Thermal Stress and Ca-Independent Contractile Activation in Mammalian Skeletal Muscle Fibers at High Temperatures

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ABSTRACT Temperature dependence of the isometric tension was examined in chemically skinned, glycerinated, rabbit Psoas, muscle fibers immersed in relaxing solution (pH ~ 7.1 at 20°C, pCa ~ 8, ionic strength 200 mM); the average rate of heating/cooling was 0.5–1°C/s. The resting tension increased reversibly with temperature (5–42°C); the tension increase was slight in warming to ~25°C (a linear thermal contraction, $-\alpha$, of ~0.1%/°C) but became more pronounced above ~30°C (similar behavior was seen in intact rat muscle fibers). The extra tension rise at the high temperatures was depressed in acidic pH and in the presence of 10 mM inorganic phosphate; it was absent in rigor fibers in which the tension decreased with heating (a linear thermal expansion, α, of $\sim 4 \times 10^{-5}$/°C). Below ~20°C, the tension response after a ~1% length increase (complete <0.5 ms) consisted of a fast decay ($\sim 150 \cdot s^{-1}$ at 20°C) and a slow decay ($\sim 10 \cdot s^{-1}$) of tension. The rate of fast decay increased with temperature (Q_{10} ~ 2.4); at 35–40°C, it was $\sim 800 \cdot s^{-1}$, and it was followed by a delayed tension rise (stretch-activation) at $30–40 \cdot s^{-1}$. The linear rise of passive tension in warming to ~25°C may be due to increase of thermal stress in titin (connectin)-myosin composite filament, whereas the extra tension above ~30°C may arise from cycling cross-bridges; based on previous findings from regulated actomyosin in solution (Fuchs, 1975), it is suggested that heating reversibly inactivates the troponin-tropomyosin control mechanism and leads to Ca-independent thin filament activation at high temperatures. Additionally, we propose that the heating-induced increase of endo-sarcomeric stress within titin-myosin composite filament makes the cross-bridge mechanism stretch-sensitive at high temperatures.

INTRODUCTION

Elasticity of resting muscle has been studied for over a century, and some of its characteristics are well documented (see references in Meyer and Picken, 1938). Thus, at moderate stretch, resting muscle possesses long-range elasticity and gives out heat when stretched, and the temperature coefficient (β, see Materials and Methods) for isometric tension in resting muscle is positive; on the basis of such features, the elasticity of resting muscle has been likened to that of rubber (Meyer and Picken, 1938; Wöhlisch and Grüning, 1942; Guth, 1947; see review by Hill, 1952). However, from a study on intact frog sartorius muscle, Hill (1970) reported that, although the increase of resting isometric tension on warming was linear (as in rubber—see Guth, 1947; Matsubara, 1975) at temperatures between 0–23°C, the tension increase became more pronounced at the higher temperatures; he suggested an "active" origin for the increased resting tension at high temperatures.

Recent findings show that the elasticity in resting muscle fibers is mostly resident within myofibrils (Magid and Law, 1985); evidence suggests that the resting tension in chemically skinned muscle fibers is largely or entirely borne by titin (connectin)-containing gap filaments (Horowits and Podolsky, 1987 and references therein; Funatsu et al., 1990) that connect the myosin thick filaments to the Z-disc in a sarcomere (Maruyama et al., 1989 and references therein). Among various observations made on titin, which relate to muscle resting tension, are the following. a) The isometric tension of a length of isolated titin has a positive temperature coefficient (Maruyama et al., 1977). b) As a protein, titin contains little α-helix but a high random coil content (see Trinick et al., 1984). c) Titin has been described as very long, flexible, and filamentous, and gap filaments have been shown to have a stretch-dependent thickness (Wang et al., 1984). d) The passive tension in stretched skinned relaxed muscle fibers, at 20°C, is insensitive to high pressure (Ranatunga et al., 1990). In general, these features would be consistent with a net "rubber-like" elastic behavior (Flory, 1956; Davis and Harrington, 1987) in the gap filament and, consequently, with respect to relaxed muscle fiber tension. In the intact sarcomere, a single titin polypeptide spans half a sarcomere and consists of two mechanical segments: the segment between the Z-line and the edge of A-band ("gap filament") is extensible, whereas the segment within the A-band is anchored and prevented from stretching (see Wang et al., 1991 and references therein). The elegant experiments of Wang et al. (1993) clearly show that the elastic behavior of the titin-myosin composite filament (at constant temperature) can fully account for the force-extension characteristics of a muscle fiber.

The present study examines the temperature dependence of isometric tension in short segments of single, chemically skinned, rabbit psoas muscle fibers over a wide temperature range (0–45°C). The results show that the resting tension increase is approximately linear in warming to ~25°C, but it becomes more marked within the temperature range of

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30–40°C. It is suggested that the linear increase of tension is due to heating-induced changes in the titin-myosin composite filament, whereas the marked tension rise at high temperatures is due to heating-induced, Ca-independent contractile activation.[1]

MATERIALS AND METHODS

Trough system

The trough system consisted of a series of ~50-μl troughs milled in a block of titanium that was mounted on a microscope stage. It had an especially designed end-trough that was lined with aluminum foil and surrounded by a number of small Peltier devices. The temperature of the whole trough system was set typically at ~15°C by a separate large Peltier device, whereas the temperature of the end-trough could be independently varied between and clamped at temperatures ranging from 5 to 50°C; a small diameter (~0.4 mm) thermistor mounted in the trough provided feedback to the control circuit and, in early experiments, the temperature signal. In later experiments, a 0.2-mm thermocouple mounted on the spindle of a DC motor was positioned directly above the muscle fiber to monitor temperature and also to stir the solution in a trough. For a step command signal, a temperature increase of 10°C was complete in ~2.5 s and, at steady state, the thermal gradient along the length of the trough was <0.25°C. The trough system could be moved by a lever system to place the muscle fiber in different troughs.

The tension transducer was an AE 801 strain gauge element (Akers, Horten, Norway) housed inside a brass box: a second AE 801 element was mounted by its side to form a complete resistance bridge to reduce thermal sensitivity of the output. The uncertainty associated with thermal drift in the tension transducer output was <5 μN for temperature changes between 5 and 40°C.

A Piezo-electric element was used for applying small-length steps (<12 μm) (AT-23K, Piezo ceramic transducer, STC Electronic, UK); the element was activated by 0.01–1 s voltage pulses (±10–180 V) from two serially connected isolated stimulators. The output of an inbuilt feedback electrode was monitored as a length signal. In control experiments, the time course of a step response was examined by monitoring the hook movement with a photo-diode; a step was complete in <0.4 ms.

The stainless steel transducer hooks used in the preliminary experiments (Ranatunga, 1990) were replaced, in the present experiments, with hooks made of ~150-μm diameter silica fibres, because the linear coefficient of thermal expansion of silica ($\sim 6 \times 10^{-7} \cdot K^{-1}$) is about 1/20 that of steel ($1.2 \times 10^{-5} \cdot K^{-1}$). The results obtained after the modification were qualitatively similar, but the estimated linear coefficient of thermal expansion in rigor fibers is significantly lower in the present experiments (see below).

