Effect of joule temperature jump on tension and stiffness of skinned rabbit muscle fibers

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ABSTRACT The effects of a temperature jump (T-jump) from 5–7°C to 26–33°C were studied on tension and stiffness of glycerol-extracted fibers from rabbit psoas muscle in rigor and during maximal Ca^{2+} activation. The T-jump was initiated by passing an alternating current pulse (30 kHz, up to 2.5 kV, duration 0.2 ms) through a fiber suspended in air. In rigor the T-jump induces a drop of both tension and stiffness. During maximal activation, the immediate stiffness dropped by $(4.4 \pm 1.6) \times 10^{-3}/1°C$ (mean + SD) in response to the T-jump, and this was followed by a monoexponential stiffness rise by a factor of 1.59 ± 0.14 with a rate constant $k_s = 174 \pm 42\ s^{-1}$ (mean ± SD, $n = 8$). The data show that the fiber stiffness, determined by the cross-bridge elasticity, in both rigor and maximal activation is not rubberlike. In the activated fibers the T-jump induced a biexponential tension rise by a factor of 3.45 ± 0.76 (mean ± SD, $n = 8$) with the rate constants 500–1,000 s^{-1} for the first exponent and $167 \pm 39\ s^{-1}$ (mean ± SD, $n = 8$) for the second exponent. The data are in accordance with the assumption that the first phase of the tension transient after the T-jump is due to a force-generating step in the attached cross-bridges, whereas the second one is related to detachment and reattachment of cross-bridges.

INTRODUCTION

Recently, some investigators have recorded tension transients in response to a T-jump in skinned muscle fibers (Smith et al., 1984; Goldman et al., 1985, 1987; Bershitsky and Tsaturyan, 1985, 1986*a* and *b*, 1988; Davis and Harrington, 1987*a*; Tsaturyan and Bershitsky, 1988). In rigor the T-jump induces an immediate tension drop. The drop is induced by the thermal expansion of contractile proteins (Smith et al., 1984; Bershitsky and Tsaturyan, 1985, 1986*a*; Goldman et al., 1987; Davis and Harrington, 1987*a*). The fibre stiffness in rigor also drops after the T-jump (Bershitsky and Tsaturyan, 1986*a*). As the fiber stiffness is mainly determined by the cross-bridge stiffness (Ford et al., 1981; Tawada and Kimura, 1984), the stiffness drop in response to the T-jump suggests that the cross-bridge thermoelasticity in rigor is not rubberlike.

In the Ca^{2+}-activated fibers the T-jump induces a tension drop (Goldman et al., 1987; Davis and Harrington, 1987*a*) followed by a biexponential tension rise (Bershitsky and Tsaturyan, 1986*b*, 1988; Goldman et al., 1987; Davis and Harrington, 1987*a*). The tension rise is induced by some force-generating process in the attached cross-bridges ("perestroyka") and by the cross-bridge reattachment.

The present study of stiffness changes in glycerol-extracted rabbit muscle fibers was made with the aim of examining cross-bridge behavior during the tension rise at maximal Ca^{2+}-activation. These stiffness changes during maximal Ca^{2+}-activation are compared with those in the rigor state.

MATERIALS AND METHODS

Fiber preparation, solutions, and trough

Glycerol-extracted rabbit psoas muscles were kept at −18°C for 2–4 wk. A single muscle fiber segment (60–80 μm in diameter) was separated in the storage solution (50% glycerol and 50% relaxing solution [Table 1]) at 0–5°C. The fiber was transferred to the experimental trough and its ends were placed on the dried nickel tube electrodes attached to the force transducer and the length change generator. Next the ends were moistened by droplets (~0.05 μl) of shellac dissolved in alcohol. Then the trough was filled with the relaxing solution. As the alcohol diffused into solution, the shellac hardened quickly and the fiber segment (1.5–2.5 mm in length) was reliably fixed to the electrodes. The surface of the segment was inspected in two projections under a microscope and the sarcomere length homogeneity was checked at different fiber lengths in the relaxing solution.

Then the fiber was bathed for 30–40 min in the relaxing solution with 0.5–1% vol/vol solution of the nonionic detergent Triton X-100 for reliable destruction of the sarcolemma and the membranes of the sarcoplasmatic reticulum. The "Ca^{2+} jump method" of Moisescu (1976) was used for the fiber activation. The composition of solutions is shown in Table 1.

