Endothermic Force Generation in Fast and Slow Mammalian (Rabbit) Muscle Fibers

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ABSTRACT Isometric tension responses to rapid temperature jumps (T-jumps) of 3–7°C were examined in single skinned fibers isolated from rabbit psoas (fast) and soleus (slow) muscles. T-jumps were induced by an infrared laser pulse (wavelength 1.32 μm, pulse duration 0.2 ms) obtained from a Nd-YAG laser, which heated the fiber and bathing buffer solution in a 50-μl trough. After a T-jump, the temperature near the fiber remained constant for ~0.5 s, and the temperature could be clamped for longer periods by means of Peltier units assembled on the back trough wall. A T-jump produced a step decrease in tension in both fast and slow muscle fibers in rigor, indicating thermal expansion. In maximally Ca-activated (pCa ~4) fibers, the increase of steady tension with heating (3–35°C) was approximately sigmoidal, and a T-jump at any temperature induced a more complex tension transient than in rigor fibers. An initial (small amplitude) step decrease in tension followed by a rapid recovery (τ_1; see Davis and Harrington, 1993) was seen in some records from both fiber types, which presumably was an indirect consequence of thermal expansion. The net rise in tension after a T-jump was biexponential, and its time course was characteristically different in the two fibers. At ~12°C the reciprocal time constants for the two exponential components (τ_2 and τ_3, respectively) were ~70·s^{-1} and ~15·s^{-1} in fast fibers and ~20·s^{-1} and ~3·s^{-1} in slow fibers. In both fibers, τ_2 ("endothermic force generation") became faster with an increase in temperature. Furthermore, τ_3 was temperature sensitive in slow fibers but not in fast fibers. The results are compared and contrasted with previous findings from T-jump experiments on fast fibers. It is observed that the fast/slow fiber difference in the rate of endothermic force generation (three- to fourfold) is considerably smaller than the reported differences in the "phosphate release steps" (>30-fold).

INTRODUCTION

The maximum isometric force of mammalian muscles and muscle fibers increases severalfold in warming from low (<10°C) to high (>30°C) physiological temperatures (Hajdu, 1951; Ranatunga and Wylie, 1983; Stephenson and Williams, 1985: Ranatunga, 1994); additionally, the force-shortening velocity relation has been found to be greatly affected by temperature (Ranatunga, 1984). Consequently, rapid temperature jumps (T-jumps) have been used as a perturbation technique to elucidate the temperature-sensitive steps in the cross-bridge cycle (Davis and Harrington, 1987b, 1993; Goldman et al., 1987; Bershitsky and Tsaturyan, 1989a, 1992). From such experiments on maximally Ca-activated skinned fibers, there is general agreement that a T-jump induces a biexponential (τ_2 and τ_3; see Davis and Harrington, 1993) rise in tension, where τ_2 represents an endothermic force generation in attached cross-bridges.

More recently, Davis and Rodgers (1995a,b) have made detailed studies using this experimental approach and proposed a comprehensive model to accommodate most of the findings. Their key conclusions are that endothermic force generation (τ_2) corresponds to a slow component of the mechanical power stroke (T_1-T_2 transition; Ford et al., 1977), cross-bridge force generation is entropy driven, the τ_3 is the rate-limiting step in the cross-bridge cycle and is temperature insensitive, and that release of inorganic phosphate in the ATPase cycle occurs before and is only indirectly coupled to force generation. The Davis and Rodgers scheme clearly deserves further examination. Additionally, there are a number of discrepancies among the T-jump studies by different groups (see Discussion) that require clarification. These were the aims of the present study.

Previous studies have shown that the temperature dependence of the steady-state characteristics (e.g., force-velocity relation and maximum active tension; Ranatunga, 1982, 1984; Stephenson and Williams, 1985) is essentially similar between fast and slow fibers, but that responses of slow fibers have slower kinetics and would provide better time resolution in analyses. Thus, experiments were done on both fast (from rabbit psoas) and slow (from rabbit soleus) skinned fibers. Results show that the laser T-jump induces a qualitatively similar tension transient in the two fiber types, but its time course is three- to fivefold slower in soleus fibers. The combined behavior between the fiber types is compared and contrasted with previous findings from fast fibers listed above.

MATERIALS AND METHODS

Trough system

A trough system mounted on an optical microscope was used in this study. It was a modified version of one described previously (Ranatunga, 1994) in which the front trough was used as the experimental trough. The front trough had glass windows in front and on the bottom; its back wall was lined with aluminium foil, behind which were assembled small Peltier

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modules for use in clamping the trough temperature. The Peltier modules could heat the trough solution at a rate of ~2.5°C/s, and their operation was under feedback control from a small-diameter (0.4 mm) thermistor placed within the trough. The temperature of the entire trough system was kept at 5–10°C by passing a cold antifreeze-water mixture through a jacket that was attached to the assembly, and the temperature of the front trough was clamped at a required starting temperature (range 3–35°C) by the Peltier module system.

T-jump technique

The principle of the T-jump method is similar to that described by Goldman et al. (1987) and Davis and Harrington (1987a). A near-infrared laser pulse (from a Nd-YAG pulsed laser; Schwartz Electro-optics) entered through the front window and heated ~50 μl of the buffer solution and muscle fiber contained within the trough. The wavelength of the laser pulse radiation was 1.32 μm (similar to that of the iodine laser used by Davis and Harrington, 1987a,b). The water extinction length at this wavelength is 10 mm (as opposed to 0.3 mm for a wavelength of ~2 μm in a Ho-YAG laser used by Goldman et al., 1987), so that the pulse radiation passed across the trough solution (~3 mm) and was reflected back through the solution by the aluminum-lined back wall. Consequently, although the energy absorption was less, the uniformity of heat distribution across the trough was considerably better than in the experiments of Goldman et al. (1987) (see Davis and Harrington, 1987b); the attenuation across the 75-μm width of a fiber was estimated to be ~1%. The laser pulse passed through a long-focal-length (300 mm) cylindrical lens so that, at the level of the muscle fiber, the pulse cross section was an elipse with a minor width of 2 mm. The temperature variation along the long axis of muscle fiber (length <3 mm) was not more than 1°C.