Fiber preparations and solutions

Rabbit psoas muscle fibers were chemically skinned and glycerinated using standard procedures (Fortune et al., 1989). The experimental solutions were similar to those used in the temperature-jump experiments (Goldman et al., 1987). Thus, the standard relaxing solution contained 5 mM MgATP, 20 mM EGTA, 20 mM creatine phosphate and 1 mg/ml creatine phosphokinase, 10 mM glutathione, and 15 mM β-glycerol phosphate as the pH buffer (ionic strength 200 mM); pCa level is likely to be ~8, because the apparent binding constant of Ca to EGTA is $\sim 5 \times 10^6\ M^{-1}$ at different temperatures (see Stephenson and Williams, 1985). A solution pH was set to 7.1 at room temperature (19–24°C), and in control experiments the pH change in warming from 5 to 50°C was −0.007/°C.

A few experiments were done on intact fiber bundles dissected from the extensor digitorum longus (a fast muscle) of 10-week-old male rats (see Ranatunga, 1984); the trough system was similar to that described previously (Ranatunga, 1982), except that the muscle chamber was small (~2 ml). Physiological saline containing bicarbonate buffer and gassed with 95% CO_2 and 5% O_2 flowed through the muscle chamber at a rate of 0.5 ml/min. The saline in the chamber was warmed or cooled slowly (0.1–0.2°C/s) to allow for muscle temperature equilibration, and saline temperature was measured by a thermocouple placed above the bundle.

Experimental protocols, data recording, and analyses

In an experiment, a fiber segment of 1–3 mm was mounted (using nitrocellulose glue) between the two silica hooks of the tension transducer and the Piezo-ceramic element, and fiber dimensions and sarcomere length were determined by microscopical examination. The fiber was then transferred to the end-trough (already filled with the appropriate solution and temperature-clamped at ~15°C). By manual resetting of the control unit, the end-trough temperature was first lowered to ~5°C, then increased typically in 5°C steps to 35–45°C, and finally lowered to 15–20°C. Average rate of warming was 0.5–1°C/s. In many of the later experiments, responses were recorded within a narrower temperature range (15–40°C) or at a few temperatures only. In determining the force-extension relation, a fiber was stretched and subsequently released in a number of length steps (0.125–0.25 mm) applied manually using the micrometer on which the tension transducer was mounted.

A Tandon computer (Target 386 SX-40) with a CED 1401 laboratory interface (Cambridge Electronic Design Ltd., Cambridge, UK) was used to digitize and store the tension transducer output and the thermistor and/or thermocouple outputs. The curve drawn through each set of tension data represents an equation of the form $P = (A + B) \cdot \exp(kT)$, where P = tension, T = temperature (°C), and A, B, and k are constants, fitted by using a nonlinear curve-fitting program (FIG-P, Biosoft). To compare temperature sensitivity of tension, the linear thermal coefficient of tension (β, as $(1/P)(\delta P/\delta T)$) was calculated (Wöhlisch and Grüning, 1942; Hill, 1952) using the fitted equation. The coefficient of thermal expansion (α, as $(1/L_o)(\delta P/\delta T)/(\delta P/\delta L)$ where L = muscle fiber length) was estimated in a comparable manner using, in addition, the force-extension relation. The vertical (v) and horizontal (h) widths of a fiber were measured under the microscope, and the fiber cross sectional area (X^2) was estimated as $\pi vh/4$: these were done at rest length (L_o) where the average sarcomere length (S.L.) was 2.3–2.5 μm.

RESULTS

Temperature dependence of resting tension

Intact muscle fibers

Fig. 1 *a* shows two superimposed tension records made from a stretched intact fiber bundle (rat) when it was slowly warmed and cooled between 20 and 40°C. The tension increased markedly in warming to high temperature, and the change was reversible, confirming Hill's (1970) observation on frog muscle. Data collected from another preparation set at three different initial lengths are plotted in Fig. 1 *b*. The data in Fig. 1 show that in mammalian fibers the resting tension increase occurs in the physiological temperatures (30–40°C).

Skinned muscle fibers

The tension (*upper*) and temperature (*lower*) traces recorded from a single skinned muscle fiber immersed in the standard

[1] An abstract based on this study was presented to the Physiological Society (Ranatunga, 1992).

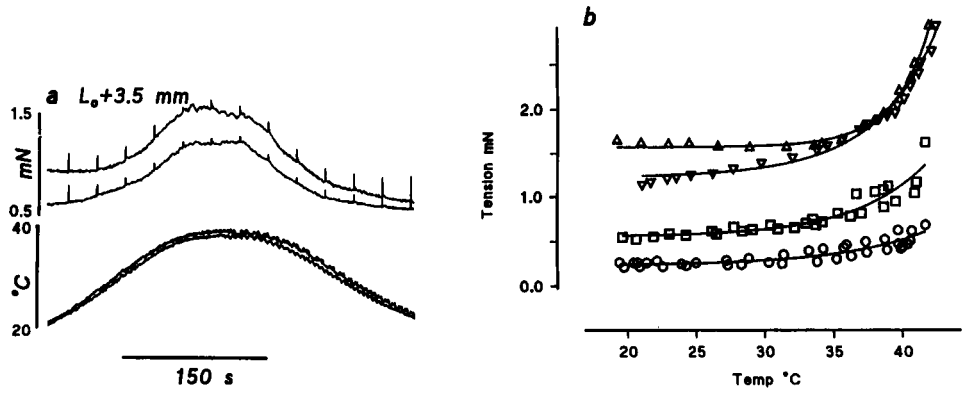

FIGURE 1 (*a*) Sample records of tension (*upper*) and temperature (*lower*) from an intact fiber bundle (rat extensor digitorum longus); the records are from two consecutive warming/cooling procedures (spikes at regular intervals represent electrically evoked twitch contractions). Bundle width = 240 μm, L_0 = 10 mm. Based on previous studies (see Elmubarak and Ranatunga, 1984), the increased tension would be ~5–10% of maximum tetanic tension. (*b*) Tension versus temperature data and fitted curves (see Materials and Methods) from another preparation (L_0 = 12.5 mm) at: L_0 + 2 mm (○), L_0 + 3 mm (□), and L_0 + 4 mm (△); data from both warming and cooling are plotted, and at +4 mm the tensions recorded after cooling were lower (▽). L_0 to L_0 + 4 mm corresponds to sarcomere length range of ~2.5 to 3.4 μm.

relaxing solution are shown in Fig. 2, *a–c*. The tensions measured at different steady temperatures during warming and cooling are plotted in Fig. 2 *d*. As in intact rat muscle fibers, the resting tension at a range of initial lengths increased with warming, and the increase was more pronounced at the higher temperatures (30–40°C). It is seen also that, at longer sarcomere length (e.g., ~3.2 μm), the tension increase occurred at a lower temperature (~30°C).

