J. Physiol. (1987), **392**, *pp.* 71–95
With 13 *text-figures*
Printed in Great Britain

TRANSIENT TENSION CHANGES INITIATED BY LASER TEMPERATURE JUMPS IN RABBIT PSOAS MUSCLE FIBRES

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(*Received 28 August 1986*)

SUMMARY

1. A technique was developed to generate 2–8 °C step temperature perturbations (T-jumps) in single muscle fibres to study the thermodynamics of muscle contraction. A solid-state pulsed holmium laser emitting at 2·065 μm heated the fibre and surrounding solution in approximately 150 μs. The signal from a 100 μm thermocouple fed back to a heating wire maintained the elevated temperature after the laser pulse.

2. Tension of glycerol-extracted muscle fibres from rabbit psoas muscle did not change significantly following T-jumps when the fibre was relaxed.

3. In rigor, tension decreased abruptly on heating indicating normal (not rubber-like) thermoelasticity. The thermoelastic coefficient (negative ratio of relative length change to relative temperature change) of the fibre was estimated to be −0·021 at sarcomere lengths of 2·5–2·8 μm. Rigor tension was constant after the temperature step and returned to the original value on recooling.

4. In maximal Ca^{2+} activation, tension transients initiated by T-jumps had several phases. An immediate tension decrease suggests that thermoelasticity during contraction is similar to that in rigor. Active tension then recovered to the value before the T-jump with an apparent rate constant of approximately 400 s^{-1} (at 10–20 °C). This rate constant did not have an appreciable dependence on the final temperature. Finally, tension increased exponentially to a new higher level with a rate constant of approximately 20 s^{-1} at 20 °C. This rate constant increased with temperature with a Q_{10} of 1·4.

5. At submaximal Ca^{2+} activation the tension rise was followed by a decay to below the value before the T-jump. This decline was expected from the temperature dependence of steady pCa–tension curves. The final tension decline occurred on the 1–5 s time scale.

6. The value and amplitude dependence of the rate constant for the quick recovery following T-jumps were similar to those of the quick recovery following length steps during active contractions. The enthalpy change associated with the quick tension

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recovery following temperature-step perturbations was estimated to be positive suggesting that the recovery process is an endothermic reaction. Slower reaction steps on the 10–30 ms timescale, as well as reactions corresponding to the quick recovery, may contribute to the cross-bridge power stroke.

INTRODUCTION

Many physiological responses of muscle are temperature sensitive, including the twitch and shortening velocity (Woledge, Curtin & Homsher, 1985, and references therein; Ranatunga, 1982, 1984; Ranatunga & Wylie, 1983) and heat production during muscle activation and contraction has been extensively characterized (Fenn, 1923; Hill, 1938; Woledge *et al.* 1985). In such a temperature-sensitive reaction system, if the temperature is changed abruptly, the kinetics of approach to the new steady state provides information on the rates and, in some cases, may be used to identify the reaction steps involved (Bernasconi, 1976). The temperature dependence of the equilibria between kinetically identified intermediate states can provide information on the energetics of the reactions (Edsall & Gutfreund, 1983). With that rationale, we used a near-infra-red pulsed laser (Smith, Goldman, Hibberd & McCray, 1987) to rapidly jump the temperature (within 150 μs) of muscle fibres during relaxed, rigor and contracting conditions, and studied the kinetics of the resulting tension and stiffness changes.

The advantages of the temperature jump (T-jump) method for studying the energetics of muscle contraction as compared to measurements of heat production, are that the time resolution is enhanced and that the experiments can be performed on skinned muscle fibres in which the biochemical and ionic milieu of the myofibrils can be set independently. Some of the results have been briefly reported (Smith, Goldman, Hibberd, Ligouri, Luttman & McCray, 1984; Goldman, McCray & Ranatunga, 1985).

METHODS

Principle of the method

Radiation near wavelengths of 2 μm is absorbed by water leading to a rapid temperature increase (Goodall & Greenhow, 1971; Fornes & Chaussidon, 1978; Holzwarth, Eck & Genz, 1985). In order to step change the temperature of a muscle fibre, a 150 μs, 100–700 mJ pulse of 2·065 μm infra-red radiation from a solid-state holmium laser (Smith *et al.* 1987) was directed at a small trough containing the fibre. The absorptivity of water at this wavelength is 37 cm^{-1}, so the fibre and water near the front of the trough were heated during the 150 μs pulse of laser radiation.

The muscle fibre was suspended in an appropriate buffer for the relaxed, rigor or contracting conditions of the experiment and its tension was recorded at one end while the temperature was suddenly increased. A small thermocouple next to the fibre recorded the temperature change. Some of the transient tension changes occurred on the approximate timescale of the passive cooling after the laser pulse. This led us to construct a feed-back loop in the trough using a constantan heating wire to maintain the elevated temperature steady after the laser pulse.

Fibre preparation and solutions

Glycerol-extracted single fibres were prepared from rabbit psoas muscle as described by Goldman, Hibberd & Trentham (1984). After dissection, T-shaped aluminium foil clips were attached to the ends of the fibre segment and the fibre was transferred to the experimental trough. Fibre dimensions were measured as described by Goldman & Simmons (1984).

The solutions bathing the fibres are shown in Table 1. The solutions are similar to the activation and rigor media previously described (e.g. Dantzig & Goldman, 1985). However, the pK_a of the pH buffer previously used (TES, *N*-tris (hydroxymethyl)methyl-2-aminosulphonic acid) had too large a temperature coefficient (−0·02 log units /°C; Good, Winget, Winter, Connolly, Izawa & Singh, 1966) for the present experiments. In a T-jump, the temperature dependence of the pK_a would be expected to cause a decrease in pH. The Ca^{2+} affinity of EGTA is strongly dependent on pH, so an increase in Ca^{2+} concentration would also be expected. The Ca^{2+} affinity of EGTA and troponin are also directly temperature dependent.

TABLE 1. Composition of the solutions used in the experiments

Solution	$MgCl_2$	Na_2ATP	EGTA	Ca–EGTA	EDTA	Na_2CP	GSH	G2P
Relaxing solution (REL)	8·14	5·48	20	—	—	19·6	10	15
Pre-activating solution (PRE)	7·52	5·48	0·1	—	19·9	19·9	10	15
Activating solution (ACT)	7·39	5·52	—	20·0	—	19·9	10	15
Rigor solution	3·50	—	49·0	—	—	—	10	15

Concentration of each chemical is given in mM; GSH and G2P refer, respectively, to glutathione and β-glycerol phosphate. The pH of each solution was adjusted to 7·1 at 20 °C by titrating with 1 M-KOH. The ionic strength of all experimental solutions was 200 mM. Creatine phosphokinase (1 mg/ml) was added prior to an experiment to the solutions containing creatine phosphate (Na_2CP). Activating solutions with submaximal Ca^{2+} concentrations were prepared by mixing ACT and REL solutions.

In preliminary experiments using TES buffer at submaximal Ca^{2+} concentrations a slow (~ 500 ms) tension increase followed a T-jump laser pulse. Control experiments using steady contractions at various temperatures showed that the slow tension increase was caused by Ca^{2+} release from EGTA following proton release from TES. Therefore a less temperature-sensitive pH buffer was required. β-Glycerol phosphate was chosen as the pH buffer in all of the present experiments because its pK_a has an unusually low temperature coefficient (Ashby, Crook & Datta, 1954) with a broad minimum ($pK_a = 6·65$) in the 0–30 °C temperature range. Replacement of 100 mM-TES buffer with 15 mM-β-glycerol phosphate (ionic strength being maintained by alterations of other constituents) did not appreciably affect the maximal active tension or the relation between tension and Ca^{2+} concentration. (M. Astion, G. A. Liguori & Y. E. Goldman, unpublished observations).

Mechanical and recording instrumentation

Muscle fibre tension was measured using an oil-damped semiconductor strain gauge as described by Goldman & Simmons (1984). Quick length changes were applied by a piezoelectric stack as described by Goldman *et al.* (1984). Stainless-steel hooks on the tension transducer and length driver were passed through holes in the T-clips to hold the fibre in the experimental trough (Goldman & Simmons, 1984). The transducer and length driver were mounted on manipulators to position the fibre in the laser beam and to adjust the striation spacing. The tension, stiffness and temperature signals were recorded on a storage oscilloscope, chart recorder, and in digital form on flexible diskettes (Goldman *et al.* 1984).

Figure 1 schematically shows the trough assembly. It consisted of two metal blocks (B_1 and B_2) which were thermally insulated from each other by a Teflon partition. Block B_1 contained a cooling chamber and the front trough (25 μl) was constructed with glass and fused silica microscope slides. During T-jumps the fibre was positioned in the front trough, and the laser beam was directed through the fused-silica window to heat the solution and the fibre. A 100 μm chromel–alumel thermocouple was positioned directly under the fibre to record the temperature transient. The thermocouple signal was amplified and fed back to a resistive (Joule) heating element behind the front trough to maintain the temperature of the fibre constant after the laser pulse. The front trough was mounted on an aluminium block (B_1) that was cooled to 6–7 °C by a circulating cold

ethanol–water mixture. The combination of the cold block and the Joule heating servo clamped the fibre temperature to within ±0·1 °C of a predetermined value. The response time (10–90 %) of the resistive heating servo in the front trough was 2·4 s with an overshoot of less than 5 %.