ATP and CP were from Reanal (Budapest, Hungary). EGTA was

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0006-3495/89/11/809/08 $2.00

TABLE 1 Composition of solutions

Solution	$MgCl_2$	Na_2ATP	EGTA	$CaCl_2$	HDTA	NaN_3	Na_2CP	CPK
								mg/ml
Relaxing	6	5	15	—	—	3	20	1
Preactivating	6	5	0.1	—	15	3	20	1
Activating	6	5	15	15	—	3	20	1
Rigor	1	—	15	—	—	3	—	—

The concentration of each chemical except for creatine phosphate kinase (CPK) is given in millimolar. CPK activity was 100 U/mg.

from Ferak (Berlin, FRG), HDTA was from Fluka AG (Buchs, Switzerland), cacodilate and NaN_3 were from Sigma Chemical Co. (St. Louis, MO), $MgCl_2$ and $CaCl_2$ were from Khimreaktiv (Moscow, USSR). CPK was kindly provided by Dr. V. Saks (Center of Cardiology, Academy of Medical Science of the USSR, Moscow).

All the solutions contained 100 mM cacodilate pH buffer, pH 7.0 at 25°C. The temperature coefficient of pH for the activating solution in temperature range 5–30°C, measured by the EV-74 ionomer with ESA-43-07 electrode, was about −0.0045 log units per 1°C. So, the T-jump by 20–25°C induced a decrease in pH by only 0.1. The ionic strength was adjusted by KCl to 245 mM.

An experimental chamber with a 0.9-ml volume solution trough was made of anodized aluminum. The chamber was placed into a thermostat. The uncoated transistor of ~0.5 mm diameter was placed 1 mm from the fiber and used as a thermometer. Its accuracy was 0.1°C, time constant in air was ~2 s at 5–7°C.

The trough had the in and out quartz windows for the He-Ne laser beam to pass through the fiber. The out window was a dividing prism which allowed measurement of the fiber dimensions in two perpendicular projections by a long working distance microscope. The fiber cross-section area, A, was calculated by the formula $A = \pi d_1 d_1/4$, where d_1 and d_2 the fiber widths in the two projections. An accuracy of the fiber dimensions measurement was about +5 μm.

Mechanical measurements

The tension was measured by the hybrid force transducer consisting of a mechanoelectric valve gauge (0–40 Hz bandwidth) and a piezoelectric high-frequency transducer fixed on the gauge level (Bershitsky and Tsaturyan, 1985). The piezoelectric transducer was similar to that described by Chiu et al. (1978). Its natural frequency was 6.2 kHz, the noise was 2 μN peak-to-peak, and the time constant of the charge drain was ~3 s. The transducer was damped by a drop of unhardened epoxy resin. A double screen was used to diminish an artifact of the heating current pulse on the transducer output.

The moving-coil length generator (Bershitsky and Tsaturyan, 1986*a*) was similar to that described by Ford et al. (1977). The photo-optic length transducer measured changes of the fiber length with an accuracy of ~0.2 μm. Small sinusoidal length changes (2.3 kHz, 1–2.5 nm/half-sarcomere in amplitude) were used for the stiffness measurement. The sarcomere length (2.5–2.8 μm) was measured by the He-Ne laser beam diffraction.

Joule T-jump method

Fig. 1 shows schematically the T-jump instrumentation. The T-jump was induced by passing an alternating current pulse (30 kHz, up to 2.5 kV, 0.2 ms in duration) produced by the heating pulse generator (HPG) through the fiber segment. In <5 s before the T-jump the solution was removed from the trough. So, the fiber was a single conductor between the active electrode attached to the length change generator (LCG) and the ground electrode attached to the force transducer (FT). The amplitude of the T-jump, ΔT, was calculated from the Joule heat produced in the fiber using the formulae,

$$T(1 - \gamma\Delta T/2) = W/(\rho cALR), \quad W = \int_0^\tau u^2(t)\,dt,$$

where $u(t)$, τ are the voltage and the duration of the heating pulse, ρ = 1,070 kg/m^3 and c = 3.65 kJ/(kg °C) are the density and the specific thermal capacity of the fiber, A and L are the fiber cross-sectional area and length, R is the fiber resistance at 5–7°C, and γ = 0.02/1°C is the temperature coefficient of the fiber resistance in both rigor and activation solutions.

