The Control of Myocardial Contraction with Skeletal Fast Muscle Troponin C*

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The present study describes experiments on the myocardial trabeculae from the right ventricle of Syrian hamsters whose troponin C (TnC) moiety was exchanged with heterologous TnC from fast skeletal muscle of the rabbit. These experiments were designed to help define the role of the various classes of Ca\(^{2+}\)-binding sites on TnC in setting the characteristic sensitivities for activations of cardiac and skeletal muscles. These trabeculae were skinned and about 75% of their troponin C extracted by chemical treatment. Tension development on activations by Ca\(^{2+}\) and Sr\(^{2+}\) was found to be nearly fully blocked in such TnC extracted preparations. Troponin C contents and the ability to develop tension on activations by Ca\(^{2+}\) and Sr\(^{2+}\) was permanently restored after incubation with 2–6 mg/ml purified TnC from either rabbit fast-twitch skeletal muscle (STnC) or the heart (CTnC, cardiac troponin C). The native (skinned) cardiac muscle is characteristically about 5 times more sensitive to activation by Sr\(^{2+}\) than fast muscle, but the STnC-loaded trabeculae gave response like fast muscle. Attempts were also made to exchange the TnC in psoas (fast-twitch muscle) fibers, but unlike cardiac muscle tension response of the maximally extracted psoas fibers could be restored only with homologous STnC. CTnC was effective in partially extracted fibers, even though the uptake of CTnC was complete in the maximally extracted fibers. The results in this study establish that troponin C subunit is the key in setting the characteristic sensitivity for tension control in the myocardium above that in the skeletal muscle. Since a major difference between skeletal and cardiac TnCs is that one of the trigger sites (site I, residues 28–40 from the N terminus) is modified in CTnC and has reduced affinity for Ca\(^{2+}\) binding, the possibility is raised that this site has a modulatory effect on activation in different tissues and limits the effectiveness of CTnC in skeletal fibers.

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1 The abbreviations used are: TnC, troponin C; TnI, troponin I; TnT, troponin T; STnC, skeletal fast-twitch muscle TnC; CTnC, cardiac TnC; LCs, light chains (C-LC1 and C-LC2 of cardiac myosin) and S-LC3 of fast skeletal myosin); EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N',N"-tetraacetic acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; pK, pCa or pSr for half-maximal activation; P", tension made in pCa 4 (180 mM ionic strength) by the native preparations.
of the Ca$^{2+}$-specific sites in defining the activation characteristics in skeletal and cardiac muscles and thereby to gain further insights into the nature of the conformational changes in TnC moiety needed to initiate muscular contraction. The present study with myocardium seemed particularly attractive because recent similar attempts, in the converse experiment using skeletal muscle with C-TnC, gave variable results and led to conclusions diametrically opposite of each other (Kerrick et al., 1985; Moss et al., 1986). Several new studies of TnC exchange were also made with skeletal fibers in an effort to seek plausible explanations for these differences.

Our results at near physiological ionic strength (180–190 mM) demonstrate the possibility of significant plasticity in the activation mechanism of the cardiac muscle contractile apparatus. On the basis of these findings the differences in the half-maximal activations for tension generation by divalent metal ions in different tissues could be positively assigned to the possible variations in the properties of the regulatory sites of TnC subunits. In addition, the modification of a trigger site (I) in cardiac TnC appeared to be critical for the tension control in skeletal muscle in 180 mM salt.