The solution temperature in the front trough was monitored by the thermistor as well as by a separate small thermocouple (20–100 diameter in different experiments) placed very close to the muscle fiber. The laser pulse duration (T-jump rise time) was 0.2 ms and, after a pulse, the temperature measured at the level of muscle fiber remained elevated for >5 s. Fig. 1 *a* shows a temperature record from a control experiment. It is seen that there is an initial spike accompanying the laser pulse and the temperature then decays by about 50% in 5 s. The initial spike in the thermocouple output has been observed in previous studies (see Goldman et al., 1987; Davis and Harrington, 1987a) and is probably due to direct absorption by the thermocouple. Fig. 1 *b* shows that the solution temperature in the trough remains approximately constant for ~500 ms. By appropriate timing of the Peltier heating, the temperature could be clamped for longer periods (5–10 s after a laser pulse), as shown by Fig. 1 *c*. The laser pulse energy could be adjusted (maximum ~2 J) to obtain T-jump amplitudes of 3–8°C.

The design of the tension transducer was given previously (Ranatunga, 1994); its natural resonant frequency was 6–7 kHz. The transducer hooks were made of 50–100-μm-diameter Invar wires (an alloy of steel and Nickel; gift from Goodfellow, Cambridge, England), because the thermal coefficient of expansion of Invar is low ($1.7–2 \times 10^{-6}$ K^{-1}). To prevent direct absorption by the metal hooks attached to the transducers, the transducer hooks were shadowed from the laser beam by placing aluminum flags on the front window. Such precautions were not strictly adhered to in our previous laser T-jump experiments (Goldman et al., 1987), and as suggested by Bershitsky and Tsaturyan (1989a), this may have given the high values for apparent thermal expansion in rigor and initial tension drop in active fibers obtained in that study. The compositions of the buffer solutions used were the same as those used in our previous studies (Goldman et al., 1987; Ranatunga, 1994), and they contained 15 mM β-glycerol phosphate as the pH buffer, chosen because of its low temperature sensitivity.

Experimental details

Muscle fibers from rabbit psoas (fast) and soleus (slow) muscles were chemically skinned and glycerinated as described previously (Fortune et al., 1989a). In an experiment, a fiber segment of 0.7–2 mm was mounted

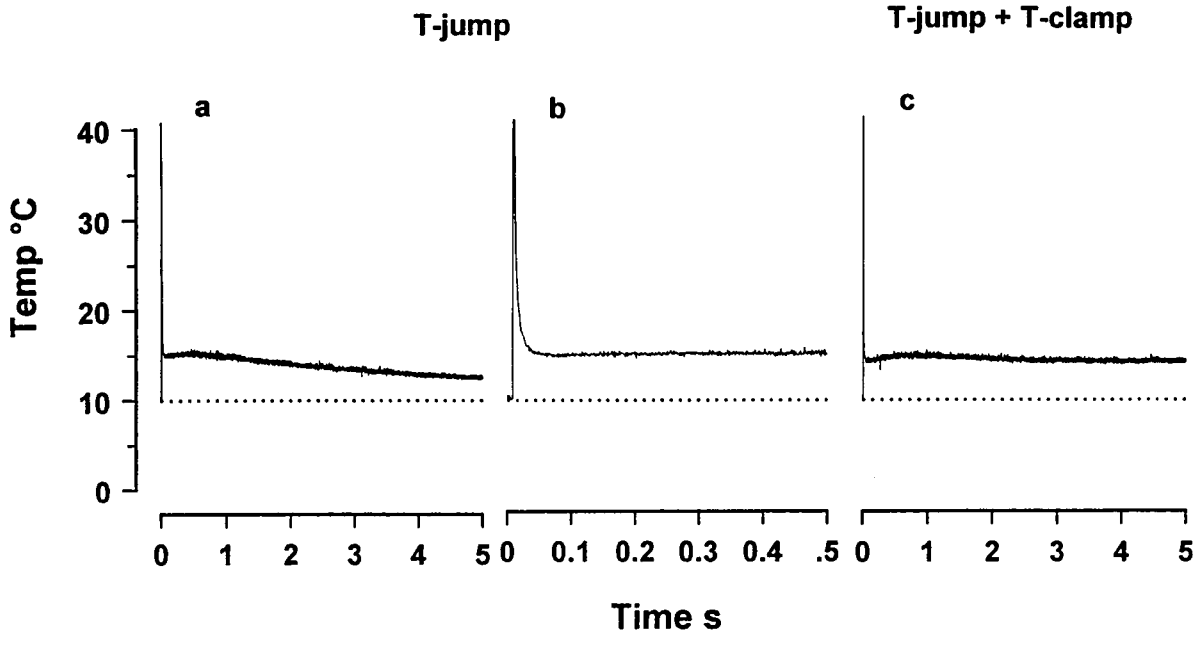

FIGURE 1 Records of the thermocouple output in response to two laser pulses of the same intensity. The trough was filled with the buffer solution, the thermocouple was near the middle of the muscle fiber, and the laser was flashed 10 ms after the begining of the trace. The initial spike coincident with the laser pulse in the records is due to direct absorption and does not represent the solution temperature (see Goldman et al., 1987; Davis and Harrington, 1987a; also, see rigor tension response in Fig. 2). (*a*) The T-jump amplitude after a laser pulse decays by about 50% in ~5 s. (*b*) The same record as in *a*, to show that the temperature remains constant for the first 0.5 s after the laser pulse. (*c*) A response due to a laser pulse coupled with the Peltier temperature control (see Materials and Methods). The slow decay of temperature can be prevented for longer periods by using Peltier T-clamping.

