Length dependence of Ca^{2+} sensitivity of tension in mouse cardiac myocytes expressing skeletal troponin C

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1. Beat-to-beat performance of myocardium is highly dependent on sarcomere length. The physiological basis for this effect is not well understood but presumably includes alterations in the extent of overlap between thick and thin filaments. Sarcomere length dependence of activation also appears to be involved since length–tension relationships in cardiac muscle are usually steeper than those in skeletal muscle.

2. An explanation recently proposed to account for the difference between length–tension relationships is that the cardiac isoform of troponin C (cTnC) has intrinsic properties that confer greater length-dependent changes in the Ca^{2+} sensitivity of tension than does skeletal troponin C (sTnC), presumably due to greater length-dependent changes in the Ca^{2+}-binding affinity of cTnC. To test this hypothesis, transgenic mice were developed in which fast sTnC was expressed ectopically in the heart. This allowed a comparison of the length dependence of the Ca^{2+} sensitivity of tension between myocytes having thin filaments that contained either endogenous cTnC or primarily sTnC.

3. In myocytes from both transgenic and normal mice, the Ca^{2+} sensitivity of tension increased similarly when mean sarcomere length was increased from approximately 1·83 to 2·23 μm. In both cases, the mid-point (pCa_{50}) of the tension–pCa (i.e. $-\log[Ca^{2+}]$) relationship shifted 0·12 $\pm$ 0·01 pCa units (mean $\pm$ S.E.M.) in the direction of lower Ca^{2+}.

4. We conclude that the Ca^{2+} sensitivity of tension in myocytes changes as a function of sarcomere length but is independent of the isoform of troponin C present in the thin filaments.

According to the Frank–Starling relationship, cardiac output is highly dependent on the end-diastolic volume of the ventricle. The physiological basis for this effect is thought to involve, at least in part, well-characterized variations in isometric tension with sarcomere length. Sarcomere length–tension relationships for lengths up to the optimum for tension development (i.e. the ascending limb) are much steeper in intact cardiac muscle than in skeletal muscle fibres (Gordon, Huxley & Julian, 1966; ter Keurs, Rinsburger, van Hueningen & Nagelsmit, 1980). However, since cardiac muscle cannot be tetanized under physiological conditions, comparisons of length–tension relationships between cardiac and skeletal muscles should be considered on the basis of twitch contractions in living muscles or steady activations of skinned muscle preparations. Length–tension relationships obtained from maximally activated preparations of skinned cardiac trabeculae, single cardiac myocytes and mammalian skeletal muscle fibres (Fabiato & Fabiato, 1975; Kentish, ter Keurs, Ricciardi, Bucx & Noble, 1986; Allen & Moss, 1987) were all similar, although cardiac myocytes exhibited less steep length–tension relationships below ~1·7 μm.

Length–tension relationships in both skinned skeletal and cardiac muscle are altered considerably when obtained at submaximal Ca^{2+} concentrations. At low Ca^{2+} concentrations, the sarcomere length for optimal tension is increased to lengths that lay on the descending limb of relationships obtained during maximal activation. In addition, the Ca^{2+} sensitivity of tension increases as sarcomere length is increased, since increases in sarcomere length shift the pCa_{50} (i.e. $-\log[Ca^{2+}]$ at which tension is half-maximal) to higher pCa (lower free Ca^{2+}) in both skeletal and cardiac muscle (Hibberd & Jewell, 1982; Stephenson & Williams, 1982; Allen

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& Moss, 1987; Martyn & Gordon, 1988). Length–tension relationships obtained at a given submaximal Ca^{2+} concentration appear to be steeper in skinned cardiac muscle (Allen & Kentish, 1985) than in skinned skeletal muscle (Allen & Moss, 1987). Consistent with this observation, the length dependence of the Ca^{2+} sensitivity of tension was reported to be twofold greater in skinned cardiac trabeculae than in fast skeletal muscle fibres (Gulati, Sonnenblick & Babu, 1990). A molecular mechanism proposed to explain this difference is that the cardiac isoform of troponin C (cTnC) may have intrinsic properties that result in greater length- dependent changes in the Ca^{2+} sensitivity of tension than does skeletal troponin C (sTnC) (Babu, Sonnenblick & Gulati, 1988). This idea was based on the finding that replacement of endogenous cTnC with sTnC in skinned cardiac muscle reduced the length-dependent shift of the tension–pCa relationship. Moss, Nwoye & Greaser (1991) later published results suggesting that cTnC is not the sole mediator of the greater length dependence of Ca^{2+} sensitivity. They found that complete replacement of sTnC with cTnC in skinned psoas fibres resulted in length-dependent changes in Ca^{2+} sensitivity, similar to those of control fibres containing sTnC. However, the possibility remains that interactions of cTnC with cardiac thin filament proteins is essential for the greater length dependence of the Ca^{2+} sensitivity of tension. To test this idea, transgenic mice were developed which ectopically expressed the fast skeletal isoform of TnC in the heart. This provided a means to examine the length dependence of Ca^{2+} sensitivity in single cardiac myocytes having thin filaments that contained either endogenous cTnC or ectopically expressed sTnC.

