Effects of troponin C isoforms on pH sensitivity of contraction in mammalian fast and slow skeletal muscle fibres

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1. The effects of troponin C (TnC) isoforms on the acidic pH-induced rightward shift in the tension–pCa ($-\log[Ca^{2+}]$) relationship were examined in slow soleus and fast psoas skeletal muscle fibres. Endogenous TnC was partially extracted from skinned single fibres and the extracted fibres were subsequently reconstituted with purified TnC. The pCa producing one-half maximal tension (pCa_{50}) was determined at pH 7·00 and 6·20 in each fibre and then the pH-induced shift in pCa_{50} (ΔpCa_{50}) was calculated.

2. In control fast fibres which express fast skeletal TnC (sTnC), the ΔpCa_{50} was $0{\cdot}64 \pm 0{\cdot}02$ pCa units ($n = 10$), and this increased significantly to $0{\cdot}78 \pm 0{\cdot}04$ pCa units ($n = 8$) following extraction and reconstitution with cardiac TnC (cTnC). In each fibre, the reconstituted ΔpCa_{50} was subtracted from the control ΔpCa_{50} which yielded a significant shift of $-0{\cdot}13 \pm 0{\cdot}05$ pCa units ($n = 8$; $P < 0{\cdot}05$). Thus, the pH sensitivity of contraction was increased in the cTnC-reconstituted psoas fibres.

3. In extracted psoas fibres that were reconstituted with fast sTnC the pH sensitivity of contraction was unchanged, indicating that the above effects were related to the TnC isoform and not a non-specific effect of the extraction procedure.

4. In a second series of experiments cTnC was specifically extracted from slow soleus fibres which were subsequently reconstituted with purified fast sTnC. Skeletal TnC reconstituted soleus fibres demonstrated a significant decrease in pH sensitivity. In each fibre, the reconstituted ΔpCa_{50} (mean, $0{\cdot}58 \pm 0{\cdot}02$ pCa units) was subtracted from the control ΔpCa_{50} (mean, $0{\cdot}63 \pm 0{\cdot}02$ pCa units) which yielded a significant shift of $0{\cdot}05 \pm 0{\cdot}01$ pCa units ($n = 4$; $P < 0{\cdot}05$). The pH sensitivity was not altered in cTnC-reconstituted soleus fibres ($-0{\cdot}01 \pm 0{\cdot}01$ pCa units, $n = 4$).

5. These findings indicate that TnC isoforms alter the pH sensitivities of contraction in slow and fast skeletal muscle fibres. However, the magnitude of the change in pH sensitivity is muscle lineage dependent, indicating that differential expression of other myofilament protein isoforms, together with TnC, is necessary to confer full pH sensitivity of contraction in striated muscles.

It is well known that Ca^{2+}-activated contractile function is markedly altered by acidic pH in both cardiac and skeletal muscle. The contractile dysfunction induced by acidic pH includes inhibition of maximum force, reduction in unloaded shortening velocity, altered kinetics of tension redevelopment, and a rightward shift in the tension–pCa relationship indicating a marked desensitization of the contractile apparatus to activation by Ca^{2+} (Donaldson & Hermansen, 1978; Fabiato & Fabiato, 1978; Metzger & Moss, 1987, 1990; Cooke, Franks, Luciani & Pate, 1988; Chase & Kushmerick, 1988; Godt & Nosek, 1989).

The extent to which contractile function is perturbed by acidic pH is highly muscle lineage dependent. For example, acidic pH induces a significantly greater rightward shift in the tension–pCa relationship in cardiac than in skeletal muscle (Donaldson & Hermansen, 1978; Fabiato & Fabiato, 1978; Metzger *et al.* 1993). Because this effect is demonstrated in permeabilized preparations during controlled Ca^{2+} activations, the variation in pH sensitivity must be due to the expression of different myofilament protein isoforms among these muscle types. It is known that myofilament protein isoforms of the thick filament, including myosin heavy and light chains, and the thin filament, including actin, troponin, and tropomyosin, are differentially expressed in cardiac and skeletal muscles (Nadal-Ginard & Mahdavi, 1989). The specific role of the

different contractile and regulatory protein isoforms in determining the pH sensitivity of contraction in striated muscle is of great interest but is not yet completely understood.

There is evidence that subunits of the troponin complex, namely TnC and troponin I, are involved in defining the pH sensitivities of striated muscle. Solaro and colleagues showed that neonatal myocardium is less sensitive to acidosis than adult myocardium, and further provided evidence that this difference is due to the developmental transition from slow skeletal troponin I isoform to the cardiac troponin I isoform in the heart (Solaro, Kumar, Blanchard & Martin, 1986). Solution studies show that acidic pH decreases Ca^{2+} binding to TnC, with the effect being greater for the cardiac than for the fast skeletal TnC isoform (Solaro, El-Saleh & Kentish, 1989; Palmer & Kentish, 1994). In addition, the magnitude of this pH effect increases when TnC is complexed with troponin I (Solaro *et al.* 1989). These findings support the idea that TnC and troponin I isoforms may play a central role in conferring pH sensitivity of Ca^{2+}-activated contraction in muscle.

