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Skeletal troponin C reduces contractile sensitivity to acidosis in cardiac myocytes from transgenic mice

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ABSTRACT **Depressed contractile function plays a primary role in the pathophysiology of acute myocardial ischemia. Intracellular acidification is an important factor underlying the inhibition of force production in the ischemic myocardium. The effect of acidosis to depress contractility is markedly greater in cardiac as compared to skeletal muscle; however, the molecular basis of this difference in sensitivity to acidosis is not clearly understood. In this report, we describe transgenic mice that express the fast skeletal isoform of troponin C (sTnC) in cardiac muscle. In permeabilized single cardiac myocytes the shift in the midpoint of the tension–pCa relationship (i.e., pCa_{50}, where pCa is $-\log[Ca^{2+}]$) due to lowering pH from 7.00 to 6.20 was 1.27 ± 0.03 (n = 7) pCa units in control cardiac TnC (cTnC) expressing myocytes and 0.96 ± 0.04 (n = 11) pCa unit in transgenic cardiac myocytes ($P < 0.001$). The effect of pH to alter maximum Ca^{2+}-activated tension was unchanged by TnC isoforms in these cardiac myocytes. In a reciprocal experiment, contractile sensitivity to acidosis was increased in fast skeletal muscle fibers following extraction of endogenous sTnC and reconstitution with purified cTnC *in vitro*. Our findings demonstrate that TnC plays an important role in determining the profound sensitivity of cardiac muscle to acidosis and identify cTnC as a target for therapeutic interventions designed to modify ischemia-induced myocardial contractile dysfunction.**

It is well known that force generation diminishes markedly during ischemia in the heart (1–3). In contrast, studies on isolated whole heart or isolated papillary muscles using NMR, fluorescent indicators, or the photoprotein aequorin reveal that the Ca^{2+} transient of contraction is generally little changed during the time period of ischemia when force is declining markedly (2). Thus, the basis of contractile dysfunction in the ischemic heart appears to involve events in the excitation–contraction coupling scheme distal to Ca^{2+} release from the sarcoplasmic reticulum. During ischemia in the heart, metabolic byproducts of ATP hydrolysis and the glycolytic energy pathway, including H^+, inorganic phosphate, and ADP, accumulate within the myoplasm and these byproducts have been implicated in mediating the observed altered contractile function of the failing heart in ischemia (2). For example, it has been shown that intracellular pH declines from about 7.00 to 6.20 during ischemia in the heart (2, 4). Studies using the permeabilized muscle preparation, in which the sarcolemma is chemically or mechanically disrupted to allow access and direct control of the intracellular solution bathing the otherwise intact contractile apparatus, provide evidence that low pH has a direct effect to depress Ca^{2+}-activated contraction in cardiac and skeletal muscle, with the effect being greater in cardiac muscle (refs. 5 and 6; present study). Taken together these results suggest that the basis of pH-induced inhibition of force generation resides in desensitization of the contractile myofilaments to activation by Ca^{2+}.

Troponin C (TnC), the Ca^{2+} binding subunit of the thin filament, plays a central role in regulating excitation–contraction coupling in cardiac and skeletal muscle. There are two isoforms of TnC that are encoded by distinct single copy genes and that are differentially expressed in the cardiac and skeletal muscle lineages. Slow/cardiac TnC (cTnC) is the only isoform expressed in cardiac and slow skeletal muscle, whereas fast skeletal TnC (sTnC) is expressed exclusively in adult fast skeletal muscles in mammals (7). Considering the central role of TnC in excitation–contraction coupling and its differential pattern of expression in cardiac and fast skeletal muscle, it is possible that different isoforms of TnC might be involved in conferring the distinct pH sensitivities of contraction in cardiac and skeletal muscle. In this report transgenic mice were produced in which sTnC was expressed ectopically in the heart to test the role of TnC isoforms in acidosis-mediated myocardial contractile dysfunction.