Reversibility

Fig. 3 *a* shows a sample record (*inset*) and plots of data from two fibers illustrating the rapid loss of tension when heated above a certain temperature. The temperature at which the tension reached an upper limit was, in different preparations, between 43 and 45°C at pH ~7. Long exposure to such temperatures was typically avoided in experiments because it resulted in "irreversible" changes such as increase of stiffness, existence of residual tension after subsequent cooling, and in some cases, loss of sarcomere appearance.

Fig. 3 *b* shows tension data from a fiber in which four sets of warming-cooling procedures were carried out at the same initial length but at different times during an experiment; they show that, if a temperature of ~42°C was not exceeded, essentially the same tension responses could be repeatedly obtained from a fiber under standard conditions (S.L. < 3 μm). At longer sarcomere lengths, the steady tension at low temperatures was lower after a warming-cooling procedure (see Fig. 2, *c* and *d*); this may be due to slow stress relaxation in structures that support high passive tension at low temperatures. The maximum Ca-activated tension recorded in muscle fibers (at ~20°C; S.L. 2.3–2.8 μm; n = 10) that were exposed to warming-cooling procedures between 5 and 40°C was 156 (± 17) kN/m² (mean ± SEM); this is similar to the mean tension

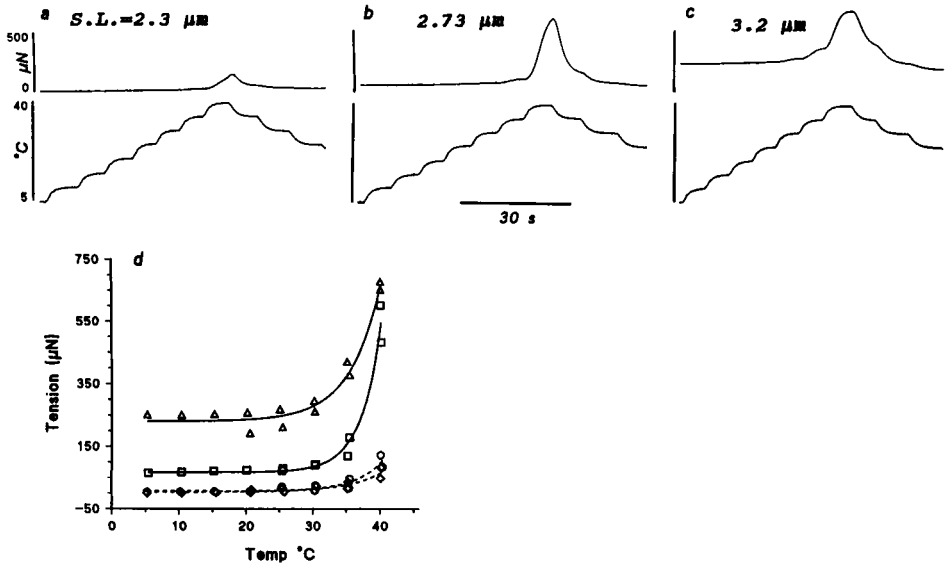

FIGURE 2 (*a–c*) Sample records from a skinned (rabbit psoas) muscle fiber in the standard relaxing solution; each frame contains a tension record (*upper trace*) and a temperature record (from the thermistor, *lower trace*). Note the sharp, reversible increase of tension in warming to high temperatures (complete cycle of cooling is not illustrated). (*d*) Tension versus temperature plots and the fitted curves from the same fiber, including the data obtained at L_0 (S.L. = 2.3 μm) at the end. Each curve contains data from warming and cooling. The fiber cross sectional area (X^2 at L_0) was 7600 μm²; sarcomere lengths were 2.3 μm (○ and ◇), 2.73 μm (□), and 3.2 μm (△); note that sharp tension rise occurs at a lower temperature when sarcomere length is longer.

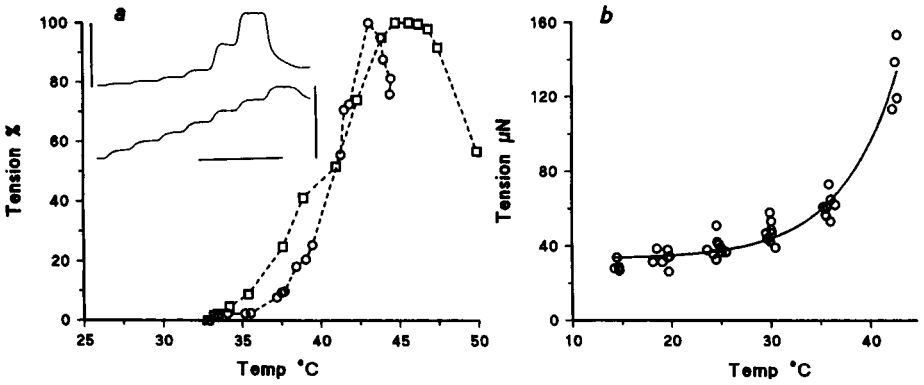

FIGURE 3 (*a*) (*Inset*) Tension and temperature records from a muscle fiber (L = 2.4 mm, X^2 = 5150 μm^2; calibration bars: time = 30 s, temperature = 15–50°C, tension = 100–250 μN). The plots show percentage tension versus temperature from two fibers. Data show the drop in tension in warming above 43–47°C; the fibers showed signs of irreversible damage after such exposures. (*b*) Tension versus temperature data from a fiber (L = 2.26 mm, S.L. ~ 2.75 μm, X^2 = 2890 μm^2), collected at the same extended length from four separate warming-cooling procedures; data show the reversiblity of the tension changes, when 42°C is not exceeded.

obtained (~160 kN/m^2) at 20°C in our T-jump experiments in which fibers were not exposed to temperatures higher than 30°C (see Goldman et al., 1987). Thus, chemically skinned rabbit psoas muscle fibers are not irreversibly affected in warming to high physiological temperatures.

The temperature coefficients of tension (β) for 20 and 37°C (see Materials and Methods) were calculated from each of 34 experimental trials (11 fibers). The mean (±SEM) coefficient of tension was 10.9 (±2.5) × 10^{-3}/°C at 20°C; it was >10 times larger, 149 (±24.6) × 10^{-3}/°C at 37°C. β at 20°C was not correlated with sarcomere length, whereas β at 37°C decreased at longer sarcomere lengths. β_{37} was 214 (±77) × 10^{-3}/°C at sarcomere lengths of 2.3–2.6 μm (n = 10), but 82 (±12) × 10^{-3}/°C at sarcomere lengths of 3.3–3.6 μm (n = 5). If the relaxed fiber tension is assumed to be elastic in origin (as assumed by most previous investigators), the extent of thermal contraction (negative thermal expansion, α) that can account for the tension changes may be estimated (see Materials and Methods) using the force-extension relation (at 15°C) of each fiber; the linear thermal coefficient of expansion (per °C) was −1.06 (±0.3) × 10^{-3} at 20°C and −15.0 (±2.9) × 10^{-3} at 37°C (n = 30).

Effect of pH, inorganic phosphate, etc.