Further evidence that TnC isoforms are involved in myofilament pH sensitivity comes from a recent study in which the fast skeletal TnC (sTnC) gene was expressed ectopically in the myocardium of transgenic mice (Metzger *et al.* 1993). The acidic pH-mediated shift in the Ca^{2+} sensitivity of tension was markedly less in cardiac myocytes isolated from transgenic mice than in those from control mice. However, this finding that TnC isoforms alter the pH sensitivity of contraction in cardiac muscle appears to differ from an earlier study which concluded TnC isoforms do not influence pH sensitivity of contraction when fast sTnC is exchanged into hamster trabeculae preparations (Gulati & Babu, 1989).

Due to this apparent controversy and the physiological significance of this problem the present study was undertaken to examine further the possible role of TnC isoforms on the pH sensitivity of contraction in striated muscle. The hypothesis tested is that the cardiac isoform of TnC confers increased pH sensitivity of contraction relative to the skeletal isoform in striated muscles. To address this possibility, rabbit psoas fibres, which express the fast skeletal isoform of TnC, and rat slow soleus fibres, which express the cardiac isoform of TnC, were examined in the present study. Fibres were studied in the control state and following partial TnC extraction and reconstitution with purified TnC isoforms. The purpose of this comparative approach was to determine if effects of TnC isoforms on pH sensitivity of contraction are a general feature of striated muscle contractility. It is known that slow soleus fibre contractility is rather less affected by acidic pH than cardiac muscle, even though they express many of the same contractile isoforms. For example, the magnitude of the acidic pH-induced shift in the mid-point of the tension–pCa relationship is greater in cardiac than in slow soleus muscle (Metzger & Moss, 1987; Metzger *et al.* 1993). However, the possible effects of TnC exchange on the pH sensitivity of contraction in soleus fibres has not been determined. Because fast sTnC isoform expression in cardiac myocytes has a marked effect on pH sensitivity it was of interest to test if pH sensitivity could be reduced further in soleus fibres reconstituted with fast sTnC.

METHODS

Muscle fibre preparations and experimental apparatus

Fast-twitch skeletal muscle fibres were obtained from psoas muscles of adult male New Zealand rabbits as detailed previously (Metzger, Greaser & Moss, 1989). These fibres contain fast isoforms of contractile and regulatory proteins as determined previously (Metzger & Moss, 1990). Slow skeletal fibres, expressing the slow isoforms of contractile and regulatory proteins (Metzger & Moss, 1987), were obtained from the soleus muscles of adult female rats. Prior to isolation of muscles, animals were killed by lethal dose of sodium pentobarbitone (100 mg kg^{-1} I.P.) under guidelines approved by the University of Michigan Unit for Laboratory Animal Medicine. Bundles of approximately fifty fibres were dissected from each muscle while in relaxing solution (for composition, see below) and tied with surgical silk to glass capillary tubes. Bundles were stored for up to 3 weeks at −20 °C in relaxing solution containing 50% (v/v) glycerol.

The single fibre attachment procedure involved mounting the fibre between a force transducer (model 400A; Cambridge Technology, Inc., Watertown, MA, USA; noise level at the output equivalent to 1 mg wt peak-to-peak) and a high performance moving-coil galvanometer (model 6350; Cambridge Technology, Inc.). Sarcomere length was set at 2·50–2·60 μm. Complete details of the mounting procedure and experimental set-up have been reported elsewhere (Metzger *et al.* 1989).

Relaxing and Ca^{2+}-activating solutions

Relaxing and activating solutions contained (mM): 7 EGTA, 1 free Mg^{2+}, 4 MgATP, 14·5 creatine phosphate, 20 imidazole, and sufficient KCl to yield a total ionic strength of 180 mM. Solution pH was adjusted to 7·00 or 6·20 using KOH/HCl at 15 °C. The pCa (i.e. $-\log [Ca^{2+}]$) of the relaxing solution was 9·00, while the pCa of the solution for maximal activation was 4·50. A. Fabiato's computer program (1988) was used to calculate the final concentrations of each metal, ligand, and metal–ligand complex, employing the stability constants listed by Godt & Lindley (1982). The apparent stability constant for Ca-EGTA was corrected for ionic strength, pH and experimental temperature (Fabiato, 1988).

Troponin C extraction and reconstitution protocol

In each single fibre segment studied data were collected prior to extraction and after extraction and reconstitution, thus permitting each fibre to serve as its own control. TnC was specifically extracted from the troponin complex using a solution containing 5 mM EDTA, 10 mM Hepes and 500 μM trifluoperazine dihydrochloride (TFP; Smith, Kline & French Laboratories, Philadelphia, PA, USA) as described previously (Metzger *et al.* 1989). The extracting solution was derived from Cox, Compte & Stein (1981). Following extraction of TnC each preparation was washed multiple times in relaxing solution to completely remove TFP

because it has been shown that Ca^{2+}-activated contraction is altered in the presence of TFP (Kurebayashi & Ogawa, 1988).

Psoas fibres were reconstituted with purified cardiac TnC (cTnC) or fast sTnC (0·1–0·3 mg ml^{-1}) by incubating the extracted fibre for 10–20 s in a TnC-containing relaxing solution (Metzger *et al.* 1989). Repeated exposures to the TnC solution were continued until tension at pCa 4·5 reached a plateau. In psoas fibres, the total time of exposure to the TnC-containing solution was approximately 2–3 min. Soleus fibres were reconstituted with purified fast sTnC (from rabbit fast muscle) or cTnC (from chicken heart) (0·1 mg ml^{-1}) using a similar protocol. The cTnC and fast sTnC were kindly provided by Drs Richard Moss and Marion Greaser (University of Wisconsin, WI, USA) and Dr Jeffery Leiden (University of Chicago, IL, USA).