(using nitrocellulose glue) between two hooks, one of which was attached to the tension transducer, and fiber dimensions and sarcomere length were determined by optical microscopical examination. The facility to make optical microscopical examination of the mounted preparation was considered essential for this type of experiment, to ensure that the fibers used in the experiments were not twisted, had uniform sarcomeres, and remained in good condition during the course of an experiment. The initial sarcomere length in different experiments was 2.3–2.5 μm.

The outputs of the tension transducer and the thermocouple were examined on a digital cathode ray oscilloscope and recorded using a PC-based computer (486, CENCE systems) with a CED 1401 (plus) laboratory interface (Cambridge Design, Cambridge, England). A continuous record of the entire experiment was also made on a dual-channel paper chart recorder (Lectromed, Letchworth Garden City, England). The curve fitting to tension transients was done using a nonlinear curve-fitting program (FIG-P, Biosoft). In most cases, a biexponential curve of the form $P_h - a_2 \cdot \exp(-(t-d)/\tau_2) - a_3 \cdot \exp(-(t-d)/\tau_3)$ was used, where P_h is the steady tension at high temperature (after the T-jump), $a_{2,3}$ and $\tau_{2,3}$ are amplitudes and time constants of the two exponential components, t is time, and d is the time delay to the laser pulse. Phases 2 and 3 correspond to T-jump relaxations 2 and 3, or τ_2 and τ_3, of Davis and Harrington (1993) and Davis and Rodgers (1995a,b). P_l will be used to denote pre-T-jump tension (i.e., at low temperature). In some cases from either fiber type, a T-jump resulted in a step decrease in tension followed by an initial fast recovery and required an additional exponential component (a_1 and τ_1); $1/\tau_1$ and a_1 were variable and temperature insensitive and did not contribute to tension rise above pre-T-jump level. This component will not be examined in detail here, because it is likely to be an indirect consequence of thermal expansion (see Davis and Harrington, 1993).

Some general considerations

The typical experimental protocol adopted in recording active tension (and transients) at different temperatures was essentially similar to that reported by Ranatunga (1994). A fiber was activated at low temperature (<15°C), and laser T-jumps were induced at different steady temperatures that were "rapidly" achieved by means of the Peltier heating/cooling. Interestingly, Pate et al. (1994) found that activation of skinned fibers at low temperature (<2°C), followed by rapid heating, reduced the development of sarcomere nonuniformity and disorder at high temperatures. However, because continuous monitoring of sarcomere length was not included in the present experiments, we could not assess whether our data were affected by sarcomere disorder at high temperatures. Because of this uncertainty, quantitative comparison of fast-slow fiber types will be made using the low-temperature (<15°C) data. All of the active tension transients shown will contain a fitted curve superimposed on them; additionally, differences between the actual data values and their values predicted from the fitted equation will be given as a residual plot. In examining the temperature dependence, the data will be plotted against reciprocal absolute temperature (as $10^3 \cdot K/T$), as done in our previous study, and the temperature sensitivity will be expressed as Q_{10}, calculated for 20/10°C using the slope of the regression fitted to the data (see Ranatunga, 1984).

RESULTS

Fig. 2 illustrates the general features of the "temperature change" (thermocouple output, top trace) and of the tension responses (lower three traces) from an experiment on a fast (psoas) muscle fiber; the fiber was exposed to three identical T-jumps when it was relaxed (bottom trace), when it was in rigor (middle tension trace), and when it was maximally Ca-activated (top tension trace). The initial spike on the temperature trace is not representative of solution temperature and is due to direct absorption of radiation by the thermocouple (see Materials and Methods); after the initial decay, the temperature remained steady for several hundred milliseconds. The middle tension record indeed shows that rigor tension abruptly decreased to a steady level, as would be expected if the laser pulse induced a step increase in