**MATERIALS AND METHODS**

**Fiber Preparations—**Skinned preparation of adult (6-9-month old) Syrian hamsters (Strain RB, Dr. M. J. Sole, University of Toronto) consisted of 60–150 μm (width) by 1–3 mm (length) trabeculae from the right ventricle. The skeletal single fiber preparation (30–80 μm wide) was from the psoas muscle. Skinning was accomplished by 30-min treatment at 10°C with 0.5% Lubrol-WX detergent in 140 mM KCl, 10 mM imidazole, 5 mM MgCl$_2$, 5 mM ATP, 5 mM EGTA, 5 mM creatine phosphate. The relaxing and activating solutions contained about 100 mM KCl, 20 mM imidazole, 5 mM MgCl$_2$, 5 mM ATP, 20 mM creatine phosphate and 250 units/ml of creatine phosphokinase, and either EGTA or Ca-EGTA, Sr-EGTA. The pH of each solution was adjusted to 7.00 ± 0.01 at the appropriate temperature. The ionic strength of the solutions was kept between 180–190 mM, which is close to the physiological range.$^2$ Free Mg$^{2+}$ was kept at 1 mM, which is also close to the in vivo value (Gupta and Moore, 1981; Baylor et al., 1982).

**Preparation of TnC-extracted Fibers—**To achieve TnC extraction from both skeletal and cardiac preparations, they were first transferred from the relaxing solution at 4°C to a rigor solution containing 20 mM imidazole, 165 mM KCl, 2.5 mM EGTA, 2.5 mM EDTA, and pH 7.0. The temperature was raised to 30°C and, after 5 min, the preparations were placed in the relaxing solution (5 mM EDTA, 10 mM imidazole, and pH 7.2) at 30°C (Babu et al., 1986). The preparations were returned to the relaxing solution and checked for tension response in pCa$_4$ (20°C), at intervals of 5 min in the extraction solutions; extraction was stopped when the tension was down to 0–10% of the native fiber. This is referred to as “maximal” extraction in the present study. Extraction time for the trabeculae in the present study was 20–50 min, and for the skeletal fibers from 5–30 min. In a few cases, the skeletal fiber preparation was extracted at 4°C and this is pointed out in the text when applicable. Reconstitution was attempted by 30–120-min incubation with 2–6 mg/ml TnC in the relaxing solution at 15–20°C. We made activations at 20°C in pCa$_4$ and pSr$_4$ except where indicated. The sarcomere length was adjusted to 2.2 (and in a few cases to 2.5 μm) for psoas fibers and 2.2 μm for the trabeculae, using laser diffraction, and was monitored throughout the experiment.

**Selection of Fast-Twitch Fibers—**Hamster psoas is a mixed muscle (80% dark (fast-twitch) and 20% light (slow-twitch)) by histochemical inspection of the known values for the major intracellular constituents contributing to ionic strength (Table 6.2 in Kernan, 1972) gives an estimate for the intracellular ionic strength for mammalian fibers as at least 170 mM (assuming full activity; Palmer and Gulati, 1978). Also, under maximal activations, skinned frog fibers were found to be more stable in 180–200 mM salt than 100–140 mM in the 0–25°C temperature range (Thames et al., 1974; Gulati and Podolsky, 1981; Gulati and Babu, 1985b).

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$^3$ J. Spiro, A. Babu, and J. Gulati, unpublished data.

![FIG. 1. TnC extraction and reconstitution of fast-twitch skeletal muscle fibers from a psoas muscle. Activations were made by pCa$_4$ (A) or pSr$_4$ (B). First trace in each set shows the tension response prior to extraction, 2nd trace after extraction, and 3rd trace after reloading. Note nearly complete elimination of tension development by the extracted fiber, indicating effectively full TnC extraction. (horizontal bar, 10 s; vertical bar, 50 kN/m$^2$). (C) 15% SDS-PAGE runs on three skin fiber segments: control (native), TnC-extracted, STnC-loaded. Silver stained. The identification of TnI, TnT, and tropomyosin (TM) bands was similar to Schachat et al. (1985).](image-url)
almost fully on loading with purified skeletal TnC (3rd trace in each set in Fig. 1). After the experiment, the fibers were subjected to SDS-PAGE (panel C in Fig. 1; see "Gel Electrophoresis" below for technical details), confirming both the loss of TnC on extraction and reconstitution following incubation with purified TnC.