Determination of protein composition by gel electrophoresis

Each skeletal fibre segment was placed in a 0·5 ml microfuge tube containing sodium dodecyl sulphate (SDS) sample buffer (10 μl (mm segment length)$^{-1}$) and stored at −80 °C for analysis of contractile and regulatory protein content by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE), and scanning densitometry, as described previously (Moss, Lauer, Giulian & Greaser, 1986; Metzger & Moss, 1991). Briefly, the gel electrophoresis procedure utilized a multiphasic buffer system that incorporated the following features: (1) acrylamide : *N,N'*-methylene-bis-acrylamide ratio of 200 : 1; (2) pH 9·3 for running gel buffer; and (3) running gel buffer molarity of 0·75 M. The acrylamide content of the running gel was 12%. Gels were fixed with glutaraldehyde overnight, washed, silver stained, and dried

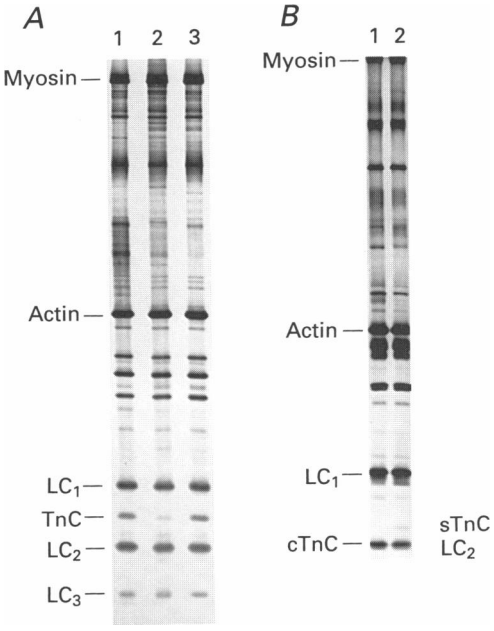

Figure 1

A, SDS–polyacrylamide gels obtained from segments of the same psoas fibre before (lane 1) and after (lane 2) partial extraction of TnC, and following reconstitution with fast sTnC (lane 3). To quantify the extent of extraction of TnC, the TnC/(LC_1 + LC_3) value was determined for control and extracted fibres by measuring the areas under the peaks corresponding to these protein using a densitometer; LC_1 and LC_3 are myosin light chain isoforms. The ratio obtained from the extracted fibre was then divided by the control value to determine the amount of TnC extracted (Metzger & Moss, 1991). Calculated this way, 79% of endogenous TnC was extracted. The LC_2/(LC_1 + LC_3) value was highly comparable in the control (0·64), extracted (0·69), and reconstituted (0·64) state, indicating that the extraction was specific for TnC. *B*, SDS–polyacrylamide gels of segments of the same soleus fibre before extraction of TnC (lane 1) and after partial extraction and subsequent reconstitution with fast sTnC (lane 2). Under these conditions, the cTnC isoform co-migrated with LC_2. However, the presence of the fast sTnC isoform is readily apparent in the reconstituted fibre shown in lane 2. Densitometric scans were performed as described above and showed that the LC_2/(LC_1 + LC_2) value was similar in the control (0·61, lane 1) and extracted/reconstituted (0·58, lane 2) state. This is evidence that the extraction protocol was specific for TnC as was the case for fast fibres as shown above in *A*. In other experiments it was possible to separate the TnC and LC_2 bands by extending the gel run time. In one such experiment the TnC/(LC_1 + LC_2) value was determined for control and extracted fibres, and the ratio obtained from the extracted fibre was divided by the control value to estimate the amount of TnC extracted. Calculated this way, 70% of endogenous TnC was extracted, and the LC_2/(LC_1 + LC_2) value was essentially unchanged by extraction (0·52 for control and 0·51 for extracted).

between mylar and cellophane sheets. In some instances fibre segments were obtained at each stage of the experiment (control, extracted, and reconstituted) for subsequent analysis of protein composition by SDS–PAGE. Gels were analysed by measuring the areas under the peaks corresponding to TnC, and myosin light chains using an Ultroscan XL densitometer (Pharmacia, Piscatawy, NJ, USA). Figure 1 shows gels obtained from segments of a single psoas fibre in the control, extracted and sTnC-reconstituted states. Figure 1 also shows gels of segments of a single soleus fibre obtained prior to extraction of TnC and after TnC extraction and fast sTnC reconstitution. These results indicate that the extraction procedure was specific for the removal of TnC in both fast and slow fibres. Similar results were obtained in experiments in which extracted psoas fibres were reconstituted with cTnC and in extracted soleus fibres reconstituted with cTnC (data not shown).

Curve fitting and statistics

Curve fitting. To derive values for the mid-point (termed pCa_{50} or K) and Hill coefficient (n_H) from the tension–pCa relationships data were fitted using the Marquardt–Levenberg non-linear least-squares fitting algorithm using the Hill equation in the form:

$$P_r = [Ca^{2+}]^{n_H}/(K^{n_H} + [Ca^{2+}]^{n_H}),$$

where P_r is the fraction of maximum tension obtained at pCa 4·5.

Statistics. Analysis of variance (ANOVA) was used to determine significant differences between the multiple groups studied (e.g.

