Tension Relaxation after Stretch in Resting Mammalian Muscle Fibers: Stretch Activation at Physiological Temperatures

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ABSTRACT Tension responses to ramp stretches of 1–3% L_o (fiber length) in amplitude were examined in resting muscle fibers of the rat at temperatures ranging from 10°C to 36°C. Experiments were done using bundles of ~10 intact fibers isolated from the extensor digitorum longus (a fast muscle) and the soleus (a slow muscle). At low temperatures (below ~20°C), the tension response consisted of an initial rise to a peak during the ramp followed by a complex tension decay to a plateau level; the tension decay occurred at approximately constant sarcomere length. The tension decay after a standard stretch at ~3–4 · L_o/s contained a fast, an intermediate, and a (small amplitude) slow component, which at 10°C (sarcomere length ~2.5 μm) were ~2000 · s^{-1}, ~150 · s^{-1}, and ~25 · s^{-1} for fast fibers and ~2000 · s^{-1}, ~70 · s^{-1}, and ~8 · s^{-1} for slow fibers, respectively. The fast component may represent the decay of interfilamentary viscous resistance, and the intermediate component may be due to viscoelasticity in the gap (titin, connectin) filament. The two- to threefold fast-slow muscle difference in the rate of passive tension relaxation (in the intermediate and the slow components) compares with previously reported differences in the speed of their active contractions; this suggests that "passive viscoelasticity" is appropriately matched to contraction speed in different muscle fiber types. At ~35°C, the fast and intermediate components of tension relaxation were followed by a delayed tension rise at ~10 · s^{-1} (fast fibers) and 2.5 · s^{-1} (slow fibers); the delayed tension rise was accompanied by sarcomere shortening. BDM (5–10 mM) reduced the active twitch and tetanic tension responses and the delayed tension rise at 35°C; the results indicate stretch sensitive activation in mammalian sarcomeres at physiological temperatures.

INTRODUCTION

From a study on intact frog sartorius muscle, D. K. Hill (1970) reported that the increase of resting isometric tension on warming became more pronounced at temperatures higher than ~23°C; he suggested an "active" origin for the temperature-induced increase of tension in resting muscle at high temperatures. Hill (1972) also showed that a similar marked increase of resting tension occurs in intact rat soleus at temperatures close to 40°C. More recently, Hill's basic observations were confirmed in a detailed study made on single skinned fast fibers from rabbit muscle (Ranatunga, 1994); additionally, evidence was presented that the contractile mechanism or its activation in mammalian sarcomere may be stretch sensitive at high temperature. Although stretch activation is well known in insect muscle fibers (see references in White and Thorson, 1975), and some data are available from maximally Ca-activated skinned mammalian muscle fibers (Galler et al., 1994), the type of stretch activation reported by Ranatunga (1994), i.e., in relaxed muscle fibers at high physiological temperatures, has not been reported in intact mammalian muscle fibers.

The present study examines stretch-induced tension responses in intact mammalian (rat) fast and slow muscle fibers at temperatures ranging from 10°C to 36°C and shows that, at physiological temperatures, a tension response contains a characteristic delayed tension rise that may be indicative of stretch activation. Interesting differences are also observed between fast and slow fibers in their passive viscoelasticity.

Abstracts based on this study were presented to the Physiological Society (Mutungi and Ranatunga, 1994a,b).

MATERIALS AND METHODS

Adult male rats (body weight 230 ± 2.5 g) were killed with an overdose of sodium pentobarbitone (Sagatal; May and Baker) injected intraperitoneally, and the extensor digitorum longus (e.d.l.) and soleus muscles from each hind limb were carefully removed. Small bundles of muscle fibers were isolated under a dissecting microscope fitted with dark-field illumination. Considerable care was taken in removing damaged fibers to ensure that those which extended from end to end in a bundle were intact and electrically excitable. In different experiments a preparation contained 2–12 intact fibers. Because histochemical analyses showed that in terms of the cross-sectional area occupied by the fiber types, the adult rat e.d.l. contains >90% type 2 fibers and soleus contains ~80% type 1 fibers (Ranatunga and Thomas, 1990), the e.d.l. and soleus preparations will be referred to as fast and slow fibers, respectively.

A preparation was set up in a flow-through stainless steel chamber (volume ~2 ml). The chamber was fitted with a glass window at the bottom and mounted on an optical microscope assembly. The preparation was mounted between two stainless steel hooks, one attached to a force transducer and the other to a servo motor using aluminium foil clips, and was perfused (0.5 ml/min) with Ringer's solution containing (in mM): NaCl, 109; KCl, 5; $MgCl_2$, 1; $CaCl_2$, 4; $NaHCO_3$, 24; NaH_2PO_4, 1; sodium pyruvate, 10; and 200 mg/liter bovine fetal serum. The solution was continuously bubbled with 95% O_2 and 5% CO_2.

