HEAT CHANGES DURING TRANSIENT TENSION RESPONSES TO SMALL RELEASES IN ACTIVE FROG MUSCLE

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ABSTRACT Tension and heat production were measured in frog sartorius muscles in response to small shortening ramps (releases) at high and moderate speed. Transient tension responses to fast releases (0.1 to 0.4 mm in 1 or 4 ms) were similar to the tension transients length-clamped single fibers. Tension time courses during releases at 25 mm/s were like fiber responses calculated from the first two phases of the step responses (Ford et al., 1977). We conclude that similar crossbridge transitions produce tension transients observed in whole muscles and single fibers. Heat was absorbed during rapid tension recovery after fast releases and during the later part of releases at 25 mm/s. Variation of heat absorption with release size was compared with that of crossbridge movement predicted by the Huxley-Simmons hypothesis of force generation (Huxley and Simmons, 1971). Agreement between the two supports the conclusion that heat is absorbed by the crossbridge transitions responsible for rapid tension recovery after release. The results indicate that the entropy change of these transitions is positive.

INTRODUCTION

Energy transduction in active muscles occurs by the cyclic interaction of myosin crossbridges with actin filaments (see reviews by Huxley, 1980; Goldman and Brenner, 1987; Goldman, 1987). Fruitful experimental approaches to elucidating the mechanism have been to measure transient tension responses to step changes in length (e.g., Ford et al., 1977), temperature (e.g., Goldman et al., 1987), or concentrations of ligands that bind to the contractile proteins (e.g., Goldman et al., 1984; Lacktis and Homsher, 1987; Dantzig et al., 1987). The response to the step is produced by transitions that are momentarily favored because of their sensitivity to changes in the stepped parameter. Huxley and Simmons (1971, 1973) attributed early phases of the tension transients observed after shortening steps to transitions involving attached crossbridges. Subsequent studies have supported this interpretation (Ford et al., 1977, 1981, 1985, 1986), and insight into the nature of the underlying processes can be gained by simultaneous measurements of other parameters.

We report here measurements of heat changes during tension transients produced by sudden releases. The finding that early rapid tension recovery absorbs heat provides thermodynamic information about associated crossbridge transitions. Some of the experiments were reported previously (Gilbert and Ford, 1986, *a* and *b*).

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METHODS

Experimental Procedure

Most procedures and apparatus were described previously (Gilbert and Ford, 1986*c*; Ford and Gilbert, 1987). Pairs of sartorius muscles from *Rana temporaria* were mounted one on each side of a thermopile and the pelvis held in a clamp attached either to the thermopile frame or to a photoelectric force transducer (Gilbert and Ford, 1986*c*) fixed to the frame. Muscle temperature was maintained at 0°C by suspending the chamber containing the thermopile in an ice and water bath. During the experiment and for at least an hour beforehand the muscles were bathed in oxygenated Ringer solution (composition, mM, NaCl 113.5, KCl 2.0, $CaCl_2$ 1.8, phosphate buffer 2.0, and pH at room temperature 7.0). The muscles were stimulated for 2–2.5 s every 5 min. Contractions were isometric either throughout stimulation or for 1.2 s, when a length change was applied (Fig. 1). A randomly assigned sequence of the different types of contractions was repeated in forward and reverse order and records from like contractions averaged at the end of the experiment.

Apparatus

Two thermopiles were used for heat measurements. H1M, insulated with a layer of kapton and a layer of mylar, was described by Ford and Gilbert (1987). H2 was identical to it except that it was insulated with a single layer of mylar. At the beginning of the experiment, temperature was recorded separately from each of three recording sections of the thermopile to detect misalignments of the muscles and nonuniform draining of solution. These produced anomalous responses to length changes which varied from one recording section to another and could be eliminated in most cases by reorienting the preparation or attaching a small wick to the pelvic clamp to facilitate uniform draining. Heat measurements from preparations that did not give similar heat responses from all three sections were discarded.

The thermopile signal was amplified by a 15C-3a chopper-stabilized amplifier (Ancom, Royston, England) (gain $1.2 \cdot 10^5$, chopper frequency

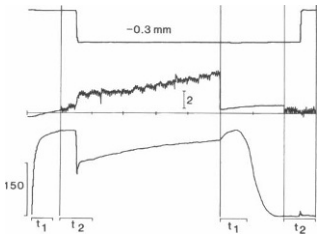

FIGURE 1 Record of length change, heat production, and tension during a contraction with a rapid release. Each channel of the record contains 1,000 points. Sampling frequency and heat amplifier gain were changed at times indicated by vertical lines. Electrical artifacts from the stimulus apparent ~50 and 160 ms into the second time section were also present in isometric records and were removed when isometric heat was subtracted (see text). Heat calibration, 2 mJ/g, is only for sampling at high gain. Other calibrations: tension 150 kN/m^2, time t_1 1 s, t_2 40 ms.