In experiments on three muscle fibers, the temperature dependence of tension was examined with the relaxing solution pH set (at 20°C) to 7.7, 7.1, and 6.5. Data were collected for the three pH values at different sequences and, in all three fibers, the tension rise at high temperature was depressed at the lower pH. The records and plots from one muscle fiber are shown in Fig. 4; the tension rise occurred at ~30–35°C with the solution pH at 7.7, whereas it was considerably depressed with the pH at 6.5, and the pH effects were reversible. Interestingly, the temperature at which the tension reached an upper limit was also pH-dependent; it was 42–45°C at pH ~7 (see Fig. 3) but 35–37°C at pH ~7.7.

The effect on the heat-induced tension rise of 10 mM added phosphate in the relaxing solution (pH 7.1, ionic strength 200 mM) was examined in experiments on four

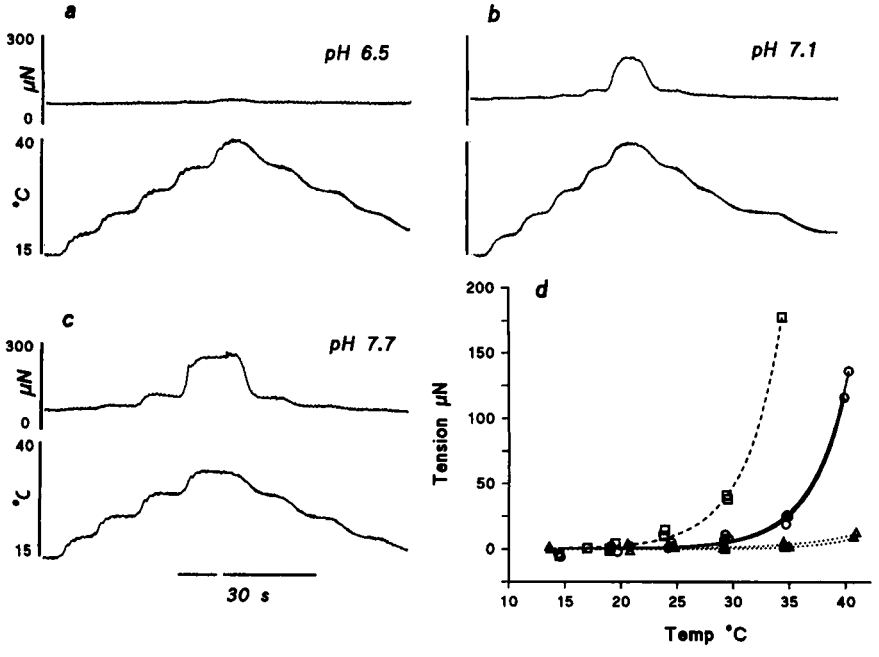

FIGURE 4 Effect of pH. (*a–c*) Records from a muscle fiber bathed in relaxing solutions of pH ~6.5 (*a*), pH ~7.1 (*b*), and pH ~7.7 (*c*). (*d*) Plots from *a–c* and two others from the same fiber: pH 7.1 (○), pH 6.5 (△ and ▲), pH 7.7 (□); (S.L. = 2.7 μm, X^2 = 4233 μm^2); steady tensions at low temperature removed. Tension increase is depressed in acidic pH and it occurs at a lower temperature at pH 7.7.

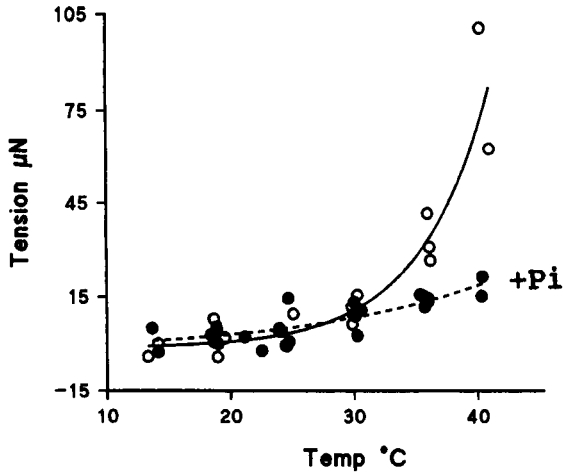

FIGURE 5 Effect of 10 mM inorganic phosphate in the relaxing solution. Tension-temperature data from four warming-cooling procedures on a muscle fiber (S.L. = 2.9 μm, X^2 = 6700 μm^2); steady tensions at low temperature removed; standard, no added phosphate (○), with 10 mM phosphate (●). Tension increase is depressed with added phosphate.

fibers. Fig. 5 shows experimental data from one fiber; the sharp increase of tension in warming above ~30°C was considerably reduced (Fig. 5, *filled symbols*) in the presence of 10 mM phosphate.

Rigor fiber tension

Fig. 6 shows data from a muscle fiber that was put in rigor at 15°C; Fig. 6 *a* shows tension responses to small-length perturbations, and Fig. 6 *b* shows its subsequent tension record in response to stepwise temperature change between 5 and 40°C. As illustrated in the plots in Fig. 6, *c* and *d*, both the force-extension relation and the tension-temperature relation are linear. Similar experiments were done on four other muscle fibers (number of trials, n = 7), and linear thermal expansion was evident irrespective of whether the rigor condition was established at rest length or an extended length.

The mean (±SEM) thermal stress estimated from such experiments, at sarcomere lengths of 2.4–3.2 μm, was −0.912 (±0.215) kN/m^{-2}/°C (n = 7). From the slopes of the linear regressions fitted to the data (Fig. 6, *c* and *d*), the mean (±SEM) linear thermal coefficient of expansion (estimated as $\alpha = (1/L)(\delta P/\delta T)/(\delta P/\delta L)$) was 3.96 (±0.19) × 10^{-5} (n = 7). This is clearly smaller than an estimate given in our previous study (~7 × 10^{-5}; Goldman et al., 1987) in which steel transducer hooks were used; the value, however, is within the range obtained in the T-jump experiments of Bershitsky and Tsaturyan (1989).