METHODS

Transgenic animals

Three independent lines of transgenic mice were produced using a cardiac-specific expression vector (pMHCsTnC). The expression vector was constructed using a 4 kb murine genomic sTnC fragment containing all six exons of the sTnC gene (Parmacek, Bengur, Vora & Leiden, 1990) under the transcriptional regulation of a 650 bp cardiac-specific α-MHC promoter fragment (MHC Pr) (Field, 1988), which was cloned into an *Eco*RI/*Hind* III-digested pUC19. One of the three transgenic lines ectopically expressed high levels of the fast skeletal isoform of TnC in the heart and therefore was used for all experiments. Transgenic mice did not show any deleterious effects of expression of sTnC in the heart. Both transgenic mice and control littermates were housed and cared for under the supervision of a veterinarian, according to the guidelines of the institutional animal care and use committee.

The expression of sTnC in transgenic and control animals was determined using Northern blot analysis as previously described in detail (Parmacek & Leiden, 1989; Parmacek, Bengur, Vora & Leiden, 1990). RNA was prepared from non-transgenic littermates (control) or transgenic mouse organs using lithium chloride–urea. Ten micrograms of whole RNA from organs of control littermates or transgenic organs were equalized by ethidium bromide staining on non-denaturing gels, and were then subjected to Northern blot analysis. The probes used were a 501 bp murine skeletal troponin C cDNA and the 504 bp murine cardiac troponin C cDNA. All probes were radiolabelled with ^{32}P using random hexanucleotide priming. The detailed molecular characterization of this transgenic line has been reported previously (Metzger, Parmacek, Barr, Field & Leiden, 1993). Briefly, Northern blot analysis demonstrated high levels of sTnC mRNA in both heart and skeletal muscle from the transgenic animals. In contrast, control littermates expressed sTnC only in skeletal muscle. As expected, neither transgenic nor control animals expressed sTnC mRNA in the liver. Hearts from both transgenic and control animals expressed equivalent levels of cTnC mRNA, indicating that expression of the sTnC transgene did not appear to influence the expression of the endogenous cTnC gene.

The expression of the transgene protein was examined by Western blot analysis using a sTnC-specific polyclonal rabbit antiserum. This antiserum recognized recombinant sTnC but failed to cross-react with recombinant cTnC (Metzger *et al.* 1993). Equal amounts of crude organ homogenates were blotted and probed with the sTnC-specific rabbit antiserum and peroxidase-conjugated goat anti-rabbit second antibody using a commercially available kit (Amersham International, UK). Western blots of tissue homogenates from transgenic and control animals using an sTnC-specific antiserum demonstrated high levels of sTnC protein in the hearts of transgenic, but not control animals. As expected, sTnC was expressed in skeletal muscle but not in the liver of both control and transgenic animals.

Preparation

Hearts were excised from either transgenic mice or control littermates, killed by decapitation. The hearts were placed in ice-cold relaxing solution, and minced to yield 2–3 mm pieces. These pieces were placed in 12 ml relaxing solution and were further disrupted for 20 s using a polytron homogenizer (Kinematica, Luzern, Switzerland). The resulting suspension of cells and cell fragments was centrifuged for 15 s at 20 *g* and then at 165 *g* for an additional 45 s to remove debris. The cells were subsequently skinned by suspending the pellet in 0·3% ultrapure Triton X-100 (Pierce Chemical Co., Rockford, IL, USA) for 4 min. The skinning process also disrupted any remaining sarcoplasmic reticulum and improved the contrast of the striation pattern observed by light microscopy. The pellet was then washed twice with relaxing solution at 4 °C. The remaining pellet was resuspended in 10 ml relaxing solution and stored at 4 °C for 1 day.

The experimental apparatus and cell attachment procedure were similar to those described previously by Sweitzer & Moss (1990), with some modifications. The experimental chamber consisted of a flat aluminum plate with three thermo-electric devices mounted along one edge for temperature control. The central portion of the chamber was made of a separate stainless steel piece, containing four circular holes (each 5 mm diameter) with glass floors for illumination. Solution changes were made by translating the chamber laterally via a stage manipulator, such that another solution-containing trough was brought beneath the cell.

Single myocytes were attached to the apparatus via the tips of glass micropipettes which were fastened to the active elements of a force transducer and piezoelectric translator. The force

transducer (Model 403, Cambridge Technology, Inc., Cambridge, MA, USA) and piezoelectric translator (Physik Instruments GmbH & Co., Waldbronn, FRG) were mounted on micromanipulators for precise three-way positioning. Micropipettes were pulled from borosilicate glass to create tip diameters of 10–20 μm. The tips were coated with small drops of silicone adhesive (Dow Corning, Midland, MI, USA) and were then lowered onto each end of the skinned cell. Following 30–40 min curing, the cell was tightly adherent to the pipettes. For mechanical measurements, the piezoelectric translator was used to impose length changes of 0–25 μm in <1 ms. The displacement of the translator was driven by a Kepco power supply controlled by a pulse generator (series 1800; World Precision Instruments, New Haven, CT, USA). The signals from the pulse generator and force transducer were recorded on a digital oscilloscope (model NIC-310; Nicolet Instrument Corp., Madison, WI, USA) and stored on magnetic disk for later analysis.