MATERIALS AND METHODS

Generation and Characterization of Transgenic Mice. Transgenic mice that expressed sTnC ectopically in the heart were produced using a cardiac-specific sTnC expression vector (pMHCsTnC) that contained exons 1–6 of the murine sTnC gene (7) cloned 3′ of the 650-base-pair (bp) cardiac-specific (8) α-myosin heavy chain (α-MHC) promoter in pUC19. A synthetic oligonucleotide linker (5′-CCTCTAGAGTCGACGTCGCCAGCAACCATGG-3′) (L) was used to join the α-MHC promoter and the sTnC gene and to introduce a consensus translation initiation codon. To generate transgenic mice, the α-MHC-sTnC transgene was linearized at a unique *Nde* 1 site and microinjected into inbred C3HeB/FeJ (Jackson Laboratories, Bar Harbor, ME) zygotes using standard methodologies (9). Microinjected embryos were cultured *in vitro* to the two-cell stage and then reimplanted into pseudopregnant SW Taconic (Taconic Farms, Germantown, NY) female mice. Transgenic animals, as determined by diagnostic PCR (10), were then used to establish lineages of transgenic mice by crossing with C3HeB/FeJ inbred mice.

Cardiac Myocyte Preparation. Cardiac myocytes were mechanically dissected from the ventricles of whole hearts as described (11). The experimental apparatus and the cardiac myocyte attachment and Triton X-100 procedure were similar to those described in an earlier study (11). Briefly, single permeabilized cardiac myocytes were attached to borosilicate glass micropipettes pulled to tip diameters of about 1 μm using a silicone adhesive. The microelectrodes were connected to an output tube of a force transducer (Cambridge

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Abbreviations: TnC, troponin C; cTnC, slow/cardiac TnC; sTnC, fast skeletal TnC; α-MHC, cardiac α-myosin heavy chain.

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Technology, Cambridge, MA) and a high-performance moving coil galvanometer (Cambridge Technology). Sarcomere length was set at 2.20 μm. Average dimensions of the cardiac myocyte preparation were 73 μm (length) and 27 μm (width). Details of the methods used for the skinned single skeletal muscle fiber have been published (12). Relaxing and activating solutions contained (in mmol/liter): EGTA, 7; free Mg^{2+}, 1; MgATP, 4; creatine phosphate, 14.5; imidazole, 20; and sufficient KCl to yield a total ionic strength of 180 mmol/liter; temperature was set at 15°C. Solution pH was adjusted to 7.00/6.20 with KOH/HCl (13). A computer program (13) was used to calculate the final concentrations of each metal, ligand, and metal–ligand complex, employing the stability constants indicated previously (12). An analysis of variance test was used to determine significance using Bonferroni adjusted P values to correct for multiple comparisons, $P < 0.05$.