The W value was calculated by a special analog device consisting of a voltage divider, an analog multiplier (used for $u^2[t]$ calculation), an integrator, and a sample and hold circuit (SHC). The fiber dimensions were measured in air before and during the experiment. The fiber resistance R (the active component of the fiber impedance, Z) was

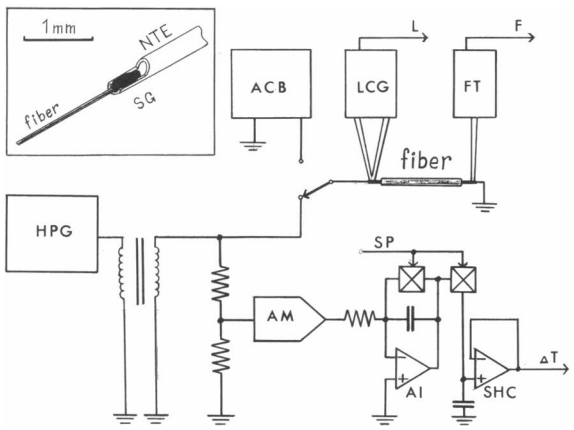

FIGURE 1 Schematic diagram of Joule T-jump apparatus. Inset shows the fiber glued by shellac glue (*SG*) to the nickel tube electrode (*NTE*). Tube was 0.3 mm in diameter and 0.1 mm wall thickness. The electrodes were attached to the force transducer (*FT*) and the length change generator (*LCG*). The heating pulse generator (*HPG*) produced an AC pulse applied to the fiber. Pulse voltage was divided, squared by an analog multiplier (*AM*), integrated by an analog integrator (*AI*), and then remembered by a sample and hold circuit (*SHC*). AI and SHC were controlled by analog keys switched by the switch pulse (*SP*). In 2–3 s after the T-jump the fiber resistence (active component of the impedance) was measured by an AC bridge (*ACB*). *L*, *F*, and *T* denote force, length change, and T-jump outputs, respectively.

measured by an AC bridges BM-484 (Tesla, Prague, Czechoslovakia) at 1.6 kHz in 2–3 s after each T-jump. A separate study showed that R at this frequency (300–700 kΩ) is practically the same as that at 30 kHz. In some cases R was measured in 2–3 s before the T-jump as well as after it. In all cases the changes in the R value during the experiment were less than 10%.

RESULTS

T-Jump effects in rigor

A series of experiments was performed to examine the T-jump effects in rigor. The rigor state was initiated by bathing the fiber at 0–2°C for 2–4 min in each activating solution containing 0.1 mM MgATP and 30 μM Ca^{2+} and then in rigor solution (Table 1). With this procedure a high level of rigor tension (64 ± 23 kN/m^2, mean ± SD, n = 5) is obtained, which is close to the maximal active tension at 5–7°C (57 ± 11 kN/m^2). The heating pulse was applied to the fiber suspended in air within 5 s after removing the rigor solution.

Records in Fig. 2 show typical effects of the T-jump in rigor. Both mean tension and stiffness dropped almost immediately when temperature was increased from 5.8 to 27°C. The mean tension was calculated as the mean of the maximal and minimal tension values during a cycle of sinusoidal length change. The mean tension dropped from P_i = 81 kN/m^2 to P_T = 72 kN/m^2. The fiber stiffness dropped from S_i = 24.5 MN/m^2 to S_T = 20.5 MN/m^2, i.e., by 7.6 × 10^{-3}/°C. The drop of tension and stiffness in response to the T-jump was followed by a slow recovery to initial values (not shown) with the rate constant ~7 s^{-1}. The rate constant is close to that calculated for fiber cooling (see Discussion).

The results of the experiments performed on five fibers were used for the calculation of the coefficient of thermal expansion, α, and the temperature coefficient of fiber stiffness, β, using the formulae $\alpha = (P_T - P_i)/(S_i\Delta T)$, $\beta = (S_T - S_i)/(S_i\Delta T)$. The average values were α = −(3.2 ± 0.7) × 10^{-5}/°C and β = −(7.7 ± 2.5) × 10^{-3}/1°C, respectively (mean ± SD, n = 8, negative sign represents normal, i.e., not rubberlike, thermoelasticity).

T-Jump effects at Ca^{2+} activation

In a series of eight experiments the effects of the T-jump on tension and stiffness were studied at maximal activation. To initiate an active contraction the relaxing solution was replaced by the preactivating one at 5–7°C for 3–5 min. Then the preactivating solution was removed and the trough was filled by the activating solution (Table 1). When the tension achieved its steady level, the activating solution was removed from the trough. In a few seconds the fiber temperature increased from 0–2°C to 5–7°C. The increase in temperature was accompanied by a tension increase. Not later than 5 s after removing the activation solution, the heating pulse was passed through the fiber suspended in air. 0.2 s before the T-jump, small sinusoidal length changes (2.3 kHz, 1–2.5 nm/half-sarcomere in amplitude) were switched on to measure the fiber stiffness.