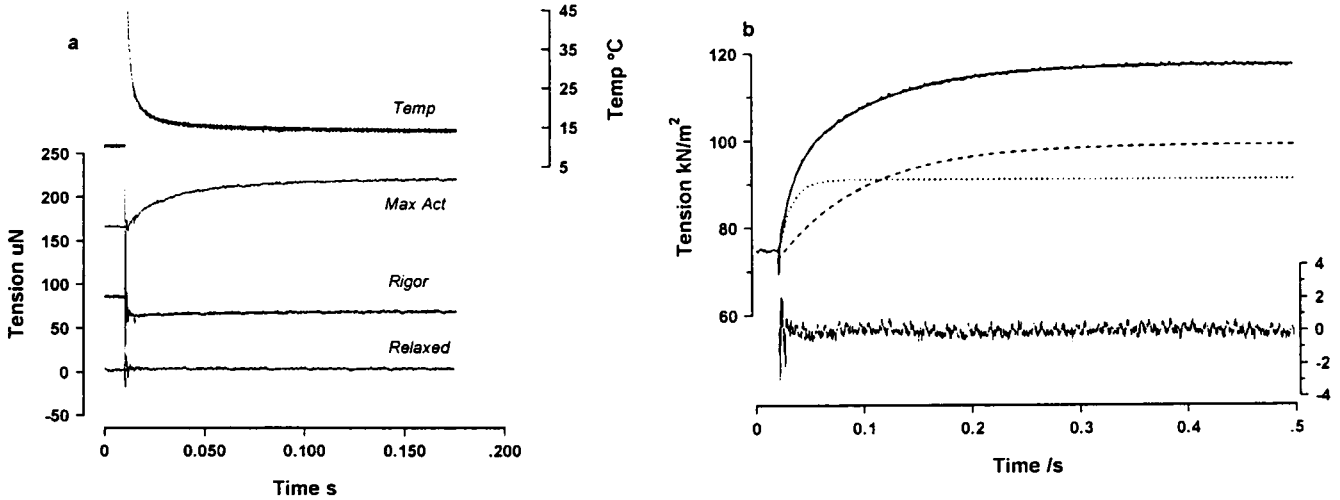

FIGURE 2 (*a*) Tension responses induced by a laser T-jump from a single fast (psoas) fiber preparation, when it was relaxed (bottom tension trace), when it was in rigor (middle tension trace), and when it was maximally Ca-activated (top tension trace). The T-jump was the same (10–15°C), and one thermocouple trace is shown (right ordinate). Note that, apart from the transient oscillations synchronous with the laser pulse, the relaxed fiber tension is not altered appreciably, the rigor tension is decreased abruptly to a new steady level, and the active tension is increased along a characteristic time course to a new steady level. (*b*) The analysis of the tension transient of a maximally Ca-activated psoas fiber. The T-jump was from 8.5 to 14.1°C. The tension transient is fitted with a biexponential function (solid curve through the tension trace), and the two exponential curves are shown separately by the dotted (phase 2 or τ_2, endothermic force generation) and dashed (phase 3 or τ_3) curves. As found by all previous workers, there was a small drop in tension coincident with the T-jump in a few cases, and this was followed by an initial fast recovery. This component, however, did not contribute to a tension rise above the pre-T-jump level and could be an indirect consequence of thermal expansion. The bottom trace is a plot of the residuals after the curve fitting.

temperature. The tension record obtained from a nonmuscle elastic element (carbon fiber) was qualitatively similar (not illustrated). The T-jump amplitude was estimated by measurement of thermocouple output at 200–400 ms after the laser pulse. The resting tension trace (bottom) only shows transient oscillations, whereas the tension trace from the active fiber shows a characteristic rise in tension to a new steady level. Fig. 2 *b* is a tension transient from a maximally Ca-activated psoas fiber, with a double exponential function (see Materials and Methods) fitted to obtain numerical data to characterize the tension response. The two components are shown separately by the interrupted curves, and the residuals associated with the fitting are shown by the bottom trace.

Fig. 3 shows active tension transients to different T-jumps (see figure legend) from two slow (soleus) muscle fibers, where the presentation is basically similar to that in Fig. 2 *b*. The transients from fiber 1 (Fig. 3, *a* and *b*) could be fully described by two exponentials (as in Fig. 2 *b*). Those of fiber 2 (Fig. 3, *c* and *d*) show a drop in tension before tension rise, and a full transient required inclusion of an initial exponential component (not shown). The records show that the time course of tension rise is clearly longer and that phase 2 (τ_2) leads to a tension rise above the pre-T-jump level (dotted curves; see legend).

Fig. 4 *a* shows data from a fast fiber, where the steady tension recorded after a slow temperature change (circles) and the tension reached biexponentially after a T-jump (squares) are plotted against temperature. Fig. 4 *b* shows data from a similar experiment on a slow fiber, and Fig. 4 *c* shows pooled, normalized data from three slow fibers (see legend for details). The data show a number of points. First, the data are basically similar between fast and slow fibers. Second, data from T-jumps (squares) and those from slow temperature changes are within the same range, indicating that, within experimental error, the biexponential tension rise after a T-jump fully accounts for the temperature dependence of steady active tension. Third, to a first approximation, the relationship between active tension and temperature is sigmoidal, with a 50% tension at 15–17°C. Compared to the tension at ~5°C, the tension at ~20°C is threefold higher and the tension at physiological temperatures (>30°C) is four- to fivefold larger.

Fig. 5 *a* shows, from one fast muscle fiber experiment, the reciprocal time constants for phase 2 ($1/\tau_2$, circles) and for phase 3 ($1/\tau_3$, squares) plotted against the post-T-jump

