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Expression of tropomyosin- κ induces dilated cardiomyopathy and depresses cardiac myofilament tension by mechanisms involving cross-bridge dependent activation and altered tropomyosin phosphorylation

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Abstract

Tropomyosin-kappa (TPM1- κ) is a newly discovered tropomyosin (TM) isoform that is exclusively expressed in the human heart and generated by an alternative splicing of the α -TM gene. We reported that TPM1- κ expression was increased in the hearts of patients with chronic dilated cardiomyopathy (DCM). To increase our understanding of the significance of this shift in isoform population, we generated transgenic (TG) mice expressing TPM1- κ in the cardiac compartment where TPM1- κ replaces 90% of the native TM. We previously showed that there was a significant inhibition of the ability of strongly bound cross-bridges to induce activation of TG myofilaments. Here, we compared the force- Ca^{2+} relations in detergent extracted (skinned) fiber bundles isolated from non-transgenic (NTG) and TG-TPM1- κ hearts at two sarcomere lengths (SLs). Our data demonstrated a significant decrease in the Ca^{2+} sensitivity of the myofilaments from TG-TPM1- κ hearts with no change in the maximum developed tension, length-

dependent activation, and the ratio of ATPase rate to tension. There was also no difference in the affinity and cooperativity of Ca^{2+} -binding to troponin in thin filaments reconstituted with either TPM1- κ or α -TM. We also compared protein phosphorylation in NTG and TG-TPM1- κ myofilaments. There was a decrease in the total phosphorylation of TPM1- κ compared to α -TM, but no significant change in other major sarcomeric proteins. Our results identify a novel mode of myofilament desensitization to Ca^{2+} associated with a DCM linked switch in TM isoform population.

Keywords

Calcium sensitivity; Tension cost; Thin filament regulation; ATPase; Sarcomeric proteins

Introduction

In experiments reported here we have compared mechanisms by which myofilaments are regulated by α -tropomyosin (α -TM) and a novel isoform designated as TPM1- κ (Denz et al. 2004). Expression of the TPM1- κ isoform results from alternative splicing of the α -TM gene, in which the striated muscle exon 2b is replaced with the smooth muscle exon 2a. We previously demonstrated that TPM1- κ is normally expressed at the protein level in the human heart (3–5% of the total TM isoform population) and that its expression is approximately doubled in patients with DCM (Rajan et al. 2010). We also reported that TG mice expressing TPM1- κ in the heart (~90% of TM population) developed a DCM phenotype with altered cardiac dynamics characterized by a slowing of relaxation kinetics.

Detailed mechanisms of how TPM1- κ affects myofilament function is critical for the design of therapies for both familial and acquired disorders of the heart. In our studies (Rajan et al. 2010) comparing myofilaments prepared from TG-TPM1- κ and NTG controls, a significant finding was a decreased Ca^{2+} sensitivity of tension development. Moreover, preliminary investigation of the mechanism for this depression revealed a decreased ability of strong cross-bridges in the form of *N*-ethyl maleimide modified myosin heads (NEM-S1) to activate the thin filament. This finding demonstrated a unique and poorly understood mechanism linking sarcomeric dysfunction to DCM (see editorial comment, VanBuren and Palmer 2010). Near neighbor interactions between thin filament functional units (actin–TM–Tn in a 7:1:1 ratio) forms a prominent theory related to the common demonstration that the steady-state tension–pCa relation in heart myofilaments is steeper than predicted by a non-cooperative binding isotherm expected from control of tension by a single regulatory binding site on cardiac troponin (cTn) C. A second mechanism is a cooperative promotion of cTnC Ca^{2+} binding by interactions of cross-bridges within a functional unit. In both mechanisms there is a communication among proteins between or within a functional unit, where TM is strategically located and possesses end to end interactions for communication along the thin filament.