The motor was built using a permanent ring magnet from a loudspeaker; the former was made of aluminum foil, it was held by plastic hinges, and its axial displacement was monitored photoelectrically. The motor was

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capable of producing ramp length steps of upto ~300 μm complete in <0.5 ms. The design of the force transducer was as described previously (Ranatunga, 1994); it consisted of two AE 801 elements (AME, Horten, Norway) mounted side by side to reduce the temperature sensitivity of its output, and its natural resonant frequency was ~5 kHz.

The cross-sectional area of a bundle was estimated from two width measurements orthogonal to each other (by optical microscopy). A diffractometer, assembled using a 5 mW He-Ne laser, was used to monitor average sarcomere length; using an assembly of cylindrical and biconvex lenses (similar to that described by Goldman and Simmons, 1984), a rectangular beam of ~2 mm (along the fiber axis) by 0.2 mm across was projected onto the fiber bundle near the force transducer end. Equatorially scattered light was collected on a photodetector by a cylindrical lens to monitor the position of the first-order diffraction. The photodetector was a one-dimensional position-sensitive detector (Hamamatsu Photonics, S3932) coupled to an analog divider circuit, and it provided a voltage signal that was proportional to the position of the first-order diffraction. In an experiment, a preparation was first set up at low temperature (<20°C) at a length where the active (twitch) tension was maximum; the sarcomere length at this initial length was 2.4–2.7 μm in e.d.l. and ~2.8–3.0 μm in soleus.

A fiber bundle was stimulated with single supramaximum stimuli at a rate of 1/60–90 s, and small stretches were interposed between twitches during some cycles. The resting tension responses to ramp stretches of 100–300 μm in amplitude (1–3% L_o, fiber length) and completed in ~1 ms, or at standard stretch velocities, were examined at temperatures ranging from 10°C to 36°C. The temperature variation and control (±0.1°C) were done by means of a Peltier device fitted beneath the muscle chamber, and it was monitored with a thermocouple placed inside the muscle chamber.

A Tandon computer (Target 386 SX-40) with a CED 1401 laboratory interface (Cambridge Electronic Design, Cambridge, England) was used to digitize and store the tension signal, the length signal (from the motor), and in some experiments, the sarcomere length signal (from the diffractometer). Additionally, the tension transducer output and the thermocouple amplifier output were continuously recorded on a chart recorder (Devices). To obtain numerical data to define tension relaxation after stretch, the tension decay signal was fitted to the sum of two or three exponentials, using a nonlinear curve-fitting program (FIG-P, Biosoft). From a visual examination of the tension decay trace with the fitted curve, a two-exponential curve was judged to be inadequate to define the full tension decay at low (<20°C) and high (>30°C) temperatures; a three-exponential function was adequate, and the differences between the three components in their rate and amplitude remained statistically significant.

It is pertinent to mention that the detailed methodology and techniques adopted in these experiments were such that a small increase of steady resting tension in warming (see Ranatunga, 1994) could not be accurately assessed. Nevertheless, in 15 of 19 e.d.l. preparations, the steady resting tension at 35°C was higher than that at 10°C; the mean (±SEM) tension increase was 2.8 (±0.57) kN/m^2 (range 0.5–6.8 kN/m^2). This average tension increase is considerably lower than that obtained in single skinned fibers (~10 kN/m^2) under more controlled conditions (see figures 8 and 9 in Ranatunga, 1994). The apparent low sensitivity and the variability are likely to be due to the heating-induced expansion in the stainless steel hooks, Al-clips, and tendon used in muscle attachment in the present experiments. Estimation of thermal resting tension was not a main concern of this study, and hence, except in Fig. 1, the steady tension will be removed in the data presentation.

RESULTS

Fig. 1, *a*, *b*, and *c*, shows the tension responses recorded from an intact fast fiber bundle in response to a standard, moderately fast stretch applied to its tendon; it is seen that the tension rises to a peak during the stretch but then decays to a steady level at the extended length. Comparison of the records at low temperature (20°C; Fig. 1, *a* and *c*) with that at 35°C (Fig. 1 *b*), however, shows the occurrence of a delayed tension rise at the high temperature. Indications of a delayed tension rise at ~35°C were seen in every preparation, but its amplitude was variable in different preparations. Fig. 1, *d*, *e*, and *f*, shows records from a preparation where the delayed tension rise was particularly pronounced; records in Fig. 1 also show that changes seen on warming from 15–20°C to 35°C are largely reversible on cooling