The second metal block (B_2) contained three further troughs (70 μl each) used for exchanging

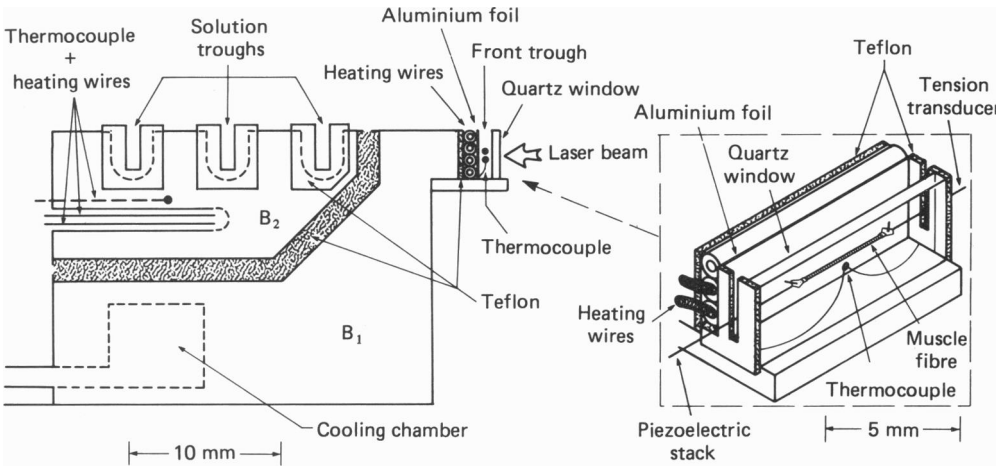

Fig. 1. Schematic diagram of the muscle-fibre trough assembly. It consisted of two metal blocks (B_1 and B_2) separated by a Teflon partition. The cold front block (B_1, aluminium) carried the front trough built with glass microscopic slides and a fused silica window facing the laser beam. The other block (B_2, stainless steel) had three troughs milled in it and a separate thermocouple and constantan heating wire. These three troughs normally contained the relaxing and pre-activating solutions. A detailed view of the front trough is shown on the right. The front trough had a permanently mounted thermocouple below the fibre and constantan heating wires within 1 mm ceramic tubes behind the fibre. The ceramic tubes were glued to a 0·4 mm Teflon partition on B_1. A sheet of aluminium foil in front of the ceramic tubes facilitated vertical heat equilibration. The muscle fibre was mounted, as shown, between a tension transducer hook and another hook attached to a piezoelectric stack.

experimental solutions. A second independent thermocouple and Joule heating servo maintained the temperature of B_2 constant to within ±0·5 °C in the range 10–20 °C. Surface tension at Teflon end-pieces held the solution in all four troughs. During solution changes, the fibre was held in place by the transducer and length driver hooks. The trough was lowered, moved horizontally and raised to immerse the fibre in the new solution (Goldman *et al.* 1984).

A third thermocouple was mounted on a vibrating shaft directly above the fibre to stir the bathing solution. This thermocouple could be moved within the trough by a three-axis manipulator to scan the spatial distribution of T-jump amplitudes and time courses in the region of the muscle fibre.

Transient temperature changes

The holmium laser heated the solution and fibre in the trough during the 150 μs pulse of infrared radiation. The absorptivity of water at the 2·065 μm laser wavelength is 37 cm^{-1} so the spatial profile of the laser pulse heating decayed exponentially from the front of the trough with a 1/e distance of 1/37 cm (= 0·27 mm). The fibre was placed 0·3 mm from the front window of the trough. A recording of the temperature at the position of the fibre by the 100 μm thermocouple (Fig. 2*A* and *B*) shows several phases when the laser was triggered without the Joule heating feed-back. An electrical spike due to the high-voltage trigger circuit was followed by a rapid increase of the signal and then a partial decay within 10 ms. Temperature then decreased to the original level during the next 10 s (Fig. 2*A*).

In order to obtain a constant temperature after the T-jump, the command signal for the Joule heating servo was adjusted to complement the laser heating. Addition of a heating phase 1 s before the laser pulse and adjustment of the feed-back parameters produced the combined heating record shown in Fig. 2*C*. In some experiments it was also necessary to apply an extra pulse of Joule heating after the laser trigger. These adjustments were made before each experiment to compensate for variations in laser output and for variations in performance of the set-up from different starting temperatures. It was possible to obtain 2–8 °C T-jumps with the elevated temperature clamped steady to within 15% of the temperature step 10 ms after the laser pulse.

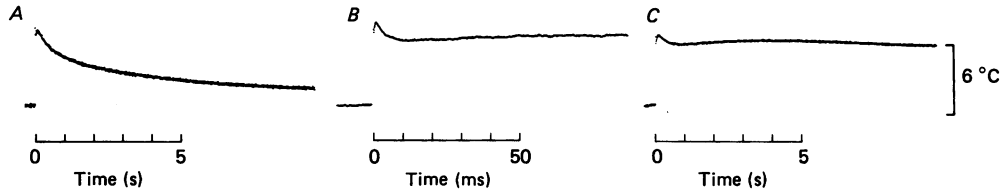

Fig. 2. Traces illustrating the time course of temperature perturbations in the front trough. The thermocouple was in the front trough at the position of a muscle fibre. *A* and *B*, recordings, at two different sweep speeds, of ~ 6 °C temperature jumps induced by a laser pulse alone. *C*, a temperature clamp obtained by appropriate adjustment of the Joule heating servo. The command voltage was increased the equivalent of 24 °C, 1 s prior to the laser pulse.

The temperature jump was also monitored spectrophotometrically in a separate experiment by measuring the T-jump-induced change of pH in a Tris buffer with phenol red as indicator. No overshoot was observed in this case indicating that the 10 ms overshoot on the thermocouple signal was due to heating of the thermocouple wire by direct absorption of laser light (see also Results). Thus thermal equilibration of the thermocouple with the surrounding water occurred in approximately 10 ms although the spectroscopic measurements indicated that the new temperature level is reached within 1 ms. Computer simulations of the heat flow in the trough showed that with the fibre at 0·31 mm from the front, the new temperature was constant to within 3% during the first 100 ms following the T-jump (Smith *et al.* 1987). This maintenance of temperature occurred because the exponential temperature gradient produced by the laser pulse provided heat flux that compensated for heat loss at the back of the trough.

Spatial distribution of heating

The infra-red beam was approximately 3 mm in diameter on exit from the laser aperture and was expanded along the fibre axis by a cylindrical fused silica lens. In order to monitor the spatial uniformity of the T-jump, a thermocouple was positioned by a manipulator along the position normally taken by a fibre. T-jump amplitudes showed a trapezoidal position dependence with full-width at a half-maximum of approximately 5·5 mm. Within the plateau region of the intensity profile the variability of the T-jump amplitude was ±18% (S.D.) of the mean. Fibres of length near 2·5 mm were positioned within this region.

Variations across the fibre diameter were not measured directly, but can be estimated. In the vertical direction the laser beam was much wider than the fibre, so variations in T-jump amplitude would be small compared to the other two dimensions. Along the optical axis, attenuation of the laser beam due to absorption by water would be approximately 25% across a 75 μm diameter fibre. The computer simulations showed that this temperature gradient is constant for the first 30 ms, then decreases to half by 100 ms following the laser pulse.

RESULTS

Steady-state experiments

The temperature-jump and temperature-clamp experiments reported here were performed at starting temperatures ranging from 6 to 22 °C. A number of steady-state experiments were therefore performed over a wider temperature range of 5–

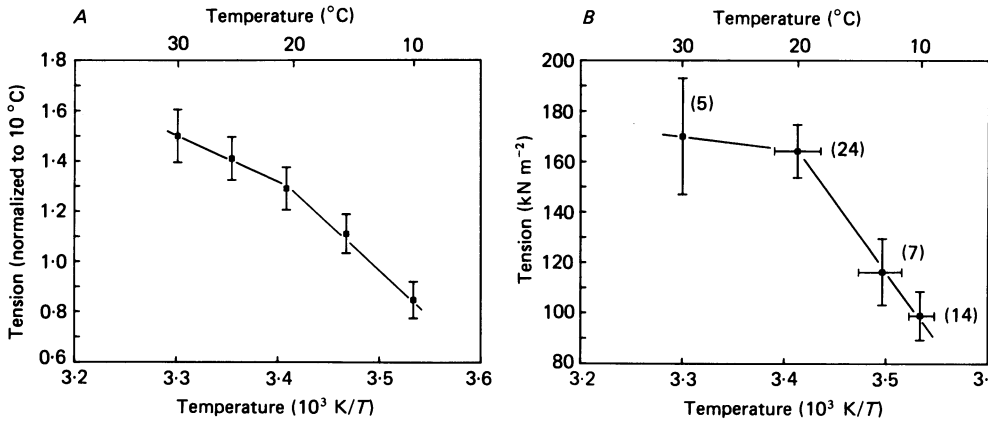

Fig. 3. Variation with temperature of the maximal Ca^{2+}-activated tension. *A*, data from three muscle fibres in which recording was first made at 10 °C and then at ~ 5 °C temperature intervals in an increasing temperature sequence and then a decreasing temperature sequence. Tensions recorded from a given fibre were normalized to that first recorded at 10 °C prior to changing temperatures; the datum at 10 °C includes the initial and final series of measurements. Symbols denote the mean tensions and the vertical lines, ± S.E. of mean. Horizontal axis is reciprocal absolute temperature. The lines were drawn by eye. *B*, pooled data from twenty-seven muscle fibres each of which was examined at one or two temperatures. Tension per cross-sectional area (kN m^{-2}) is plotted on the ordinate and reciprocal absolute temperature on the abscissa. Each symbol is the mean tension from a number of fibres (shown within parentheses), vertical bars are S.E. of mean and horizontal bars represent the temperature ranges. The lines were drawn by eye.