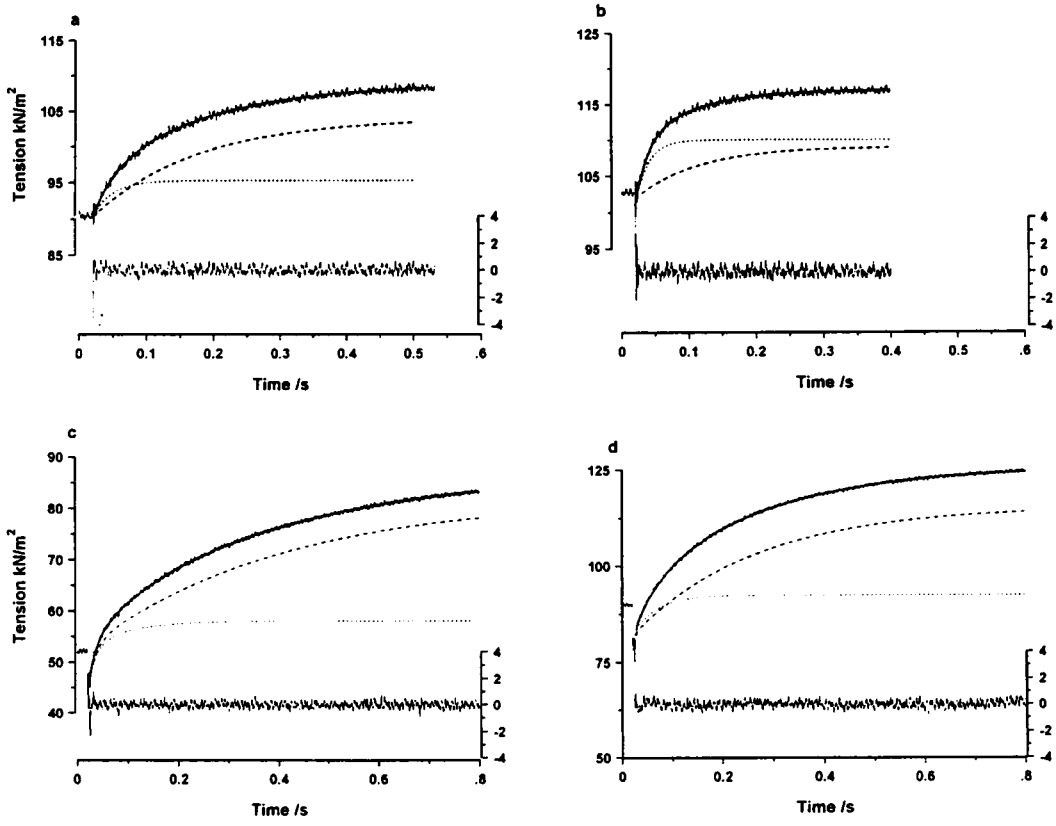

FIGURE 3 A selected number of tension transients to T-jumps from two maximally Ca-activated soleus fibers. (*a* and *b*) Fiber 1; (*c* and *d*) fiber 2. The bottom trace in each frame is a plot of the residuals after the curve fitting. In fiber 1, the T-jumps were 14.3 to 20.5°C (*a*) and 21.8 to 27.5°C (*b*), and two exponentials were adequate for the full tension transient, as in Fig. 2 *b*. Fiber 2 is an example where the tension rise began from below the pre-T-jump tension, presumably because of thermal expansion. The T-jumps were from 4.5°C to 10.5°C (*c*) and 10.7°C to 16.9°C (*d*). The curve fitted to the tension trace is a triexponential function, the dotted curve represents the initial plus phase 2 (τ_2), and the dashed curve represents the initial plus phase 3 (τ_3). Note that tension rises above the pre-T-jump level during phase 2 in all cases, and the tension rise is slower than in psoas (Fig. 2 *b*).

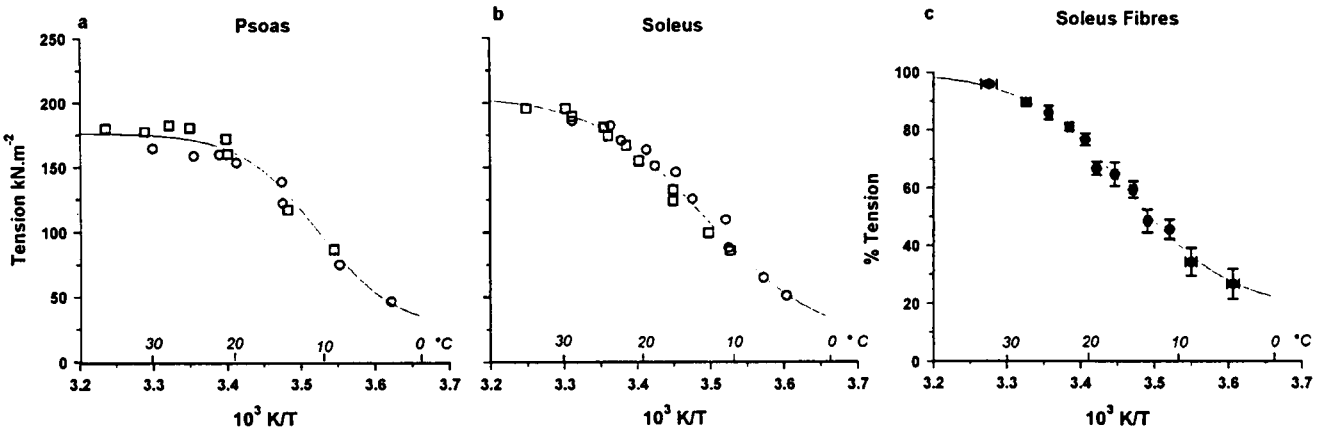

FIGURE 4 Temperature dependence of maximally Ca-activated steady tension; curves are fitted by eye. (*a*) Psoas fiber. ○, pre-T-jump tension; □, post-T-jump tension. The pre-T-jump temperature was changed by the Peltier system. (*b*) Soleus fiber; presentation similar to that in *a*. (*c*) Data similar to those in *b*, but pooled from three soleus fibers. For each fiber, the tensions recorded at other temperatures were represented as percentages of that at >30°C before the data were pooled. Each symbol is the mean (n = 3–5), with bars representing ±SEM.

temperature. The phase 2 is temperature sensitive, with a Q_{10} of 2.69, whereas phase 3 is less temperature sensitive (Q_{10} of 1.38). Similar sets of data from a slow muscle fiber experiment are shown in Fig. 5 *b*; both component rates are temperature sensitive, with Q_{10} of 1.67 (and 1.53). The data show that the fast fiber rates for both phases are severalfold larger than in slow fibers.

Fig. 6 *a* (fast fibers) and Fig. 6 *b* (slow fibers) show the pooled data from all of the experiments for the reciprocal time constants of phase 2 ($1/\tau_2$, open circles) and phase 3 ($1/\tau_3$, filled circles). The calculated regression lines correspond to Q_{10} of 2.75 and 1.66 for $1/\tau_2$ in fast and slow fibers; the Q_{10} for the $1/\tau_3$ were 1.15 and 1.83. The regression for fast muscle $1/\tau_3$ data, however, was not significant, presumably because of the low temperature sensitivity and/or pooling of data from different preparations. For ~12°C (range 9–15°C), the mean (±SEM) values for $1/\tau_2$ and $1/\tau_3$ were 68.9 (± 8.4, n = 6)·s^{-1} and 13.9 (± 1.0)·s^{-1} in fast fibers and 19.1 (± 2.48, n = 7)·s^{-1} and 3.3 (± 0.42)·s^{-1} in slow fibers.