400 Hz). Its frequency response was 3 dB down at 100 Hz. Its step response was exponential with a time constant of 2 ms and was not affected by the relation of the applied step to the chopper period, step size over a range from 0.2 to 50 μV nor the DC voltage level on which the input step was superimposed. Amplifier delay reduced the theoretical step responses of the two thermopiles at 5 ms (see Gilbert and Mathias, 1988) by <20% of the input. The output of the Ancom was passed to a second-stage conventional amplifier (combined gain 10^6) and to a pulse-activated switch that selected between the first- and second-stage outputs recorded by the computer (see Fig. 1).

Length changes were imposed by a servo-controled motor (0.4 mm in 1 ms, overshoot <10%). Tension was measured by a strain gauge attached to the tibial tendons and in some experiments by a second photoelectric fiber-optic transducer attached to the pelvis. The latter was used to detect friction between the muscles and the thermopile, which produces a tension difference between the two ends of the preparation in response to a length change (Gilbert and Ford, 1986*c*). Friction was associated with the kapton insulation used on some thermopiles (compare left and center panels of Fig. 2) and was eliminated when the kapton was replaced with mylar (Fig. 2, right panel). The tension difference with mylar-insulated thermopiles was <10% of the drop in tension observed during a 1-ms release and the calculated heat dissipation <0.02 mJ/g. No heat dissipation attributable to friction was observed in response to 0.2-mm stretches in 1 ms, which produced the expected thermoelastic heat absorption (Fig. 3). The resonance of the pelvic transducer was excited by 1-ms length changes, and slower length changes were therefore used in experiments with that transducer.

The compliance of the apparatus was 114 μm/N and linear from 0 to 3

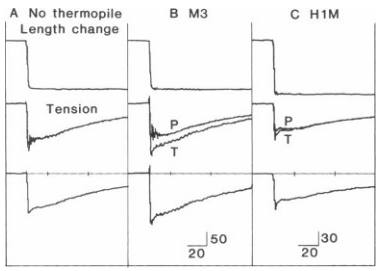

FIGURE 2 Tension responses to 1-ms releases recorded from tibial and pelvic transducers. (*A*) A pair of muscles on a thermopile frame without thermopile, nerve stimulation. (*B*) Same preparation on thermopile M3, insulated with one layer of kapton, direct muscle stimulation. (*C*) Different preparation on thermopile H1M, insulated with layer of mylar over a layer of kapton, muscle stimulation. (*Top*) Length change, 0.3-mm release. (*Center*) Tension measured by tibial (*T*) and pelvic (*P*) transducers. (*Bottom*) Tibial tension alone. Lowermost horizontal line shows tension baseline for bottom records. Tensions normalized to isometric level just before release, 334 kN/m^2 for *A* and *B*, 187 kN/m^2 for *C*.

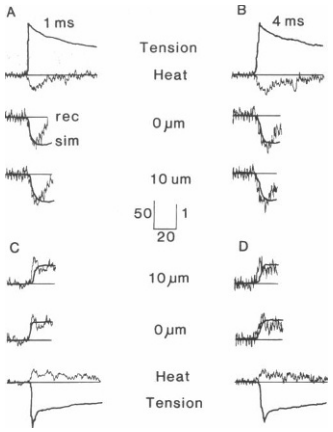

FIGURE 3 Comparison of muscle records with simulated thermopile responses to thermoelastic heat produced by 0.2-mm stretches and releases. Thermopile H2, single layer of mylar insulation. First 60 ms of tension and heat responses are shown by records at top and bottom. First 20 ms of heat responses at twice the gain are shown in the center (rec), superimposed on thermopile responses simulated as described in text for solution layers 0 and 10-μm thick (sim). *A*, *B* – 1- and 4-ms stretches; *C*, *D* – 1- and 4-ms releases. Calibrations: tension 50 kN/m^2, time 20 ms, heat 1 mJ/g (*outer traces* 2 mJ/g).

N. Compliance of the preparation and apparatus was estimated from the slope of plots of applied length change against tension during quick stretches and releases (0.1–0.2 mm, complete in 1 ms). It was ~280 μm/N and linear within ±30% of isometric tension (average 1.36 N). The net compliance of the preparation in that tension range was therefore 166 μm/N or 1% muscle length (average 28 mm at 2.2 μm/sarcomere) for 1.36 N of isometric tension.