Maximal Ca-activated fiber tension

The maximal Ca-activated tension in a fiber increased with temperature (as found in steady-state experiments, see Stephenson and Williams, 1985; Goldman et al., 1987), but the tension-temperature (5–40°C) relation was approximately S-shaped, with the 50% tension being at 17.1°C (±2.4°C, n = 7) and the tension increasing only slightly at temperatures higher than ~30°C; the results were basically similar to those reported previously (Ranatunga, 1990). Whether the nonlinear (S-shaped) dependence of active tension is due to development of sarcomere nonuniformity under these conditions (as suggested by Bershitsky and Tsaturyan, 1992) remains uncertain. Fig. 7 shows data from two fibers for comparing the temperature dependence of maximally Ca-activated tension (*filled symbols*, pCa ~ 4.5) and that of Ca-independent ("resting") tension (pCa ~ 8); the discrepancy between Ca-activated tension and Ca-independent

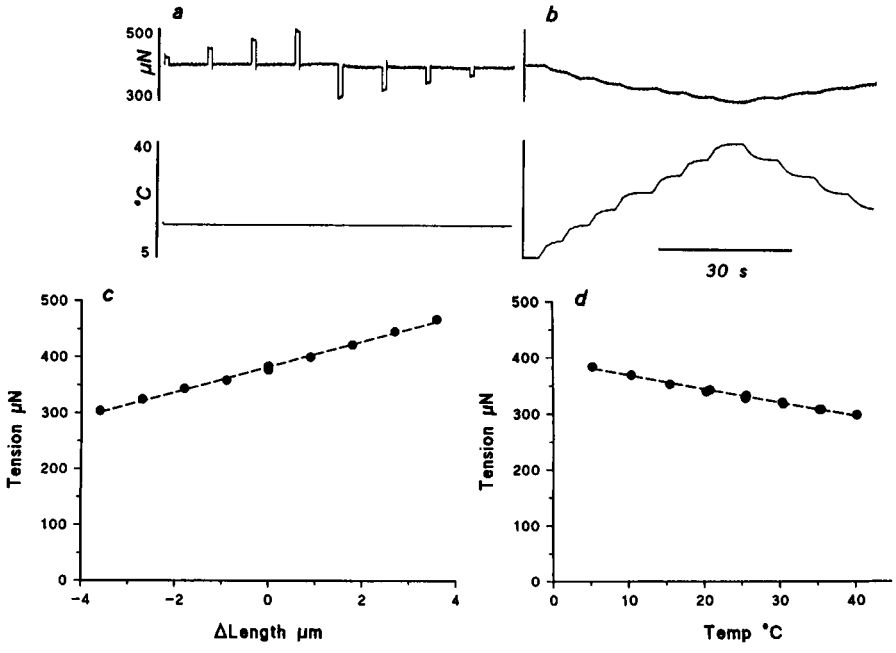

FIGURE 6 Rigor tension: sample records and data from the same fiber as in Fig. 2, in rigor state (S.L. = 3.2 μm). (*a*) Tension responses to small stretches and releases at 15°C. (*b*) Tension response to stepwise warming from 5 to 40°C. (*c*) Force-extension relation. (*d*) Steady tension-temperature data. The coefficient of thermal expansion (α) calculated from the slopes of the linear regressions was 3.23 × 10^{-5}/°C (see Materials and Methods).

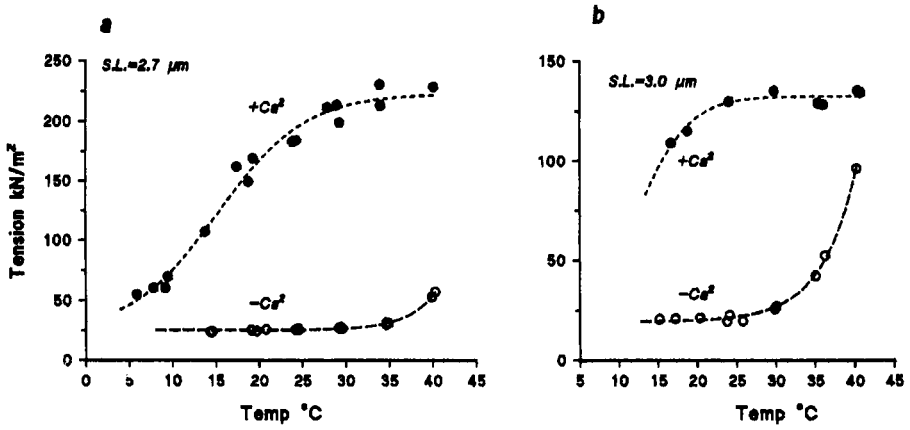

FIGURE 7 Steady tension-temperature data from two muscle fibers (*a* and *b*) in the presence of maximal level of Ca^{2+} (pCa ~ 4; ●) and in the absence of Ca^{2+} (pCa ~ 8; ○). Note that at longer sarcomere length, the heat-induced tension, at 40°C, approaches Ca-activated tension.

tension at a given high temperature (e.g., 40°C) is less at the longer (Fig. 7 *b*) than at the shorter sarcomere length (Fig. 7 *a*). This is in accordance with the observations that, at the longer sarcomere length, the heat-induced tension rise occurs at a lower temperature (see Fig. 2) and that the actual maximal active tension would be less due to reduction in filament overlap.

Tension increase in relation to sarcomere length

The pooled data for tension difference between high (37–40°C) and low (20°C) temperatures (from tension versus temperature curves as in Fig. 2) are plotted against the sarcomere length in Fig. 8 *a*. Corrections made for the small linear increase of tension in warming (using β_{20}) neither decreased the scatter nor changed the bell-shaped relation appreciably. The data show that the nonlinear increase of tension in warming to 37–40°C is maximal (20–50 kN/m^2) at sarcomere lengths of 3–3.4 μm, but this may be due to pooling of data from different fibers.

Fig. 8 *b* illustrates the protocol adopted for a different type of experiment; the force-extension curves were determined at a low (15°C) and at a high (39°C) temperature. The high temperature minus low temperature difference-tension versus extension was bell-shaped in all such determinations (n = 5); curves from two other fibers are shown in Fig. 9 *a* (*open symbols*). In a few such experiments, the tension responses to a small stretch (see Fig. 9 *b*) were also recorded; the high temperature minus low temperature stiffness decreased with extension (Fig. 9 *a*, *filled circles*). These experiments showed that the nonlinear tension rise in warming to 37–40°C was maximal at sarcomere lengths of 2.6–3.0 μm, which is longer than the optimal range for maximal active tension (~2.2–2.6 μm in mammalian muscle, see Elmubarak and Ranatunga, 1984). This discrepancy may be partly because the fibers were not length-clamped in these experiments. Also, the fit may be better if a temperature higher than 39–40°C was used. First, the temperature at which the sharp tension rise occurs is dependent on sarcomere length (i.e., higher temperatures for shorter sarcomere lengths; see Fig. 2). Second, the temperature coefficient of tension at 37°C (β_{37}) decreased at longer (>2.6 μm) sarcomere lengths (see above). The interaction of such factors is indicated in the data from two fibers illustrated in Fig. 9: the optimal length range is shifted to lower sarcomere lengths with increase of tension at higher temperatures.

Fig. 10 shows tension records from a fiber illustrating the time course of tension rise when the temperature is raised from 34°C and clamped at 39°C; the records are superimposed responses from two trials at two extensions (see Fig. 10 *b*) at which the difference in steady tension at 34°C has been removed. Such experiments showed that the onset and the rate of tension rise on heating to high temperature were slow; the half-times of tension rise ranged between 1 and 3 s.