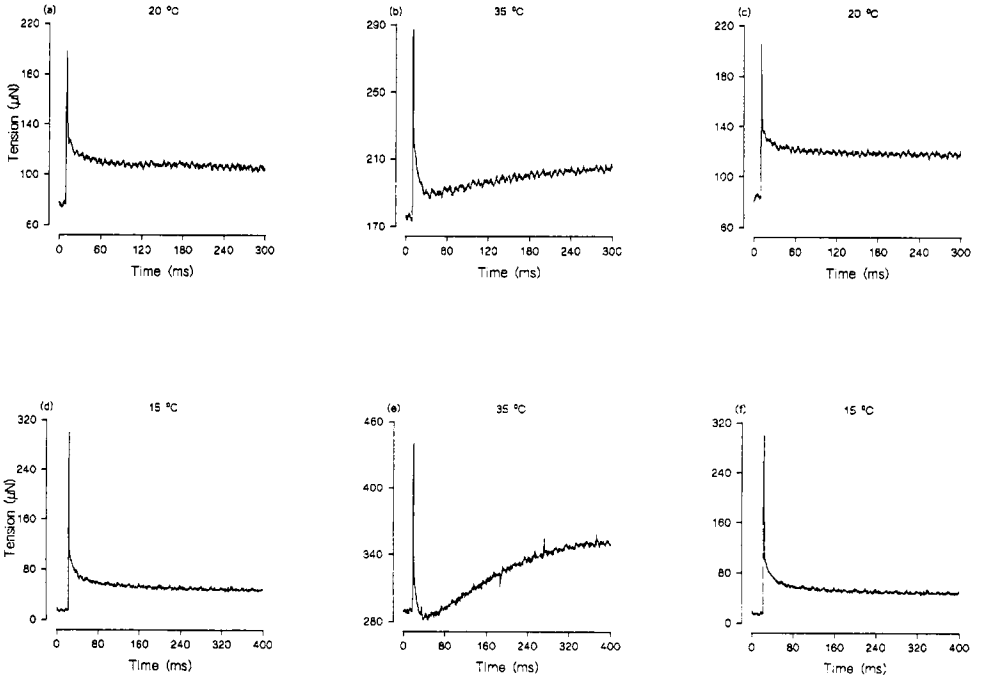

FIGURE 1 Stretch-induced tension responses recorded in series from two fast fiber preparations (*a*, *b*, *c* and *d*, *e*, *f*) at the temperatures indicated. L_o was 12.0 mm for *a*, *b*, *c* and 14.4 mm for *d*, *e*, *f*, and the stretch amplitude in all cases was 186 μm. Note the delayed tension rise in *b* and *e* (at 35°C).

back to low temperature (compare Fig. 1, *a* and *c*, and *d* and *f*).

It is seen from Fig. 1 *e* that, at the onset of delayed tension rise, the tension is decreased to a level below the pre-stretch tension. According to our previously reported observations on skinned fibres and the interpretations given to them (see Ranatunga, 1994), the resting tension of mammalian muscle fibers increases with heating, and at high temperatures (>~35°C) it may contain an active component (due to cycling cross-bridges; see Discussion). Thus, a reduction below steady level may be due to stretch-induced decrease of active tension (cross-bridge detachment); indeed, similar stretch-induced tension records were obtained in skinned fibers (see figure 11b in Ranatunga, 1994), particularly at temperatures of 38–40°C, that were higher than the highest temperature typically used in the present experiments (~36°C).

The sarcomere length change induced by the applied stretch was monitored in a 2-mm region near the tension transducer end of a bundle. Fig. 2 shows some sample records where the bottom trace in each frame is the diffractometer signal. It is seen that the complex tension decay at the low temperature occurs at approximately constant sarcomere length, as had been reported from intact resting frog muscle (Magid and Law, 1985). This indicates that the tension changes are not due to sarcomere shortening and/or lengthening. In contrast, the delayed tension rise at high temperature is accompanied by sarcomere shortening (Fig. 2 *b*); because transient sarcomere shortening was observed during active twitch and tetanic responses (not illustrated), the above observation indicates that the delayed tension rise may result from "contractile activation."

The tension record after the initial rapid tension rise, which occurred in phase with length change, could be fitted with a triple-exponential function (see Materials and Methods), thus resolving the tension transient at stretched length into a fast (phase 1), an intermediate (phase 2), and a slow (phase 3) component. Fig. 3 shows the tension responses and fitted curves at ~10°C from an e.d.l. fiber bundle, where the same record is shown at two different time scales. Fast, intermediate, and slow exponential components in the resting tension decay at low temperatures, with rates of $\sim 2000 \cdot s^{-1}$, $\sim 150 \cdot s^{-1}$, and $\sim 20 \cdot s^{-1}$, were obtained in experiments on a large number of e.d.l. preparations (see Mutungi and Ranatunga, 1994b). Phase 1 was by far the most dominant component (contributing ~50% to the total decay amplitude); its amplitude was directly proportional to the velocity of the ramp stretch (data not shown), indicating that it is due to decay of a viscous force.