Data Acquisition and Analysis

Analog signals of length, change, tension, and heat production were digitized by a Nova 4/S minicomputer (Data General Corp., Westboro, MA) and stored on disk. As shown in Fig. 1, signals were recorded in four time sections at different sampling frequencies. In subsequent figures only the second time section is shown (1.18 s after stimulation, data acquisition at 2.5 kHz per channel). Heat changes associated with the tension transients were magnified during rapid sampling by a computer-operated switch that selected between the first- and second-stage amplifiers of the thermopile signal.

Records from like contractions were averaged and isometric heat subtracted from records with length changes. Noise from the Ancom chopper was removed by a smoothing routine in which points $i-1$, i, and $i+1$ were averaged for the ith point and the procedure repeated several times. Its effect is seen by comparing records before and after smoothing (Figs. 3 and 4). The records were then corrected for heat diffusion delay

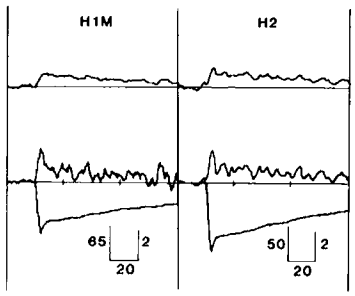

FIGURE 4 Heat records from thermopiles with different speeds due to differences in insulation (*top panel*). Release 0.3 mm in 4 ms, different preparations, thermopiles H1M (*left*) and H2 (*right*). (*Bottom*) tension; (*center*) results obtained from correction of records in top panel for heat-diffusion delay (see text). Isometric tension 178 (H1M) and 230 (H2) kN/m^2. Calibrations heat 2 mJ/g, tension 65 (H1M) or 50 (H2) kN/m^2, time 20 ms.

as described below. Results were independent of whether smoothing or the correction was carried out first.

Heat Diffusion Delay

As demonstrated in the preceding paper (Gilbert and Mathias, 1988), delay of the thermopile signal with respect to muscle heat production is described mathematically by a transfer function that depends on the thicknesses of thermopile components and the thermal properties of the thermal junctions, the muscles and intervening layers. That analysis was used to estimate the thickness of a layer of solution adhering to the muscle and to correct records for delay.

Solution-layer thickness was estimated as described by Ford and Gilbert (1987), except that the transfer function used here was that derived by Gilbert and Mathias (1988). Families of simulated responses were computed by convolving known heat inputs from the muscle with transfer functions having different thicknesses of solution layer. These were compared with thermopile records obtained when the muscle produced those heat inputs, to determine the solution-layer thickness producing the best match. The known heat inputs used were the initial parts of thermoelastic responses that occur during quick stretches and releases (0.1 and 0.2 mm in 1 and/or 4 ms), calculated by multiplying the concurrent tension change by the thermoelastic coefficient measured in rigor muscles (Gilbert and Ford, 1986*c*; Goldman et al., 1987).[1]

Fig. 3 illustrates two examples of matching for each of four responses. Tension and heat changes are shown in the top and bottom panels. The first 20 ms of the heat changes shown in the center panels (at twice the amplification) are superimposed on responses simulated for solution layers 0- and 10-μm thick. The 10-μm simulations lag the records, indicating that the solution layer thickness was nearer to 0 than to 10 μm.

The effects of delay on responses of the two thermopiles are compared in the top panel of Fig. 4. The record on the left is from H1M insulated with two layers of polymer film and the faster record on the right from H2 with one layer. The slower thermopile smoothed the sudden changes apparent in the record from the fast one. For example, the fast record on the left shows a transient peak followed by precipitate cooling, which in the slow record appears as a broader maximum followed by a gradual decline.

The records were corrected for delay by deconvolution of the muscle response with the appropriate transfer function. The corrected records from the two thermopiles (center of Fig. 4) are similar in size and time course and show about the same speed and amount of rapid cooling during early tension recovery. Records from one experiment were corrected with transfer functions computed for solution layer thicknesses of 0, 5, and 10 μm. The principal effect of increasing solution layer thickness was to increase the apparent magnitudes of heat changes associated with the fall in tension and subsequent rapid tension recovery.

RESULTS

Tension Responses to Small Releases

Tension records in Figs. 5 and 6 exhibit several features of the tension transients described in single-muscle fibers (Huxley and Simmons, 1971, 1973). Four phases are apparent: tension drop during release (phase 1), rapid recovery (phase 2) to a momentary tension plateau (phase 3), and slow recovery to the initial isometric level (phase 4). The rate of rapid recovery increased with release size, and the plateau tension level was independent of release speed (horizontal lines of Fig. 5). Records in Fig. 5 are from muscles attached to a frame with no thermopile, those in Fig. 6 from a different preparation mounted on a thermopile. The responses are similar, indicating that the thermopile did not affect the transients.