A typical mechanical response to the T-jump from 6.7

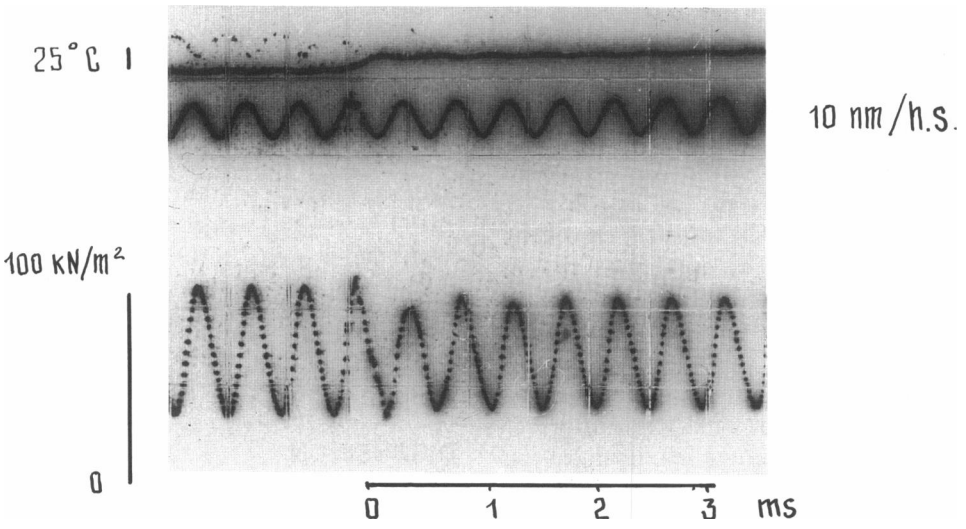

FIGURE 2 Tension and stiffness response to T-jump in rigor. The upper trace is the calculated value of the T-jump amplitude (from 5.8 to 27°C); the middle trace is the length change (2.3 kHz, 4 μm peak-to-peak); the lower trace is tension. Sarcomere length, 2.6 μm; fiber length, L = 1.63 mm; cross-sectional area, A = 4.65 × 10^{-9} m^2; fiber resistance, R = 465 kΩ at 6°C.