Ca\(^{2+}\) and Sr\(^{2+}\) Activations: Relation between Skeletal and Cardiac Muscles—Fig. 2 compares the pCa-force and pSr-force relationships for the hamster skeletal (at sarcomere length, 2.2 \(\mu\)m) and cardiac preparations (also 2.2 \(\mu\)m). The data and the computer fits of Hill’s equations are indicated. Sensitivities for Ca\(^{2+}\) activations appear to overlap for the two tissues at these sarcomere lengths (left-hand plot) but the marked difference in the sensitivities of skeletal and cardiac muscles is brought out by Sr\(^{2+}\) activations. The native (skinned) cardiac preparation of the hamster is found to be about 5 times more sensitive to Sr\(^{2+}\) than the skeletal fiber (pK = 4.4 for psoas, 5.0 for the trabeculae). The disparity in the Sr\(^{2+}\) relationships between skeletal and cardiac muscles greatly facilitated testing of the effects of TnC exchange in these tissues.

Gel Electrophoresis—To establish the extraction procedure for trabeculae, cardiac bundles (5–10 times the sample size used for mechanical measurements) were used for initial gel runs. The treated bundles were dissolved in SDS sample application buffer with the addition (25 \(\mu\)g urea and analysed by SDS-PAGE according to the method of Laemmli (1970). Fig. 3 shows the results. These gels (15%) were stained with Coomassie Brilliant Blue R250 and scanned on a Beckman DU-8 spectrophotometer at 584 nm to detect the peak for apparent molecular weight determinations and for quantitation of troponin C. To correct for unequal loading of the gel lanes, densitometric scans of the lanes in these initial runs showed that 80–90% of the TnC had been extracted (the tension response of the extracted bundles (pCa4) was 0.07 Po). Proteins in the TnC band were the major ones extracted with the exception of one other band that had an apparent molecular weight 11,000. No effort was made to identify this low molecular weight component.

The gel runs (10 or 15%) of all other experimental fibers and trabeculae were silver stained for improved sensitivity (the expected TnC was 2.2 pm. At 2.5 pm, the activation curves for psoas fibers were shifted to the left by 0.15 units (not shown) and such shifts are consistent with results in the literature (Stephenson and Wendt, 1984). The data for both Ca\(^{2+}\) and Sr\(^{2+}\) activations are computer-fitted by Hill’s equation: relative force = \([Me]/([K]+[Me])\), where Me is either Ca\(^{2+}\) or Sr\(^{2+}\). Fitted parameters for Ca\(^{2+}\) (left panel): pK = 4.4, n = 6.0 on psoas (circles) and trabeculae (triangles); for Sr\(^{2+}\) (right panel), pK = 5.0, n = 4.4 on trabeculae (triangles). Note the marked disparity in Sr\(^{2+}\) activations of the two preparations, and there was close similarity in our Hill’s coefficient (n, for Ca\(^{2+}\)) on hamster trabeculae with Kentish et al. (1986) on rat.

Fig. 2. Comparison of activation characteristics for fast-twitch muscle fibers and cardiac muscles. Number of skeletal fiber preparations was four and cardiac five, and the sarcomere length shifted to the left by 0.15 units (not shown) and such shifts are consistent with results in the literature (Stephenson and Wendt, 1984). The data for both Ca\(^{2+}\) and Sr\(^{2+}\) activations are computer-fitted by Hill’s equation: relative force = \([Me]/([K]+[Me])\), where Me is either Ca\(^{2+}\) or Sr\(^{2+}\). Fitted parameters for Ca\(^{2+}\) (left panel): pK = 4.4, n = 6.0 on psoas (circles) and trabeculae (triangles); for Sr\(^{2+}\) (right panel), pK = 5.0, n = 4.4 on trabeculae (triangles). Note the marked disparity in Sr\(^{2+}\) activations of the two preparations, and there was close similarity in our Hill’s coefficient (n, for Ca\(^{2+}\)) on hamster trabeculae with Kentish et al. (1986) on rat.