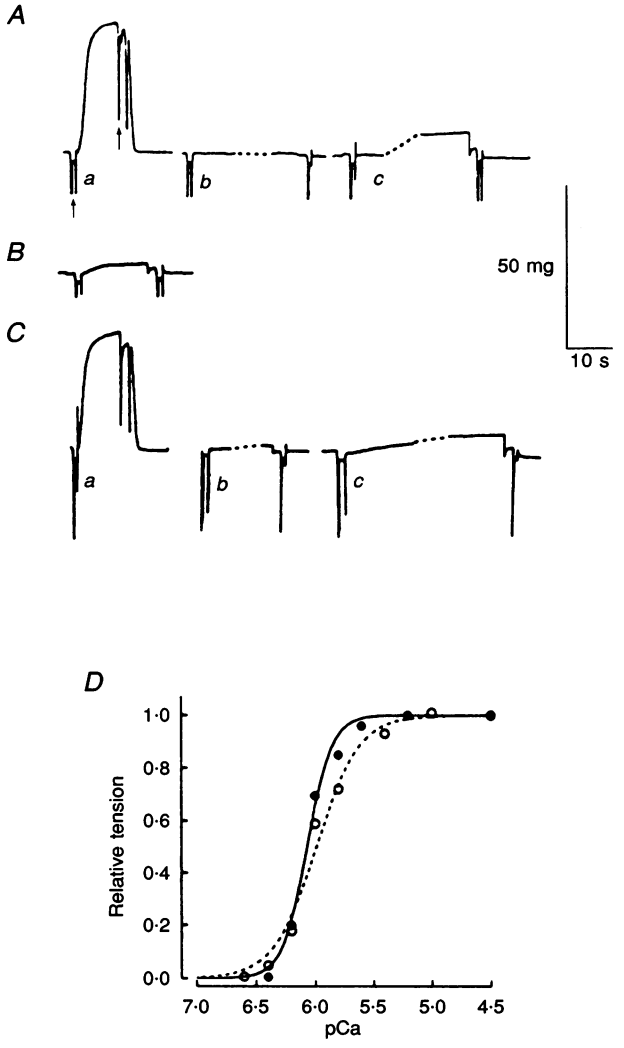

Figure 2. Mechanical effects of cTnC exchange in a skeletal fibre at pH 7·00

A–D are from the same psoas fibre. *A–C*, slow time base recordings of isometric tension obtained from a single psoas fibre. *A*, control activations at pCa 4·5 (*a*, first arrow indicates change of solution from pCa 9·0 to 4·5, second arrow indicates release of fibre length to determine tension baseline which is not resolved on these records), pCa 6·4 (*b*), and pCa 6·2 (*c*). *B*, tension at pCa 4·5 following partial extraction of TnC. $P/P_0 = 0·06$, P/P_0 was determined by dividing tension obtained at pCa 4·5 by the control tension at pCa 4·5 in the same fibre. *C*, tension records after reconstitution with cTnC. $P/P_0 = 0·95$. The pCa values for parts *a–c* are 4·5, 6·4, and 6·2, respectively. Dotted lines in records in *A* and *C* represent about 1–2 min. *D*, tension–pCa relationship in the same fibre shown in parts *A–C*; ●, control ($pCa_{50} = 6·07$, $n = 4·1$); ○, reconstituted ($pCa_{50} = 5·99$, $n = 2·4$); fibre length, 2·03 mm; sarcomere length, 2·50 μm; P/P_0, 219 kN m^{-2}.

Table 1. Mechanical properties of single psoas fibres following extraction of sTnC and reconstitution with cTnC at pH 7·00

	P/P_0	pCa_{50}	n_H	$\Delta pCa_{50(7-7)}$	$\Delta n_{H(7-7)}$
Control	1·00	5·99 ± 0·06	2·8 ± 0·2	n.a.	n.a.
TnC extracted	0·12 ± 0·02	n.a.	n.a.	n.a.	n.a.
Reconstituted	0·99 ± 0·02	6·04 ± 0·02	2·2 ± 0·1 †	−0·02 ± 0·05	0·58 ± 0·20 *

Values are means ± S.E.M., $n = 8–10$. P/P_0 was determined by dividing tension obtained at pCa 4·5 by the control tension at pCa 4·5 in the same fibre. $\Delta pCa_{50(7-7)}$ was calculated by subtracting the reconstituted pCa_{50} at pH 7·00 from the control pCa_{50} at pH 7·00 in the same fibre. $\Delta n_{H(7-7)}$ was calculated by subtracting the reconstituted n_H at pH 7·00 from the control n_H at pH 7·00 in the same fibre. Thus a $\Delta n_{H(7-7)}$ of 0·00 indicates no change following reconstitution. * Significantly different from 0·00 ($P < 0·05$). † Hill coefficient significantly lower in reconstituted than in control group ($P < 0·05$). n.a., not applicable.

maximum tension in control, extracted and reconstituted fibres). When interactions among the groups were indicated by ANOVA, Student's two-tailed t test was used as a *post-hoc* test to determine significant differences between two mean values using Bonferroni corrected values for multiple comparisons. Unless indicated otherwise values are reported as means ± S.E.M. A probability level of $P < 0·05$ was selected as indicating significance.