Myocytes were observed with an inverted microscope (Carl Zeiss, Inc., Thornwood, NY, USA) having a ×40 objective lens, ×15 eyepiece, and a ×1·6 intermediate lens. Using a video camera and monitor, sarcomere length (SL) and SL uniformity were monitored at ×1700 magnification, while the myocyte was both relaxed and activated. Video recordings were made during every activation to permit subsequent measurement of striation spacing. In some experiments, a 35 mm Nikon camera was used to take photomicrographs of the cell both while relaxed and during steady activation. Developed negatives were projected with a photo-enlarger and sarcomere length was measured at a magnification of ×1400. These measurements were similar to sarcomere length measurements obtained from video recordings during subsequent activations of the same cell. The active sarcomere lengths presented in Table 1 are mean values obtained during activations to tensions greater than 75% P_0 (peak tension).

Solutions

Relaxing and activating solutions for skinned myocyte preparations are described by Sweitzer & Moss (1990). Solution composition was as follows: 7 mM EGTA, 1 mM free Mg^{2+}, 20 mM imidazole, 4·73 mM MgATP and 14·5 mM creatine phosphate, pH 7·0, various free Ca^{2+} concentrations between 10^{-9} M (relaxing solution) and $10^{-4.5}$ M (activating solution) or various free Sr^{2+} concentrations between 10^{-9} and 10^{-4} M, and sufficient KCl to adjust ionic strength to 180 mM. The final concentrations of each metal, ligand and metal–ligand complex were determined with the computer program of Fabiato (1988).

Experimental protocol

Cell selection. The selection of myocytes from transgenic animals consisted of an initial screening of cells for sTnC expression using a protocol developed by Metzger *et al.* (1993). The screening protocol (Fig. 1) was based on the observation that activation of contraction by Sr^{2+} differs markedly between cardiac and skeletal muscles (Kerrick *et al.* 1980), a difference that is thought to be due to characteristics inherent to the two isoforms of TnC (Hoar, Potter & Kerrick, 1988). Skinned cells were suspended in a solution containing 10–16 μM free Sr^{2+} (pSr 5·0–4·8). At the mean sarcomere length of approximately 1·8 μm assumed by relaxed cells, this protocol distinguished two populations of cells, relaxed or contracted, which were easily observed using bright-field microscopy (Fig. 1*B*). Cells that remained relaxed in the Sr^{2+}-containing screening solution (defined as transgenic (+) myocytes) were found to have tension–pSr relationships that were shifted significantly to the right, compared to those of control cardiac myocytes, and were very similar to those obtained from skeletal muscle fibres (Fig. 1*A* and *C*). Final selection of a myocyte for attachment was determined on the basis of its overall size (60–130 μm long × 15–30 μm wide) and the uniformity of its striation pattern.

Tension–pCa/pSr relationships

Two tension–pCa relationships and one tension–pSr relationship were characterized for each myocyte. A tension–pCa relationship was first determined at short sarcomere length (approximately 1·85 μm) and a second relationship was determined at a sarcomere length of approximately 2·23 μm. A tension–pSr relationship was then determined at the long sarcomere length. Tension measurements at each sarcomere length involved the initial activation of the cell in a solution of pCa 4·5 and assessing the sarcomere length. The overall length of the cell was then adjusted to achieve the appropriate sarcomere length during subsequent activations. Tension–pCa/pSr relationships were characterized by first maximally activating the cell and subsequently transferring the preparation into a series of higher pCa/pSr solutions. At each pCa/pSr, a steady tension was allowed to develop, and the cell was then rapidly released to determine total tension. Upon release, tension dropped to zero and the myocyte immediately began to shorten under zero load while taking up the imposed slack (Fig. 2*C*). The cell was then quickly transferred back to relaxing solution and was re-extended to its original length. The amount of active tension generated at each pCa/pSr was calculated as the difference between total tension and passive tension, as assessed by slackening the cell while in the relaxed state. In order to monitor any decline in tension-generating capability, each cell was maximally activated after every third or fourth activation. In the determination of a given curve, all cells maintained at least 75% of initial maximum tension. Tensions in submaximally activating solutions were expressed as fractions of peak tension (P_0) measured at the same sarcomere length. The P_0 value used to normalize submaximal tensions was obtained by linear interpolation between successive maximal activations.

The form and the mid-point (pCa_{50}) of the tension–pCa relationship were determined by Hill plot analysis of the data (Shiner & Solaro, 1984). Two separate straight lines were fitted to tension data above and below $0{\cdot}5P_0$ by least-squares analysis using the following equation:

$$\log[P_r/(1 - P_r)] = n(\log[Ca^{2+}] + k),$$

where P_r is tension as a fraction of P_0, n is the Hill coefficient, and k is the pCa_{50}. The phases of the Hill plot above and below pCa_{50}, are represented by n_1 and n_2 respectively. The lesser of the abscissal intercepts of the two straight lines that fitted to the data was taken as pCa_{50} since the greater intercept did not lie upon the tension–pCa relationship.

Statistical analysis

Student's paired t tests were used to determine whether there were significant differences in myocyte properties and in tension–pCa relationships as a function of changes in sarcomere length. Student's unpaired t tests were used to assess differences in these variables between control and transgenic groups for a given sarcomere length. Values of $P < 0{\cdot}05$ were chosen as indicating significance.