Fig. 3. A composite of various lanes of 15% SDS-polyacrylamide gels from cardiac trabeculae, rabbit cardiac troponin C, and molecular weight standards. Lane a is trabecular muscle and lane b another trabecular extract for troponin C. The purified troponin C used in the reconstitution experiments is shown in lane c with protein molecular weight standards in lane d of 45,000, 31,000, 21,000 and 12,300. Loading was different for lanes a and b. The bottom bands are close to the dyefront and contain material of 7 kDa or less. The 38- Da band probably contains TnT.

the expected values (normalization in these cases was generally to LC1 band, by area of the densitometer peak). The results of analysis from the experimental preparations are discussed below.

Statistics—All data are given as mean ± S.E. Curve fittings, wherever appropriate, were computed by the method of least squares on a microcomputer (Hewlett Packard-85).

RESULTS

Extraction of Troponin C from the Myocardium and Reconstitution—Fig. 4 shows the tension responses to Ca\(^{2+}\) activations on two typical trabecular preparations before and after exposure to the TnC extraction procedure. Like skeletal fibers, TnC was extracted until force was close to zero (middle traces in Fig. 4, a and b). The last two traces in Fig. 4, a and b show the reconstitution with purified cardiac and skeletal TnC, respectively. Nearly full recovery of the tension was found on the trabeculae with both types of TnC. Resting tension in the relaxing solution was not affected by TnC extraction.

The gel runs (silver stained) on these preparations in Fig. 4c show the presence and absence of cardiac TnC, and the pooled quantitative data from gel scans on successful skeletal and cardiac preparations is summarized in Table I. Fig. 4c clearly shows the restored C(TnC) band, but the presence of STnC is difficult to ascertain in the third lane as STnC runs in the same spot as cardiac LC2. These results show 1) that tension development in cardiac muscle drops to practically zero when 75% or more of the native TnC is extracted, 2) that nearly complete reconstitution of the trabeculae is made on incubation with purified TnC, and 3) that the loss of material other than TnC (e.g., the 11,000-dalton component seen in the runs in Fig. 3 and a possible loss of at most 10% C-LC2 in Table IB) during the extraction procedure was not important for tension generation by contractile apparatus in the cardiac muscle (S-LC2 loss was significant in psoas fibers, as explained under "Discussion"), and 4) that any differences in the skeletal and cardiac TnCs are not critical in the reconstituted myocardium to develop the original level of cardiac tension. Additional studies to determine the effects of various TnCs on the sensitivity to Sr\(^{2+}\) are given below.
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**Trabeculae (Ca²⁺ activation)**

- **a.** (+) cardiac TnC
- **b.** native (-) TnC (+) skeletal TnC
- **c.** S TnC - C TnC - LC1 - LC2

**FIG. 4. Trabeculae loaded with cardiac TnC (a) and skeletal TnC (b).** Note nearly full tension recovery (pCa4) with either type of TnC. (horizontal bar, 20 s; vertical bar, 10 kN/m²). c. 10% gels on CTnC- and STnC-loaded trabeculae (silver stained). The CTnC band was close to that in the native trabeculae when the data were normalized to the intensity of the LC1 band (see Table I). Also, note the near absence of CTnC in the 3rd lane.

Further evidence against the possibility of deleterious effects of the extraction procedure was derived from the stiffness measurements in rigor, since this parameter gives an index of the maximum possible number of cross-bridge attachments in the fiber. Table II compares the rigor stiffness of native and TnC-extracted preparations. For both the skeletal fiber and the myocardium, rigor stiffness was found to be unchanged by TnC extraction (Table II).