RESULTS

Effects of cTnC exchange on the tension–pCa relationship in psoas fibres

The effects of cTnC exchange on Ca^{2+}-activated tension at pH 7·00 in a single psoas fibre are shown in Fig. 2. In this experiment TnC was extracted such that tension at pCa 4·5 decreased by 94% relative to the value at pCa 4·5 prior to extraction. The fibre was then reconstituted with purified cTnC and tension at pCa 4·5 increased to $0·95P_0$, where P_0 is the maximum tension obtained in the untreated fibre. In this fibre the steepness of the tension–pCa relationship decreased from an n_H value of 4·1 to 2·4 following extraction and reconstitution, a result which was confirmed in additional experiments (Table 1). This effect of cTnC exchange in psoas fibres at pH 7·00 is in general agreement with earlier results obtained by Moss *et al.* (1986).

Qualitatively similar differences in the shape of the tension–pCa relationship in control compared with reconstituted psoas fibres were also apparent at pH 6·20. Steady-state isometric tensions were generally lower at intermediate concentrations of Ca^{2+}, resulting in a reduced steepness in the tension–pCa relationship in reconstituted (n_H, 2·8 ± 0·4; $n = 8$) compared with control (n_H, 3·4 ± 0·4; $n = 9$) psoas fibres at pH 6·20.

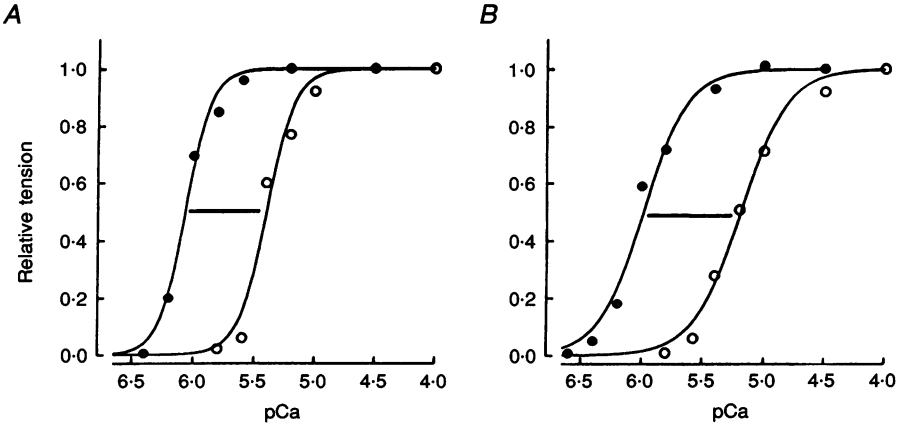

Figure 3. Alterations in pH-mediated shift in the tension–pCa relationship due to cTnC exchange in a single psoas fibre

A, effects of altered pH on the tension–pCa relationship in a control fibre. *B*, effects of altered pH on the tension–pCa relationship after cTnC reconstitution (same fibre as in *A*). pH values are: ●, 7·00; and ○, 6·20. The pH-dependent shift in pCa_{50} (ΔpCa_{50}, indicated by horizontal line) was 0·67 pCa units in *A* and 0·80 pCa units in *B*. After partial TnC extraction maximum isometric tension was $0·06P_0$. Maximum tension increased to $0·95P_0$ after reconstitution with cTnC. Sarcomere length, 2·50 μm; end-to-end length, 2·03 mm.

Table 2. Mechanical properties of single soleus fibres following extraction of cTnC and reconstitution with fast sTnC at pH 7·00

	P/P_0	pCa_{50}	n_H	$\Delta pCa_{50(7-7)}$	$\Delta n_{H(7-7)}$
Control	1·00	6·05 ± 0·01	1·7 ± 0·2	n.a.	n.a.
TnC extracted	0·56 ± 0·12	n.a.	n.a.	n.a.	n.a.
Reconstituted	0·86 ± 0·05	6·01 ± 0·04	1·6 ± 0·1	0·06 ± 0·03	0·06 ± 0·17

Values are means ± S.E.M., $n = 4$. P/P_0 was determined by dividing tension obtained at pCa 4·5 by the control tension at pCa 4·5 in the same fibre. $\Delta pCa_{50(7-7)}$ and $\Delta n_{H(7-7)}$ calculated as described in Table 1.

A comparison of the effects of acidic pH on the tension–pCa relationship before and after cTnC reconstitution of a psoas fibre is presented in Fig. 3. One of the main effects of TnC exchange was an increase in the pH-mediated shift in pCa_{50} (ΔpCa_{50}, calculated by subtracting the pCa_{50} at pH 6·20 from the pCa_{50} at pH 7·00 in each fibre). In this fibre, ΔpCa_{50} increased from a control value of 0·67 to 0·80 pCa units following reconstitution with cTnC.

In additional experiments it was shown that the increase in ΔpCa_{50} observed in cTnC-reconstituted psoas fibres was significant. In each fibre the postexchange ΔpCa_{50} (reconstituted) was subtracted from the control ΔpCa_{50} (native) to calculate $\Delta pCa_{50(n-r)}$ (Table 3). The mean $\Delta pCa_{50(n-r)}$ was −0·13 ± 0·05 pCa units ($n = 8$), a value significantly less than zero ($P < 0·05$). In summary, these results indicate an increase in the pH sensitivity of contraction in cTnC-reconstituted compared with control psoas fibres.