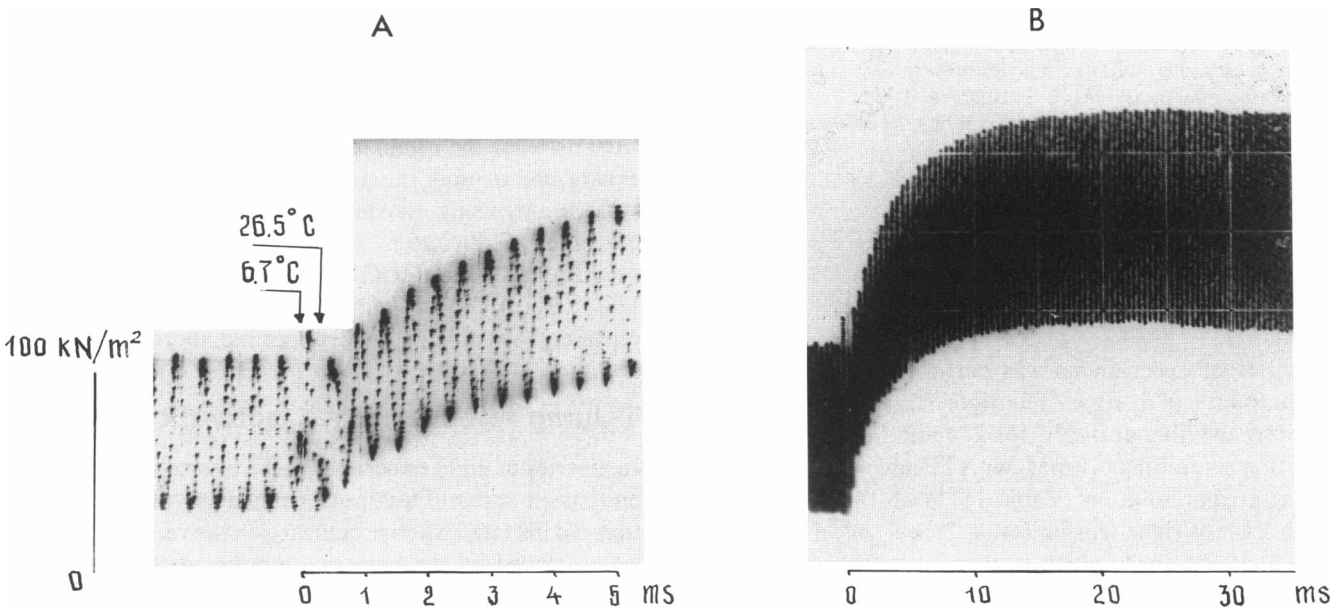

FIGURE 3 Tension and stiffness response to T-jump in maximally activated fiber. *A* and *B* are the same tension transient at different sweep speeds. The arrows show the beginning and the end of the T-jump. Sinusoidal length changes (2.3 kHz; 4 μm peak-to-peak) were used for the stiffness measurement. Sarcomere length, 2.75 μm; fiber length, L = 1.82 mm; cross-sectional area, $A = 6.25 \times 10^{-9}$ m²; fiber resistance, R = 560 kΩ at 7°C.

to 26.5°C in the experiment is shown in Fig. 3. The T-jump induced an increase of the mean tension from P_i = 67 kN/m² to P_o = 171 kN/m², i.e., by a factor of 2.55, followed by a slow tension return to its initial value (data not shown). The rate constant of the tension decrease was ~7 s^{-1}, and this was close to that of the fiber cooling. An artifact of the current pulse precluded the measurement of the mean tension and stiffness earlier than in 0.5–1 ms after the T-jump. That is why we could not find out whether the T-jump induces an immediate tension drop followed by the tension rise or not.

In response to the T-jump fiber stiffness dropped from S_i = 29 MN/m² to S_T = 26 MN/m² (S_T is the fiber stiffness 1 ms after the T-jump). The transient rise of the mean tension was accompanied by a stiffness rise to S_o = 37 MN/m², i.e., by a factor of 1.42.

For eight fibers the average values of the mean tension and stiffness at the starting temperature 5–7°C were P_i = 57 ± 11 kN/m² and S_i = 23 ± 4 MN/m² (mean ± SD), respectively. At the final temperature of 30 + 2.5°C (mean ± SD) the average maximal tension was P_o = 192 ± 20 kN/m² (mean ± SD). The average stiffness drop 1 ms after the T-jump was (4.4 ± 1.6) × 10^{-3}/1°C (mean ± SD). The average ratio of the maximal stiffness, S_o, to its value 1 ms after the T-jump, S_T, was 1.59 ± 0.14 (mean ± SD). Thus, the tension to stiffness ratio at 30°C was more than two times greater than that at 5–7°C.

Kinetics of tension and stiffness transients

A temporal development of the tension and stiffness transients for the experiment shown in Fig. 3 is presented in Fig. 4. The stiffness transient is approximated by a single exponential function (Fig. 4 *A*), whereas the tension transient is approximated by the biexponential function $P(t) = P_o - P_1 \exp(-k_1 t) - P_2 \exp(-k_2 t)$, where $P_o - P_1 - P_2$ is close to P_i (Fig. 4 *B*). The rate constant of the first (fast) exponent, k_1, in eight experiments was 500–1,000 s^{-1}. Its amplitude was 50–100% of P_i. The average rate constants in the experiments were k_s = 174 ± 42 s^{-1} (mean ± SD) for the stiffness transients and k_2 = 167 ± 39 s^{-1} (mean ± SD) for the second (slow) exponent of the tension transients. The average ratio of the rate constants was k_2/k_s = 0.98 ± 0.31 (mean ± SD).

DISCUSSION

Joule T-jump method

Possible artifacts of the Joule T-jump method as well as errors of the T-jump amplitude calculation were briefly discussed by Bershitsky and Tsaturyan (1985). It was shown that the direct effect of the heating electric field on the cross-bridges and a field nonuniformity due to the skin