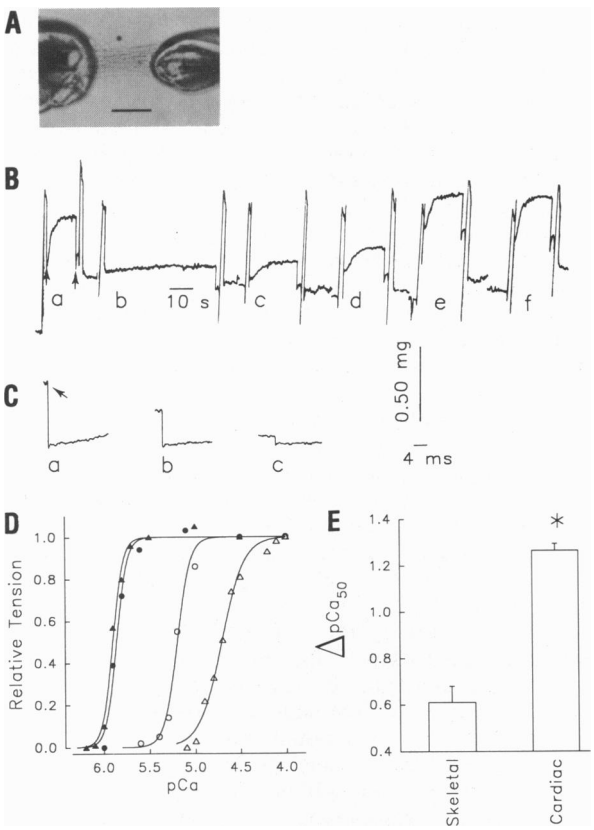

FIG. 1. Single cardiac myocyte preparation and effects on Ca^{2+}-activated isometric tension due to reduced pH in control mice. (*A*) A single cardiac myocyte attached to the tips of glass microelectrodes. Cardiac myocyte length is 70 μm and average sarcomere length is 2.2 μm. (Bar = 50 μm.) (*B*) Slow time-base records of Ca^{2+}-activated isometric tension at pH 7.00 from a control mouse cardiac myocyte. At the first arrow, the cardiac myocyte was transferred (tension spikes are due to the cardiac myocyte crossing the solution–air interface) from relaxing solution to maximal activating solution (pCa 4.5). At the second arrow, cardiac myocyte length was rapidly released to obtain a tension baseline (see records in *C*) and transferred back to relaxing solution in which cardiac myocyte length was reextended. In parts b–f, the pCa of the activating solution was set at 5.8, 5.7, 5.6, 4.5, and 5.0, respectively. (*C*) Fast time-base records of tension. Record a. The cardiac myocyte was activated at pCa 4.5/pH 7.00 and steady isometric tension developed, at which point the cardiac myocyte was rapidly shortened (arrow) to obtain a tension baseline; thus from the peak to the baseline is the total tension. Record b shows a fast time-base record obtained at pCa 4.0/pH 6.20. Record c shows a fast time-base record at pCa 9.0 to obtain resting tension, which was then subtracted from the total tension value (i.e., record a) to obtain the active tension generated by the cell. (*D*) Effects on the tension–pCa relationship due to reduced pH in a cardiac myocyte (triangles) and a fast skeletal fiber (circles) from normal mice. Filled symbols are pH 7.00 and open symbols are pH 6.20. Tension values are normalized to the maximum tension value obtained in each cell at the same pH value. (*E*) Summary of ΔpCa_{50} values in skeletal and cardiac muscle. Values are mean ± SE (n = 4–7). The asterisk indicates the cardiac value is significantly greater than the skeletal value ($P < 0.001$).

RESULTS

Fig. 1 shows the effects on the tension–pCa relationship (where pCa is $-\log[Ca^{2+}]$) in permeabilized single cardiac myocytes and skeletal muscle fibers from normal mice due to reduction in pH from 7.00 to 6.20. The pH-induced shift in the Ca^{2+} sensitivity of tension, termed ΔpCa_{50} (the difference in pCa_{50} values obtained at pH 7.00 and pH 6.20, where pCa_{50} is the pCa that results in one-half maximum tension), was 0.61 ± 0.07 (n = 4) pCa unit in mouse fast skeletal muscle fibers compared to 1.27 ± 0.03 (n = 7) pCa units in mouse cardiac myocytes ($P < 0.001$).

To directly test the role of TnC in conferring pH sensitivity of contraction in cardiac muscle we used a cardiac-specific sTnC expression vector to produce transgenic mice in which sTnC was expressed ectopically in cardiac muscle (Fig. 2). Three independent lines of transgenic mice were produced.