In control experiments, TnC was extracted from psoas fibres and the fibres were subsequently reconstituted with fast sTnC. Here, maximum Ca^{2+}-activated tension was 0·15 ± 0·07P_0 after extraction, and 0·98 ± 0·02P_0 after reconstitution with fast sTnC ($n = 4$). Effects of acidic pH on the tension–pCa relationship in a TnC-extracted, fast sTnC-reconstituted psoas fibre are shown in Fig. 4. Results indicate that at both pH 7·00 and pH 6·20 the tension–pCa relationships are virtually identical in the control and reconstituted state. In these control studies the $\Delta pCa_{50(n-r)}$ was 0·026 ± 0·025 ($n = 4$), a value not significantly different from zero (Table 3). This provides good evidence that the observed effects of cTnC exchange on ΔpCa_{50} are due to the TnC isoform and not due to a non-specific effect of the extraction–reconstitution procedure on fibre contractility.

Effects of fast sTnC exchange on the tension–pCa relationship in slow soleus fibres

Rat soleus muscles were used in these experiments which have approximately 90% slow type I fibres. As was done previously, the isolated soleus fibres were run on SDS–polyacrylamide gels to verify that these fibres expressed the slow isoforms of contractile and regulatory proteins, including cTnC (Metzger & Moss, 1987).

In comparison to the psoas fibre study, a reduced amount of TnC was extracted from soleus fibres as judged by the residual tension during pCa 4·5 activation (Table 2) and by gel analysis (Fig. 1). Reconstitution of the extracted fibres with fast sTnC significantly increased tension during maximum Ca^{2+} activation (0·86 ± 0·05P_0, $n = 4$). Although greater apparent extraction of TnC was possible in these soleus fibres (tension < 0·20P_0), subsequent reconstitution with fast sTnC did not sufficiently restore function (< 0·40P_0). These fibres were not used in the analysis due to the possibility of irreversible or non-specific effects of the extraction procedure on mechanical function (Moss, 1992).

Results showed that fast sTnC-reconstituted soleus fibres exhibited a small but statistically significant decrease in their sensitivity to acidic pH. More specifically, the magnitude of the acidic pH-induced shift in the tension–pCa relationship was significantly reduced in the reconstituted soleus fibres (Fig. 5). In this fibre ΔpCa_{50} decreased from a

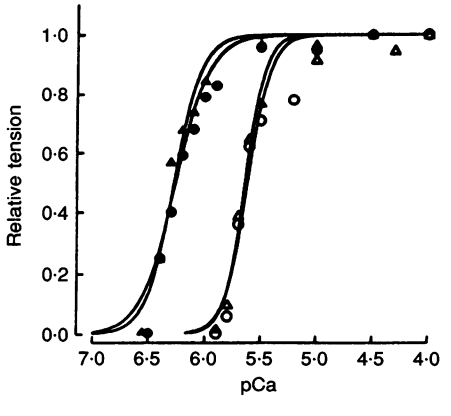

Figure 4. Control experiment examining effects on the tension–pCa relationship in a psoas fibre reconstituted with fast sTnC

The pH-mediated shift in the tension–pCa relationship before (circles) and after (triangles) reconstitution with fast sTnC in a single psoas fibre is shown. Filled symbols are results at pH 7·00, open symbols are results at pH 6·20. In this experiment the control ΔpCa_{50} value minus the reconstituted ΔpCa_{50} value was −0·026.

Table 3. Ca^{2+}-activated tension development in psoas and soleus skeletal fibres following extraction of TnC and reconstitution with either sTnC or cTnC

	P/P_0			
	Control	Extract	Reconstituted	$\Delta pCa_{50(n-r)}$
Psoas				
sTnC extract/ cTnC reconst.	1·00	0·07 ± 0·09 (8)	1·003 ± 0·002(8)	−0·130 ± 0·050 (8)*†
sTnC extract/ sTnC reconst.	1·00	0·15 ± 0·07 (4)	0·98 ± 0·02 (4)	0·026 ± 0·025 (4)
Soleus				
cTnC extract/ sTnC reconst.	1·00	0·56 ± 0·12 (4)	0·86 ± 0·05 (4)	0·05 ± 0·01 (4)*†‡
cTnC extract/ cTnC reconst.	1·00	0·56 ± 0·17 (4)	0·93 ± 0·05 (4)	−0·01 ± 0·01 (4)
cTnC extract	1·00	0·83 ± 0·04 (4)	n.a.	−0·02 ± 0·01 (4)

Values are mean ± S.E.M. (n). P/P_0 was determined by dividing tension obtained at pCa 4·5 in the control, extracted or reconstituted (reconst.) state, by the control tension at pCa 4·5 in the same fibre. $\Delta pCa_{50(n-r)}$ was calculated in each fibre by subtracting the reconstituted ΔpCa_{50} from the native value, with the exception of the soleus cTnC extract value where ΔpCa_{50} after extraction was subtracted from control in each fibre. * Significantly different from 0·00 ($P < 0·05$). For psoas fibres, † indicates values significantly different from sTnC extract/sTnC reconstituted value ($P < 0·05$). For soleus fibres, † indicates values significantly different from cTnC extract/cTnC reconstituted value, and ‡ indicates values significantly different from cTnC extract value ($P < 0·05$). n.a., not applicable.

control value of 0·67 pCa units to 0·60 pCa units following reconstitution with fast sTnC. In reconstituted fibres, $\Delta pCa_{50(n-r)}$ was 0·05 ± 0·01 pCa units ($n = 4$), a value significantly different from zero ($P < 0·05$; Table 3). This indicates a decrease in pH-mediated alterations in pCa_{50} in the fast sTnC-reconstituted slow soleus fibres.