In order to further explore possible mechanisms for the alterations induced by expression of TPM1- κ , we extended our studies of myofilaments regulated by TPM1- κ . We tested for differences in alterations in cross-bridge dynamics, length dependent activation (LDA), and sarcomeric protein phosphorylation. Our data indicate that TPM1- κ decreases Ca^{2+} sensitivity of the myofilaments through a mechanism that does not involve alterations in LDA, in the affinity and cooperativity of Ca^{2+} binding to cTnC, and in the tension cost (ratio of ATP hydrolysis to force generation). We do however demonstrate that TPM1- κ phosphorylation is significantly reduced compared to α -TM, which suggests a role of the altered TPM1- κ phosphorylation in the mechanical defects observed in TG hearts. We conclude that the increased levels of TPM1- κ in the TG hearts induce a unique modification

in thin filament activation that is dominated by modification of cross-bridge dependent activation potentially modified by the state of phosphorylation.

Experimental procedures

The experiments were carried out according to guidelines instituted by the Animal Care and Use Committee at the University of Illinois at Chicago. Animals were housed at the University of Illinois Biological Resources Laboratory, and the protocols were approved by the Animal Care and Use Committee of the University of Illinois.

Protein purification of TM and cTn complex reconstitution

Mouse α -TM and TPM1- κ cDNA constructs were cloned into a pET-3d vector and designed to include an N-terminal alanine-serine (AS) dipeptide to mimic acetylation of endogenously expressed TM (Monteiro et al. 1994). We expressed and purified TM as previously described by Monteiro et al. (1994) with minor modifications. Recombinant mouse cTnC and cTnI were expressed and purified as described previously (Kobayashi et al. 1999). Recombinant mouse cTnT was expressed and purified as described previously (Sumandea et al. 2003). The reconstitution of the cTn complex was accomplished by mixing equimolar amounts of the cTn components followed by sequential dialysis to remove urea and decrease the salt concentration. The cTn complex was purified using a Resource-Q column (1 ml, Amersham Biosciences) (Sumandea et al. 2003).

Measurements of Ca^{2+} -dependent activation of force and ATPase activity

Fiber bundles, approximately 250 μm in diameter and 2–4 mm in length, were dissected from papillary muscles of NTG and TG-TPM1- κ hearts and were detergent-extracted (skinned) in a high relaxing buffer (HR) containing 1% Triton X-100. HR buffer contained 10 mM EGTA, 41.89 mM K-Prop, 100 mM BES, 6.75 mM MgCl_2 , 6.22 mM Na_2ATP , 10 mM Na_2CrP , 5 mM NaN_3 . The following protease inhibitors were added to all solutions: pepstatin A (2.5 $\mu\text{g}/\text{ml}$), leupeptin (1 $\mu\text{g}/\text{ml}$), and phenylmethylsulfonyl fluoride (PMSF) (50 $\mu\text{mol}/\text{l}$). Solutions with varying pCa ($-\log [\text{Ca}^{2+}]$) values were prepared by mixing HR with HR at pCa 4.5. The concentration of salts, buffers, and ATP in solution to achieve desired pCa values and a 5 mM MgATP concentration were computed using a computer program (Fabiato 1988). The skinned fiber bundles were mounted between a force transducer and micro-manipulator with cellulose-acetate glue. The sarcomere length (SL) was initially set at 1.9 μm using laser diffraction. The myofilaments were incubated in HR then were subjected to sequential solutions (pCa 8–pCa 4.5) and isometric tension was recorded on a chart recorder (Wolska et al. 1999). At the end of the experiment, SL was increased to 2.3 μm and another round of pCa activation was performed. All experiments were carried out at 22°C. In a second series of experiments we carried out simultaneous measurements of steady-state isometric tension and Mg-ATPase activity in skinned fiber bundles as a function of pCa as previously reported (de Tombe and Stienen 1995, 2007). The SL was adjusted to 2.2 μm using laser diffraction. All experiments were done at 20°C. In all experiments only fiber bundles retaining more than 80% of their initial maximum tension were included in the analysis.

Ca^{2+} binding measurements

We determined Ca^{2+} binding properties of the regulatory site of TnC in reconstituted thin filaments by employing iodoacetic acid derivative of 2-(4'-iodoacetamido)anilinonaphthalene-6-sulfonic acid (IAANS) as previously described (Kobayashi and Solaro 2006). Ca-binding was reported by changes in fluorescence emission intensity of the IAANS probe covalently attached to cysteine (Cys)-53 in a single Cys mutant cTnC (C35S/T53C/C84S) as described by Davis et al. (2007). The ratio of actin/

tropomyosin/troponin was 5:2:1. The solutions conditions were 100 mM NaCl, 5 mM MgCl₂, 20 mM MOPS, 2 mM EGTA at pH 7.0, 25°C. pCa values were calculated with WEBMAXC program (Patton et al. 2004).