35 °C to determine the temperature dependence of isometric tension development in rabbit skinned muscle fibres. Such experiments have been reported by previous workers (see Stephenson & Williams, 1981, 1985), but not in the particular solutions used for the temperature jump (T-jump) experiments or with the low-temperature-coefficient pH buffer β-glycerol phosphate (see Methods).

Maximal active tension

Figure 3*A* shows the pooled data from three muscle fibres in which the maximal Ca^{2+}-activated tensions were recorded first at 10 °C and then at a number of other temperatures during warming and subsequent recooling and finally at 10 °C again. For each fibre the tension values recorded at each temperature were normalized to that recorded initially at 10 °C and then averaged (Fig. 3*A*). The results show that the tension in a maximally Ca^{2+}-activated fibre is greater at higher temperatures and tension is more temperature sensitive below 20 °C.

Values for maximal Ca^{2+}-activated tension were also available from a number of other experiments in which tension recordings were made at one or two steady temperatures in the range of 7–30 °C. In order to pool the data, tension per cross-sectional area was calculated and plotted against temperature in Fig. 3*B*. These

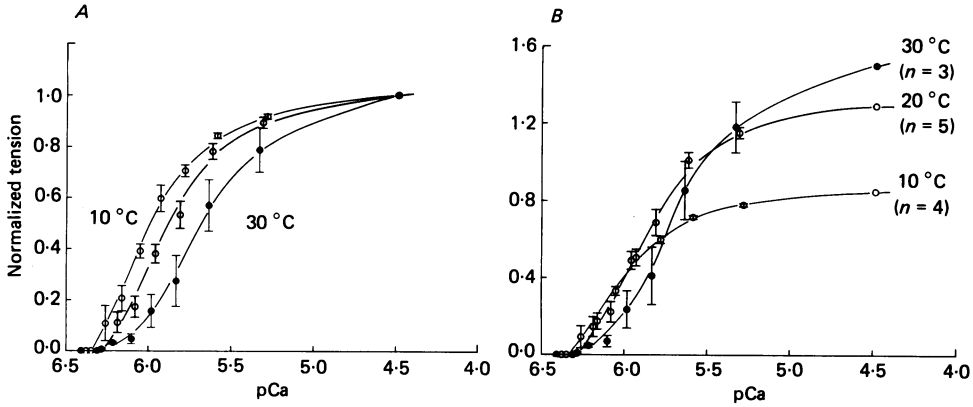

Fig. 4. Temperature dependence of Ca^{2+} sensitivity of tension development. Tension is plotted on the ordinate and pCa is plotted on the abscissa. Pooled results obtained from a number of fibres at three different temperatures are shown. A symbol represents the mean tension and the vertical lines ± the S.E. of the mean. *A*, tensions recorded at lower Ca^{2+} levels were normalized to that recorded with maximal Ca^{2+} activation at the same temperature. Note that the pCa–tension relation is shifted to the right at the higher temperatures. *B*, the relations shown in *A* were redrawn after adjustment for the temperature dependence of the maximal tension level according to the average data in Fig. 3*A*. The lines were drawn by eye.

specific tension data are from a total of twenty-seven muscle fibres. The specific tension is approximately 160 kN m^{-2} at 20 °C, as previously reported (Dantzig & Goldman, 1985). The tension is much lower, around 100 kN m^{-2}, at 10 °C and is slightly higher, around 170 kN m^{-2}, at 30 °C. The temperature dependence of tension illustrated by these data from different preparations is similar to that shown in Fig. 3*A*.

Submaximal active tension

A series of experiments was also carried out to examine the temperature dependence of the calcium sensitivity of tension development in β-glycerol phosphate buffer at 10, 20 and 30 °C. At a given temperature, the tension development was examined in each fibre at maximal Ca^{2+} activation (~ 30 μM-Ca^{2+}) and at a number of lower Ca^{2+} concentrations. The data at submaximal activation were normalized to the tension recorded at maximal activation and the data from different fibres at each temperature were pooled. The normalized pCa–tension relation (Fig. 4*A*) is shifted to the right with an increase of temperature as has been previously reported in several skeletal muscle fibre preparations (Stephenson & Williams, 1981, 1985; Godt & Lindley, 1982).

In relation to some of the experiments reported below, it was of interest to determine how the tension would vary if the temperature was increased during a

submaximal contraction. The data in Fig. 4*A* were therefore scaled according to the temperature dependence of tension during maximal activation (Fig. 3*A*) and re-plotted in Fig. 4*B*. This plot shows that in the Ca^{2+} concentration range between pCa 6·2 and 5·7, tension of a submaximally activated fibre will *decrease* with a rise of

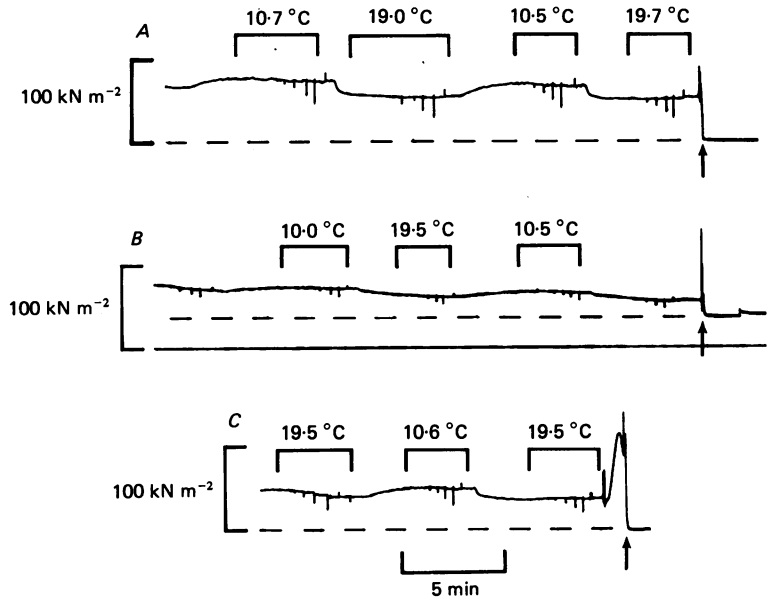

Fig. 5. Tension changes during cooling and warming of a muscle fibre in rigor. The average sarcomere lengths were 2·8, 3·9 and 2·7 μm, respectively, for *A*, *B* and *C*. For each panel the fibre was put into rigor several minutes before the beginning of the trace. The fibre was relaxed at the end (arrows). The temperature changes of approximately 10 °C were done by means of the servo-controlled Joule heating system in the front trough. Note that the rigor tension increases with cooling and decreases with warming. Vertical lines of varying amplitudes in each trace represent tension changes resulting from small length steps (1 s duration, various amplitudes) applied to the fibre. The horizontal interrupted line indicates the tension level in relaxing solution. In *B* (3·9 μm sarcomere length) there was substantial resting tension; the zero tension level is indicated by a continuous horizontal line.

temperature, especially above 20 °C. However, at maximal activation a tension *increase* is expected on increasing temperature over the whole temperature range.

Rigor state: coefficient of thermal expansion

Some of the temperature jump experiments were performed with the muscle fibre in rigor, so the effects of steady temperature changes on rigor tension were examined. The rigor state was initiated at about 20 °C by bathing the fibre for 2–3 min each in relaxing solution containing 0·1 mM-Mg–ATP and then at least two washes with rigor solution (Table 1). The tension record in Fig. 5*A* was obtained with a fibre in rigor initially at 20 °C and the temperature was decreased to 10·7 °C by altering the set-point of the Joule heating servo (see Methods). Tension increased to a new steady value when the temperature was lowered and decreased again on rewarming to 19 °C.

These changes were reversible on further warming and cooling cycles. Such temperature-dependent changes in tension were not observed when a fibre was in the relaxed state, either with negligible passive tension (at sarcomere lengths of 2·5–2·7 μm) or with substantial passive tension (at sarcomere lengths of 3·6–3·9 μm).