$pCa$-Force and $pSr$-Force Relationships of Reconstituted Fibers and Trabeculae: Loading with the Native-type (Homologous) TnC—Fig. 5 shows results on the reconstituted preparations over the entire activation range. The top two plots give the data (circles) on psoas fibers loaded with STnC following extraction. These experiments with psoas fibers serve as controls for the studies on trabeculae loaded with STnC (see below, Fig. 6). The lower plots in Fig. 5 (triangles) are on the trabeculae loaded with the homologous CTnC. The solid lines are transferred from Fig. 2 on native fibers and trabeculae, respectively, and they seem to adequately describe the data on both preparations extracted and reloaded with homologous TnCs.

**FIG. 5. Activation characteristics of psoas and cardiac muscle preparations loaded with the homologous TnCs.** The solid lines are the same as determined on unextracted (native) preparations in Fig. 2.

| Table II |
|---|
| **Fiber stiffness in rigor** |
| Stiffness was measured by stretching the fiber by 0.5% of the length in 750 ms. Temperature 5 °C. $P_o$ was measured with pCa4 prior to TnC extraction. |
| | Native TnC | (-) TnC |
| | | | |
| Psoas ($n = 5$) | 0.19 ± 0.09 | 0.20 ± 0.07 |
| Trabeculae ($n = 4$) | 0.17 ± 0.04 | 0.17 ± 0.03 |

**TABLE I**

| Relative amounts of the various thick- and thin-filament subunits |
|---|
| | A. Hamster psoas single-fiber segments |
| | | Fiber treatment* |
| | | STnC | CTnC | TnI | LC3 | LC2 | LC1 |
| Native (skinned) (8) | 0.26 ± 0.03 | 0.31 ± 0.01 | 0.26 ± 0.04 | 1.24 ± 0.11 | 1.0 |
| (-) TnC (5) | 0.07 ± 0.01 | 0.30 ± 0.03 | 0.28 ± 0.05 | 1.08 ± 0.07 | 1.0 |
| (+) STnC (4) | 0.27 ± 0.04 | 0.31 ± 0.03 | 0.31 ± 0.03 | 0.83 ± 0.06 | 1.0 |
| (+) CTnC (4) | 0.05 ± 0.03 | 0.27 ± 0.08 | 0.36 ± 0.01 | 0.24 ± 0.02 | 0.97 ± 0.11 | 1.0 |
| B. Thin trabeculae segments |
| | | Treatment | STnC | CTnC | LC2 | LC1 |
| Native (skinned) (7) | 0.32 ± 0.05 | 0.80 ± 0.07 | 1.0 |
| (-) TnC (5) | 0.07 ± 0.02 | 0.74 ± 0.06 | 1.0 |
| (+) CTnC (3) | 0.30 ± 0.04 | 0.81 ± 0.06 | 1.0 |

* Numbers in parentheses indicate the number of segments analyzed individually. These segments were the same on which tension measurements were made.
the entire pSr-tension relationships. The data are given in Fig. 6. They show that the force response of the STnC-loaded myocardium was shifted to the right by 0.7 pSr units (compare filled triangles with half-filled triangles for CTnC loaded trabeculae in Fig. 6). The shifted pK value (4.3) is similar to that found from the typical skeletal fiber pSr-force response (Fig. 2). From the fact that the skeletal fiber loaded with purified STnC gave normal response (filled triangles in Fig. 6), it is very unlikely that the shift in the STnC-loaded trabeculae was due to a modification of STnC on purification.

Studies with Skeletal Fibers Reconstituted with Cardiac Troponin C—This was done next to test the efficacy of CTnC in a converse situation from the STnC myocardium at 180 mM salt. To our initial surprise the skeletal fibers, extracted so that the tension in pCa4 was between 0–10% (10–30-min extraction period; residual TnC 20–30%, Table I), showed only marginal recovery of Ca2+ and Sr2+-activated tensions on loading with CTnC. These results are summarized in Fig. 7 (top left panel). The same fibers could recover nearly full tension (mean value, 0.8 Po) with STnC (compare bars 3 and 4 in the top left panel of Fig. 7) indicating that the extraction procedure was not deleterious. Also, the same solution of CTnC used on skeletal fibers was fully effective in restoring the tension responses in the trabeculae, indicating that the limitation of CTnC-loaded skeletal fiber was not due to inactivity of CTnC on purification.