Sarcomeric protein phosphorylation

Determination of sarcomeric protein phosphorylation was carried out as described previously (Warren et al. 2008). We labeled 50 µg of total protein from NTG and TG-TPM1-κ samples randomly with 100 pmol of either Cy3 or Cy5. In addition, an internal standard was also included in the two dimensional difference in gel electrophoresis (2D-DIGE) experiments, which consisted of mixing all the samples (NTG and TG) equally prior to labeling. Then, the mixed internal standard was labeled with Cy2 at the same ratio of 50 µg of protein to 100 pmol of dye. The inclusion of internal standard proteins as a reference reduces variability from gel to gel allowing for more accurate measurements. The labeling reaction was done at room temperature for 2 h in the dark and then quenched by adding 0.2 µl of 10 mM lysine. NTG control, TG-TPM1-κ, and internal standard samples were adjusted to 15 µg each and then added to IEF buffer. The protein mixture was added to an 18 cm IPG strip (GE Healthcare), pH 4–7 or 7–11 NL, and re-hydrated for 12–16 h in the Bio-Rad Protean IEF cell using a linear method (250 V rapid ramp for 15 min; 10,000 V slow ramp for 2 h; 10,000 V rapid ramp for 45,000 Vh). After equilibration (Warren et al. 2008), the IPG strips were placed on top of the 12% SDS-PAGE gel and run at 25 mA/gel until the dye reached the bottom of the gel in a Hoefer SE600 unit. Gels were then scanned on a Typhoon 9410 Imager (GE Healthcare) with Cy3 (excitation: 532 nm, emission: 580 nm BP 30), Cy5 (excitation: 633 nm, emission: 670 nm BP30), and Cy2 (excitation: 488 nm, emission: 520 BP 40) parameters. The 2D-DIGE images were analyzed by PDQuest version 8.0 advanced.

Data processing and statistical analysis

The relation between tension and IAANS fluorescence change with pCa were fit to the Hill equation using Prism software (GraphPad Ver.4). Tension cost was determined by linear fit to the tension-ATPase data. Statistical analyses were performed by applying Student *t*-test. *P* < 0.05 was considered statistically significant. Data are presented as means ± SEM.

Results

TPM1-κ depresses myofilament sub-maximum tension development independent of LDA

Previous studies of effects of mutation in α-TM linked to DCM demonstrated an alteration in the Ca²⁺-dependence of tension generation on SL. In view of the possible mechanism of LDA involving thin filament cooperative activation and its significance as the basis for the Frank–Starling relation, we compared the pCa–tension relation in skinned fiber bundles from NTG and TPM1-κ hearts at two SLs. Our data demonstrated that at SL = 1.9 µm, there was a significant decrease in the Ca-sensitivity of tension development for myofilaments from TPM1-κ hearts compared to NTG controls (Fig. 1). There was no difference in maximum tension induced by TPM1-κ expression. However, there was a significant increase in Hill coefficient in the TG hearts indicative of an increase in the cooperativity. A similar desensitization to Ca²⁺ was obtained when SL was increased to 2.3 µm (Fig. 1). The shift in pCa₅₀ (half maximally activating pCa) when SL was increased from 1.9 to 2.3 µm was same for myofilaments controlled by α-TM and by the TPM1-κ isoform. Data obtained in our studies of LDA are summarized in Table 1.