Table 1 shows pooled data from four e.d.l. preparations in which the same standard stretch ($\sim 3 \cdot L_o/s$) was used at 10°C and 35°C; the data show that the reciprocal time constant for phase 1 was $\sim 2000 \cdot s^{-1}$ at both temperatures, but its amplitude decreased ~25% with warming to 35°C. Data for phase 2 show that its amplitude decreased ~50% and its rate increased on warming; this probably represents decay of a viscoelastic force. Phase 3, which contributed to tension decay ($\sim 25 \cdot s^{-1}$) at the low temperature, is not evident at the high temperature but is replaced by a tension rise at $\sim 10 \cdot s^{-1}$. From other experiments, in which tension response to a standard stretch was examined at various temperatures, it was found that a phase 3 as a tension decay component was not discernible at temperatures between 20 and 25°C, and indications of a delayed tension rise were evident at temperatures higher than ~28°C. Because the tetanic tension for intact rat fast muscle at 35°C is ~200 kN/m^2 (see Ranatunga, 1984), the amplitude of delayed tension rise (phase 3 at 35°C) under these conditions corresponds to ~2% of maximal Ca activated tension in fast fibers. The extent to which this amplitude is reduced by sarcomere shortening remains unknown.

BDM (2,3-butanedione monoxime), in millimolar concentrations, is known to reversibly depress active tension in intact muscle fibers by reducing Ca release and suppressing cross-bridge cycling (Fryer et al., 1988). We therefore examined the effects of 5–10 mM BDM on the stretch-induced tension responses.

Fig. 4 shows a part of the chart recording of tension (*lower*) and temperature (*middle*) from an experiment. Twitch contractions were elicited at regular intervals and tetani at infrequent intervals while the temperature was raised from 16°C to 35°C; during the period indicated by the horizontal bar, the preparation was exposed to Ringer's solution containing 5 mM BDM. It is seen that at 16°C, the twitch is almost completely abolished and the tetanic tension markedly depressed on exposure to BDM; the effects are less pronounced at 35°C and are reversible. In experiments on four fiber bundles, 5 mM BDM nearly abolished the twitch response and reduced the tetanic tension to ~30% (at 16°C). However, the presence of 5–10 mM BDM was found to have no discernible effect on the

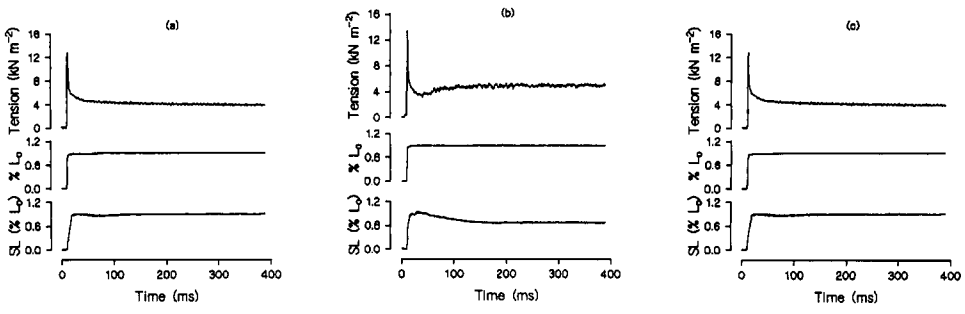

FIGURE 2 The tension response (*top trace*) and the sarcomere length change (*bottom trace*) induced by a ramp stretch (*middle trace*): records are from one fast muscle fiber bundle at 10°C (*a*), after warming to 35°C (*b*), and on cooling back to 10°C (*c*). Note that the delayed tension response is accompanied by sarcomere shortening (*b, bottom trace*).