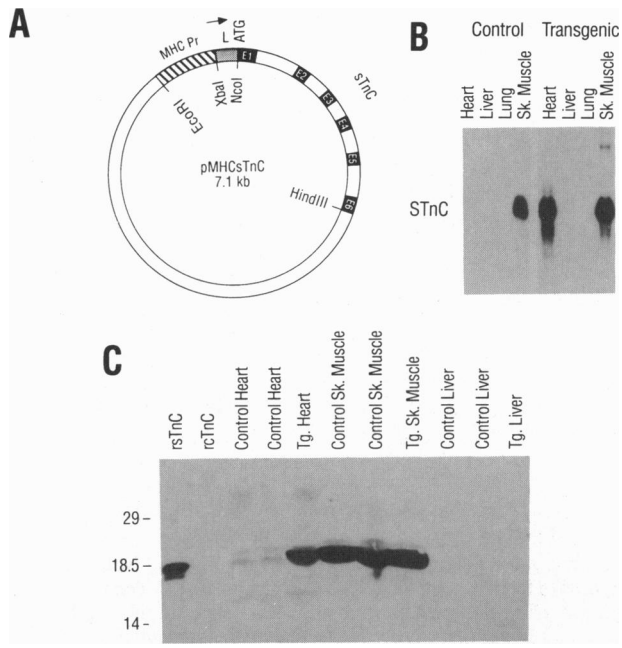

FIG. 2. Production and characterization of sTnC transgenic mice. (*A*) Structure of the MHC-sTnC transgene. The 4-kb genomic sTnC fragment (7) and 650-bp α-MHC promoter fragment (MHC Pr) were cloned into *Eco*RI/*Hin*dIII-digested pUC19 (7). The six exons (E1–E6) of the sTnC gene are labeled. (*B*) Northern blot analysis of sTnC expression in transgenic and control animals. RNA was prepared from nontransgenic littermates (control) or transgenic (transgenic) mouse organs using lithium chloride/urea (7). Ten micrograms of whole RNA from nontransgenic littermates (control) or transgenic organs, equalized by ethidium bromide staining on nondenaturing gels, was subjected to Northern blot analysis (7). Sk., skeletal. Probes included the 501-bp murine sTnC cDNA (7) and the 504-bp murine cTnC cDNA (7). All probes were radiolabeled with ^{32}P using random hexanucleotide priming as described (7). The size of the major sTnC mRNA was ≈700 bp, in good agreement with previous results (7). (*C*) Western blot analysis of sTnC expression in transgenic (Tg.) and control animals. Western blots containing equal amounts of crude organ homogenates were probed with a sTnC-specific rabbit antiserum and goat anti-rabbit/peroxidase-conjugated second antibody using a commercially available kit (Amersham International, Buckinghamshire, U.K.). Size markers are indicated in kDa. r is 100 ng of recombinant s(c)TnC.