TPM1- κ induces a proportional depression of tension development and rate of ATP hydrolysis without affecting the ratio of ATPase to tension

In order to assess whether the presence of TPM1- κ affected cross-bridge kinetics, we measured tension development and simultaneously the rate of ATP hydrolysis at SL = 2.2 μm . Figure 2a compares the force-pCa relation in the skinned fiber bundles from NTG and TG-TPM1- κ hearts. There were no differences in maximum tension or Hill coefficient between both groups. However, as expected from the previous studies compared to the controls, there was a rightward shift of the pCa-tension relation of fiber bundles from TG-TPM1- κ hearts indicating a decrease in Ca^{2+} sensitivity. Our measurement of ATPase rate during the development of steady-state tension showed the same changes as determined for tension (Fig. 2b). ATPase and tension data are summarized in Table 2. This proportional change in ATPase rate as tension increased is emphasized in Fig. 2c, which illustrates a plot of the dependence of ATPase rate on tension (Wannenburg et al. 1997). The slope of this relation (tension cost) was the same in fiber bundles isolated from NTG and TG-TPM1- κ hearts. One interpretation of the lack of effect of TPM1- κ on tension cost is that there was no effect on the cross-bridge detachment rate (Wannenburg et al. 1997).

The affinity of thin filament cTnC and the cooperativity of Ca^{2+} binding are unaffected by TPM1- κ

In order to determine whether the observed decrease in Ca^{2+} sensitivity of the TG-TPM1- κ myofilaments is due to a change in Ca^{2+} affinity of TnC induced by the switching of TPM1- κ for α -TM, we employed reconstituted thin filaments with cTn containing the IAANS-labeled mutant cTnC (C35S/T53C/C84S) as previously described (Kobayashi and Solaro 2006) and outlined in the methods section. Results of measurements of Ca^{2+} binding to the regulatory site of TnC in thin filaments are shown in Fig. 3. Controls, reconstituted with α -TM, and thin filaments reconstituted with TPM1- κ showed the same TnC Ca-binding affinity as indicated by the pCa₅₀ values (Table 3). In both cases the Ca^{2+} -binding was cooperative with Hill coefficients in excess of 1.0, but there was no significant difference in Hill coefficient (n_H) between the groups. These results indicate that although myofilaments regulated by TPM1- κ have reduced sensitivity to Ca^{2+} there is no associated alteration in Ca^{2+} binding to the regulatory binding site of TnC.

TPM1- κ is relatively dephosphorylated in TG hearts compared to NTG Controls

Our previous studies revealed a significant change in phosphorylation of the mutant α -TM-E54K that we hypothesized is functionally significant in the progression to DCM. We therefore employed the same 2D-DIGE approach here in order to determine the effect of TPM1- κ on sarcomeric protein phosphorylation. 2D-DIGE provides a method to separate and compare proteins from different samples on the same gel, which limits variability between gels. Quantification of phosphorylated TM is shown in Fig. 4. Our results show that TPM1- κ phosphorylation in the TG hearts was significantly decreased compared to that of α -TM in NTG ones (TPM1- κ : 24.1 ± 2.9 ; α -TM: 37.5 ± 4.2 ; $P = 0.02$) (Fig. 4b). This change in TPM1- κ phosphorylation was also significant when compared to phosphorylation of endogenous α -TM in TG-TPM1- κ hearts (TPM1- κ : 24.1 ± 2.9 ; endogenous α -TM: 35.7 ± 3.5 ; $P = 0.02$) (Fig. 4c). As a result, the total TM phosphorylation in TG hearts was significantly lower compared to that in NTG ones (TPM1- κ + α -TM: 25.5 ± 2.5 ; α -TM: 37.5 ± 4.2 ; $P = 0.03$). These results indicate the presence of structural alterations that may affect TPM1- κ as a substrate for kinases and/or phosphatases. There may also be secondary changes that induce alterations in the activity of the kinases and phosphatases in association with the transgenesis.

Discussion

The major finding of experiments reported here is the identification of a novel mode of myofilament desensitization to Ca^{2+} associated with a disease promoting modification in TM isoform population. The mechanism for this desensitization appears to involve an inability of TPM1- κ to engage in the process of cooperative activation of thin filaments. Moreover, our evidence indicates that a specific reduction in TM phosphorylation contributes to the loss of communication among the thin filament proteins required for cooperative activation. In previous studies (Rajan et al. 2010), we probed this communication by treating skinned fiber bundles with NEM-S1 and measuring the ability of these strongly bound cross-bridges to recruit endogenous tension generating cross-bridge–actin interactions. Compared to controls, promotion of tension development by NEM-S1 binding in low Ca^{2+} was significantly depressed in myofilaments from TG-TPM1- κ hearts. There was no effect of the strongly bound cross-bridges at saturating levels of Ca^{2+} inasmuch as the titration of actin sites with NEM-S1 is insufficient to compete with endogenous force generating cross-bridges and the cooperative process is fully developed at maximum activation.