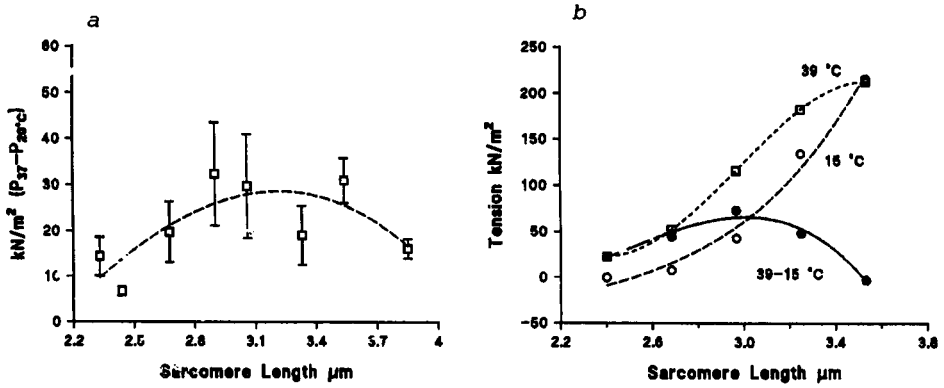

FIGURE 8 (*a*) Data for tension difference between 37 and 20°C, calculated from different trials (like the trials in Fig. 2), from 10 fibers (n = 40). A curve is fitted by eye to the mean (±SEM) data. (*b*) Force-extension curves from a muscle fiber at 15 (○) and at 39°C (□). The 39–15°C tension difference (●). The relation between the increased tension at the high temperature and sarcomere length is bell-shaped.

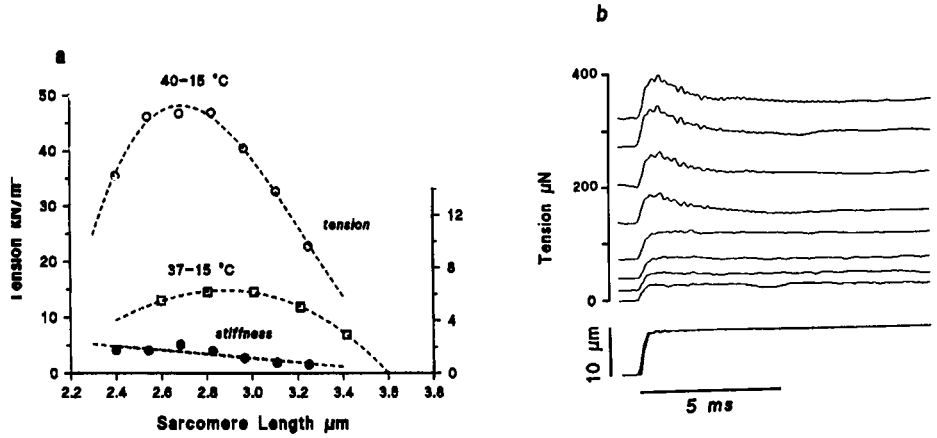

FIGURE 9 (*a*) 40–15°C difference tension (○, *left vertical axis*) and 37–15°C difference-tension (□, *right vertical axis* in kN/m^{-2}) data from two fibers; curves were fitted by eye. Note that the descending limb extrapolates approximately to a sarcomere length of ~3.6 µm and that optimal length is shifted to the left at higher temperatures. (*b*) Sample records of tension responses to a small stretch (superimposed length records: *bottom* trace) at four different initial lengths; the lower four records were made at 15°C, and the upper four records were made at 40°C. 40–15°C stiffness-difference was estimated as the tension difference at 300 µs, and plotted in *a* (●; *right vertical axis* in kN/m^2/0.5% L_o).

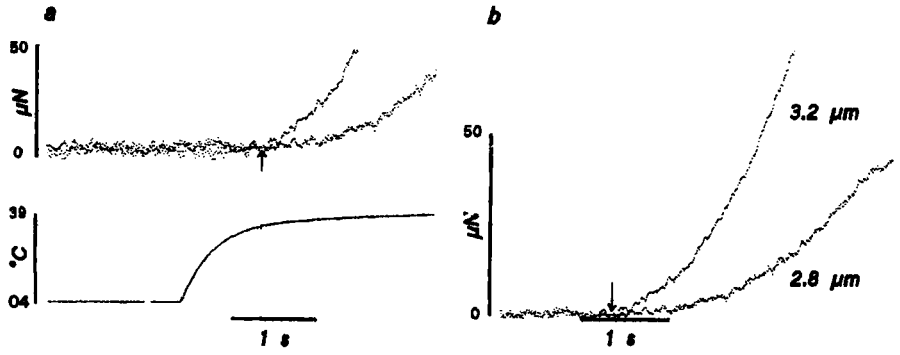

FIGURE 10 (*a*) Tension responses from a muscle fiber at two extensions when a temperature clamp (34–39°C) was applied; the tension responses are shown at higher amplification in *b*. The difference in steady tension at 34°C was removed for superimposing the tension traces. Note that the tension rise on heating is slow, with half-times of 1–2 s.

Tension responses to small-length perturbations

Fig. 11, *a* and *b* show, at a longer time scale (>100 ms), the tension responses to the stretch from another fiber. It is seen that, although the tension responses at 15°C are essentially visco-elastic in nature, the responses at 38°C are considerably more complex and show an apparent activation on stretch and recovery on release. The tension responses of similar magnitude produced by length perturbation in a rigor fiber did not change with temperature.

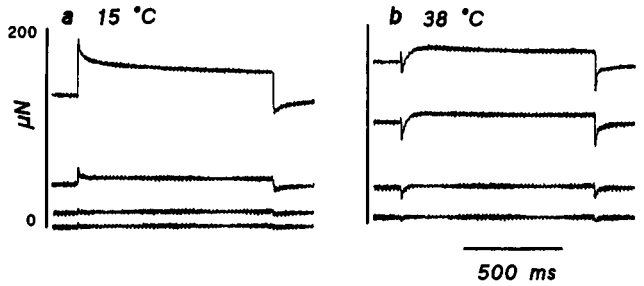

FIGURE 11 Tension responses, at a longer time scale, to the same 9-µm stretch from a fiber at 15°C (*a*) and at 38°C (*b*). The four traces in each frame were at S.L. of 2.4 µm (*bottom* trace; L_o = 1.06 mm; ; X^2 = 3260 µm^2), 2.7 µm, 3 µm, and 3.2 µm. Note the occurence of delayed tension rise at high temperature.