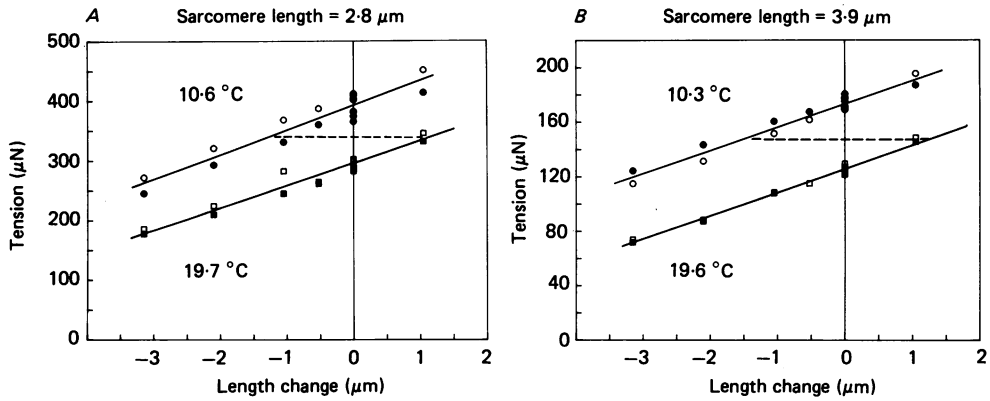

Fig. 6. Data for force–extension relations from a single muscle fibre which was put into rigor at two different sarcomere lengths (*A* and *B*). In each case, the force–extension relations were determined at ~ 20 °C (squares) and at ~ 10 °C (circles) in two cooling–rewarming cycles (filled and open symbols). Regression lines were fitted for the pooled data at ~ 20 °C and ~ 10 °C. The thermal expansion (dashed line) due to heating of the fibre corresponds to 2·4 μm at a sarcomere length of 2·8 μm (*A*) and to 2·65 μm at a sarcomere length of 3·9 μm (*B*).

The reversible changes in rigor tension when temperature was altered are consistent with thermal expansion of a normal (not rubber-like) material. To quantitate the thermal expansion, the tension response to imposed quick length changes of the same order was measured. Tension in a fibre in the rigor state changed in proportion to the length step and there was little tension recovery following the length step (Goldman & Simmons, 1977; Yamamoto & Herzig, 1978).

The force–extension curves of a fibre in rigor at sarcomere length 2·8 μm and at ~ 10 °C and ~ 20 °C are shown in Fig. 6*A*. The different symbols refer to separate series of length steps and lines were drawn through them by linear regression. The results show that the relation between rigor tension and length step is shifted to the right with increased temperature by about 2·4 μm in this fibre (horizontal dashed line). Similar experiments were performed on three fibres at sarcomere lengths ranging from 2·5 to 2·8 μm and the horizontal shift of the force–extension curves (Δl) were converted to coefficients of thermal expansion (α) using the formula $\alpha = \Delta l/(l\,\Delta T)$ where l = fibre length and ΔT = temperature change. The average value for the coefficient of thermal expansion was $\alpha = (7{\cdot}4 \pm 0{\cdot}3) \times 10^{-5}/°C$ (mean ± S.E. of mean, $n = 11$ measurements in three fibres).

The values for α were converted to dimensionless thermoelastic coefficients (R) by multiplying $-\alpha$ with the mid-range temperature (see Woledge *et al.* 1985, p. 241). The mean thermoelastic coefficient was $-0{\cdot}021 \pm 0{\cdot}001$ (S.E. of mean, $n = 11$; the negative sign represents normal thermoelasticity) at sarcomere lengths of 2·5–2·8

μm. This value is within a factor of two of the average value (−0·013) obtained by Gilbert & Ford (1986) from thermal measurements on frog muscle in rigor. The thermoelastic coefficient was slightly larger (−0·024 ± 0·001, $n = 6$) when calculated from data obtained on warming the fibres than that obtained (−0·018 ± 0·001, $n = 5$) on cooling the fibres. Stress relaxation due to high rigor tension during stretches at the low temperature may explain this difference.

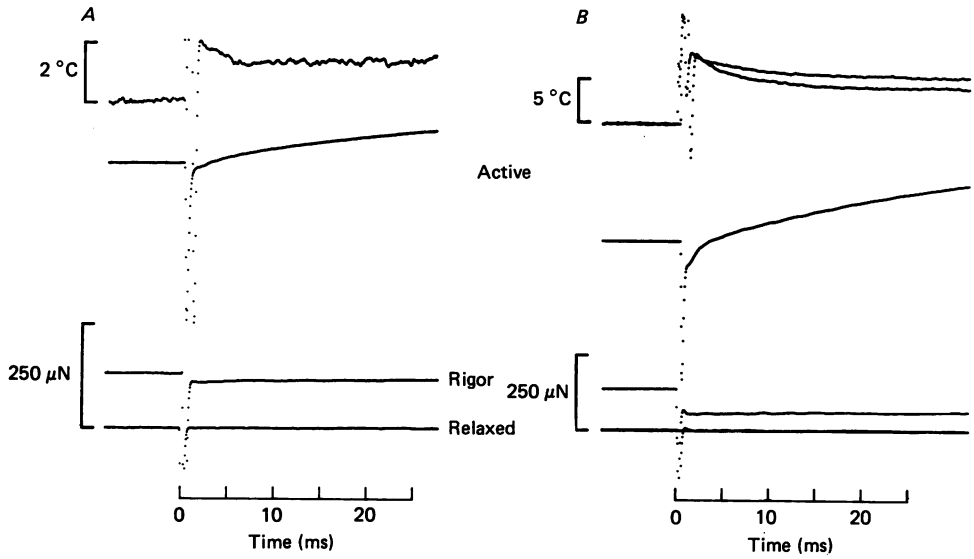

Fig. 7. Tension transients in response to T-jumps. *A* and *B* are from separate experiments. The tension traces shown in each panel were obtained in separate trials when the fibres were relaxed (lowest tension traces), in rigor (middle tension traces) and in maximal Ca^{2+} activation (uppermost tension traces). The temperature was recorded with a thermocouple placed close to the fibre. The starting temperature was 7–8 °C in both experiments and the T-jump amplitude was ~ 2 °C in *A* and ~ 5 °C in *B*. Although temperature traces were recorded with each individual T-jump, only one is illustrated in *A*; the two temperature traces illustrated in *B* indicate the extent of variability seen in a given experiment. The vertical calibration bars for tension represent 250 μN for all of the tension traces except for the rigor state in *A* where the bar represents 200 μN. There are oscillations accompanying the laser pulse in each trace. Following this electrical disturbance the tension was flat in the relaxed state, it abruptly decreased (in < 1 ms) in the rigor state and it changed in a complex manner in active contraction.

Experiments were performed in two fibres at longer sarcomere lengths (3·5 and 3·9 μm) to investigate whether the thermal expansion occurs in the filaments or in the cross-bridges. The fibres were stretched to the longer sarcomere lengths in relaxing solution and then put into rigor. Smaller reversible rigor tension changes were obtained during temperature changes at the longer sarcomere length (see Fig. 5*B*). Figure 5*C* shows that rigor tension recovered on restoring the fibre to its original sarcomere length.

Force–extension curves determined at longer sarcomere lengths (Fig. 6*B*) show that the absolute expansion (2·6 μm) for a 10 °C temperature increase was very

similar to that obtained at shorter sarcomere lengths. If the thick and thin filaments have equal coefficients of thermal expansion, the overall thermal expansion of the fibre would be expected to increase in proportion to the change in sarcomere length (1·4×). The change in thermal expansion was much less, suggesting that the thermoelasticity is not evenly distributed between the thick and thin filaments. This point is taken up again in the Discussion.

Temperature-jump, temperature-clamp experiments

Tension in active and rigor contractions

Figure 7 shows the typical characteristics of the tension transients which accompany a T-jump, where the uppermost traces are the thermocouple outputs (temperature) and the other three traces in each panel are the tension transducer outputs (muscle fibre tension) during an active contraction, rigor and relaxation (top to bottom respectively). When a T-jump was performed on a muscle fibre in the relaxed state (lowest trace), there were oscillations due to the electrical spike from the laser trigger circuit in both the thermocouple and the transducer outputs, but no significant tension transients occurred in relaxation.

When the fibre was in rigor, tension decreased abruptly on heating (middle tension traces). Tension remained lower while temperature was clamped at the higher level and then increased to its original value when temperature was returned to its initial setting. These results indicate that the tension decrease was a reversible effect of the heating rather than a mechanical artifact due to the laser radiation. The tension decrease on laser heating was quantitatively equivalent to the thermoelastic expansion observe on slower heating (Figs 5 and 6). Rigor tension was constant within 1 ms of the laser pulse indicating that the temperature step was complete at this time. Thus the overshoot of the thermocouple signal lasting 5–10 ms is not a valid indication of the solution temperature and is probably caused by direct heating of the thermocouple by absorption of the laser radiation.

The corresponding tension response to a T-jump during active contraction is a sudden decrease followed by relatively fast recovery and a slower rise to a new higher tension level (top tension traces in Figs 7 and 8). There was no evidence for a pause or delay between the fast recovery and the slower component of tension rise. Tension remained at the increased level if the temperature was clamped at the high value (Goldman *et al.* 1985). Tension returned to the previous level when the clamp was turned off and the trough cooled to its original temperature.