RESULTS

Mechanical characterization of skinned cardiac myocytes

Light photomicrographs of a myocardial preparation used in this study are shown in Fig. 2. In this case, sarcomere length was 2·27 μm in the relaxed state (Fig. *A*, pCa 9·0). Comparison of panels *A* and *B* in Fig. 2 (pCa 5·6) indicates that sarcomere length was minimally affected by activation. The tension generated by this myocyte in a solution of pCa 5·6 (Fig. 2*B*) was 81% of that obtained during maximal activation (Fig. 2*C*). Figure 2*C* shows fast time base records of tension obtained during characterization of a tension–pCa relationship.

Table 1 summarizes the pCa_{50} and Hill coefficient values of control and transgenic myocytes at both long and short sarcomere lengths. Tension–pCa relations and Hill plots for

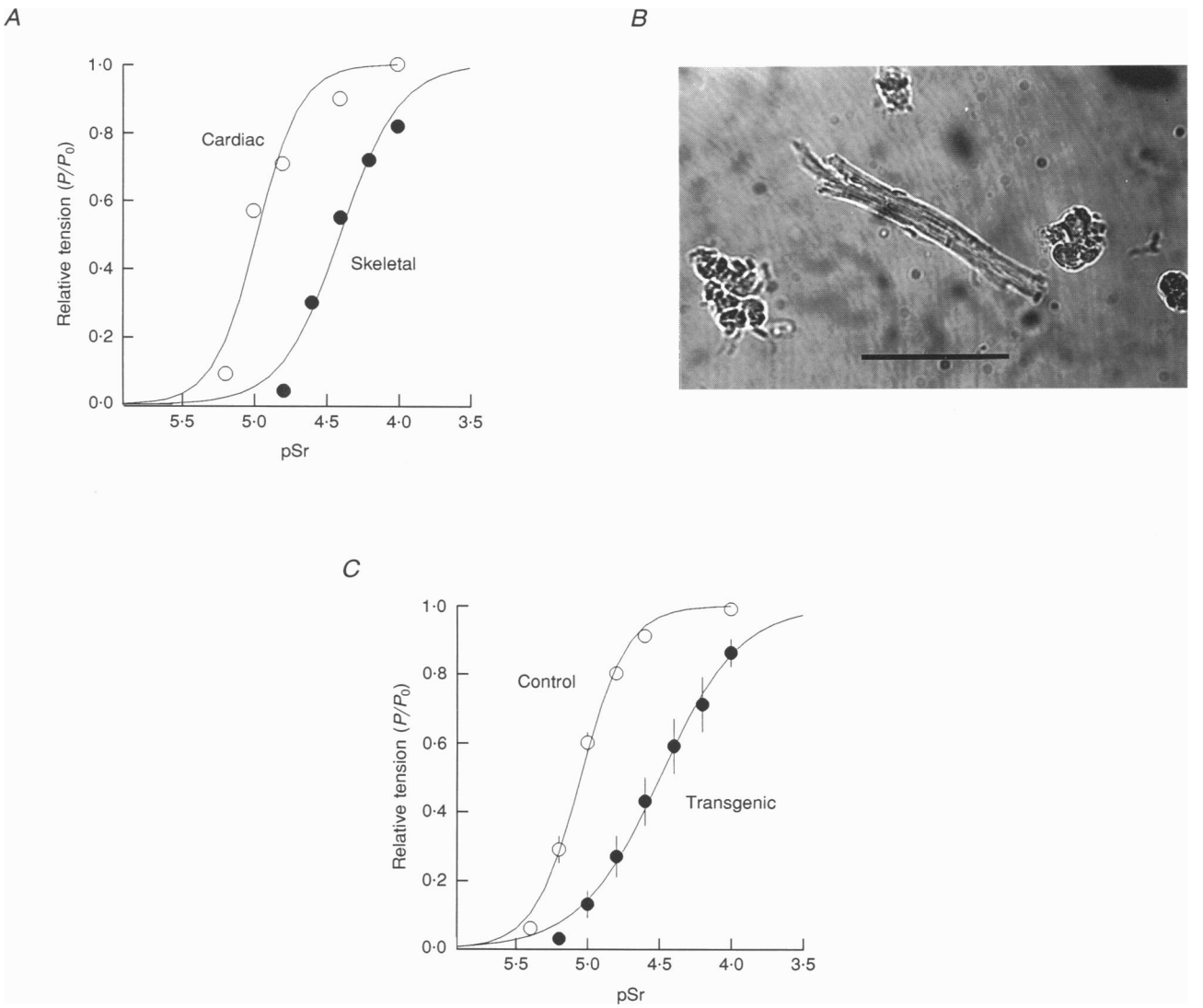

Figure 1. Screening of cardiac myocytes from transgenic mice for expression of sTnC

A, tension–pSr relationships from a control cardiac myocyte and a single skeletal muscle fibre. The pSr for half-maximal activation (pSr_{50}) differed between the cardiac myocyte and skeletal muscle fibre by approximately 0·60 pSr units. *B*, light photomicrograph showing cardiac myocytes from a transgenic mouse that were screened for sTnC expression using a solution of pSr 4·8. Some myocytes in the Sr^{2+}-containing screening solution remained relaxed while others contracted, forming myoballs. Maintained relaxation of myocytes in solution of pSr 5·0–4·8, a solution which activates all control myocytes, was used as a marker for sTnC expression. Myocytes that were insensitive to solution of pSr 5·0–4·8 were defined as transgenic (+) myocytes. Scale bar represents 100 μm. *C*, mean tension–pSr relationships for control and transgenic (+) myocytes in which the length dependence of Ca^{2+} sensitivity was characterized. The difference between pSr_{50} values from control and transgenic myocytes was approximately 0·60 pSr units.