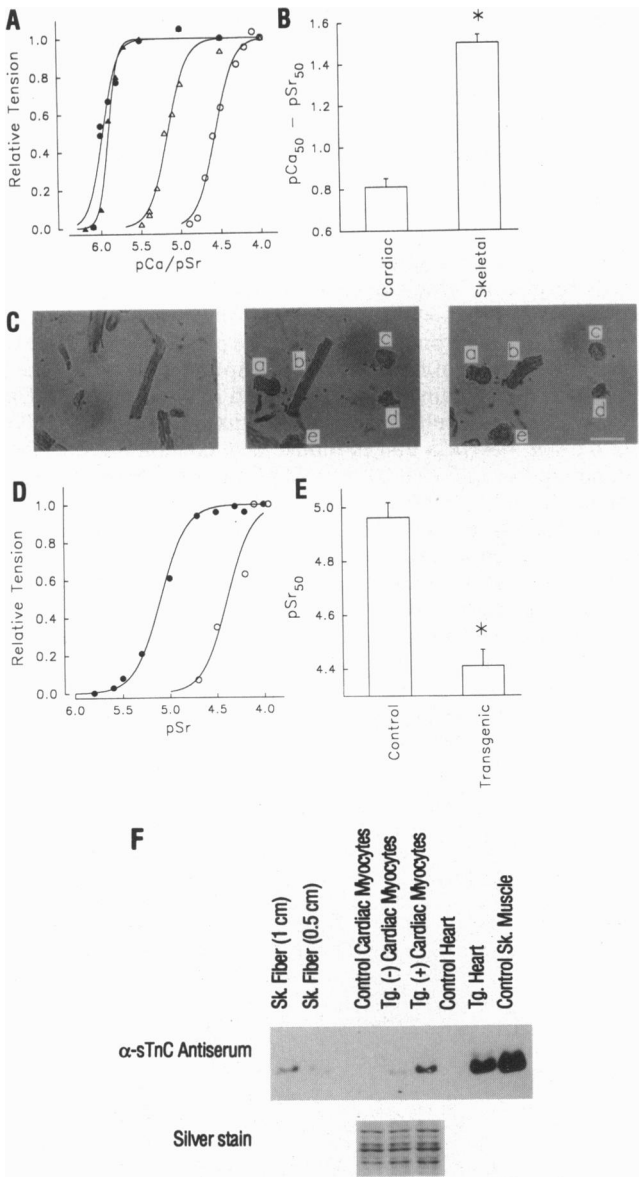

FIG. 3. Screening for expression of sTnC in isolated cardiac myocytes from transgenic mice with strontium. (*A*) Tension–pCa and tension–pSr relationships in a single fast skeletal muscle fiber (circles) and a cardiac myocyte (triangles) from control mice. Filled symbols are Ca^{2+} data and open symbols are Sr^{2+} data. Data are normalized to maximum Ca^{2+}- or Sr^{2+}-activated tension value. (*B*) Summary of the magnitude of difference in the midpoint of activation by Ca^{2+} and Sr^{2+} between skeletal fibers and cardiac myocytes from control mice. In each experiment (cardiac or skeletal) pCa_{50} and pSr_{50} values were obtained and the difference value was then derived by subtracting the pSr_{50} value from the pCa_{50} value. The values are mean ± SE (n = 4–7). The asterisk indicates the skeletal value is greater than the cardiac value ($P < 0.001$). (*C*) Photomicrographs of cardiac myocytes from a transgenic mouse that were isolated in solutions of varied Sr^{2+} concentration. (*Left*) pSr 9.0; cardiac myocytes are rod shaped and relaxed. (*Center*) pSr 5.0; cardiac myocytes labeled a and c–e are contracted [termed transgenic (−) cardiac myocytes] whereas cardiac myocyte b remained relaxed [termed transgenic (+) cardiac myocyte]. (*Right*) Upon increasing Sr^{2+} to pSr ≈ 4.5, cardiac myocyte b became activated and contracted. (Bar = 100 μm.) (*D*) Tension–pSr relationships in a representative control (filled circles) and a transgenic (+) (open circles) cardiac myocyte. (*E*) Summary of pSr_{50} values for control and transgenic (+) cardiac myocytes. Values are expressed as mean ± SE (n = 7–11 per group). The asterisk indicates that the transgenic (+) value is significantly less than the control value ($P < 0.001$). (*F*) Western blot and silver staining. Approximately 1000 single control, transgenic [Tg. (−)], and transgenic [Tg. (+)] cardiac myocytes were selected as described in *C*. Differences in protein loading among the lanes were less than ±17% as determined by scanning densitometry of the upper portion of the silver-stained gel used in the Western blot. Skeletal (Sk.) single fiber samples were from rabbit psoas muscle.

One of these lines, which expressed high levels of sTnC mRNA and protein in cardiac muscle, was used for all further experiments. Northern blot analyses demonstrated sTnC transgene mRNA in the hearts of transgenic but not control littermates (Fig. 2*B*). As expected, transgene expression was restricted to cardiac muscle and was not observed in liver or lung, in agreement with our previous characterization of this promoter in transgenic animals (8). The level of myocardial transgene expression was similar to that observed for the endogenous sTnC gene in skeletal muscle as evidenced by Northern blot analysis (Fig. 2*B*). Moreover, transgene expression did not negatively influence expression of the endogenous cTnC gene, as similar levels of cTnC mRNA were observed in heart homogenates from transgenic mice and their nontransgenic siblings (data not shown). In a second set of experiments, Western blots of tissue homogenates were probed with a sTnC-specific polyclonal rabbit antiserum (Fig. 2*C*). sTnC was detected in skeletal muscle from transgenic and control animals. In addition, sTnC was detected in transgenic cardiac homogenates but was not observed in cardiac homogenates prepared from two nontransgenic control littermates. In control experiments, sTnC protein was also not observed in liver homogenates from either transgenic or control animals. These experiments demonstrated cardiac-specific expression of the sTnC transgene at the mRNA and protein levels.

We also determined transgene protein expression at the cellular level. To accomplish this a screening protocol was developed that utilized Sr^{2+} rather than Ca^{2+} to activate contraction of cardiac myocytes. The screening protocol was based on the observation that Ca^{2+} activation of contraction is rather similar in normal cardiac and skeletal muscle, whereas activation by Sr^{2+} differs markedly in these two striated muscle types (Fig. 3 *A* and *B*). This difference is determined in part by the differential expression of TnC isoforms in these tissues (15, 16). We found that isolating single cardiac myocytes from sTnC transgenic animals in solutions in which the free concentration of Sr^{2+} was set at pSr 5.0 resulted in two populations of cells, relaxed or contracted, that were easily distinguished visually using conventional bright-field light microscopy (Fig. 3*C*). In control experiments cardiac myocytes from normal animals were uniformly contracted upon exposure to the same concentrations of Sr^{2+}. Single sTnC transgenic cardiac myocytes that remained relaxed in the Sr^{2+} screening solution displayed a tension–pSr relationship that was significantly right-shifted (i.e., less Sr^{2+} sensitive), compared to that obtained from control cardiac myocytes, but similar to that obtained from control fast skeletal muscle fibers (Fig. 3 *D* and *E*). Importantly, Western blot analysis of cardiac myocytes from transgenic mice that were relaxed in the Sr^{2+} screening solution demonstrated directly that these isolated cardiac myocytes expressed high levels of sTnC (Fig. 3*F*). Results showed a >25-fold increase in sTnC protein levels in the relaxed (termed transgenic +) as compared to the contracted (transgenic −) cardiac myocytes from transgenic mice. Thus, the Sr^{2+} screening protocol demonstrated differences in transgene expression at the cellular level and, more importantly, allowed us to select and then subsequently study those individual cardiac myocytes expressing high levels of the sTnC transgene.

It was also determined whether transgene expression had an effect on the ultrastructure of the cardiac myofilaments or altered the expression of other contractile protein isoforms.