In control experiments the pH sensitivity of contraction was determined in TnC-extracted and cTnC-reconstituted soleus fibres. In these experiments the relative tensions in the extracted and reconstituted condition were 0·56 ± 0·17P_0 and 0·93 ± 0·05P_0 ($n = 4$), respectively, values highly comparable to those obtained for the fast

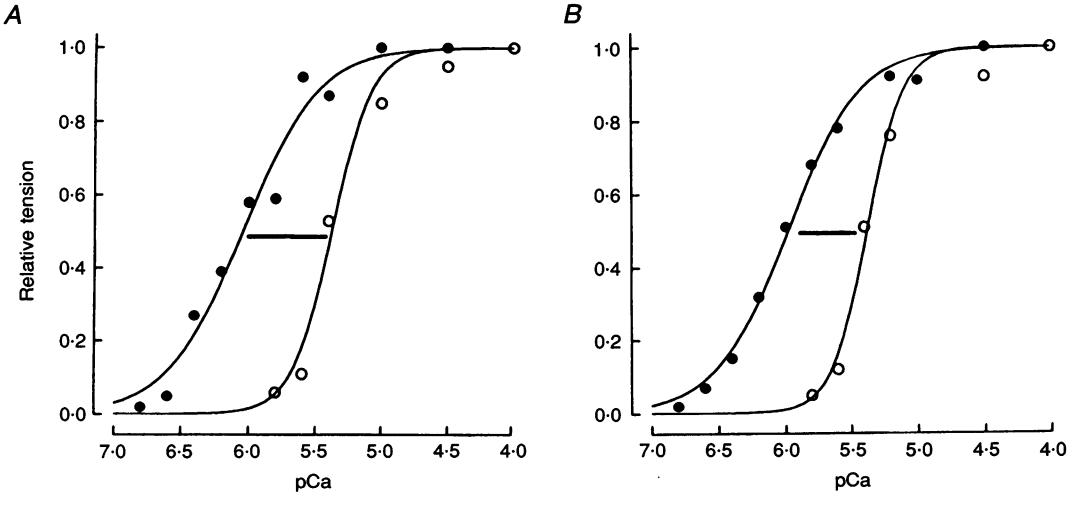

Figure 5. Alterations in pH-mediated shift in the tension–pCa relationship due to partial fast sTnC exchange in a single slow soleus fibre

A, effects of altered pH on the tension–pCa relationship in a control fibre. *B*, effects of altered pH on the tension–pCa relationship after fast sTnC exchange (same fibre as in *A*). ●, results at pH 7·00; ○, results at pH 6·20. The pH-dependent shift in pCa_{50} (ΔpCa_{50}, indicated by horizontal line) was 0·67 pCa units in *A* and 0·60 pCa units in *B*. After partial TnC extraction maximum isometric tension was 0·78P_0. Maximum tension increased to 0·94P_0 after reconstitution with fast sTnC. Sarcomere length, 2·50 μm; end-to-end length, 1·7 mm.

sTnC-reconstituted soleus fibres (Table 3). The value obtained for native ΔpCa_{50} minus cTnC-reconsituted ΔpCa_{50} was $-0{\cdot}01 \pm 0{\cdot}01$ pCa units ($n = 4$), which was significantly different from the value obtained for native ΔpCa_{50} minus sTnC-reconstituted ΔpCa_{50} (Table 3).

In a second series of control experiments TnC was extracted from soleus fibres so that the relative tension after extraction approximated the level of tension that was obtained in the cTnC-extracted and fast sTnC-reconstituted soleus fibres (Table 3). In these partially TnC-extracted fibres, the value for native ΔpCa_{50} minus extracted ΔpCa_{50} was not different from zero (Table 3). Taken together, these results are evidence that the altered pH sensitivity of contraction obtained in fast sTnC-reconstituted soleus fibres is not due to a non-specific effect of the extraction–reconstitution procedure or due to incomplete TnC reconstitution.

DISCUSSION

The results of this study indicate that the magnitude of pH-mediated alterations in the Ca^{2+} sensitivity of tension is influenced, at least in part, by TnC isoforms in both fast psoas and slow soleus skeletal muscle fibres. The acidic pH-induced shift in the tension–pCa relationship is significantly greater in psoas fibres reconstituted with cTnC. In keeping with this finding, the pH sensitivity of contraction is reduced in fast sTnC-reconstituted slow soleus fibres. The pH sensitivity of contraction was not significantly altered in either cTnC-reconstituted soleus fibres or fast sTnC-reconstituted psoas fibres. Taken together, these findings provide evidence that TnC isoforms play a role in acidic pH-mediated contractile dysfunction in striated muscle fibres.

The mechanism by which TnC isoforms alter the pH sensitivity of contraction is not known. One possibility is that the extent of acidic pH-mediated reduction in Ca^{2+} binding to the low-affinity, regulatory binding site(s) on TnC is isoform dependent. Studies on isolated regulatory proteins and on isolated myofilaments indicate that Ca^{2+} binding to the low-affinity, regulatory site(s) on TnC is reduced upon lowering pH in the physiological range (El-Saleh & Solaro, 1988; Solaro *et al.* 1989). Reduced Ca^{2+} binding to TnC could account for the observed decrease in the Ca^{2+} sensitivity of tension due to acidic pH. Solution studies further provide evidence that acidic pH decreases Ca^{2+} binding affinity of TnC to a greater extent in the isolated cardiac isoform than in the skeletal isoform of TnC (Solaro *et al.* 1989; Palmer & Kentish, 1994). This effect may provide the basis of the TnC isoform-dependent effects reported here in skinned single fibres. Acidic pH may exert this effect by altering the three-dimensional structure of TnC. It has been shown that upon reduction in pH from 6·8 to 5·0 the distance between the N-terminal and C-terminal globular domains of the TnC molecule increases (Wang, Zhan, Tao & Gergely, 1987). Thus pH-mediated alterations in the structure of TnC could provide a mechanism of altered Ca^{2+} binding properties of this protein under acidic conditions.