The abrupt tension decrease in active contractions is similar in amplitude to the thermoelastic tension drop in rigor (Fig. 7*A* and *B*) indicating that the initial tension drop during active contraction is also caused by thermal expansion.

The recovery and rising phase of such tension responses could be well fitted with a double-exponential function of the following form:

$$P = P_0^{\mathrm{H}} - a_1 \, \mathrm{e}^{-k_1 t} - a_2 \, \mathrm{e}^{-k_2 t}, \tag{1}$$

where P_0^{H} is the asymptote of the tension at high temperature, a_1 and a_2 are amplitude coefficients, k_1 and k_2 are rate constants and t is time from the laser pulse. The squares superimposed on the tension traces in Fig. 8*A* and *B* represent the fitted sum of two exponential functions. Extrapolation of the slower component back to the time of

the laser pulse is shown in Fig. 8*A* by small squares. This back-extrapolation nearly matches the pre-laser-pulse tension suggesting that the faster component is merely a quick recovery from the thermoelastic tension decline.

The tensions calculated by back-extrapolation of the slower phase, relative to the

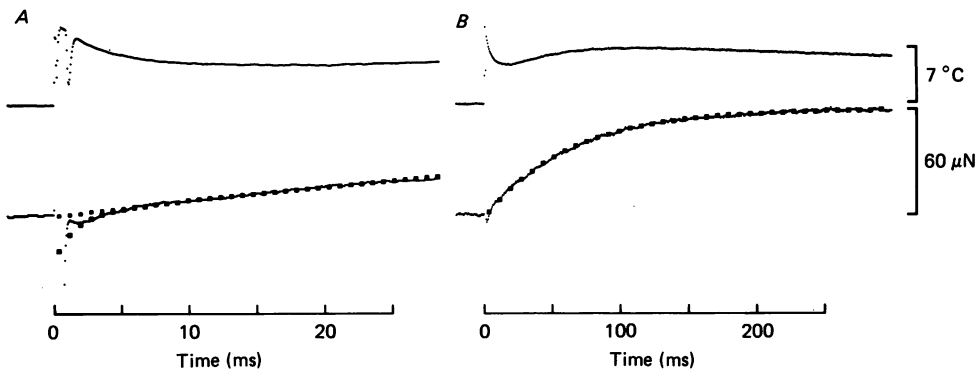

Fig. 8. Analysis of the tension response following a T-jump in a maximally Ca^{2+} activated muscle fibre at 6·6 °C. The same event is shown in *A* and *B* at two different sweep speeds. The upper trace is the temperature and the lower trace is the muscle fibre tension. The tension response was fitted with a double-exponential function (eqn (1) in text). The larger squares show the fit. Extrapolation of the slower exponential components back to the time of the laser trigger is shown by small squares in *A*. Note that the back-extrapolation falls close to the pre-T-jump tension.

tensions before the laser pulse (P_0^L), are plotted in Fig. 9*A* against amplitude (a_1) of the thermoelastic tension decline as a percentage of P_0^L. The data indicate that for amplitudes of thermoelastic tension decline less than 10% of P_0^L, tension recovered fully during the quick phase to P_0^L or higher. Although there is scatter in the data, on average the back-extrapolated tension was $1{\cdot}023 \pm 0{\cdot}004$ of P_0^L (S.E. of mean, $n = 20$) for the data with $a_1/P_0^L < 10\%$. At a_1/P_0^L values $> 10\%$ there is a suggestion that the tension recovery is less complete.

The amplitude of the net tension increase (final tension value minus initial tension, $P_0^H - P_0^L$) in the same set of T-jump experiments is shown in Fig. 9*B*. The net increase of tension per °C, is plotted along the ordinate as a percentage of the tension before the laser pulse. Plotted on the abcissa is the mid-temperature. The results show that the relative amplitude of the tension increase per °C of T-jump is considerably greater at low temperatures than at high temperatures. These data are consistent with steady-state contraction experiments illustrated in Fig. 3. When eqn (1) was fitted to the tension transient, the amplitude (a_2) of the slower component was almost identical to the increase in steady tension shown in Fig. 9*B*.

The temperature dependence of the two rate constants (k_1 and k_2) is illustrated by an Arrhenius plot in Fig. 10. The lower rate (k_2, closed squares) is approximately 20 s^{-1} at 20 °C and increases with temperature with a Q_{10} of approximately 1·4. The higher rate (k_1, open squares) is approximately 400 s^{-1} at 10–20 °C and has no appreciable dependence on temperature.

The amplitude (a_1) of the faster component was quite variable and showed no clear trend when plotted against temperature. This variability might be expected if, as

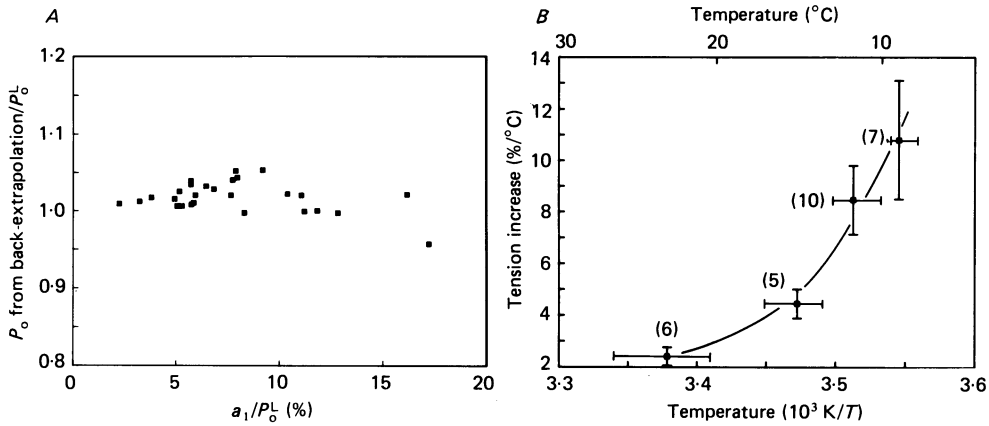

Fig. 9. *A*, relationship between the tension level following the quick recovery and the amplitude of the thermoelastic tension decline. Tension values calculated by back-extrapolation of the slower phase of the transient (as shown in Fig. 8*A*), relative to tensions before the laser pulse (P_o^L), are plotted on the ordinate. Amplitudes of the thermoelastic tension decline as a percentage of P_o^L are plotted on the abscissa. *B*, increment of steady tension obtained by a T-jump is plotted against reciprocal absolute temperature. Tension increment per °C of T-jump is plotted on the ordinate as a percentage of the pre-T-jump tension ($100 \times (P_o^H - P_o^L)/[(T^H - T^L)\, P_o^L]$) where T^L and T^H are the temperatures before and after the T-jump, respectively, and the mid-temperature ($(T^L + T^H)/2$) is plotted as 10^3 K/T on the abscissa. The symbols denote the means and the vertical lines, the S.E. of mean. The number of observations is given in parentheses. Horizontal bars show temperature ranges of grouped data. T-jump amplitudes ranged from 1·5 to 7 °C. The line was drawn by eye.

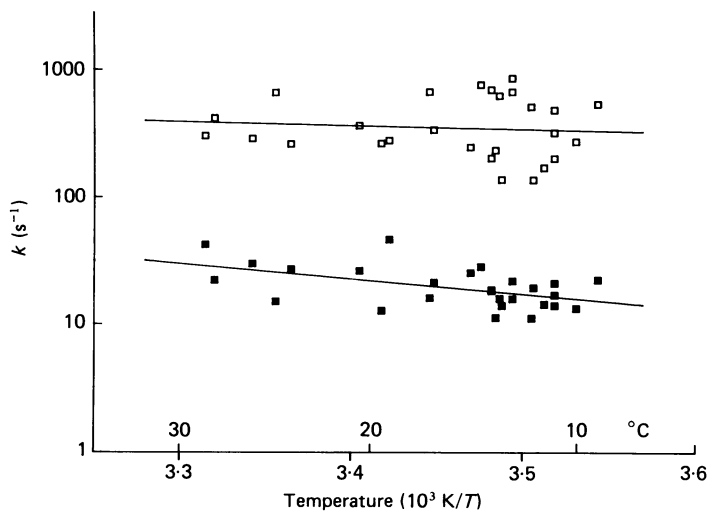

Fig. 10. Arrhenius plots of the two exponential rate constants, k_1 (□) and k_2 (■). The rates are plotted on a logarithmic ordinate against the reciprocal absolute temperature (T^H) on the abscissa. Straight lines represent the calculated regressions for each set of data points. For the faster component the line represents the dual regression, as discussed in the text, plotted at the average amplitude, 7·9% P_o^L. For the slower component the line represents the simple linear regression. Note that k_2 (■) increases with temperature, whereas k_1 (□) shows no significant temperature dependence. Data are from eight muscle fibres ($n = 27$).

discussed above, the fast component is recovery from the thermoelastic expansion since end-compliance would strongly influence the amplitude. In that case the recovery after an equivalent length change would have similar kinetics. Figure 11 shows a comparison of T-jump and length-step perturbations during active contractions of