Temperature dependence of the amplitudes of phase 2 (a_2, open circles) and phase 3 (a_3, filled circles) is illustrated in Fig. 7, *a* (fast) and *b* (slow) fibers. Plotted on a logarithmic ordinate is $(a/\delta T) \times (100/P_h)$, where δT is the T-jump amplitude. It is seen that the normalized amplitude for phase 3 decreases with temperature in both fibers (Q_{10} of 0.43 and 0.52 for fast and slow fibers, respectively); there is little (Q_{10} of 0.73, fast) or no (slow) decrease in the normalized amplitude for phase 3. Consequently, the ratio a_2/a_3 increased with temperature in both fiber types (not illustrated). The results in Fig. 7 indicate that the increase in steady tension with temperature is paralleled by a change in a_2, so

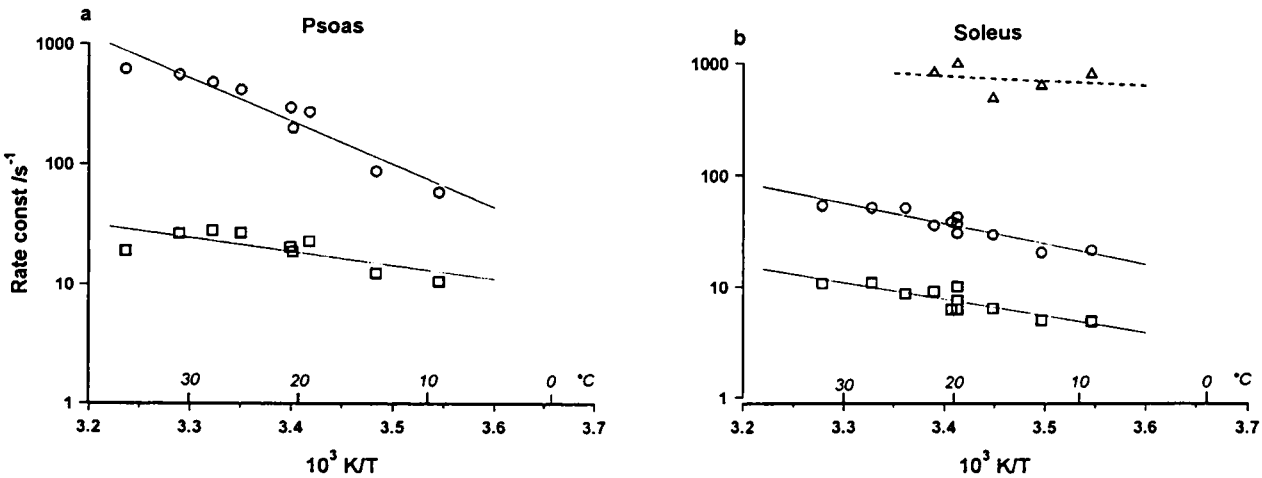

FIGURE 5 The reciprocal time constants, obtained by curve fitting as in Figs. 2 and 3, for τ_2 (○) and for τ_3 (□) of the T-jump-induced tension rise. (*a*) Psoas fiber. The calculated regressions (lines through the points) correspond to a Q_{10} of 2.69 for τ_2 and 1.38 for τ_3. (*b*) Soleus fiber; presentation similar to that in *a*. The Q_{10} are 1.67 for τ_2 and 1.53 for τ_3. Some of the transients contained an initial component due to thermal expansion (see Fig. 3). The data for the initial component are shown by the triangles. There was no correlation with temperature.

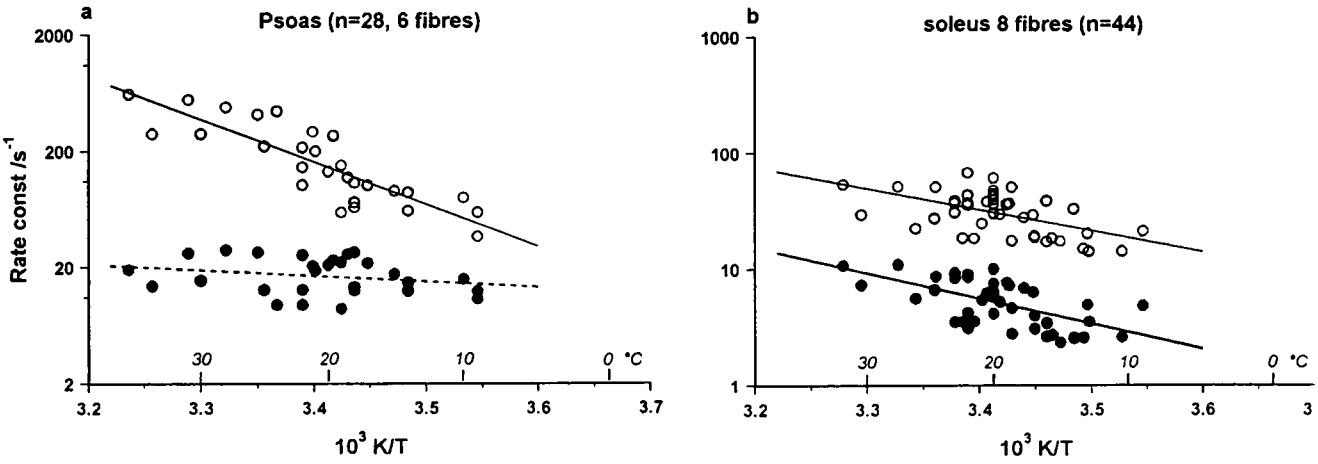

FIGURE 6 Pooled data for reciprocal time constants of τ_2 (○) and τ_3 (●). (*a*) Data from six psoas fibers (n = 28). The regression lines correspond to a Q_{10} of 2.75 for τ_2 and 1.15 for τ_3. The dotted line through τ_3 data, however, is not significant ($p > 0.05$). (*b*) Data from eight soleus fibers (n = 44). Q_{10} is 1.66 for τ_2 and 1.83 for τ_3.

that the a_2/P_h ratio remains approximately constant in either fiber type.