Based on SDS/PAGE analysis on single isolated cardiac myocytes, there was no apparent difference in expression of myosin heavy chain, actin, or myosin light chain 1 and 2 protein isoforms in transgenic (+) compared to control isolated cardiac myocytes (Fig. 4). Similar results were obtained in nine additional comparisons between control and transgenic cardiac myocytes. Further, if transgene expression altered expression of thin filament regulatory protein isoforms—for example, TnT or tropomyosin isoforms—the steepness tension–pCa relation at pH 7.00 would be expected to be markedly altered (17). In the present study the steepness of the tension–pCa relationship was not statistically different in control as compared to transgenic (+) cardiac myocytes at pH 7.00. In addition, there were no apparent differences in myofilament ultrastructure based on comparisons of electron micrographs obtained from individual control and transgenic (+) cardiac myocytes (Fig. 4). Furthermore, no apparent differences in structure between control (Fig. 1*A*) and transgenic (+) cardiac myocytes (Fig. 3*C*) were apparent at the light microscope level. Taken together, these findings provide evidence against the possibility that sTnC transgene expression altered the expression of other regulatory protein isoforms or affected the ultrastructure of the myofilaments.

Transgenic cardiac myocytes expressing high levels of sTnC were used to test the hypothesis that TnC confers the varying pH sensitivities of contraction observed in skeletal and cardiac muscles (Fig. 1). Fig. 5*A* compares representative tension–pCa relationships from a control cardiac myocyte to that of a transgenic (+) cardiac myocyte. The shift in pCa_{50} upon lowering pH from 7.00 to 6.20 was 0.80 pCa unit in the transgenic (+) cardiac myocyte as compared to 1.24 pCa units in the control cardiac myocyte. The basis of the differing ΔpCa_{50} values was primarily acidic pH-dependent because the tension–pCa relationships of the transgenic (+) and control cardiac myocytes were relatively comparable at pH 7.00.

A summary of the effects of reduced pH on the Ca^{2+} sensitivities of contraction in transgenic (+) and control cardiac myocytes is displayed in Fig. 5*B*. The mean ΔpCa_{50} values for control and transgenic (+) cardiac myocytes were

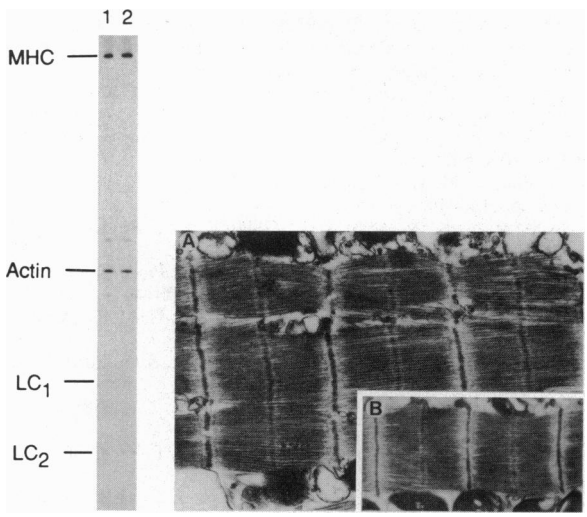

FIG. 4. Comparisons of protein expression and myofilament ultrastructure between control and transgenic single cardiac myocytes. (*Left*) SDS/PAGE (14) on a control (lane 1) and a transgenic (+) (lane 2) cardiac myocyte. Selection of individual transgenic cardiac myocytes is detailed in Fig. 3*C*. LC, light chain. sTnC expression in transgenic myocytes is demonstrated in Fig. 3*F*. (*Right*) Electron micrographs demonstrating comparable myofilament ultrastructure in a transgenic (+) (*A*, ×38,750) and control (*B*, ×28,750) cardiac myocyte.