control and transgenic (+) myocytes were averaged, and mean plots are presented in Figs 3 and 4. The Ca^{2+} sensitivity of tension, as assessed by pCa_{50}, increased in control myocytes from 5·70 ± 0·04 to 5·82 ± 0·04 (means ± S.E.M.) when sarcomere length was increased (Table 1). Similar length-dependent changes in the Ca^{2+} sensitivity of tension were obtained in transgenic (+) myocytes (pCa_{50} was 5·60 ± 0·03 at short SL *vs.* 5·72 ± 0·03 at long SL). Moreover, the mean change in pCa_{50} values between short and long sarcomere lengths was identical for control and transgenic (+) myocytes (0·12 ± 0·01). Hill coefficients were not significantly different when compared either between control and transgenic (+) myocytes or between short and long sarcomere lengths within each group. However, there was a tendency for n_1 values to decrease and n_2 values to increase when sarcomeres were lengthened, a finding consistent with previous results in skeletal muscle fibres from this laboratory (Moss, Swinford & Greaser, 1983; Allen & Moss, 1987; Moss, Nwoye & Greaser, 1991).

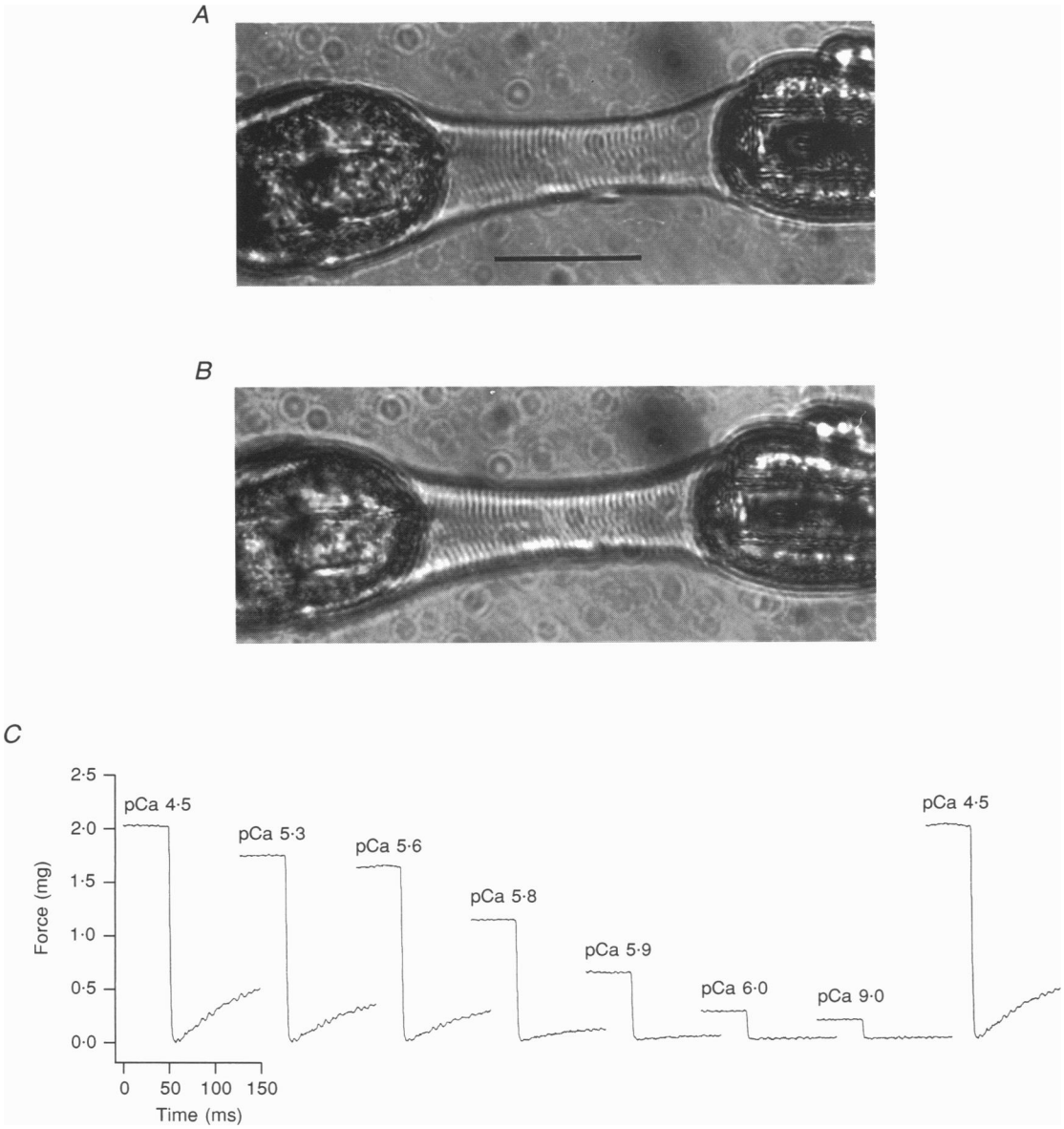

Figure 2. Cardiac myocyte preparation and measurement of isometric tension

A and *B*, light photomicrographs of a transgenic (+) myocyte while relaxed (pCa 9·0) and during activation (pCa 5·6), respectively. Relaxed sarcomere length was 2·27 μm. Sarcomere length during activation was 2·21 μm. Scale bar represents 50 μm. *C*, representative fast time base records of tension from the same cell at various pCa values. Total tension was determined at each pCa by first allowing tension to reach a steady state and then rapidly releasing the myocyte causing tension to drop to zero. The difference between total tension and passive tension (i.e. at pCa 9·0) was taken as active tension.