The results of the present study are consistent with our earlier work showing an effect of fast sTnC to reduce pH-mediated alterations in the tension–pCa relationship in cardiac myocytes from transgenic mice (Metzger *et al.* 1993). However, the decrease in the pH sensitivity of contraction is greater in the transgenic cardiac myocytes than in the fast sTnC-reconstituted soleus fibres. This difference could in part be attributed to the apparent reduced extent of fast sTnC-reconstitution into soleus fibres (see Results) compared with the transgenic cardiac myocytes. However, this comparison may not be straightforward because the precise extent of TnC isoform remodelling could not be fully determined in our earlier study (Metzger *et al.* 1993). Also, it was possible to accomplish marked cTnC isoform exchange into psoas fibres, and while the pH sensitivity was increased in these fibres, the magnitude of pH-induced alteration in tension was significantly less than that found in native cardiac myocytes (Metzger *et al.* 1993). Another observation that needs to be considered is that native soleus fibres are less affected by acidic pH than native cardiac myocytes, even though they both express the cardiac isoform of TnC (Metzger & Moss, 1987; Metzger *et al.* 1993). These findings clearly indicate that TnC isoforms alone cannot fully account for the differing pH sensitivities of contraction in cardiac, slow and fast muscles. The inability of cTnC exchange to fully confer cardiac-type pH sensitivity in fast fibres indicates that additional protein subunits, together with TnC, are required to confer full pH sensitivity of contraction in striated muscles. In this regard, Solaro and colleagues provide strong evidence that troponin I, the inhibitory subunit of troponin, plays an essential role in defining the pH sensitivity of striated muscle. They showed that troponin I potentiates the decrease in Ca^{2+} binding to TnC that is induced by acidic pH (El-Saleh & Solaro, 1988; Solaro *et al.* 1989). In addition they showed that the developmental transition from the slow skeletal to the cardiac isoform of troponin I is correlated with increased pH sensitivity of contraction in heart (Solaro *et al.* 1986). More recently, this group studied isolated troponin subunits in solution and showed that while the cardiac troponin I isoform potentiates acidic pH-induced decreases in the Ca^{2+} affinity of cTnC, the slow skeletal isoform had no such effect (Wattanapermpool, Reiser & Solaro, 1995). This indicates that isoforms of troponin I play an important role in defining pH sensitivity. One of the main differences in primary sequence between the slow skeletal and cardiac troponin I isoforms resides in the N-terminus of the molecule: the N-terminus of the cardiac isoform contains a twenty-four to thirty-two amino acid extension (depending on species) that is not present in the slow skeletal isoform.

However, recent mutagenesis experiments indicate that deletion of the thirty-two amino acid extension in murine cardiac troponin I has no effect on pH-mediated alterations in the MgATPase–pCa relationship in reconstituted cardiac myofibrils (Guo, Wattanapermpool, Palmiter, Murphy & Solaro, 1994). Whereas these findings show that the N-terminus is not involved in conferring pH sensitivity, these results do not rule out the possibility that other regions of troponin I are involved in conferring pH sensitivity to striated muscles.

Our present and earlier (Metzger *et al.* 1993) findings, which indicate a role for TnC isoforms in conferring, at least in part, pH sensitivity of contraction in striated muscle preparations, are in apparent conflict with a report in which hamster cardiac trabecula preparations were reconstituted with fast sTnC (Gulati & Babu, 1989). The latter study concluded that TnC isoforms have no effect on pH sensitivity of contraction in cardiac muscle. While the present study was nearing completion two reports were published which lend further support to the idea that TnC isoforms define, at least in part, the pH sensitivity of striated muscle. Ball, Johnson & Solaro (1994) reported an increase in the magnitude of the acidic pH-induced shift in the ATPase–pCa relationship in psoas myofibrils that were reconstituted with cTnC, a result highly comparable with our findings. Because the ATPase–pCa and tension–pCa relationships may not necessarily parallel each another under conditions of altered pH (Fig. 6*A* in Ebus, Stienen & Elzinga, 1994) it was important to show in the present study that the TnC isoforms alter the acidic pH-induced shift in the tension–pCa relationship as shown earlier for the ATPase–pCa relationship (Ball *et al.* 1994). In addition, Ding, Akella & Gulati (1995) recently showed a decrease in the pH sensitivity of cardiac trabeculae preparations that were reconstituted with fast sTnC, a result consistent with our earlier work (Metzger *et al.* 1993). These results, together with those of the present study, demonstrate that TnC isoforms alter the pH sensitivity of contraction in striated muscles. It is also apparent from these studies that muscle-type-dependent interactions between TnC and troponin I isoforms are critical in establishing the differential effect of acidic pH on Ca^{2+} sensitivity of contraction in cardiac, slow and fast muscles.

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