DISCUSSION

Comparison with previous studies: general

The temperature dependence of maximum active tension is basically similar between the two fiber types (Fig. 4). The results indicate that the change in maximum active tension with temperature is nonlinear. Although the present data were mostly collected with activations at lower temperatures (<15°C; see Materials and Methods), the possibility that the nonlinearity may be due to the development of sarcomere nonuniformity/disorder at higher temperatures (as suggested by Bershitsky and Tsaturyan, 1992) cannot be completely ruled out. It is noteworthy, though, that the nonlinearity of the temperature dependence of maximum active force has been observed not only in skinned fibers using different experimental protocols (see Stephenson and Williams, 1985; Goldman et al., 1987: Ranatunga, 1994), but also in previous studies on intact mammalian muscle (Hadju, 1951; Ranatunga and Wylie, 1983, and references therein). The simplest interpretation of the appoximate sigmoidal relation (Fig. 4) may be that there is a temperature-dependent change in the cross-bridge (myosin head) structure/conformation. Because rigor tension decreases linearly (Ranatunga, 1994) over the full temperature range, the heating-induced conversion in the structure/conformation may occur only in nucleotide-bound cross-bridges. In principle, such a change can account for the increase in active tension without a concomitant increase in stiffness, implying that each cross-bridge develops more tension at higher temperatures (Goldman et al., 1987).

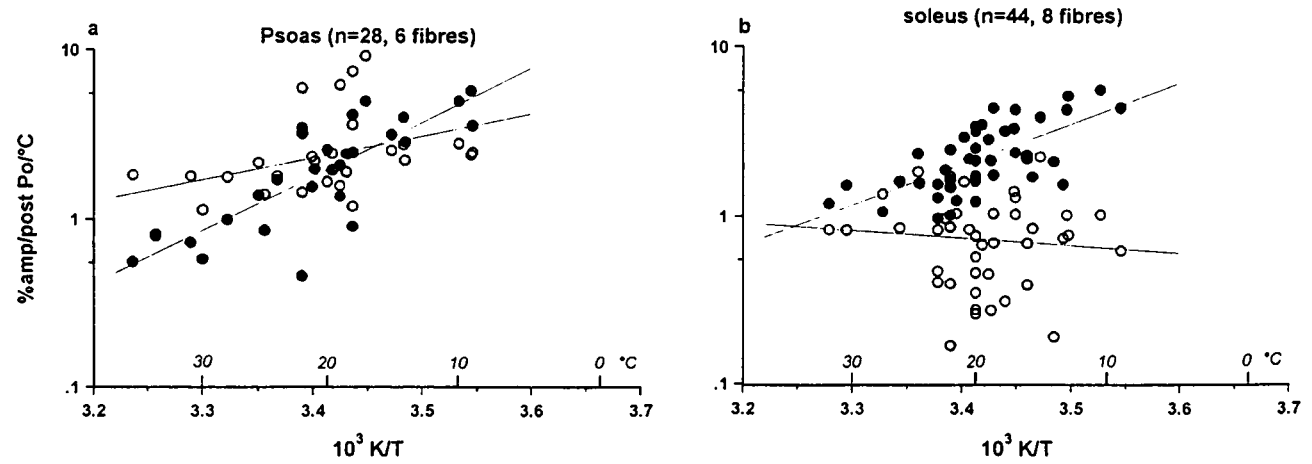

FIGURE 7 Temperature dependence of the amplitudes of τ_2 (a_2, ○) and τ_3 (a_3, ●). Each tension amplitude was normalized to the T-jump amplitude (i.e., per °C T-jump) and represented as a percentage of post-T-jump tension (i.e., $(a/\delta T) \times (100/P_h)$ is on the ordinate). (*a*) Pooled data from the psoas fibers. Both amplitudes decreased with temperature, but the decrease was less pronounced in a_2. Q_{10} for a_2 was 0.73, and for a_3 it was 0.46. (*b*) Pooled data from the soleus fibers. Q_{10} for a_3 was 0.52, but a_2 shows no significant change.

The studies of Davis and colleagues (see Davis and Rodgers, 1995a,b, and references therein) have clarified several uncertainties associated with T-jump experiments in general and the discrepancies among different T-jump studies. Thus, in our previous study (Goldman et al., 1987), phases 2 and 3 of the tension rise were not clearly and separately identified, and in studies of Bershitsky and Tsaturyan (1992), the T-jump amplitudes were probably too large to resolve individual steps in the perturbed cycle. Consequently, detailed comparison of the present data with those particular studies would be difficult and perhaps would not be appropriate. Nevertheless, the tension transients initiated by a T-jump in fast fibers were qualitatively similar in all of the studies.

The tension rise above the pre-T-jump level was biexponential, and our data from fast fibers are basically similar to those of Davis and Harrington (1993). The faster (τ_2 or phase 2) of the two components was temperature sensitive in both fibers. If this phase (τ_2) represents endothermic force generation in attached cross-bridges, then our results show that, at ~12°C, it is 3–4 times slower in the slow fibers. Our results also show that phase 3 (τ_3) in slow fibers is more temperature sensitive than in fast fibers. If, as suggested by Davis and Harrington (1993), τ_3 represents the rate-limiting step in the cross-bridge cycle (see, however, Zhao and Kawai, 1994), then the results indicate that it may be quite different in the two fiber types. Interestingly, Millar and Holmsher (1992) suggested the possibility of such a difference between fast and slow fibers from their experiments, where the exponential tension decline (k_{Pi}) was examined after rapid photogeneration of phosphate in muscle fibers. The "phosphate release step(s)" (k_{Pi}) in slow fibers, but not in fast fibers, was sufficiently slow to partially limit the ATPase turnover rate.