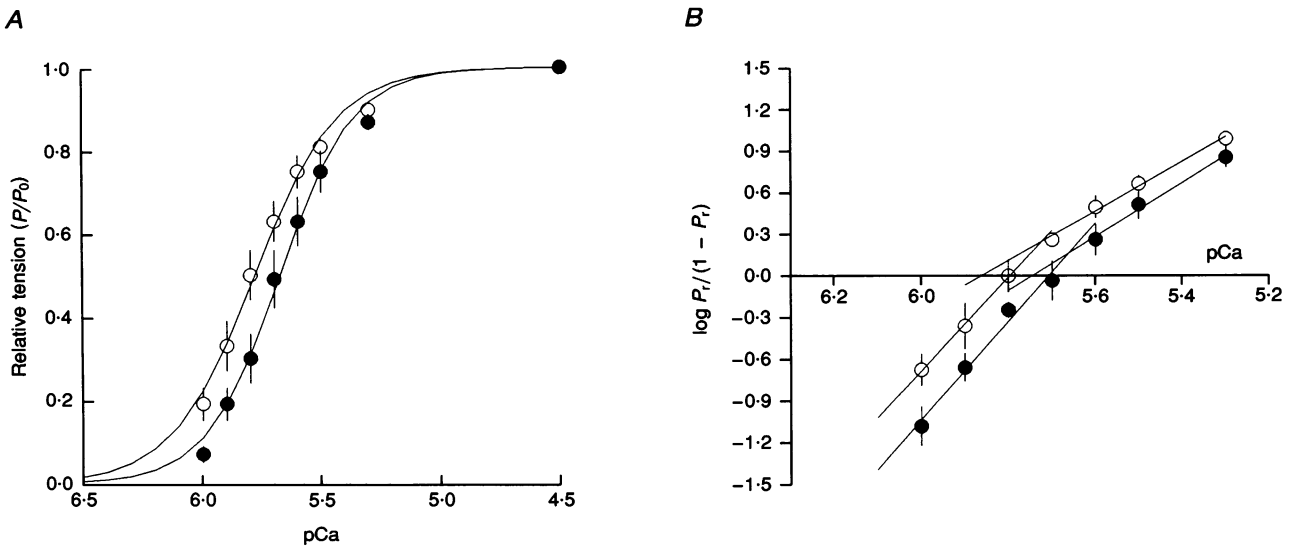

Figure 3. Plot of the length dependence of the Ca^{2+} sensitivity of tension for control myocytes
A, mean tension–pCa relationships at long (○, 2·23 ± 0·01 μm) and short (●, 1·82 ± 0·02 μm) sarcomere lengths. *B*, mean data after transformation to the linearized Hill plot.

Evidence that the thin filaments of transgenic (+) cardiac myocytes studied contained sTnC

Following the characterization of the length dependence of the Ca^{2+} sensitivity of tension, tension–pSr relationships were obtained in each myocyte at a sarcomere length of approximately 2·23 μm. Mean tension–pSr relationships from control and transgenic (+) myocytes are shown in Fig. 1*C*. As expected from the fact that the transgenic (+) myocytes remained relaxed in a screening solution of pSr 5·0–4·8, the mean tension–pSr relationship of these cells was significantly shifted in the direction of higher $[Sr^{2+}]$ compared with control myocytes. At pSr 5·0–4·8 (i.e. the pSr of the screening solution) transgenic (+) myocytes produced about 20% peak tension, since these tension measurements were obtained at long sarcomere length. However, as noted in Methods, sTnC-containing myocytes remained relaxed during the screening protocol, which was due to the much lower pSr sensitivity of tension at the sarcomere length (~1·8 μm) assumed by free-floating skinned myocytes. The pSr_{50} at long lengths differed