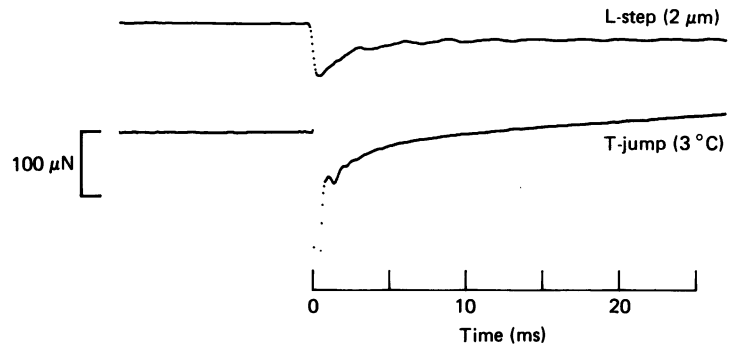

Fig. 11. A comparison of the tension responses due to a step length release (2·1 μm, upper trace) and that due to a T-jump (3 °C, lower trace) from a single muscle fibre. The fibre was maximally Ca^{2+} activated in separate contractions for the two trials. Note that the tension recovers following the two types of perturbations with similar rates. Fibre length = 2·6 mm; initial temperature = 8·0 °C.

a single fibre. A 2·1 μm release was applied to the fibre resulting in a tension decrease (top trace) similar to the thermoelastic effect of a 3 °C T-jump (lower trace). The recovery rates after the two perturbations are similar.

As previously described by several groups (Huxley & Simmons, 1971, 1973; Ford, Huxley & Simmons, 1977; Abbott & Steiger, 1977), the quick phase of tension recovery after a length change is slower for stretches than releases and faster the larger the release. If the fast component of tension recovery following a T-jump corresponds to the same process as the quick tension recovery following a length step, then the rate constant for recovery should be faster when the amplitude is larger. Figure 12 (filled circles) shows the rate constant of the faster phase (k_1 in eqn (1)) plotted on a logarithmic scale against the percentage amplitude (a_1/P_0^L). A statistically significant correlation is observed in the expected direction (continuous line); fibres having large amplitudes, corresponding to large thermoelastic tension decrease, generally had faster rates (k_1).

The open squares represent the corresponding data obtained by fitting single-exponential components to the tension traces after quick releases. A similar correlation is observed; the regression lines for the length-step and T-jump data in Fig. 12 are not significantly different from each other, suggesting that the initial components of tension recovery after length and temperature perturbations may correspond to the same process.

The data in Figs 10 and 12 were obtained over a range of temperatures, and the amplitudes of the thermoelastic tension decline varied because the T-jump amplitudes varied and because the fibres had different values of end-compliance. In order to examine interactive effects of temperature

and amplitude (a_1) on the rate (k_1), the data were analysed by partial regression. The data were fitted by the regression equation:

$$\log k_1 = 3{\cdot}48\,(\pm 2{\cdot}08) + 0{\cdot}034\,(\pm 0{\cdot}011)\,a_1/P_o^L - 0{\cdot}35\,(\pm 0{\cdot}60)\,10^3\mathrm{K}/T$$

(regression coefficients ±S.E. of mean, $n = 27$). The values calculated for k_1 on the basis of this equation were 386 s^{-1} at 10 °C and 425 s^{-1} at 20 °C when a_1 was 10% of P_o^L. This corresponds to a Q_{10} of 1·10 but statistically, Q_{10} was not significantly different from zero. Using the partial regression analysis, the correlation obtained between log k_1 and a_1/P_o^L (Fig. 12) was present, but there was no statistically significant dependence of k_1 on temperature.

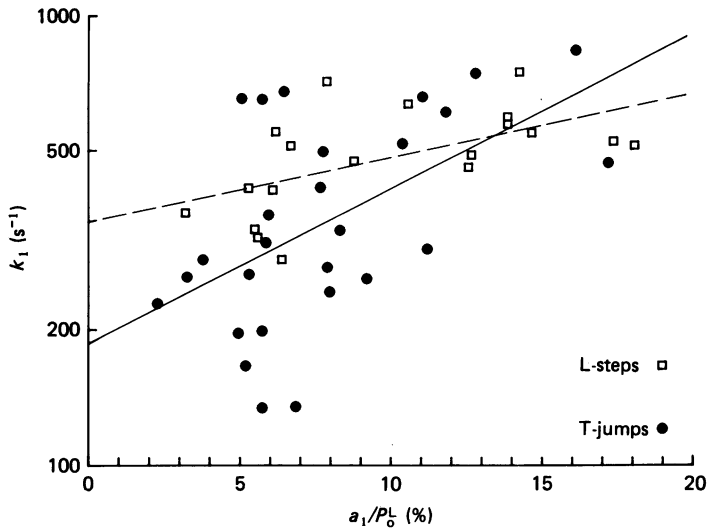

Fig. 12. Relation between the amplitude (a_1) and the rate (k_1), calculated for the phase of quick tension recovery. Amplitude is plotted as a percentage of the pre-T-jump steady tension ($100 \times a_1/P_o^L$). Rate constants are plotted on a logarithmic scale. Squares are data obtained from length-step (release) experiments. The filled circles represent data from T-jump experiments. The partial regression fit to the T-jump data as described in the text was used to calculate the continuous line at the average temperature of 16·2 °C. The dashed line was calculated by linear regression of the length-step data. Within experimental error, the rates of tension recovery from the two types of perturbations are similar and both rates increase significantly with the amplitude.

Besides the dependence of rate on amplitude, the quick recovery after length changes has the property that the extent of recovery is less for larger releases than for small releases (the T_2 curve, Huxley & Simmons, 1971, 1973). An indication of this type of behaviour is present in the data of Fig. 9*A* but there are not enough points at high amplitude to ensure that quick recovery following T-jumps is incomplete when the amplitude is high. Experiments using greater T-jumps (10–20 °C) would be required to make this comparison conclusive.

Tension in submaximal active contractions

In a separate series of experiments, the effect of a temperature clamp on tension in submaximally Ca^{2+}-activated muscle fibres was examined. Since preliminary experiments showed that there were slow tension changes, the temperature clamps in these experiments were made much longer (up to 30 s) in duration. When a muscle

fibre was submaximally activated (1–2 μM-Ca^{2+}) at temperatures around 20–23 °C and the temperature was stepped and then clamped to a value 5–7 °C higher, the tension initially rose as in maximally active fibres, and then declined slowly to a new steady level which was often lower than the pre-T-jump steady tension (Fig. 13*A*).

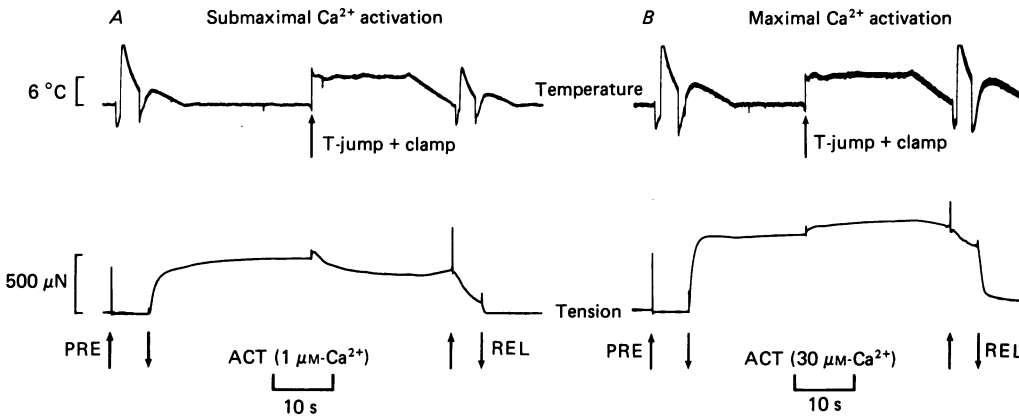

Fig. 13. Tension responses induced by a T-jump and T-clamp in a single muscle fibre preparation at submaximal Ca^{2+} activation (*A*) and at maximal Ca^{2+} activation (*B*). Temperature is the top trace and tension is the lower trace. The fibre was removed from a back trough containing pre-activating solution (PRE) at the first upward arrow and put into the front trough containing activating solution (ACT) at the first downward arrow. In the maximally activated case (*B*) the tension remained high during maintenance of the elevated temperature and declined with cooling when the high T-clamp is turned off. When submaximally activated, the T-clamp lead to a transient tension rise followed by a slow decline of tension. On cooling tension increased again. Starting temperature = 22 °C. REL = relaxing solution.

Recordings at a faster time base of the tension changes seen under these conditions were published in our preliminary abstract (Goldman *et al.* 1985). In experiments on twelve muscle fibres, the half-time of the tension decline was found to be 1–4·5 s with an average of approximately 2 s ($n = 24$). The tension recovered to the original level when the temperature was returned to the starting value. For comparison, the response obtained from the same fibre when maximally activated is shown in Fig. 13*B*. Following the T-jump tension remained elevated as long as the high temperature was maintained.