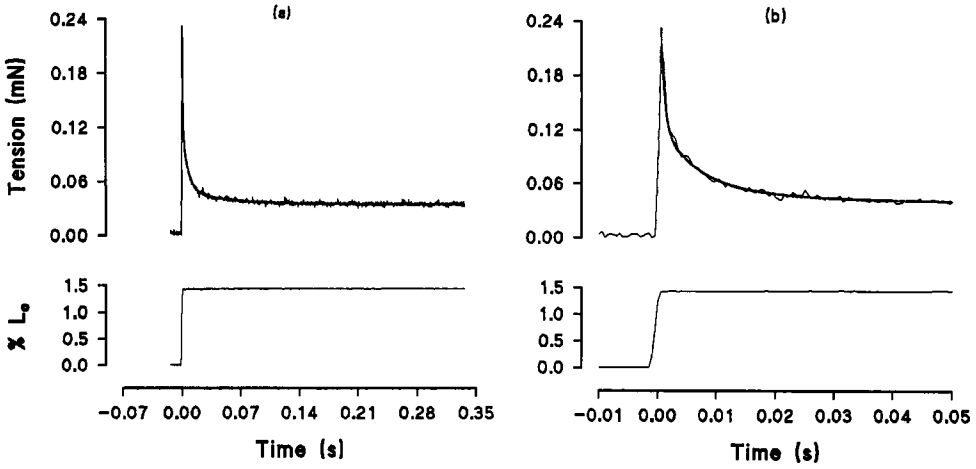

FIGURE 3 The stretch-induced tension response from a fast bundle at 10°C with a tri-exponential curve fitted; the same record is shown at two different time scales. The calculated rates (±SE in s^{-1}) for the components were 1653 (±118), 157 (±10.5), and 20.5 (±2.1).

steady resting tension or on the stretch-induced tension reponse of relaxed fibers at low temperatures; from four e.d.l. preparations, the mean (±SEM) rate data (in s^{-1}) for phases 1, 2, and 3 were 1870 (±130), 166 (±14), and 20 (±3) without BDM and 1998 (±150), 170 (±20), and 20 (±4) with BDM. On the other hand, 5–10 mM BDM reversibly depressed the delayed tension rise seen at 35°C, as illustrated by the sample tension traces from two preparations given in Fig. 5. In every preparation, the steady resting tension at 35°C was reversibly reduced in the presence of BDM; the mean (±SEM) tension reduction was 3.8 (± 1) kN/m^2 (range 0.3–6 kN/m^2) from five preparations. This supports the idea (see above) that a small component of "resting muscle tension" at high temperature is active. Interestingly, the "residual" tension at stretched lengths is higher in the presence of BDM, an effect that is difficult to explain. Whether this is somehow related to the fact that the delayed tension rise is accompanied by sarcomere shortening or is due to the fact that BDM depresses the active tension more than the stiffness (Bagni et al., 1992b; Zao et al., 1995) remains uncertain.

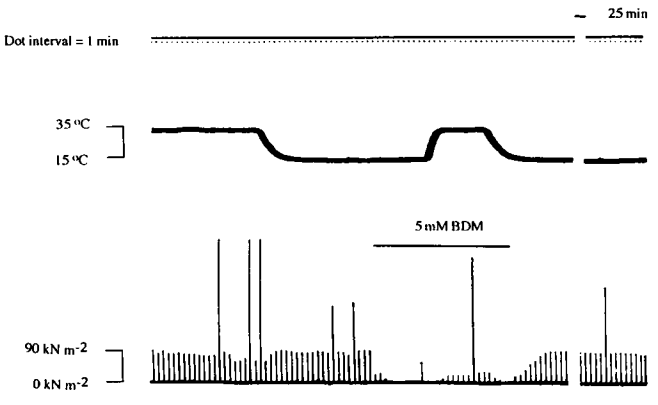

FIGURE 4 Part of a chart record illustrating the effects of 5 mM BDM on twitch and tetanic contractions (larger spikes) at 15 and 35°C (fast fiber bundle). Note that at 10°C 5 mM BDM abolishes twitch tension completely and depresses tetanic tension markedly. The effects are less but significant at 35°C and reversible at both temperatures.

The same basic observations were made in experiments on soleus muscle fiber bundle preparations. As shown by the representative records in Fig. 6 *i*, the stretch-induced tension response at 10°C is a viscoelastic one, whereas the tension response at 35°C contains a delayed tension rise that disappears on cooling back to 10°C. Fig. 6 *ii* shows tension reponses from another preparation recorded at 35°C; the delayed tension rise is abolished in the presence of 10 mM BDM. The mean (±SEM) values obtained for the rates of tension decay, from five preparations (sarcomere length ~3 μm), were 485 (± 14) · s^{-1}, 55 (± 1.2) · s^{-1}, and 7.6 (± 0.2) · s^{-1} at 10°C and 445 (± 19) · s^{-1}, 63 (± 1.9) · s^{-1}, and 2.4 (± 0.2) · s^{-1} (delayed tension rise) at 35°C; the delayed tension rise was considerably slower than in e.d.l. fibers.