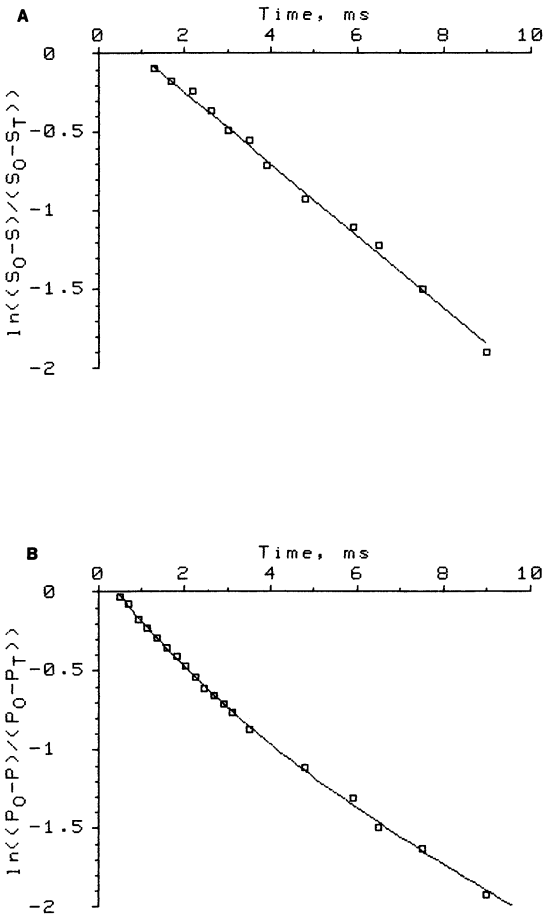

FIGURE 4 Temporal development of stiffness and tension transients. (*A*) Semilog plot of the stiffness transient for the experiment presented in Fig. 3. The straight line is the best fit by an exponential function (rate constant, k_s = 219 s^{-1}). (*B*) Semilog plot of the mean tension transient for the same experiment. The line is the best fit by the biexponential function (rate constants, k_1 = 530 s^{-1} and k_2 = 156 s^{-1}), squares are the experimental points. S and P are stiffness and tension during the transient, S_T and P_T are the same variables 1 ms and 0.5 ms after the T-jump respectively, S_0 and P_0 are the maximal values of the tension and stiffness after the T-jump.

effect (current displacement to a conductor surface at high frequency) are negligible. Because we used a 30 kHz AC, the thickness of the layer near the electrodes, where the changes of the solution composition due to an electrolysis were essential, was only ~1 μm.

The spatial temperature nonuniformity due to a variation of the cross-sectional area along the fiber does not exceed 10%.

Another possible reason of the spatial temperature nonuniformity is related to the end fiber cooling effect due to the heat loss to the cold metal electrodes after the T-jump. The length, x, of the fiber ends region where the temperature differs from that in the central region by >30% of T, can be estimated by the formula (Korn and Korn, 1961) $x = 1.47\sqrt{æt}$, where æ = 1.4×10^{-7} m^2/s is the coefficient of the temperature conductivity and t is the time after the T-jump. In 20–30 ms after the T-jump when the maximal tension was achieved, x was <100 μm. Therefore, during the tension rise the length of the cold ends does not exceed 10% of the fiber length.

As the maximal isometric tension in the rabbit psoas muscle fibers strongly depends on the temperature (Goldman et al., 1987), the cooling of the fiber ends induced, perhaps, their elongation and, therefore, a shortening of the central segment of the fiber. However, the cooling of the ends is not likely to affect in a major way the results reported in this study because of maximal tension achieved after the T-jump to 30°C (192 + 20 kN/m^2) was not less than the isometric tension at the same temperature in solution (170 + 23 kN/m^2, Goldman et al., 1987). Also fiber stiffness dropped immediately after the T-jump, when the fiber ends cooling was negligible. Furthermore tension transient after the T-jump was accompanied by a stiffness increase to the same extent as was reported for the steady-state temperature experiments (Brenner, 1986).

The rate constant of the cooling of the central segment of the fiber after the T-jump was calculated taking into consideration the thermal conductance in the fiber and air, the water evaporation from the fiber surface and the steam diffusion (Bershitsky and Tsaturyan, 1985). The calculated rate constant (6–10 s^{-1}) is close to that of the slow tension recovery after the T-jump in both rigor and Ca^{2+} activation. Because tension and stiffness achieved their maximum in 20–30 ms after the T-jump, the decrease in temperature during the rise of tension and stiffness was 15–25% of the T-jump value.

The heating pulse was applied to a fiber suspended in air within 5 s after removing the solution from the trough. The calculation showed that an increase in the ionic strength of the solution due to the water evaporation from the fiber surface was <3% in 5 s at 5–7°. At 20–25°C the calculation predicts an increase in the ionic strength by 10% in 5 s, i.e, the value close to that obtained by Ferenczi (1985).