Our interpretation of the DCM linked significance of reduced TM phosphorylation fits with data from previous experiments investigating its functional significance. Several reports have demonstrated the role of phosphorylation in modulating TM function. Phosphorylation strengthened the head-to-tail overlap of adjacent TMs as well as their interaction with cTn (Heeley et al. 1989). In addition, phosphorylated α -TM also increased the rate of ATP hydrolysis in reconstituted thin filaments (Heeley et al. 1989; Lehrer et al. 1997). Furthermore, Rao et al. (2009) employed the in vitromotility assay and a laser trap to investigate a role for TM phosphorylation in cooperative activation of thin filaments. Their data indicated that compared to de-phosphorylated TM, natively phosphorylated α -TM induced cooperative activation presumably by affecting end to end interactions between near neighbors. It is also relevant to our findings here that myofilaments from neonatal hearts with higher levels of α -TM phosphorylation than their adult counterparts (Yuan et al. 2008) are significantly more sensitive to Ca^{2+} (Solaro et al. 1988). Thus, the decrease in phosphorylation reported here in TPM1- κ would be expected to reduce myofilament Ca^{2+} sensitivity (Figs. 1, 2) and the ability of NEM-S1 to activate thin filaments as we reported in Rajan et al. 2010. There may also be an effect of altered TPM1- κ phosphorylation on TnC Ca^{2+} -affinity, which we did not determine in experiments with preparations reconstituted with recombinant proteins. Moreover, previous studies (Warren et al. 2008) from our laboratory reported a specific decrease in TM phosphorylation in myofilaments harboring the DCM-linked α -TM E54K mutation. Compared to controls there was also a decrease in Ca^{2+} sensitivity of α -TM E54K myofilaments (Rajan et al. 2007). We (Vahebi et al. 2007) also reported a correlation of decreased Ca^{2+} responsiveness of force and ATPase with decreased α -TM phosphorylation in myofilaments from TG mice expressing an upstream activator (MKK6bE) of the mitogen activated protein kinase (p38-MAPK). Thus, based on these results, the depressed Ca^{2+} sensitivity and rate of ATP hydrolysis observed in our TG mice (Figs. 1, 2) could be due, in part, to the decreased TPM1- κ phosphorylation.

The decrease in Ca^{2+} -sensitivity of myofilaments from hearts with isoform switching to TPM1- κ also occurs generally in hearts with mutations in α -TM linked to DCM as demonstrated in three DCM-linked mutations (Olson et al. 2001; Lakdawala et al. 2010). However, there are some significant differences indicative of a unique mechanism of desensitization induced by TPM1- κ . For example, in contrast to TPM1- κ , myofilaments from hearts of TG- α -TM-E54K mice develop less maximum tension than NTG controls. Moreover, whereas thin filaments controlled by TPM1- κ bind Ca^{2+} with the same affinity and cooperativity as those controlled by α -TM, thin filaments controlled by α -TM-E40K

show a decrease and those by α -TM-E54K show an increase of cTnC affinity for Ca^{2+} (Robinson et al. 2007). These changes in cTnC affinity to Ca^{2+} may arise from differences in the effects of the E54K mutation and the amino acid substitutions in TPM1- κ (Denz et al. 2004) on TM structure. Data from circular dichroism analysis indicate that TPM1- κ is more stable at physiological and higher temperatures (Rajan et al. 2010) than α -TM harboring the E54K mutation (Rajan et al. 2007). Whatever the case, these effects that are unique to each mutation indicate potentially significant differences in therapeutic targets among these sarcomeric alterations. The special nature of these alterations is pointed up by data in which isoform switching from α -TM to the γ -TM isoform induced a decrease in myofilament Ca^{2+} -sensitivity but unlike the case with a switch to TPM1- κ , there was induction of a hyperdynamic state of the heart accompanied by a decrease in length-dependent Ca^{2+} activation of the myofilaments (Pieples et al. 2002). Importantly, isoform switching to γ -TM did not induce any morphological alterations in the heart (Muthuchamy et al. 1995; Pieples et al. 2002).