Endothermic force generation and mechanical power stroke

From a general examination of the time course, both Goldman et al. (1987) and Bershitsky and Tsaturyan (1992) compared endothermic force generation with the mechanical power stroke (T_1-T_2 transition or quick tension recovery; Ford et al., 1977) obtained with length perturbation. From a more detailed analytical study, Davis and Harrington (1993) showed that the endothermic force generation corresponds to a "slower" component of the mechanical power stroke, the initial faster component being attributed to a purely mechano-elastic recovery. In principle, the latter idea would be consistent with the findings that ~50% of the compliance during quick tension recovery may reside outside the cross-bridges (Irving, 1995). Davis and Harrington (1993) suggested that τ_2 represents the basic cross-bridge force generation step and that it is entropy driven. Zhao and Kawai (1994) came to a similar conclusion from studies using a sinusoidal analysis technique, although they identified entropic cross-bridge force generation to be subsequent to the quick tension recovery (i.e., as T_3) observed in length release experiments. On the other hand, there is accumulating and definitive experimental evidence of movement and tilting of the head region of the myosin cross-bridges that can account for force generation during the quick tension recovery (Irving et al., 1992, 1995). Thus, the implication seems to be that the axial movement of the myosin head region that characterizes the mechanical power stroke may involve an "order-disorder" change (Davis and Harrington, 1993).

The fast-slow fiber difference in the endothermic force generation, reported in the present study, suggests that the time course of T_1-T_2 transition would be different between fast and slow mammalian fibers. Recent findings of Galler et al. (1996) on rat muscle fibers clearly show fiber type differences in quick tension recovery. It is relevent to note, however, that their data show a much larger fast (type IIB)-slow (Type I) fiber difference for quick tension recovery (~30-fold) than that obtained here for endothermic force generation (three- to fourfold). Additionally, whereas the time course of the T-jump force generation has been found to be Ca^{2+} insensitive (Bershitsky and Tsaturyan, 1989b), the mechanical power stroke has been shown to be affected by the level of Ca^{2+} activation (Martyn and Chase, 1995).

Endothermic force generation and pressure-release force transients

From hydrostatic pressure-release experiments on muscle fibers, we previously identified a tension transient as cross-bridge force generation and suggested that cross-bridge force generation may involve an increase in volume (Fortune et al., 1991). Like the endothermic force generation, the time course of the pressure-release tension transient is temperature sensitive (Q_{10} of 2–3; Fortune et al., 1989b) and Ca^{2+} insensitive (Fortune et al., 1994), and it is considerably slower in slow fibers (Fortune et al., unpublished observations). Molecular steps that involve an increase in volume may be associated with an increase in entropy. Thus, the important question that arises is whether cross-bridge force generation is an event of that type (as indeed suggested by Zhao and Kawai, 1994). From our more recent findings (Fortune et al., 1994), the reciprocal time constant for the pressure release force generation was ~30·s^{-1} for psoas fibers at 12°C. This is slower than that for endothermic force generation (τ_2) obtained in T-jump experiments (~70·s^{-1}, present study). More importantly, pressure-release force generation is sensitive (Fortune et al., 1991), whereas endothermic force generation (τ_2) is rather insensitive to added inorganic phosphate (Davis and Rodgers, 1995b). Indeed, Davis and Rodgers (1995b) showed the occurrence of an additional component ($\tau_{negative}$) in the T-jump tension transient in the presence of added phosphate, which they correlate with pressure release force generation (and phosphate release events). Thus, the findings

indicate that the primary force generation induced by T-jump and by pressure release may represent different steps in the cross-bridge cycle, and their rates are different between fast and slow fibers.

Endothermic force generation and the ATPase pathway

Davis and Rodgers (1995b) provided some evidence that the endothermic step represents a nonchemical, de novo entropic force generation in attached cross-bridges and that it occurs after the release of inorganic phosphate in the ATPase cycle, i.e., a transition between two actomyosin·ADP states. Present results show a three- to fivefold difference, at ~12°C, between fast and slow fibers in the time course of endothermic force generation, which is similar to the difference seen between them in many other contraction parameters (see Ranatunga, 1982, 1984). In a general sense, this could mean that the coupling between the ATPase pathway and endothermic force generation may not be indirect. According to Zhao and Kawai (1994), the step that underlies (endothermic) force generation is a transition between two attached cross-bridge states before the release of phosphate; this is indeed the suggestion made from pressure release experiments (Fortune et al., 1991) and from caged phosphate-release experiments (Dantzig et al., 1992)). As mentioned above, however, there is uncertainty as to whether force generation after T-jump and pressure release are comparable. The findings of Miller and Holmsher (1992) show that the exponential tension decline after photoliberation of phosphate (k_{Pi}, or "phosphate release steps") in slow fibers at 10–20°C is >30 times slower than in fast fibers; indeed, the fast-slow fiber difference in time course of the pressure release force generation is also considerably larger (>20-fold at ~12°C: Fortune et al., unpublished observations). Therefore, the three- to fivefold difference obtained between fast and slow fibers in the present study appears to be too small to suggest a more direct coupling between T-jump force generation (τ_2) and phosphate release.

CONCLUSION

It should be clear from the above that the differences in the T-jump-induced tension transients reported here for fast and slow muscle fibers can be broadly accommodated within the scheme proposed by Davis and Rodgers (1995b). Thus, a T-jump initiates an entropic force generation (τ_2) in active muscle fibers that appears not to be directly coupled to the "phosphate release steps" in the ATPase pathway. Nevertheless, whether there is another force-generating step in the cross-bridge cycle, a step that is closely coupled to phosphate release (Dantzig et al., 1992), is endothermic and entropy driven (Zhao and Kawai, 1994), and additionally, involves an increase of volume (Fortune et al., 1991) may be worthy of further consideration. The correlation between T-jump tension transient and the mechanical power stroke also remains to be fully elucidated.

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