With the particular experimental protocol adopted in the initial setting of a preparation (see Materials and Methods), the resting sarcomere length in the above soleus preparations was considerably longer (2.8–3.0 μm) than in e.d.l. (2.4-.2.6 μm). To compare viscoelasticity between fast and slow fibers, recordings were made in each of four fast and three slow fiber bundle preparations, at three different sarcomere lengths (range 2.4–3 μm). The results showed that the phase 2 and the phase 3 component rates remain distinct between fast and slow fibers; the full ranges for the two components were 120–180 · s^{-1} and 15–30 · s^{-1} for fast and 40–80 · s^{-1} and 5–15 · s^{-1} for slow muscle. The rate of the phase 1 component in slow fibers was lower at longer sarcomere length (full range 400-1900 · s^{-1}), whereas that of fast fibers remained between 900 and 2000 · s^{-1}. These results show that the intermediate (phase 2) and the slow (phase 3) rates of resting tension decay after a stretch, at low temperatures, is two- to threefold slower in slow soleus than in fast e.d.l. muscle fibers.

DISCUSSION

Resting viscoelasticity (low temperature)

Previous findings on skinned fibers (Ranatunga, 1994) and certain observations presented above from intact fibers indicated that the steady "resting tension" in mammalian

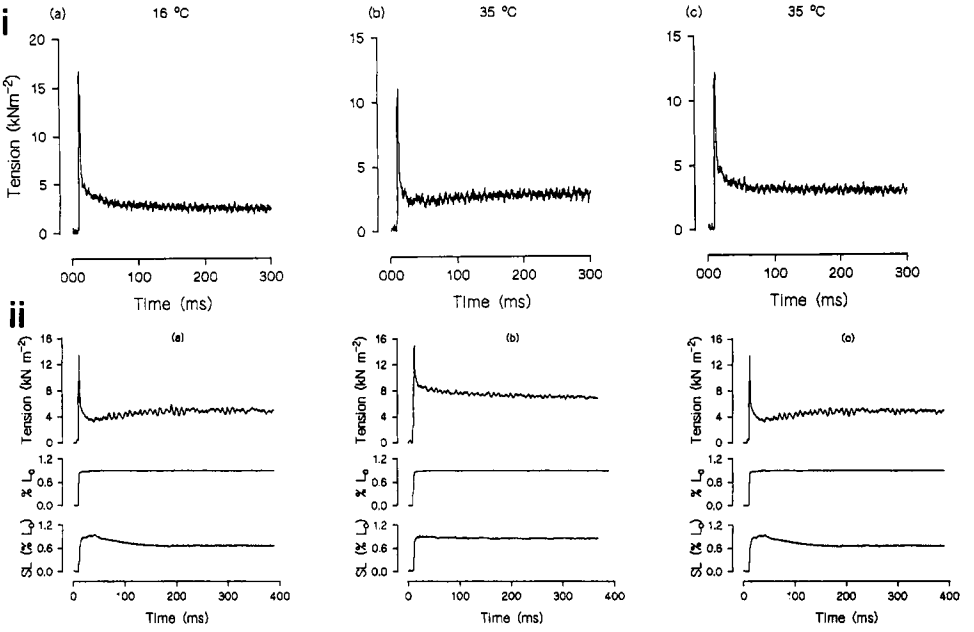

FIGURE 5 (*i*) Tension responses to a standard ramp stretch from a fast muscle fiber preparation at 16°C (*a*) and 35°C in the absence of BDM (*b*) and at 35°C in the presence of 5 mM BDM (*c*). Note that the addition of 5 mM BDM abolishes the delayed tension. (*ii*) Tension (*top trace*) and sarcomere length (*bottom trace*) responses to a ramp stretch (*middle trace*) recorded from a fast muscle fiber bundle at 35°C in the absence of BDM (*a* and *c*) and at 35°C in the presence of 10 mM BDM (*b*). Note that 10 mM BDM reversibly abolishes the delayed tension rise and sarcomere shortening.

muscle fibers at high temperatures may contain an "active" component; therefore, the resting viscoelasticity will be examined with respect to the data collected at low temperatures.

Some observations presented by Ford et al. (1977) on intact frog muscle fibers at low temperatures showed that the tension decay after a ramp stretch contained a fast ($\sim 1500 \cdot s^{-1}$) and a slow ($\sim 50 \cdot s^{-1}$) component. In our experiments on fast muscle fibers at low temperatures, the tension decay had a fast ($\sim 2000 \cdot s^{-1}$), an intermediate ($\sim 150 \cdot s^{-1}$), and a slow component ($\sim 25 \cdot s^{-1}$). From a detailed analysis made on the rising phase of the ramp stretch-induced tension responses in frog fibers, Bagni et al. (1995) showed the presence of viscous, viscoelastic, and elastic tension components in resting muscle fiber. Our findings from the analyses of tension decay at low temperatures, and from either fiber type, are basically consistent with their model. Thus, the fast phase, the amplitude of which changed directly with stretch speed, would represent decay of the viscous force. The intermediate phase, the amplitude of which decreased but the rate of which increased with temperature (see Table 1), would be decay of the viscoelastic force, and the small amplitude (third) component of decay (phase 3) may arise from some slow stress