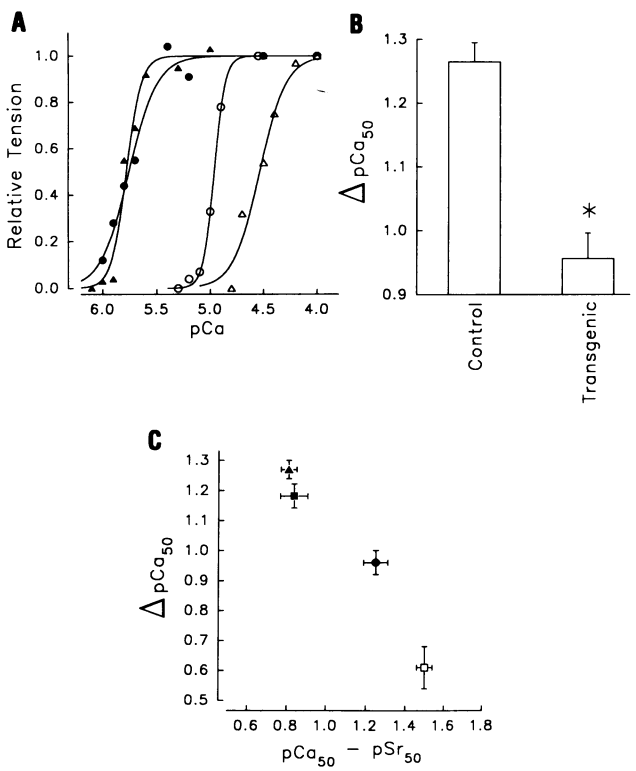

FIG. 5. Effects of reduced pH on the Ca^{2+} sensitivity of contraction in cardiac myocytes from control and transgenic mice. (*A*) Effects on the tension–pCa relationship due to reduced pH in control (triangles) and transgenic (+) (circles) cardiac myocytes. Filled symbols are pH 7.00 and open symbols are pH 6.20. Tensions are normalized to the maximum tension obtained in each cardiac myocyte at the particular pH value (7.00 or 6.20). (*B*) Summary of ΔpCa_{50} values in control and transgenic [Tg (+)] cardiac myocytes. The ΔpCa_{50} values are mean ± SE (n = 7–11). The asterisk indicates the transgenic value is significantly less than the control value ($P < 0.001$). (*C*) Summary of the relationship between ΔpCa_{50} and ($pCa_{50} - pSr_{50}$) values in control (filled triangle), Tg (+) (filled circle), and Tg (−) (filled square) cardiac myocytes and mouse fast skeletal muscle single fibers (open square). Values are mean ± SE.

1.27 ± 0.03 (n = 7) and 0.96 ± 0.04 (n = 11), respectively. Thus, the effect on the Ca^{2+} sensitivity of contraction due to reduced pH was markedly less in transgenic (+) as compared to control cardiac myocytes ($P < 0.001$). Consistent with this result, transgenic (−) cardiac myocytes, which expressed very low levels of sTnC (Fig. 3*F*), were more comparable to control cardiac myocytes in terms of the pH and Sr^{2+} sensitivities of contraction (Fig. 5*C*).

To confirm the role of cTnC in determining the greater pH sensitivity of contraction in cardiac as compared to skeletal muscle, we performed a reciprocal experiment in which endogenous sTnC was extracted from permeabilized fast psoas skeletal muscle fibers (12) and replaced with purified exogenous cTnC. As shown in Table 1, the pH sensitivity of contraction was significantly increased in skeletal muscle fibers reconstituted with cTnC ($P < 0.05$). Thus, differential expression of TnC isoforms alters the pH sensitivity of contraction in cardiac and skeletal muscle.

DISCUSSION

Our findings demonstrate in a cellular preparation that TnC isoforms play an important role in determining the distinct pH sensitivities of contraction of cardiac and skeletal muscle (Figs. 1 and 5). Our results obtained using the isolated cardiac myocyte preparation are supported by earlier biochemical studies on isolated TnC in solution (18). Here, Ca^{2+} binding

Table 1. Effects on the pH sensitivity of contraction in sTnC extracted and cTnC reconstituted fast skeletal muscle fibers

ΔpCa_{50} (pre − post)	Relative tension (P/P_o)		
	Control	sTnC extracted	cTnC reconstituted
−0.11 ± 0.02*	1.00	0.12 ± 0.03	0.99 ± 0.03

Values are mean ± SE ($n = 4$). The ΔpCa_{50} (pre − post) value was calculated by subtracting the ΔpCa_{50} value following cTnC reconstitution from the ΔpCa_{50} value obtained prior to extraction of sTnC from the same rabbit psoas fiber. In a control experiment, there was no effect on the ΔpCa_{50} (pre − post) value following sTnC extraction and sTnC reconstitution in a psoas fiber. Methods for the extraction and reconstitution of TnC have been published (12). Relative tension was obtained by taking tensions at pCa 4.5 (P) at various stages of the experiment as a function of the control tension in each fiber at pCa 4.5 (P_o).

