}\) may be explained by the RcTnT\(_{1–43}\)-mediated increase in the rigidity of Tm (Mamidi et al., 2013a). Such an impact on Tm would shift the OFF/ON equilibrium of the regulatory units (RUs; Tm–Tn) more toward the OFF state, thereby attenuating myofilament Ca\(^{2+}\) sensitivity in \(\alpha\)-MHC + RcTnT\(_{1–43}\) fibers. \(\mathrm{pCa}_{50}\) decreased significantly by \(0.13\) pCa units in \(\alpha\)-MHC + RcTnT\(_{1–43}\) fibers (Fig. 5 C) but remained unaffected in \(\beta\)-MHC + RcTnT\(_{1–43}\) fibers (Fig. 5 D), demonstrating that the increasing effect of \(\beta\)-MHC on thin filament cooperativity negated the attenuating effect of RcTnT\(_{1–43}\) on myofilament Ca\(^{2+}\) sensitivity. If this observation is true, we expect \(\beta\)-MHC to further increase myofilament Ca\(^{2+}\) sensitivity in RcTnT\(_{14–73}\) fibers. Indeed, that is exactly what we observe: for example, RcTnT\(_{14–73}\) increases \(\mathrm{pCa}_{50}\) by \(0.22\) pCa units in the presence of \(\alpha\)-MHC and by \(0.29\) pCa units in the presence of \(\beta\)-MHC. Inferences drawn from the above analysis lead us to conclude that
β-MHC affects the transition between OFF/ON states of the RU.

A shift from α- to β-MHC ablates the negative effect of RcTnT1-43α on the sudden stretch–mediated recruitment of new force-bearing XB

The effects mediated by the N terminus of cTnT on the rate of RU ON/OFF transitions may be transduced through a direct effect on the overlapping ends of two contiguous Tm dimers (Mamidi et al., 2013b). Moreover, strong XB themselves affect the balance between RU OFF and ON states through cooperative activation (Fitzsimons and Moss, 1998; Gordon et al., 2000; Razumova et al., 2000; Moss et al., 2004). These observations suggest that some aspect of RU-related cooperativity may modulate the recruitment of XB and, therefore, the length-dependent activation. One way to determine this is by estimating $E_R$ from sudden stretch experiments performed at SL of 2.3 µm. $E_R$ is the slope of the relationship between new steady-state force ($F_{ss}$) and $\Delta L$ (see Fig. 6 and supplemental text). As described previously, $E_R$ is an index of the sensitivity of sudden stretch–mediated recruitment of additional XB into the force-bearing state (Ford et al., 2010). It is interesting to note that $E_R$ was different in α- and β-MHC fibers. Specifically, α- and β-MHC isoforms have a divergent impact on $E_R$ in RcTnT1-43α fibers but not in RcTnT14-73α fibers. Note that both $pG_{30}$ and $E_R$ decrease significantly in α-MHC + RcTnT1-43α fibers (Figs. 5 C and 6 A) but not in β-MHC + RcTnT1-43α fibers (Figs. 5 D and 6 B). Conclusions drawn from this observation suggest that the β-MHC–mediated effect on the thin filament negates the attenuating effect of RcTnT1-43α on $E_R$. Thus, our results demonstrate that the interplay between the N terminus of cTnT and MHC isoform–mediated effects on the thin filament has a divergent impact on mechanisms that modulate both the sudden stretch–mediated XB recruitment and myofilament Ca2+ sensitivity. Collectively, these observations have implications for understanding how cTnT-mediated thin filament activation is modulated in hearts of different species that express either a fast or a slow MHC isoform.

Summary

Novel findings from our study demonstrate that α- and β-MHC isoforms have divergent effects on how the N terminus of cTnT modulates cardiac contractile dynamics. Inferences made from our study also have significant relevance to cardiac health and disease; for example, alterations in MHC and cTnT are known to occur via changes in isoform expression or mutations (Miyata et al., 2000; Wei and Jin, 2011).

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REFERENCES


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Mamidi and Chandra 423