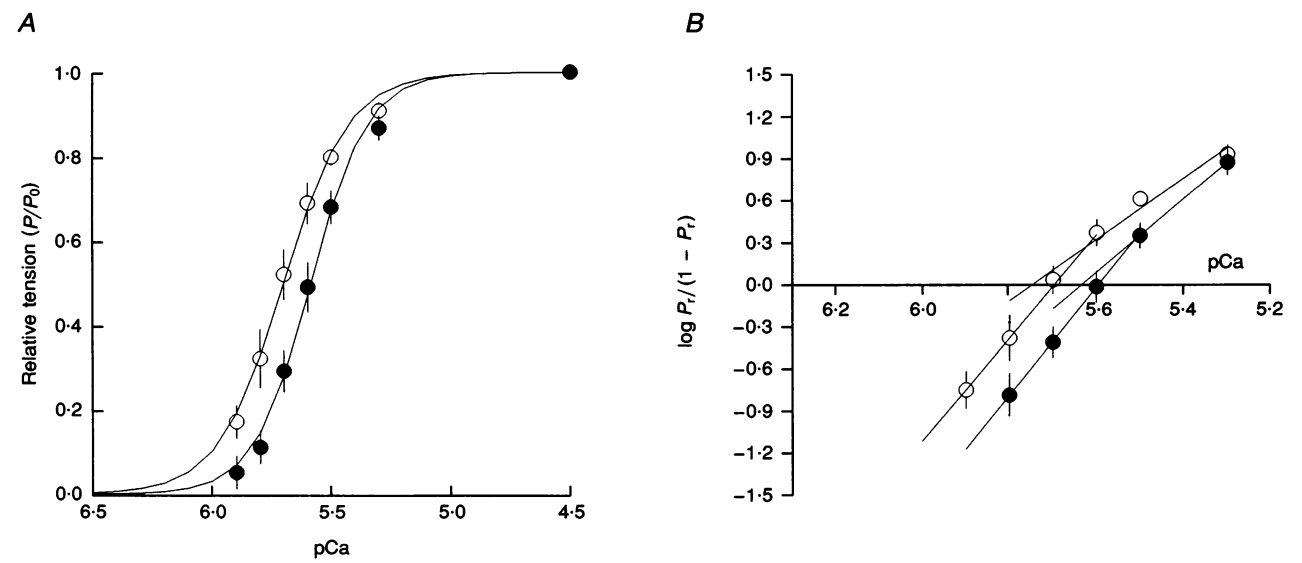

Figure 4. Plot of the length dependence of the Ca^{2+} sensitivity of tension for transgenic (+) cardiac myocytes
A, mean tension–pCa relationships at long (○) and short (●) sarcomere lengths. *B*, mean data after transformation to the linearized Hill plot.

Table 1. Summary of pCa_{50} and Hill coefficient values from mouse myocytes

		Sarcomere length (μm)	n_1	n_2	pCa_{50}	ΔpCa_{50}
Control ($n = 6$)	Short	$1{\cdot}82 \pm 0{\cdot}02$	$2{\cdot}29 \pm 0{\cdot}15$	$4{\cdot}55 \pm 0{\cdot}34$	$5{\cdot}70 \pm 0{\cdot}04$	$0{\cdot}12 \pm 0{\cdot}01$
	Long	$2{\cdot}23 \pm 0{\cdot}01$*	$2{\cdot}10 \pm 0{\cdot}18$	$4{\cdot}76 \pm 0{\cdot}04$	$5{\cdot}82 \pm 0{\cdot}04$*	
Transgenic ($n = 6$)	Short	$1{\cdot}83 \pm 0{\cdot}01$	$2{\cdot}83 \pm 0{\cdot}39$	$4{\cdot}64 \pm 0{\cdot}36$	$5{\cdot}60 \pm 0{\cdot}03$	$0{\cdot}12 \pm 0{\cdot}01$
	Long	$2{\cdot}23 \pm 0{\cdot}01$*	$2{\cdot}53 \pm 0{\cdot}39$	$5{\cdot}47 \pm 0{\cdot}70$	$5{\cdot}72 \pm 0{\cdot}03$*	

Values are means $\pm$ S.E.M. *Significant differences between corresponding values at short *versus* long lengths: $P < 0{\cdot}001$. n_1 is the slope of the line greater than pCa_{50}. n_2 is the slope of the line less than pCa_{50}.

between the control and trangenic (+) myocytes by approximately 0·60 pSr units, which is similar to the difference observed when comparing control myocytes and fast skeletal muscle fibres (Fig. 1*A* and *C*). Since the difference in Sr^{2+} sensitivity between cardiac and skeletal muscle is determined primarily by the isoform of TnC (Hoar, Potter & Kerrick, 1988), this result indicates that the thin filaments of the transgenic (+) myocytes, whose length–dependence of Ca^{2+} sensitivity was characterized, contained predominantly sTnC.

DISCUSSION

Cardiac TnC and skeletal TnC confer similar length dependence to the Ca^{2+} sensitivity of tension in mouse skinned cardiac myocytes