A number of additional experiments were performed to understand the limitation of CTnC in skeletal fibers. The possibility was considered that CTnC did not enter the fiber, but this was ruled out both with gels and with physiological studies. The gel runs in Fig. 8 compare an unextracted (native) psoas fiber segment (1st lane) with STnC-reconstituted fiber (3rd lane) and CTnC-reconstituted fiber (2nd lane). The 2nd lane indicates the loading with CTnC (physiological response of this segment is shown by the force traces in the lower left end of Fig. 9). The quantitative data from gel scans on a number of CTnC loaded fibers are summarized in Table IA and show that CTnC was accumulated to the same level as the original level of native STnC. In another experiment (Fig. 10), cardiac TnC-loaded skeletal fibers (pCa4 tension after extraction, ~0.1 Po after loading, 0.29 Po) was incubated with STnC for additional loading (for 90 min), but we found that there was no more effect on the tension level (0.29 Po) with the second loading. This is additional evidence that CTnC loads into the denuded TnC sites in the fiber. The same doubly loaded fiber was next reextracted and now reloaded directly with STnC, and the tension response was then closer to the original level (f in Fig. 10). These results suggest that partial recovery with CTnC at 180 mM ionic strength was due directly to the reduced effectiveness of CTnC in skeletal fibers.

Fig. 9 shows the results of a series of experiments on CTnC-loaded skeletal fibers where the extraction time was varied. The maximally extracted fibers (extraction time, 20–30 min; tension with pCa4 prior to CTnC-loading, 0–0.1 Po) showed relatively little tension recovery, as above. However, as the extraction time was reduced so that the tension level prior to loading with CTnC was >0.1 Po, the tension recovery following CTnC loading was progressively enhanced. These results show that the effectiveness of CTnC was increased in moderately extracted fibers which suggests the possibility of some
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The successful reconstitution of the TnC-extracted myocardium with both CTnC and STnC indicates that the intrinsic differences amongst these moieties (e.g. the additional regulatory site in STnC) do not interfere in activating the contractile proteins in cardiac muscle. On the other hand, our results showing that CTnC is less effective in skeletal muscle fibers at close to physiological ionic strength indicate that the modified site I (of the class of regulatory sites I and II, which trigger activation) is important in this situation. Despite that, however, the uptake of CTnC by the skeletal fiber was normal and the cooperative interaction between the two types of TnCs under these conditions.

DISCUSSION

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Cooperative Interaction between the two Types of TnCs under these Conditions.

The results on CTnC loaded fibers could be explained if the changes produced by Ca$^{2+}$ binding to a TnC moiety could be communicated to the adjacent TnC sites, possibly through the 410-423 Å-long tropomyosin molecules. Accordingly, the increased residual STnC in the moderately extracted fibers would exert a greater cumulative influence on the interspersed CTnC, and this cooperative effect might be sufficient to switch on the entire thin filament. If 50% tension response of the lightly extracted fiber implied that the residual STnC was at a level 50-60% of the original TnC (see Fig. 8 of Moss et al., 1985), and assuming uniform distribution on the thin filament, our results would suggest that each cardiac TnC on the average must be separated by no more than one tropomyosin molecule from the native TnC for the cooperative effects to be effective at 180 mM ionic strength. This explanation thus implies that while the various individual segments of the thin filament may be activated in an isolated fashion, ideally, during maximal activation, the entire thin filament is turned on in a concerted manner with communication between the adjacent segments.