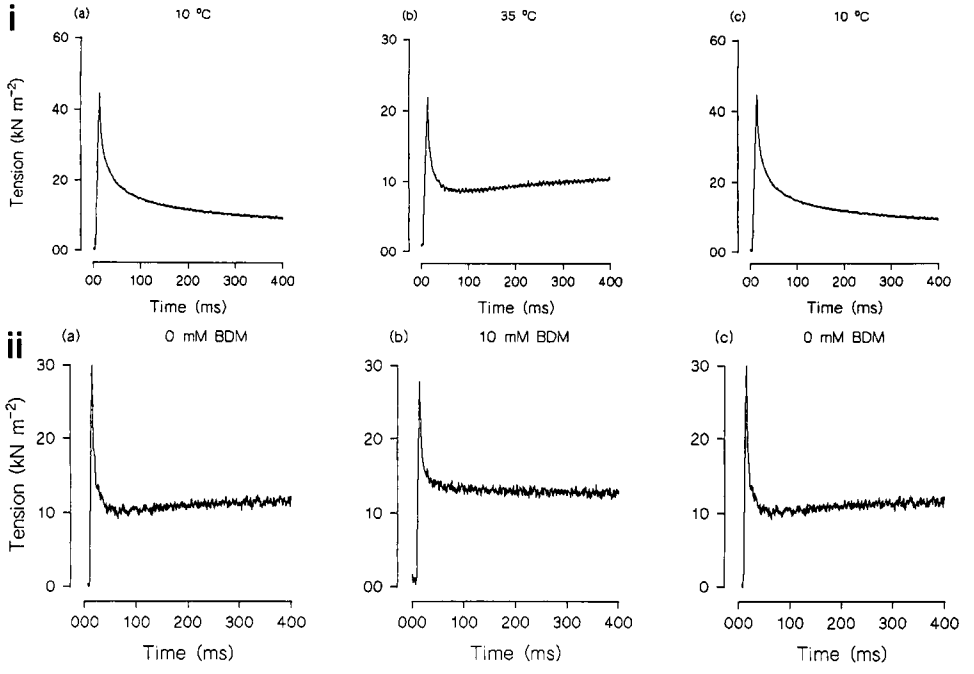

FIGURE 6 (*i*) The tension responses to a standard ramp stretch in a slow muscle fiber bundle at 10°C (*a*), after warming to 35°C (*b*), and on cooling back to 10°C (*c*) (sarcomere length ~3 μm). Note that the responses are basically similar to those observed in fast muscle in that a delayed tension rise is present at 35°C; the tension decay, however, is considerably slower than in fast muscle fibers. (*ii*) Effect of 10 mM BDM on the tension responses of a slow muscle fiber bundle preparation at 35°C (sarcomere length ~2.7 μm): (*a* and *c*) No added BDM; (*b*) with 10 mM BDM added. Note that 10 mM BDM reversibly abolishes the delayed tension rise.

TABLE 1 Characteristics of tension relaxation in fast e.d.l. muscle fibers, in response to a standard stretch (velocity ~3 · L_o/s, amplitude ~2% L_o)

	10°C	35°C
Phase 1		
Amplitude (kN · m^{-2})	17.4 (± 0.04)	12.8 (± 1.5)
Decay rate (s^{-1})	2240 (± 140)	2080 (± 136)
Phase 2		
Amplitude (kN · m^{-2})	11.8 (± 0.56)	5.82 (± 0.46)
Decay rate (s^{-1})	152 (± 15)	240 (± 29)
Phase 3		
Amplitude (kN · m^{-2})	2.49 (± 0.1)	−3.7 (± 0.08)
Decay rate (s^{-1})	25.0 (± 4)	10.0 (± 3.3)

Data are from four fiber bundles, for each of which data were obtained at both temperatures. Note that each value is the mean (±SEM) and that phase 3 at 35°C (given as a negative amplitude) represents the delayed tension rise.

relaxation in a parallel elastic component. Both the steady resting tension and the tension response to stretch were largely unaffected by the presence of BDM, indicating that they are due to passive viscoelasticity in muscle fibers; Bagni et al. (1992a, 1995) made the same conclusion from their analyses of the rising phase of the tension response in frog fibers.