The hypothesis tested in this study was that cTnC, in combination with cardiac regulatory proteins, confers greater length dependence to the Ca^{2+} sensitivity of tension in cardiac muscle when compared with skeletal muscle. Babu *et al.* (1988) previously reported that extraction of endogenous cTnC and subsequent replacement with sTnC in skinned trabeculae from the ventricles of the hamster reduced the length-dependent shift of pCa_{50}. They (Babu *et al.* 1990) later reported that substitution of cTnC for sTnC in fast skeletal fibres increased the length-dependent shift in Ca^{2+} sensitivity. Based on these findings, they proposed that a domain of cTnC possesses length-sensing properties which solely account for the differences in length dependence of the Ca^{2+} sensitivity of tension between cardiac and skeletal muscle. In analogous experiments, Moss, Nwoye & Greaser (1991) found no significant difference in the length dependence of pCa_{50} in skeletal muscle containing either sTnC or predominantly cTnC. When the sarcomere length of skeletal muscle fibres was reduced from about 2·3 μm by an average of 0·4 μm, the shift in pCa_{50} was similar both before and after extraction of most of the endogenous sTnC and complete (> 95%) reconstitution with cTnC. However, in extracted fibres in which reconstitution of cTnC was incomplete, the shift in pCa_{50} was significantly greater than control fibres or fibres that were fully reconstituted. While these results are inconsistent with the idea that cTnC alone confers unique length dependence to the Ca^{2+} sensitivity of tension, the experiments did not eliminate the possibility that cTnC may have special sensitivity to length only in the presence of cardiac regulatory and contractile proteins. To address this possibility and avoid procedural difficulties (e.g. incomplete reconstitution of extracted fibres) usually associated with reconstituting TnC into thin filaments, transgenic mice were developed in which sTnC was ectopically expressed in cardiac muscle. This allowed characterization of the length dependence of pCa_{50} in single myocytes containing either endogenous cTnC or predominantly sTnC and in which sarcomere length could be monitored throughout the entire experiment. Our finding that mean shifts in pCa_{50} were identical between control and transgenic (+) myocytes when sarcomere length was increased from about 1·83 to 2·23 μm strongly argues against the idea that cTnC imparts a unique length dependence of the Ca^{2+} sensitivity of tension even when associated with cardiac thin and thick filament proteins.

Another finding of the present study is that tension–pCa relationships were similar in myocytes containing either cTnC or sTnC. In fact, no significant differences were observed in pCa_{50} values and Hill coefficients between control and transgenic (+) myocytes at the same sarcomere length. Earlier work (Moss, Lauer, Giulian & Greaser, 1986; Gulati, Scordilis & Babu, 1988) reports that partial replacement of endogenous sTnC with cTnC in skeletal muscle fibres caused a decrease in the steepness in the tension–pCa relationship. The reason for no statistically significant effect of sTnC on the form of the tension–pCa relationships in cardiac myocytes is unknown. One possibility is that the reduction in steepness previously observed in skeletal fibres following replacement of sTnC with cTnC is due to small deficiencies in TnC content, rather than being characteristic of cTnC (see, for example, Moss, Giulian & Greaser, 1985). Alternatively, differences in Hill coefficients between skinned skeletal muscle fibres and cardiac myocytes may be due to different isoforms of subunits of troponin other than TnC, or variations in thin filament activation by strong-binding cross-bridges, ideas that have yet to be tested by experiment.

The steepness of the tension–pSr relationship appears to be reduced in transgenic myocytes compared with control, perhaps due to the presence of small amounts of cTnC in the thin filaments of these myocytes. A mixture of TnC

with differing affinities for Sr^{2+} might be expected to reduce the co-operativity by altering interactions between adjacent troponin–tropomyosin complexes. By similar argument, if the affinities for Ca^{2+} were different between cTnC and sTnC at a particular sarcomere length, transgenic myocytes would be expected to have less apparent co-operativity than controls. This was not the case, further supporting our conclusion that affinities of cTnC and sTnC for Ca^{2+} respond similarly to changes in sarcomere length.

Mechanism for possible differences in length–twitch tension relationships of living cardiac and skeletal muscle

The length–twitch tension relationships of both skeletal (Close, 1972) and cardiac muscles fall off rather rapidly when muscle length is reduced from optimum, although the falling-off of tension appears to be more rapid in cardiac muscle. Since the replacement of cTnC with sTnC did not alter the length dependence of the Ca^{2+} sensitivity of tension, it is unlikely that the steeper relationship is due to differing isoforms of TnC in these muscles. Other factors may contribute to the steeper length–twitch tension relationship in cardiac muscle. One possibility is that cardiac muscle may possess a relatively greater shortening- or activation-dependent internal load (Allen & Kentish, 1985). This would cause twitch tension to fall off more rapidly in cardiac muscle since a larger proportion of active cross-bridges would be required to overcome the force of recoil of the internal load. Also, differences in length–twitch tension relationships may result in part from greater length-dependent variations in the myoplasmic Ca^{2+} transient during the cardiac twitch (Allen, Nichols & Smith, 1988).

Possible mechanism for change in the Ca^{2+} sensitivity of tension with changes in sarcomere length

The mechanism underlying the decrease in the Ca^{2+} sensitivity of tension with reduction in sarcomere length remains to be elucidated (Stephenson & Wendt, 1984). One likely cause is the increase in fibre diameter observed in both living (Gordon *et al.* 1966) and skinned muscle when muscle length is reduced (Maughan & Godt, 1981; Moss, Lauer, Guilian & Greaser, 1986; Allen & Moss, 1987). Since increased fibre diameter in skinned skeletal muscle fibres is associated with increased interfilament lattice spacing (Rome, 1968), the likelihood of cross-bridge interaction in the zone of overlap would be reduced, thereby decreasing the the Ca^{2+} sensitivity of tension. Consistent with this hypothesis, osmotically compressed skinned skeletal fibres generated more tension at any given pCa than untreated fibres with the same sarcomere spacing (Maughan & Godt, 1981; Allen & Moss, 1987; Martyn & Gordon, 1988). Osmotic compression of skinned cardiac trabeculae (Harrison, Lamont & Miller, 1988) as well as single skinned cardiac myocytes (McDonald & Moss, 1994) also increases the Ca^{2+} sensitivity of tension at a given sarcomere length.

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