The tension record after the initial rapid tension rise, which occurred in phase with length change, could be fitted with a double exponential function, thus resolving the tension transient at stretched length into a fast (phase 1) and a slow (phase 2) component. Fig. 12, *a* and *b* show the tension responses and fitted curves at ~20 and ~35°C from a fiber (S.L. ~ 3 µm). The estimated reciprocal time constants for phase 1 (*circles*) and for phase 2 (*squares*), pooled from three fibers, are plotted against temperature in Fig. 12 *c*; the data show that the rate of fast tension decay increases from ~150·s^{-1} (141 ± 18·s^{-1}, n = 13, <20°C) at low temperatures to ~800·s^{-1} (770 ± 44·s^{-1}, n = 16) at 35–40°C (Q_{10} calculated from the regression is 2.42). The rate of the slow component contributing to tension decay at low temperatures (phase 2a, *open squares*) is ~10·s^{-1} (11.7 ± 2.6·s^{-1}, n = 13, <20°C), whereas that of the slow component contributing to delayed tension rise at high temperatures (phase 2b, *filled squares*) is 20–40·s^{-1} (34.2 ± 4.2·s^{-1}, n = 16, at 35–40°C); phase 2 could not be resolved at temperatures between 22 and 29°C. The amplitudes of the two components from the three fibers could not be pooled due to differences in tension values; the

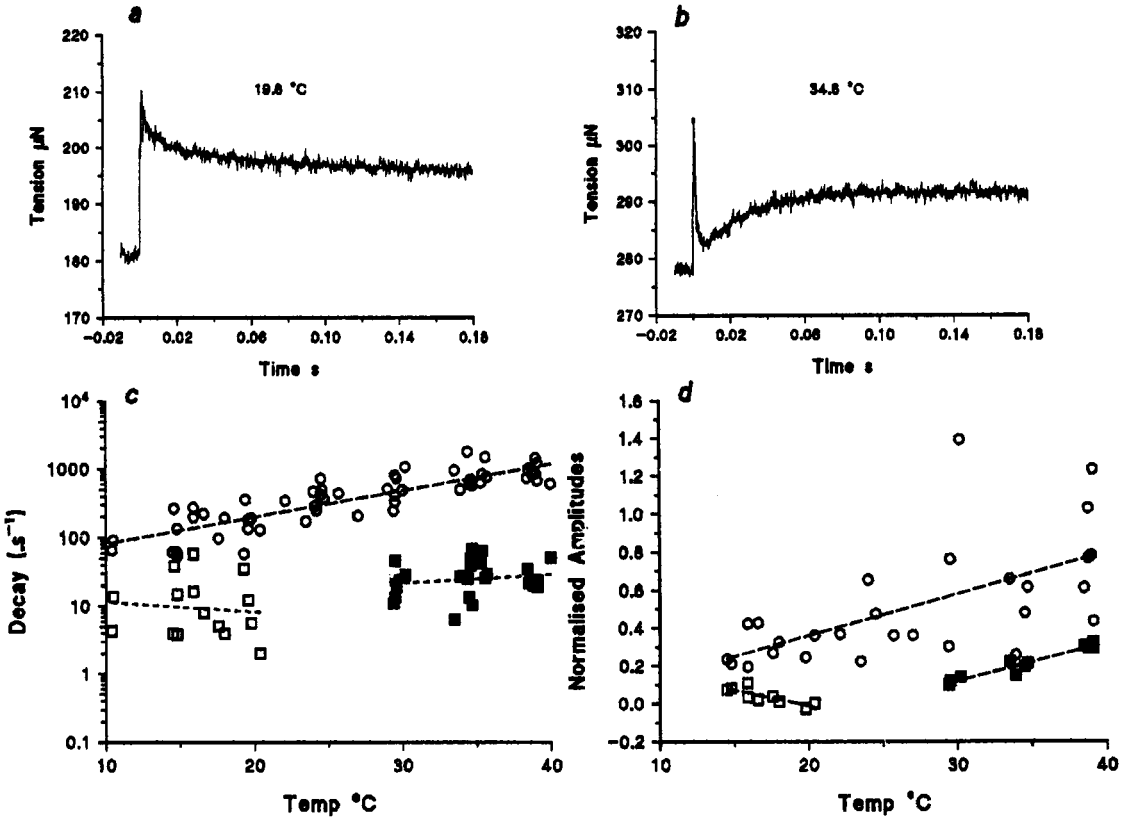

FIGURE 12 (*a, b*) Sample tension responses to the same stretch (1.4%) from a muscle fiber (S.L. ~ 3.1 μm) at ~20°C (*a*) and at ~35°C (*b*). The solid line through each tension record (after the stretch) represents a fitted double exponential function; the tension response at ~20°C consists of a fast decay (phase 1, $134 \cdot s^{-1}$) and a slow decay (phase 2a, $12 \cdot s^{-1}$), whereas at ~35°C it consists of a fast decay (phase 1, $704 \cdot s^{-1}$) and a delayed tension rise (phase 2b, $29 \cdot s^{-1}$). (*c*) The reciprocal time constants obtained from curve fitting (as in *a* and *b*) from three muscle fibers (S.L. ~ 3.0–3.2 μm; stretch 0.8–1.5%) for the initial fast decay (○), for the slow decay (□), and for the tension rise (stretch activation; ■). The fast rate of tension decay increased with temperature ($r = 0.86$; $Q_{10} \sim 2.4$), whereas the slow decay rate and tension rise were not significantly correlated with temperature. Note that within the temperature range of 22–29°C, the tension reponses were fitted with a single exponential only. (*d*) Data from one fiber to illustrate the temperature dependence of the amplitudes of the tension components (normalized to the pre-stretch steady tension).

data from one fiber shown in Fig. 12 *d*, however, illustates the finding. The amplitude of phase 1 (decay) and phase 2b (rise) increased, whereas that of phase 2a (decay) decreased with temperature.

DISCUSSION

Our results from single skinned muscle fibers confirm Hill's (1970) observations on intact frog muscle that warming increases the isometric tension in a resting muscle fiber and that the tension increase is linear at lower temperatures (<25°C), but is more pronounced at high temperatures (30–40°C). We consider below that the linear tension increase up to ~25°C originates from changes within the gap filament-thick filament complex (see Introduction), whereas the tension increase at the high temperatures arises from cycling cross-bridges.

Linear increase of resting tension

Within the range of sarcomere lengths employed (<4.0 μm), possible contributions to resting tension from exo-sarcomeric cytoskeletal components may be excluded (see Wang et al., 1993). Thus, the linear rise of resting tension in warming up to ~25°C is likely to be due to increased stress in the gap filament (extensible titin segment connecting thick filament to Z-disc). One possibility is that heating produces proportionate contraction of gap filament; gap filament tension (thermal stress) arises from a change in its intrinsic elasticity. A second possibility is that the titin-myosin anchorage is temperature-sensitive and that heating affects the nonextensible part of the titin filament in such a way as to decrease the free length of the titin segment that forms the gap filament (reverse of the process that occurs on extension of sarcomeres beyond the yield point; see Wang et al., 1993); thus, tension arises from an "effective extension" of the gap filament because a shorter titin segment length now spans the same A–Z distance. With either mechanism, the titin-myosin composite filament would develop positive thermal stress corresponding to an effective strain ($-\alpha$) of 0.1%/°C.

Thus, the increase with temperature of the fast rate of tension decay after stretch (phase 1, Q_{10} >2; see Fig. 12) would be due to either altered intrinsic elasticity of gap filament (first mechanism) or gap filament being set in an

extended part of its force-extension curve (second mechanism). At the same temperature, the tension decay became faster with larger stretch amplitude (preliminary findings), indicating that the second mechanism is feasible.