If the presence of active cross-bridges affected the properties of TnC this might also explain our results. Moderately extracted fibers on Ca$^{2+}$ activation would have a greater number of bridges as a result of the higher level of residual STnC, and these bridges could in turn exert an increased influence on CTnC improving the apparent effectiveness of CTnC. Gordon and his co-workers (Ridgway and Gordon, 1984) as well as Guth et al. (1988) have recently shown that cross-bridges may increase the Ca$^{2+}$ sensitivity of TnC, but this explanation of the present results demands in addition that, in the case of CTnC in moderately extracted skeletal fibers, the more numerous bridges should help achieve a more complete conformational change in TnC during activation. As such, our results would also raise the possibility that the overall (conformational) changes produced in TnC by Ca$^{2+}$ in native cardiac muscle are below those in native skeletal muscle.

Relation to Other Studies on TnC Exchange—Previously there was debate on the molecular origin for the marked

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A. Babu and J. Gulati, unpublished data.
increase in sensitivity to Sr²⁺ of the cardiac muscle over skeletal fast-twitch fibers, and the extent of TnC involvement was questioned. Ebashi and Endo (1968) were the first to find the results fix TnC as the main determinant of the regulation of the cardiac actomyosin ATPase activities by making superprecipitation measurements of the various composites of actomyosin, troponin, and tropomyosin. Both the skeletal and cardiac actomyosin preparations had greater sensitivity with cardiac troponin than with skeletal troponin. This is consistent with our results on skinned fibers. Indeed, since our experiments were done by replacing TnC (instead of whole troponin), the results fix TnC as the main determinant of the sensitivity of the cardiac muscle over skeletal muscle.

In the present instance, cardiac muscle was a more convenient preparation for the TnC exchange studies.

Comments on the Results from SDS-PAGE of Extracted Fibers—Close inspection of the data in Table I, A and B provides some additional interesting insights. For instance, distribution of the light chains in the native (skinned) fibers indicates LC1:LC2:LC3 of 1.6:2.0:4.1 in single fibers from hamsters, which is similar to that reported on myosin purified from rabbit muscle (1.35:2.0:6.5; Sarkar, 1972). Thus the majority of the myosin heads in the psoas fiber (about 75% after taking the difference in molecular weights) are in the LC1:LC2 configuration and the remaining few (less than 25%) of the heads are in the LC3:LC2. On the other hand, the trabeclae appear to be consistent with an equimolar LC1:LC2 configuration for all heads, which is the expected result since the myocardial tissue has no alkali LC3 moiety.

Further inspection of the data shows that TnC extraction procedure caused no significant loss of LC3 or TnI in both the myocardium and skeletal muscle. In the trabeclae there was less than 10% loss of LC2, which is within the uncertainties of gel measurements. There was a greater tendency for LC2 loss in the psoas muscle (15 to 35% decrease), which might also account for the somewhat lower mean value for the recovery in tension (0.8 Po) after reconstitution (Fig. 7). Consistent with this, in tests on four fibers we found additional recovery of tension (to 0.91 Po) with purified LC2 on top of STnC loading; LC2 also increased the tension recovery of a CTnC loaded fiber by the same amount in a separate experiment, but the final tension of this maximally extracted fiber was still far below the STnC loaded fibers.

The finding that the mean values for residual TnC in extracted fibers and trabeclae were close to 25% of the original level when the Ca²⁺-activated tension fell nearly to zero (less than 0.1 Po) may deserve a comment as well. Since the present sarcomere lengths approximately 25% of the thin filament is under nonoverlap, this could have suggested that the unextracted TnC was restricted to this region. This possibility is opposed by the findings of Yates et al. (1986) that the extractions were enhanced at long sarcomere lengths. As another possibility, the 25% residual level of TnC for zero tension may be indicative of a threshold level for thin filament activation to initiate cross-bridge cycling, which incidentally would also be consistent with the action of the thin filament as a cooperative unit.

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