Because the ATPase activity in rabbit muscle fiber at 5°C is ~0.15 mM/s (Brenner, 1986), the decrease in MgATP concentration in 5 s in fiber suspended in air was <1 mM even without taking into consideration the CP/CPK regeneration system.

The time resolution of the T-jump method is about the same as that of the laser T-jump methods (Goldman et al., 1987; Davis and Harrington, 1987*b*) and is about an order of magnitude better than the microwave radiation method (Lindley and Kuyel, 1978; Lindley and Goldthwait, 1984). The main limitation of the Joule T-jump method is the relatively rapid cooling of the fiber sus-

pended in air. For this reason we could not examine the processes which developed with rate constants $<50\ s^{-1}$. On the other hand, the temperature changes for the laser T-jump method does not exceed 8°C (Goldman et al., 1987; Davis and Harrington, 1987*b*), whereas the Joule T-jump method permits to vary the fiber temperature by 30°C or more.

Thermal expansion

There is some evidence that the drop of the rigor tension after the T-jump is due to a thermal expansion. The drop starts simultaneously with the beginning of the T-jump and finishes almost immediately after the end of the heating pulse. The tension drop is proportional to the amplitude of the T-jump and is independent of the final temperature in the physiological range (Bershitsky and Tsaturyan, 1985).

The average coefficient of thermal expansion for rabbit muscle fibers in rigor was close to that obtained by the same method for frog fibers (Bershitsky and Tsaturyan, 1986*a*) and by the thermoelastic heat measurements in whole frog muscle in both rigor (Kometani and Yamada, 1984; Gilbert and Ford, 1986) and tetani (Woledge et al., 1985). On the other hand, the value in the experiments presented here is about half of that obtained in the warming-cooling experiments in rigor and by the laser T-jump method in both rigor and Ca^{2+} activation (Goldman et al., 1987). As can be estimated from the record presented by Davis and Harrington (1987*a*), the thermal expansion in their laser T-jump experiments was also more pronounced than in ours. The difference can be explained by the thermal expansion of the steel hooks attached to the fiber ends in the laser T-jump experiments.

The infra-red laser pulse induces an increase in temperature of the solution bathing the fiber and the hooks. Therefore, the absolute thermal expansion, Δl, can be calculated from the formula $\Delta l = (\alpha_f L + \alpha_h l_h) \times \Delta T$, where α_f and α_h are the thermal expansion coefficients of the fiber and the hooks, respectively; L and l_h are the lengths of the fiber and the heated parts of the hooks. The apparent value of the thermal expansion coefficient is $\alpha_{app} = \Delta l/(L\Delta T) = \alpha_f + \alpha_h l_h/L$. Taking $L = 2$ mm, $l_h = 7$ mm and $\alpha_h = 1.2 \times 10^{-5}/1°C$ one can obtain the difference between the apparent and real α values, $\alpha_{app} - \alpha_f = -4.2 \times 10^{-5}/1°C$, which is equal to the difference between the α values obtained by Goldman et al. (1987) and by us.

The initial tension drop in the Ca^{2+}-activated fibers, which is seen in the records of the tension transients induced by the laser T-jump (Goldman et al., 1987; Davis and Harrington, 1987*a*), was absent in our experiments. The difference can be explained by several reasons. As was mentioned above, the thermal expansion in the Joule T-jump experiments was less marked than that in the laser T-jump experiments Also, the 20–26°C T-jump induced a quick tension increase of high magnitude and rate constant (Figs. 3 and 4) which would have opposed the tension drop. An artifact of the heating pulse created an additional limitation in the analysis of the very early phase of the tension transients.

Cross-bridge thermoelasticity

Because the cross-bridge length is a small fraction (~5%) of the half-sarcomere length, the measured coefficient of the thermal expansion is mainly a characteristic of the thermal expansion of the thin and/or thick filaments, rather than of the cross-bridges (Bershitsky and Tsaturyan, 1986*a*; Goldman et al., 1987). However fiber stiffness in both isometric tetani and rigor is mainly determined by the cross-bridge stiffness (Ford et al., 1981; Tawada and Kimura, 1984). The stiffness of the rubber-like materials is proportional to the absolute temperature T. When temperature is increased, the stiffness of such material increases, too, by 3.3×10^{-3} per 1°C at $T = 300°K$. On the contrary, the data presented here and earlier (Bershitsky and Tsaturyan, 1986*a*) show that the fiber stiffness decreased by $\sim 5 \times 10^{-3}$ per 1°C. Therefore, the data prove that the cross-bridge elasticity in rigor and Ca^{2+} activation is not rubberlike.