A fast component (>1000 · s^{-1}) of tension decay was not observed in our experiments on skinned fast muscle fibers (see Ranatunga, 1994); the viscous tension component was considerably reduced after skinning in frog fibers (Bagni et al., 1995). The absence (or marked reduction) of the fast tension decay in skinned fibers may arise from a decrease in interfilamentary myoplasmic viscosity resulting from skinning and from swelling of the filament lattice spacing (see Goldman and Simmons, 1986).

As suggested by Bagni et al. (1992a) and Ranatunga (1994), the viscoelasticity (the intermediate decay component) may arise from characteristics of the gap filament (+ thick filament) complex; the parallel elasticity (in fiber bundles) may be due to both exo- and endosarcomeric, noncontractile cytoskeletal filaments. Apart from the fast initial decay, the resting tension decay after a comparable stretch was two- to threefold slower in the slow fibers. It is unlikely that such differences arise entirely from non-muscle-fiber structural components in the preparations; the implication is that different fiber types may have different passive viscoelastic characteristics. Wang et al. (1991) and Horowits (1992) indeed showed differences in the "steady state, force-extension relations" between fiber types and that fast and slow fibers have different titin (connectin) size isomers forming the gap filament; clearly, the differences we observe in the rate of passive tension decay may be related. The difference between the two preparations in the rate of "passive" tension decay is similar to the difference seen between the fast and slow fiber types with respect to the speed of their "active" contractions (e.g., shortening velocity and rate of tension rise; see Ranatunga, 1984; Ranatunga and Thomas, 1990); thus, from a functional point of view, the matching of passive viscoelasticity with cross-bridge cycling rate may be an important feature in the physiological design of skeletal muscle. One may speculate that such a coupling ensures efficiency in power transfer during active muscle fiber shortening and/or lengthening.

Delayed tension rise (high temperature)

The tension decay after stretch was followed by a delayed tension rise at high temperatures in both fast and slow intact fibers. This observation compares with the delayed tension rise first reported from skinned rabbit fast fibers at high temperature (Ranatunga, 1994) and suggests that it may be an important characteristic of mammalian muscle fibers at physiological temperatures. Our finding that the delayed tension rise becomes depressed in the presence of BDM and that it is accompanied by sarcomere shortening support the suggestion that this represents a form of "stretch activation." The rate of delayed tension rise was slower in the slow fibers, as would be expected from known differences in the speed of their active contractions (see Ranatunga, 1982).

Because the observations from intact fibers herein reported are qualitatively similar to those from skinned fast fibers (which were immersed in a relaxing medium containing a Ca chelator, pCa ≈ 8; Ranatunga, 1994), the delayed tension rise is unlikely to be due to intracellular Ca release on stretch. It is well known that the Ca sensitivity of thin filament activation decreases with warming (Stephenson and Williams, 1985; Goldman et al., 1987), but how the functional state of thin filament in the absence of calcium may change with temperature remains unknown. The observations listed below clearly would be relevant to elucidating the mechanism of stretch activation observed here. First, 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. Second, according to the more recent three-state model, a thin filament exists in rapid equilibrium between blocked, closed, and open states (see Mckillop and Geeves, 1993), where only the closed-to-open transition permits weak-to-strong transition of attached cross-bridges and force generation. Interestingly, Head et al. (1995) found that the blocked state, in the absence of calcium, has reduced occupancy at high temperatures and suggested that this may account for the small level of thin filament activation in relaxed fibers as reported by Ranatunga (1994). Third, the affinity of myosin to actin is increased at high temperatures (Coates et al., 1985). Fourth, it has been shown for intact muscle by Hill (1972) and for skinned fibers in the previous study (Ranatunga, 1994) that relaxed mammalian fibers develop thermal stress on heating, presumably in the gap filament (+ thick filament) complex.

The above observations indicate that calcium regulation of thin filament may become reversibly weakened at high

temperatures and lead to formation of weakly attached cross-bridge states and some active tension development; although the effect was small, millimolar levels of BDM consistently reduced the steady resting tension at 35°C, supporting this interpretation. Thus, the delayed tension rise could be a consequence of stretch-induced cross-bridge detachment followed by synchronized attachment. The experiments by Granzier and Wang (1993) showed that weak cross-bridges exist in rabbit fibers at normal ionic strength and room temperature, and the weak bridge stiffness varies with filament overlap. It is not clear how our observations relate to their findings, because the delayed tension rise was not observed at temperatures below ~25–28°C. Nevertheless, the delayed tension rise reported in this study may be a consequence of the mechanical coupling between passive tension and cross-bridge interaction that they propose to exist even in mammalian sarcomeres. The extent to which the increased thermal stress in gap filament (see Ranatunga, 1994) plays a role in this "apparent" stretch activation and how this component of activation interacts with the more predominant Ca-dependent activation during muscle function at physiological temperatures remain to be investigated.

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