Decline of submaximal tension following a temperature rise was not observed when the starting temperature was low (10 °C, three experiments) as expected on the basis of the pCa–tension curves described above (see Fig. 4*B*). Experiments similar to those shown in Fig. 13 were also performed on two fibres in the presence of 10 mM-inorganic phosphate. The maximal tension developed was lower in the presence of phosphate (Hibberd, Dantzig, Trentham & Goldman, 1985*a*). The tension decline following T-jumps during submaximal contraction was still observed in this case and the half-time of tension decline was 2·0–2·5 s ($n = 7$), which is within the range obtained in the absence of phosphate.

In some of the experiments small sinusoidal length oscillations were applied to one

end of the fibre and the 500 Hz in-phase stiffness extracted from the tension signal as described previously (Goldman *et al.* 1984). In maximally activated contractions where a temperature clamp resulted in maintained higher tension (see Fig. 13*B*), the stiffness was found to be either slightly decreased or not changed from its value before the laser pulse. However, the stiffness decreased during the tension decline in submaximal contractions.

DISCUSSION

Laser temperature jumps greatly improve the time resolution accessible for studying the energetics of muscle contraction. Energy balance and the enthalpy of intermediate reactions have been traditionally investigated using calorimetric methods but these studies have been limited in their time resolution to greater than about 10 ms by diffusion of heat into the recording thermopiles (Gilbert & Matsumoto, 1976). Thermodynamic relationships necessarily link such calorimetric studies to temperature dependence of the reaction rates and the equilibria, but processes on a much shorter timescale can be measured using T-jumps. T-jump experiments on muscle fibres using microwave radiation have been previously reported with 10 ms time resolution (Lindley & Kuyel, 1978; Lindley & Goldthwait, 1984).

Laser T-jump, T-clamp method

The holmium laser used in the present experiments has a pulse duration of approximately 150 μs during which time the solvent and muscle fibre are heated. The small thermocouples used to monitor temperature during the T-jump transients equilibrated with the water temperature on the 10 ms timescale, so we did not directly observe the temperature profile at the 1 ms timescale of the tension transients. However, the tension drop on laser pulse heating of rigor fibres was complete and steady after 1 ms (Fig. 7*A* and *B*) strongly suggesting that the temperature of the fibre was steady after this time. Smith *et al.* (1987) spectrophotometrically measured the time course of the temperature jump using the temperature dependence of Tris pH buffer and a pH indicator dye and also found temperature to be steady 1 ms after the laser pulse. On the slower timescale of 100 ms to seconds, the Joule-heating feed-back method was optimally capable of complementing the laser pulse heating to within 15% or about 1 °C for a 5–8 °C step. Spatial non-uniformities across and along the fibre were estimated to be 25% (calculated) and 18% (measured S.D.), respectively. These factors can undoubtably be improved on further development of the method (Smith *et al.* 1987).

Temperature dependence of active tension

Control steady-state experiments were performed to check conditions and end-points for the T-jump work using the temperature-insensitive pH buffer β-glycerol phosphate. These experiments on glycerol-extracted rabbit psoas fibres confirm several findings reported by previous workers on different preparations. The maximal Ca^{2+}-activated tension was shown to increase with rise of temperature, the increase being more pronounced at the lower temperatures (Fig. 3). These results are in general agreement with those reported by Stephenson & Williams (1981, 1985) from mech-

anically skinned rat muscle fibres and also with those obtained from rat intact muscles (Ranatunga & Wylie, 1983). As expected, the tension per °C of laser pulse heating was found to be greater when T-jumps were elicited at lower starting temperatures (Fig. 9*B*). The results clearly support the view that the cooling depression of isometric tetanic tension in intact muscles and muscle fibres is largely due to this myofibrillar behaviour (Stephenson & Williams, 1985).

Submaximal activation

It is now well established that in skeletal muscle fibres, the calcium sensitivity of the thin-filament regulatory system decreases with a rise of temperature (Stephenson & Williams, 1981, 1985; Godt & Lindley, 1982; Fig. 4 of this paper). An outcome of this property is that at submaximal Ca^{2+} levels the isometric tension decreases with a temperature rise (Fig. 4*B*). However, this effect on the regulatory system is antagonized by the increase of fully activated tension as temperature rises (Fig. 3) so that a net decrease is observed only at particular Ca^{2+} concentrations and temperatures.

The interactions between these phenomena are illustrated in the experiments with T-jumps and T-clamps at partial activation at 20–22 °C (Fig. 13*A*). Following the T-jump, tension first increased and then declined to a steady lower level as previously reported by Lindley & Goldthwait (1984). The rate of the decline (half-time $\sim$ 2 s) is surprisingly slow since Ca^{2+} desorption from troponin has been estimated to occur at 20–200 s^{-1} (Potter & Zot, 1982; Rosenfeld & Taylor, 1985). The likely explanation for the slow rate of tension decline is that some Ca^{2+} dissociates from troponin promptly after the T-jump but that active force-producing cross-bridges remain attached for several hundreds of milliseconds due to a very slow step in the cross-bridge cycle. Although the identity of the rate-controlling step in the cross-bridge cycle remains unclear, the rate of T-jump-induced tension deactivation is near the range (1–3 s^{-1}) reported for steady-state ATP hydrolysis per myosin head in activated rabbit skeletal muscle fibres (Ferenczi, Homsher & Trentham, 1984; Glyn & Sleep, 1985; Hibberd, Webb, Goldman & Trentham, 1985*b*).

The above explanation of the results would imply an interaction between the Ca^{2+}regulatory system and the kinetics of the cross-bridge cycle as previously suggested by Brandt, Cox, Kawai & Robinson (1982). Those authors found that changes in the pCa–tension curve brought about by agents that modify the cross-bridge cycle (inorganic phosphate (P_i), ATP and ionic strength) could be explained if Ca^{2+} desorption does not result in immediate cross-bridge detachment. A possible difficulty with this hypothesis is that tension decreases much faster than 2 s^{-1} when Ca^{2+} is fully removed (e.g. final tension relaxation in Fig. 13*B*), so a different pathway for detachment would be required during full relaxation (see also Hibberd *et al.* 1985*a*).

Another explanation for the results obtained at submaximal Ca^{2+} concentrations is that gradual accumulation of products of the ATPase reaction (e.g. ADP, H^+) leads to the slow decline in tension. Experiments with 10 mM-P_i present lead to equivalent results so phosphate accumulation after the T-jump does not explain the slow decline. Changes in concentrations of other relevant compounds following the T-jumps have not been ruled out.

Thermoelasticity

The tension in a rigor muscle fibre was found to decrease with a rise of temperature both in steady-state and in T-jump experiments. This result was expected from previous calorimetric and mechanical studies showing that the thermoelasticity of active and rigor muscle is not rubber-like (Wöhlish & Clamann, 1931; Hill, 1953; Aubert, 1956; Woledge, 1961; Gilbert & Matsumoto, 1976; Gilbert & Ford, 1986). The tension change on heating is fully reversible on cooling to the original temperature indicating that it is due to reversible thermal expansion.

Combining length steps with temperature changes (Figs 5 and 6) enabled us to estimate the dimensionless thermoelastic coefficient (R) of the rigor fibre to be on average $-0{\cdot}021$. Previous studies on frog muscle in the cold have typically resulted in smaller values for the thermoelastic coefficient of rigor muscle: ($-0{\cdot}006$ to $-0{\cdot}013$; studies cited by Gilbert & Ford, 1986) and for active muscle ($\sim -0{\cdot}01$, Woledge *et al.* 1985, p. 238, and references therein). The differences in preparations, temperatures and techniques can probably account for this apparent discrepancy.

The absolute thermal expansion (μm/°C) in a given fibre was very similar at sarcomere lengths of 3·6–3·9 μm to that at sarcomere lengths of 2·5–2·8 μm. Although we did not investigate this point exhaustively, and non-uniformities of sarcomere length may have been substantial in the stretched fibres, the available data suggest that the thermal expansion is not evenly distributed between the two sets of filaments. If that were the case, absolute thermal expansion would have increased with sarcomere length.

If the thin filaments or cross-bridges are more compliant than the thick filaments, and the thin filaments have a much lower coefficient of thermal expansion than the thick filaments, than thermal expansion might not depend on sarcomere length. Alternatively, the cross-bridges might be considered to account for the thermal expansion. Since the cross-bridge length is a small fraction ($\sim 1/25$) of the half-sarcomere length, the coefficient of thermal expansion (α) for cross-bridge would then be $(25 \times 7{\cdot}5 \times 10^{-5}) \sim 1{\cdot}9 \times 10^{-3}$ K^{-1}. The linear coefficient of thermal expansion of a globular protein (myoglobin) has been crystallographically determined to be $1{\cdot}15 \times 10^{-4}$ K^{-1} (Frauenfelder, Hartmann, Karplus, Kuntz, Kuriyan, Parak, Petsko, Ringe, Tilton, Connolly & Max, 1987), approximately 1·5-fold greater than that of a rigor muscle fibre ($7{\cdot}5 \times 10^{-5}$ K^{-1}). Most of the thermal expansion of myoglobin is not in the α-helical segments of the molecule. Therefore $1{\cdot}9 \times 10^{-3}$ K^{-1} would be a high value to postulate for the cross-bridges in muscle. This argument suggests that the thermal expansion is not limited to the cross-bridges, but more experiments over a range of sarcomere lengths would help to place the thermoelasticity more definitively.