The difference between the β values in rigor ($-7.7 \times 10^{-3}/1°C$) and during Ca^{2+} activation ($-4.4 \times 10^{-3}/1°C$) may be explained by the nonlinear end compliance. In rigor the mean tension after the T-jump was less than that before it. During Ca^{2+} activation the mean tension 1 ms after the T-jump was greater than that at the starting temperature. For this reason after the T-jump the nonlinear elastic element was stretched to a greater extent, and therefore it was stiffer, than before the T-jump. In rigor the nonlinear element was less stiff after the T-jump than before it.

Some models assume that the cross-bridge elasticity is determined by the compliance of the melted part of the S-2 (Huxley and Simmons, 1971; Harrington, 1971, 1979). The data of the stiffness measurements in the fibers with the S-2 cross-linked to the myosin rod and with the myosin subfragment 1 (S-1) cross-linked to actin contradict this assumption (Kimura and Tawada, 1984, Tawada and Kimura, 1986). The fact that the cross-bridge elasticity is not rubberlike is an additional indication against this assumption. The temperature coefficient of the cross-bridge stiffness is close to that of the protein crystals (Morosov and Morosova, 1981). Thus, our data are in accord with the hypothesis that the cross-bridge elasticity is determined by the domains turning within S-1 (Huxley, 1974; Tawada and Kimura, 1986).

Interpretation of tension and stiffness transients

The biexponential tension transients after the T-jump in skinned muscle fibers have been described (Bershitsky and Tsaturyan, 1986*a*, 1988; Goldman et al., 1987; Davis and Harrington, 1987*a*). If the T-jump follows a stepwise shortening of the fiber, the rate constant of the fast component of the tension transient increases (Bershitsky and Tsaturyan, 1986*b*; Tsaturyan and Bershitsky, 1988). The data show that the fast phase of the tension transient after the T-jump is determined by the same force-generating process in the attached cross-bridges as the fast partial tension recovery following a length step described by Huxley and Simmons (1971) and Ford et al. (1974, 1977). The correspondence between the fast phases of the tension transients after the T-jump and length step was also pointed out by Goldman et al. (1987). An absence of significant changes in the fiber stiffness during the first phase of the tension transients after the T-jump provides additional evidence for the hypothesis that the early phase of the tension transients is induced by the force-generating process in the attached cross-bridges. The second phase of the tension transients is accompanied by a stiffness rise with the rate constant being close to that of the former process (Figs. 3 and 4). The data are an additional evidence that the second phase of the tension transients after the T-jump involves the process of the cross-bridge reattachment (Bershitsky and Tsaturyan, 1986*b*; Tsaturyan and Bershitsky, 1988).

Davis and Harrington (1987*a*) have assumed that the tension transient in response to the T-jump is induced by the melting of the hinge region between the S-2 and the myosin rod. If the assumption is correct, the additional compliance, C_T, must appear within the cross-bridge after the T-jump. Because the coiled-coil structure of S-2 is practically unstretchable, C_T can be estimated by the equation $C_T = 2nd^2/(3kT)$, where n is the number of residues in the melted part of both polypeptide chains in S-2, $d = 0.25$ nm is the length of a residuum, k is the Boltzmann constant, and T is absolute temperature. Taking $n = 100$ (the value is necessary for providing the power stroke by 13 nm) we have $C_T = 500$ m/N. There are $\sim 4 \times 10^{16}$ attached cross-bridges per square meter in each half-sarcomere in the skinned fiber at maximal activation. Consequently, the cross-bridge compliance C_o at sarcomere length 2.6 μm and the fiber stiffness 25 MN/m^2 is $C_o = 2{,}000$ m/N. This means that the cross-bridge stiffness must decrease by 25% when the S-2/myosin rod hinge is melted. Absence of the fiber stiffness decrease during the tension transients after the T-jump is the evidence that the transients are not accompanied by the hinge melting in a significant portion of the attached cross-bridges.

We thank Dr. O. N. Bershitskaya for technical assistance, Dr. A. L. Drachev for assistance in computer analysis of the experimental data, and Drs. A. L. Genin, I. V. Ostrovskaya, and Mr. I. A. Kolmanovsky for assistance in preparation of the manuscript. We thank Drs. A. E. Bukatina, N. B. Gusev, S. S. Grigoryan, V. V. Lednev, V. Ya. Isakov, and S. A. Regirer for helpful discussion.

Received for publication 1 August 1988 and in final form 22 June 1989.

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