With heating, the rigor tension decreased linearly, indicating net thermal expansion (presumably in thin filament, thick filament, and/or cross-bridges; see Goldman et al., 1987); however, the decrease of tension was similar at different sarcomere lengths, and there was no obvious evidence of thermal contraction, even at longer sarcomere lengths where a fiber had some passive tension. Evidently, the titin-myosin complex does not develop positive thermal stress in rigor fibers. It is clear that neither of the two hypotheses (mechanisms) affords a ready explanation to this observation, although more detailed experimentation on rigor fibers would be required to confirm this finding. On the other hand, it is not inconceivable that thermal contraction in gap filament (first mechanism) requires a certain minimum sarcomeric compliance and that, even with thermal expansion, it is not present in the rigor sarcomere. Alternatively, the titin-myosin anchorage (second mechanism) may be stabilized in rigor, preventing gap filament shortening.

If our interpretation that heating induces a positive thermal stress in titin-myosin composite filament is correct, then an increase of tension with temperature would not be observed in fibers where gap filament is destroyed (or in KI-treated fibers). Experiments of the type reported by Wang et al. (1993), if carried out at different temperatures, should indicate which of the above mechanisms can account for various observations.

Heat-induced activation

Observations indicating thermal instability in components of myofibrillar myosin (S-2 region) have been reported in a number of studies (Ueno and Harrington, 1986a, b; Walker and Trinick, 1986); indeed, Davis and Harrington (1987) demonstrated a thermal contraction in muscle fibers in rigor, but in a medium of low ionic strength and high pH and at temperatures higher than 41°C. However, under the conditions adopted in the present experiments, there was no evidence to suggest that such elasticity changes are the source of the pronounced increase of tension at high temperature (30–40°C): in particular, the rigor tension at similar sarcomere lengths decreased linearly within the same temperature range. Evidence indicates that the extra tension at high temperatures is "active," resulting from cycling cross-bridges; like active tension, the nonlinear tension rise was dependent ATP, depressed by acidic pH, added inorganic phosphate, and had a bell-shaped length-tension relation (the discrepancy in the sarcomere length range may be due to incomplete activation at the chosen temperature; Fig. 9).

The heat-induced tension rise is Ca-independent because the standard relaxing solutions contained EGTA (a Ca-chelator) and no added Ca^{2+}, and also the Ca-sensitivity of thin filament activation decreases with warming (Stephenson and Williams, 1985; Goldman et al., 1987). It seems unlikely that ATP-depletion in parts of the fiber (e.g., in the core) leads to thin filament activation, because the relaxing media contained creatine-phosphate/kinase system, the tension changes were largely reversible, and heat-induced tension occurs in intact fibers.

Babu and Gulati (1988) proposed a two-step regulation of cross-bridge mechanism to explain a number of observations relating to thin filament activation in muscle fibers; step 1 controls formation of weakly attached cross-bridges, and step 2 controls the transition from weak to strongly attached cross-bridge states. Step 1 normally requires Ca^{2+} (possibly for releasing steric blocking) but can proceed in the absence of Ca^{2+} at low ionic strength and low temperature (Brenner et al., 1982). The studies of Fuchs (1975) and Fuchs et al. (1975) showed evidence of "reversible inactivation" of the Ca^{2+} control mechanism in isolated actomyosin system at high temperatures (35–45°C) due to a direct effect of temperature. Thus, as a consequence of a direct heating effect on troponin and/or tropomyosin system (presumably increasing their mobility), their steric-blocking of the thin filament may become reversibly weakened at high temperature, leading to formation of weakly attached cross-bridge states. Additionally, increased affinity of myosin to actin at high temperature (Coates et al., 1985) may account for the observation that heat-induced tension develops at a lower temperature when sarcomere lengths are longer (reduced lattice spacing; see Fig. 2). Step 2 may require Ca^{2+} (see, however, Millar and Homsher, 1990) but may operate if heating induces weak-strong transition directly; indeed, increased cross-bridge force and/or force generation is evident in muscle fibers after a rapid temperature-jump at low and high temperatures (see Davis and Harrington, 1987; Goldman et al., 1987; Bershitsky and Tsaturyan, 1989, 1992). Because cross-bridge attachment and force generation would be fast, the slow onset of tension rise (Fig. 10) reflects that the heat-induced thin filament activation itself is limiting; it may be that heat-induced activation spreads from the free end of a thin filament.

The validity of our interpretation may be tested in a number of ways. First, the heat-induced activation would be more pronounced (i.e., would occur at lower temperatures) in lower ionic strength, because low ionic strength increases actin-myosin affinity and encourages weak cross-bridge attachment. Second, it would be enhanced with decrease of lattice spacing by other methods (e.g., osmotic shrinking). Third, because Ca^{2+} is not involved, heat-induced activation may not be altered in fibers from which troponin-C has been removed (e.g., by the method used by Babu and Gulati, 1988).

Stretch activation

The response to stretch showed a fast and a slow component of tension relaxation at low temperature, whereas at high temperature the fast tension decay was followed by a slow delayed tension rise (Figs. 11 and 12), reminiscent of stretch activation in insect muscle fibers (see White and Thorson,

1975 and references therein). The delayed rise of tension occurred at 30–40·s^{-1} at ~35°C, and a preliminary experiment showed it to increase (severalfold) in the presence of 10 mM added phosphate. These values are similar to the rate constant for force redevelopment reported from maximally Ca-activated muscle fibers (~50·s^{-1} at 35°C, Brenner and Eisenberg, 1986). Therefore, it seems that a 1–1.5% stretch at high temperature induces cross-bridge attachment and/or cross-bridge force generation in mammalian sarcomeres; presumably, the increased (thermal) stress in titin-myosin composite filament and changes in thin filament activation (see above) makes the cross-bridge mechanism strain-sensitive at high temperatures.

On the basis of our interpretation, a similar form of stretch activation may be observed, even at low temperature, if a fiber is set at longer sarcomere length (to stress the gap filament) and exposed to submaximal Ca-levels (for partial thin filament activation). Possible mechanism, whether similar stretch activation occurs in intact fibers, and its contribution to normal Ca-activation remain to be investigated.

CONCLUSION

Our results indicate that heating to high temperatures increases thermal stress in gap filament-thick filament complex, makes the cross-bridge mechanism strain-sensitive, and produces thin filament activation. The heat-induced activation occurs at ~40°C at sarcomere lengths considered to be physiological (<2.5 μm). Although the intramuscular temperature in humans can rise to ~39°C after exercise (Davies et al., 1982), the accompanying changes (decreased pH, increased phosphate, etc.) will supress heat-induced tension; the heat contracture in muscle (see Hill, 1970) that accompanies hyperthermia may be an accentuation of this process. The stretch-induced activation may have a physiological role; some sarcomeres in a fiber may become extended with physiological nerve-activation at submaximal frequencies, and present results show that, at physiological temperatures, extended sarcomeres will become activated and, hence, resist stretching. Thus, this would operate as a mechanism to enhance sarcomere uniformity in actively contracting in situ fibers. Moreover, the increase with temperature of endo-sarcomeric stress also implies that the mammalian sarcomeres would be more uniform at high temperatures.

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