In addition to the decrease in the Ca^{2+} sensitivity of myofilament tension, Ca^{2+} -dependent acto-myosin ATPase activity was also depressed in hearts expressing TPM1- κ (Fig. 2; Table 2). It is well established that the slope of the tension–ATPase relation corresponds to tension cost and also reflects the rate of cross-bridge detachment (g) from the myofilaments (Wannenburg et al. 1997). Data in Table 2 show that the tension cost of force development was not affected by TPM1- κ expression indicating that the new TM isoform does not alter the acto-myosin interaction dynamics. This result indicates that the depression in cardiac function (Rajan et al. 2010), is most likely not associated with a decrease in cross-bridge kinetics.

Although the change in pCa_{50} for SL dependence of activation was the same for controls and TPM1- κ myofilaments (Fig. 1; Table 1), there was a significant increase in the Hill n value especially at SL 1.9 μm . Compared to myofilaments at SL 2.3 μm (Fig. 1; Table 1) or SL 2.2 μm (Fig. 2; Table 2), at the shorter SL there was a depression in Ca^{2+} sensitivity not only due to LDA but also to the presence of TPM1- κ . Our interpretation of the mechanism for this relatively steep dependence of force on pCa in the case of myofilaments at SL 1.9 μm regulated by TPM1- κ , follows the ideas of Millar and Homsher (1990), who reported a similar increase in steepness of force– pCa relations under conditions, albeit different from ours, that also reduced Ca^{2+} sensitivity by modifying strong cross-bridge interactions. Thus, in the presence of TPM1- κ the number of strong force generating cross-bridges is low as is the communication between thin filament units. It follows that the number of active thin filament units is also relatively low. With no change in Ca^{2+} binding by the thin filaments (Fig. 3; Table 3), thin filament activation is limited by the binding of strong force generating cross-bridges, which is a cooperative process (Trybus and Taylor 1980) expected to induce an increase in Hill n . This result sheds light on current theories of cooperative activation of the thin filaments, discussed by Sun and Irving (2010). One theory emphasizes a role for strong cross-bridges in activating the thin filaments, whereas the other emphasizes a major role for cooperative Ca^{2+} binding. Here, we found no effect TPM1- κ on cooperative Ca^{2+} binding, yet there was a change in cooperative activation, which we attribute to a cross-bridge dependent mechanism. Again these data are of significance with regard to therapeutic approaches. For example, cardiotonic agents that affect the dwell time of strong cross-bridges are in development (Solaro 2009), and may be well suited in a condition in which there is a disturbance of the ability of strong cross-bridges to activate thin filaments.

In contrast with DCM, familial hypertrophic cardiomyopathy (FHC) is characterized with an increased Ca^{2+} sensitivity of the myofilaments. This defect seems to play a critical role in the development of a lethal phenotype. Previous studies done on a mouse model of FHC were successful in rescuing these mice by normalizing myofilaments Ca^{2+} sensitivity

through the co-expression of a chimeric TM isoform that was shown to desensitize the myofilaments to Ca^{2+} (Jagatheesan et al. 2007). TPM1- κ is a naturally occurring protein in the human heart (Denz et al. 2004; Rajan et al. 2010) and is expressed in equal amounts as α -TM at the mRNA but not at the protein level (Rajan et al. 2010). Thus, modulation of TPM1- κ protein expression in patients with FHC could be an efficient therapeutic approach for the treatment of this cardiovascular disease and a means to improve cardiac function.

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Fig. 1. pCa-tension relationship of skinned fiber preparations from NTG and TG-TPM1- κ hearts. TPM1- κ induced a significant decrease in the Ca^{2+} -sensitivity of the myofilaments at SLs 1.9 and 2.3 μm . Data are presented as means $\pm$ the SEM. In some cases the SEM values are smaller than the symbol size ($N = 15-17$)

Fig. 2.