T-jump-initiated transients at full activation

The various phases of the response can be related to other phenomena by their rate constants and temperature dependence. During a T-jump the tension in a fully activated fibre abruptly decreased by approximately the same amount as in a rigor fibre (Fig. 7). This result suggests that the immediate tension decrease is due to thermal expansion of the filaments (and/or cross-bridges) as in rigor. Tension then recovered during the next few milliseconds approximately to the active tension level

present before the T-jump or slightly higher. The rate of this quick recovery is within the same range and has a similar amplitude dependence as the quick recovery after step length releases. Both processes are faster when the original tension decrease is larger (Fig. 12). These comparisons suggest that in activated muscle fibres, the quick recovery after a T-jump corresponds to the same process as the quick recovery after a step length change.

The temperature dependence of the rate of quick recovery after a T-jump (k_1) may also be compared with the quick recovery following length changes. The data in Fig. 10 (open squares) show that k_1 has very little dependence on final temperature ($Q_{10} \cong 1{\cdot}0$). Ford, Huxley & Simmons (1977) obtained higher Q_{10} values (1·5–2·5) for the quick recovery after length changes in active intact frog muscle fibres near 0 °C. Abbott & Steiger (1977) found that in glycerol-extracted rabbit fibres, the earliest phase of tension recovery observed following step length changes had a low temperature coefficient ($Q_{10} = 1{\cdot}0$). The experiments of Ford *et al.* (1977) were more precise than those reported here because end-compliance was functionally eliminated using feed-back from a device monitoring the length of a central segment of the fibre and because their length steps were faster. Abbott & Steiger (1977) used length steps with a 1 ms rise-time that may have been too slow to allow observation of the fastest recovery components. Differences between the species and temperature ranges may also partly explain the difference in Q_{10} values. In the following discussion we assume that the quick recovery after T-jump perturbations corresponds to the quick recovery in the experiments of Ford *et al.* (1977), but it should be noted that this correspondence has not been proven.

The quick recovery restores tension rapidly after small length changes. Huxley & Simmons (1971, 1973) discussed evidence that the quick recovery is a reflection of the cross-bridge power stroke. They postulated that the structural change of the cross-bridge that leads to work production (often considered to be a rocking motion) occurs in a small number of discrete reaction steps and that the force is transmitted through an elastic part of the cross-bridge. These structural changes occur on the millisecond timescale and are readily reversible, so during an isometric contraction, an equilibrium is established among the structural states. A rapid length change perturbs this equilibrium and the quick recovery re-establishes the equilibrium on the characteristic millisecond timescale without cross-bridge attachment–detachment reactions. Huxley & Simmons (1973) discussed the possibility that the extra heat produced when active muscles are released partly reflects the same stepwise structural motion of the cross-bridge. Further heat measurements have not supported this assignment (Gilbert & Matsumoto, 1976; Curtin & Woledge, 1978), but have suggested that the extra heat rapidly liberated on releasing an activated muscle is the instantaneous result of the tension decrease rather than the tension recovery. The present results on thermal expansion and quick recovery confirm the latter conclusion.

If the quick recovery signals a reversible equilibrium, as postulated in the Huxley & Simmons model, and the reaction produces heat when tension is increasing, as in recovery from a quick release, then an increase in temperature would perturb the equilibrium towards *lower* tensions (e.g. Edsall & Gutfreund, 1983, p. 151). This change in equilibrium would appear in our T-jump experiment as a tension *decrease*

with a rate constant corresponding to that of the quick recovery following length steps. No such rate-controlled tension decrease occurred; instead, following the immediate decrease associated with thermal expansion, the tension change with the characteristic millisecond rate constant was an *increase* that brought tension slightly *above* the pre-T-jump level (Fig. 9*A*). This result suggests that the temperature increase perturbs the postulated equilibrium among structural states toward those generating *higher* forces, opposite to the change expected if the stepping motion produced heat when tension is increasing.

The enthalpy (ΔH°) of the reactions associated with the quick tension recovery can be estimated using the van't Hoff equation which relates ΔH° of a reaction to the temperature sensitivity of its equilibrium constant (K):

$$\frac{\mathrm{d}(\ln K)}{\mathrm{d}(1/T)} = -\frac{\Delta H^\circ}{\mathrm{R}}, \tag{2}$$

where T is the absolute temperature and R is the gas constant, 8·314 J/(K mol). Equation (2) can be rearranged to the form:

$$\frac{\mathrm{d}K}{\mathrm{d}T} = \frac{K}{T}\frac{\Delta H^\circ}{RT}. \tag{3}$$

If in steady isometric contractions the cross-bridges are distributed at an equilibrium between the states before and after the stepping motion (positions 1 and 2, respectively), then the proportion of cross-bridges (n_2) in position 2 is given by:

$$n_2 = K/(1+K). \tag{4}$$

Equations (3) and (4) can be combined to give:

$$\frac{\Delta H^\circ}{RT} = \frac{T(1+K)}{\Delta T}\frac{\Delta n_2}{n_2}. \tag{5}$$

If *only* cross-bridges in position 2 contribute force (i.e. the step corresponds to the whole power stroke) then the tension change due to perturbation of K is $\Delta n_2/n_2$. On average, the tension value reached after the quick recovery in the T-jump experiments was higher than the starting tension (Fig. 9*A*) corresponding to $\Delta n_2/n_2 = +0{\cdot}023$. Taking $K = 1$ (Huxley & Simmons, 1971) and $T/\Delta T = 289/4$, eqn (5) gives $\Delta H^\circ = +9$ kJ/mol.

The positive value for ΔH° indicates that the process leading to the quick tension recovery is endothermic, but the value given for the enthalpy change should only be considered as a guide for several reasons. We have assumed (as did Huxley & Simmons, 1971) that in isometric contractions, the cross-bridges are equally distributed between the structural states ($K = 1$) and that the stepping process returns essentially to equilibrium during the quick recovery. If the subsequent slower reactions pull the equilibrium of the stepping motion appreciably, the estimate of the enthalpy would be altered. Only two states have been assumed to be in rapid

equilibrium, although more states are probably involved (Huxley & Simmons, 1971).

It should be noted that the system considered in this thermodynamic argument is the assembly of cross-bridges including both the structures responsible for the stepwise motion and the elastic structures. During tension increase, energy is stored by stretching the elastic component, a process that absorbs heat according to the measurements showing normal thermoelasticity. Thus the process of the stepwise motion alone could liberate heat if stretching the elastic component leads to greater heat absorption.

The experiments bear on another hypothesis for the mechanism of the power stroke, a helix-to-coil transition in myosin subfragment-2 (S-2). According to this hypothesis, during active contraction part of the α-helical coiled coil of S-2 melts to the random coil form with concommitant decrease in end-to-end distance of the peptide chain (Harrington, 1979; Ueno & Harrington, 1981, 1986*a*, *b*). The quick recovery following a length decrease is postulated to result from further melting of S-2, and the cross-bridge compliance is thought to be the rubber-like entropic nature of the random coil. This model predicts that the response to a T-jump during active contraction would be an instantaneous *increase* of tension, due to the negative thermal expansion coefficient of the random coil (Florey, 1956), and then a further tension increase on the millisecond timescale, due to further melting of S-2. Neither of these events was observed, but they might be missed if the normal thermal expansion of the filaments outweighs the rubber-like thermoelasticity of the cross-bridges, or if only a small proportion of the S-2 is melted in the experimental conditions.

Temperature sensitivity of steady contraction

Since the equilibrium of the quick recovery process is not very temperature sensitive, the temperature sensitivity of the steady contraction level (Fig. 3) must be explained by another mechanism. On heating, fibre stiffness increased less than tension in our experiments confirming previous results (e.g. Ford, Huxley & Simmons, 1977) and suggesting that force per cross-bridge increases with temperature. Recent X-ray diffraction (Huxley, 1979) and mechanical stiffness measurements (Ford, Huxley & Simmons, 1986) suggest that at least during the onset of activation, reaction steps on the 10 ms timescale contribute to the kinetics of the power stroke. The quick recovery equilibrium can be integrated with such slower steps in the cross-bridge cycle either in a linear series of reactions or as parallel intermediates (Shriver, 1984; Geeves, Goody & Gutfreund, 1984; Ford *et al.* 1986). Following T-jumps, tension increased with a rate constant of 20–30 s^{-1} at 20 °C and this slower phase fully accounted for the increase of steady tension with temperature. Thus a slower step, as yet unidentified, may be the component of the power stroke that is temperature sensitive and therefore should have a substantial enthalpy change.

We thank Mr J. Pili for mechanical design and construction of the trough changer and Mr M. A. Luttmann for expert technical assistance. We thank Drs M. Geeves, S. H. Gilbert, H. Gutfreund, W. F. Harrington, E. Homsher and A. F. Huxley for helpful discussions. The work was supported by NIH grants AM26846 and AM00745, NATO grant 547/82, the Wellcome Foundation, and a Drexel University Research Scholar Award (to J.A.M.).

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