*Value significantly different from zero ($P < 0.05$).

to the low-affinity, regulatory site(s) on TnC was shown to be reduced upon lowering pH in the physiological range, with the effect being greater with cTnC than sTnC (18). The mechanism of this effect is unknown but may relate to the effect of pH to alter the tertiary structure of TnC (19). However, our findings are in apparent conflict with those of an earlier study in which endogenous cTnC was chemically extracted from permeabilized cardiac trabecula preparations and subsequently replaced with exogenous sTnC (20). In that study there was no detected effect of TnC isoforms on the ΔpCa_{50} value. The basis for the differing results obtained by us using the transgenic approach and the earlier study using the extraction/readdition method is not known. However, one possible explanation may relate to the differing range of pH values tested in the two studies. The earlier work tested a narrower range of pH values so that it would be expected that TnC-mediated differences in contractile sensitivity to pH would have been more difficult to detect. A broader pH range was tested in our study, pH 7.00 to 6.20, a pH range consistent with that obtained from working heart preparations during acute myocardial ischemia (4). This broader pH range was likely an important factor in revealing the TnC isoform-dependent effects obtained in our study.

In addition to our new findings which indicate a role for TnC isoforms in determining ΔpCa_{50} values in cardiac myocytes, previous work suggests that troponin I (TnI), the inhibitory subunit of troponin, also plays an important role in determining the sensitivity of striated muscle to acidification. For example, it has been reported that TnI potentiates the effect of low pH to reduce Ca^{2+} binding to TnC with the effect being greater in the presence of the cardiac (cTnI) as compared to the fast skeletal isoform of TnI (18). This finding could account for differences in ΔpCa_{50} between transgenic (+) cardiac myocytes and fast skeletal fibers (Fig. 5*C*). Similarly, despite the fact that both slow skeletal muscle and cardiac muscle express cTnC, the contractile function of slow skeletal fibers, which express the slow skeletal isoform of TnI (ssTnI), is markedly more resistent to acidification than that of cardiac myocytes, which express cTnI (21). This may relate to the finding that in the developing heart the transition from ssTnI to cTnI increases the pH sensitivity of contraction (22). Finally, the differential expression of TnI isoforms may also explain in part why slow and fast skeletal fibers have comparable ΔpCa_{50} values. Overall, the new findings of the present study on TnC isoforms together with early studies on TnI isoforms are consistent with a model in which interactions between specific TnC and TnI isoforms determine the distinct pH sensitivities of contraction of the different striated muscle types.

An additional feature of the effect of low pH on contractile function is that maximum Ca^{2+}-activated force decreases with the effect being greater in cardiac compared to skeletal muscle. Upon lowering pH from 7.00 to 6.20, maximum Ca^{2+}-activated tension was 0.61 ± 0.02 P_o in skeletal fibers and 0.45 ± 0.03 P_o in cardiac myocytes, a value significantly less than the skeletal value (values expressed as a fraction of the maximum tension obtained in the each cell at pH 7.00—i.e., P_o). The inhibition of maximum force appears in part to be related to pH-induced decreases in the number of cross-bridge attachments to actin (23). This is important since previous studies have shown that myosin cross-bridge binding enhances Ca^{2+} binding to TnC and thus plays a key role in activation of the thin filament (24, 25). Thus, another mechanism for the difference in ΔpCa_{50} values between skeletal fibers and transgenic (+) cardiac myocytes (Fig. 5*C*) may be related to a reduced cross-bridge-based component of thin filament activation in transgenic (+) compared to fast skeletal fibers; however, the differences in ΔpCa_{50} values observed between control and transgenic (+) cardiac myocytes appear unrelated to this aspect of activation since the effect of low pH to depress maximum tension was the same in these two groups. Thus, although TnC isoforms play a role in determining ΔpCa_{50} values, they appear to have no effect on pH-induced alterations of maximum Ca^{2+} activated force.

Current pharmacological approaches to the management of ischemic myocardial dysfunction include the use of positive inotropic agents to stimulate myocardial contractility as well as afterload reducers to modify systemic hemodynamics. The studies presented in this report indicate that components of the contractile apparatus including cTnC may represent targets for future therapies designed to treat ischemia-related contractile dysfunction. Therapies might include drugs that modify the sensitivity of cTnC to acidosis as well as genetic approaches to program the expression of sTnC or modified cTnC proteins in cardiac myocytes.

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