Myofilament Ca^{2+} sensitivity and chemo-mechanical transduction in skinned fiber bundles isolated from NTG and TG-TPM1- κ hearts. **a** Steady state isometric tension, **b** actomyosin MgATPase activity, were measured simultaneously using an enzyme-coupled assay and plotted against pCa. **c** Tension-dependent ATPase activity relationship. Tension cost was determined as the slope of the relationship between tension and ATPase activity. Data are presented as means $\pm$ the SEM. The SEM in data reported in Figs. 2a and b are smaller than the symbol size ($N = 9-12$)

Fig. 3. Ca^{2+} binding to reconstituted thin filaments with either α -TM or TPM1- κ . Relative fluorescence emission intensity changes of IAANS attached to Cys-53 of cTnC is plotted as function of pCa. TPM1- κ had no effect on neither Ca^{2+} binding affinity to the thin filament nor the Hill coefficient ($N = 4$)

Fig. 4.

TM phosphorylation. **a** Representative 2D-DIGE image showing the separation of α -TM (red) and TPM1- κ (green) from NTG and TG-TPM1- κ hearts, respectively. Only the region of interest on the gel is shown. **b** Quantification of α -TM and TPM1- κ phosphorylation in NTG and TG hearts, respectively. **c** Quantification of native α -TM and TPM1- κ phosphorylation in TG hearts. **d** Quantification of total TM phosphorylation in NTG (α -TM only) and TG-TPM1- κ (native α -TM and expressed TPM1- κ) hearts. In all cases, TPM1- κ phosphorylation was significantly less than that of α -TM. Data are presented as means $\pm$ the SEM. In some cases the SEM are smaller than the symbol size ($N = 8$). $*P \leq 0.05$

Table 1Mechanical characteristics of left ventricular papillary fiber bundles from NTG and TG-TPM1- κ hearts

	NTG	TG-TPM1- κ
SL = 1.9 μm		
pCa ₅₀	5.87 $\pm$ 0.01 **	5.73 $\pm$ 0.01 *, **
F_{max} (mN/mm ²)	28.99 $\pm$ 0.5 **	28.93 $\pm$ 0.48 **
Hill	3.92 $\pm$ 0.17 **	5.15 $\pm$ 0.19 *, **
SL = 2.3 μm		
pCa ₅₀	5.98 $\pm$ 0.01	5.82 $\pm$ 0.02 *
F_{max} (mN/mm ²)	32.14 $\pm$ 0.77	32.57 $\pm$ 0.98
Hill	3.5 $\pm$ 0.14	3.94 $\pm$ 0.11 *
ΔpCa_{50}	0.11	0.09

Measurements were made on 15–17 fibers from 6 to 9 different hearts

* $P < 0.05$ when compared to NTG** $P < 0.05$ between SL 1.9 and 2.3 μm in both groups

Table 2

Mechanical characteristics of skinned left ventricular papillary fiber bundles from NTG and TG-TPM1- κ hearts

	NTG	TG-TPM1- κ
Tension		
pCa ₅₀	5.82 ± 0.01	5.6 ± 0.01 *
$F_{\max}$ (mN/mm ²)	31.86 ± 0.98	32.56 ± 0.76
Hill	4.08 ± 0.29	3.51 ± 0.12
ATPase		
pCa ₅₀	5.88 ± 0.01	5.65 ± 0.01 *
ATPase max (pmole/mm ³ /s)	296.4 ± 9.61	298.01 ± 9.61
Hill n	4.1 ± 0.33	3.62 ± 0.11
Tension cost (ATPase/tension)	10.37 ± 0.28	10.15 ± 0.31

Measurements were done on 9–12 fibers from 6 to 9 different hearts

* $P < 0.05$ when compared to NTG

Table 3

Summary of the effect of TM isoforms on Ca^{2+} binding to the reconstituted thin filaments

TM isoform	pCa_{50}	n_{H}
α -TM	5.53 ± 0.02	2.10 ± 0.13
TPM1- κ	5.51 ± 0.02	1.87 ± 0.04

Measurements were done on four preparations in each group