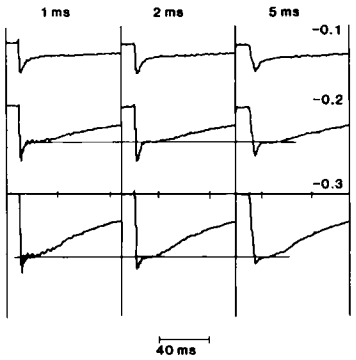

FIGURE 5 Tension responses to small releases, preparation mounted on a frame without a thermopile (nerve stimulation). Records of tibial tension; pelvic tension not shown. Durations of releases are indicated above each panel and sizes (millimeter) at the right. Plateau tension levels indicated by horizontal lines were 0.8 and 0.67 times isometric tension. Oscillations in the tension trace at lower left were caused by pelvic transducer whose resonance was excited by the length change.

Fig. 7 shows tension responses to 4-ms (*A*, *C*) and isovelocity ramps at 25 mm/s (*B*, *D*). Upward inflections appear just before the end of the largest two 4-ms releases, which are otherwise similar to the 1-ms responses in Fig. 6, and in all the responses during slower isovelocity ramps (*B* and *D*). As in Fig. 5, the plateau tension level is approximately the same after a given size of release, regardless of its speed.

Superimposed records of tension and length in Fig. 7 *C* and *D* show that the tension inflections occur at points of

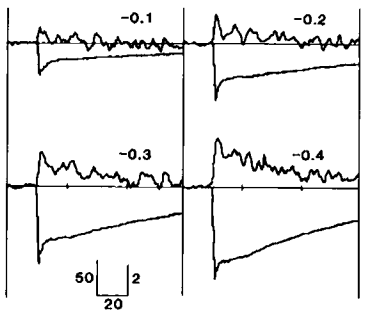

FIGURE 6 Tension and heat changes produced in response to 1-ms releases. Heat records were smoothed and corrected for diffusion delay as described in *Methods*. Calibrations tension 50 kN/m^2, heat 2 mJ/g, time 20 ms.

[1]It is assumed that no other thermally significant processes occur during the first 1–2 ms of a quick stretch or release. The assumption is supported by the observation, illustrated by records in Fig. 3, that these heat changes scale directly with the corresponding tension changes, independently of their speed, size, and direction, with a factor equal to the thermoelastic coefficient measured in rigor muscles.

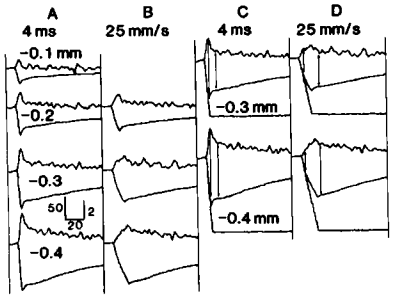

FIGURE 7 Tension and heat in response to several sizes and speeds of release. Records from three separate experiments were averaged after heat records were corrected for diffusion delay. (*A*) four releases complete in 4 ms; (*B*) three releases at 25 mm/s. (*C, D*) tension, heat and length change for two largest releases. Length record was scaled as described in the text and is approximately equal to the stiffness of the preparation and apparatus. Arrows show interval over which heat changes were measured (see text and Fig. 8). Calibrations are the same as in Fig. 6.

deviation of the two traces. The length records were scaled by a factor equal to the stiffness of the mechanical apparatus and preparation, estimated from displacement and tension measured during small quick stretches, and are therefore approximately equivalent to the tension change due to the instantaneous elasticity of the muscles and apparatus. Tension levels at the point of deviation were lower at higher velocities (compare 25 mm/s in *D*, 75 in *upper C*, 100 in *lower C*).

Several features of these records are similar to the ramp responses of single fibers observed by Armstrong and Huxley (cited by Ford et al., 1977; see their Fig. 29, p. 490) and to ramp responses calculated from the first two phases of fibers' step responses (see Fig. 30 of Ford et al., 1977). The ensemble of similarities suggests that similar crossbridge transitions produce the early tension responses to step and ramp releases in fibers and that they also occur in whole muscles. Heat changes associated with these transitions should therefore appear during rapid tension recovery after rapid releases and subsequent to the tension inflections during isovelocity ramps.

Heat Changes in Response to Release

Any heat changes associated with the crossbridge transitions responsible for the tension treansients would be superimposed on thermoelastic heat changes resulting from the normal elastic character of the muscle and produced by the tension changes as such (Hill, 1953; see Aubert, 1956, 266–267). The myofilament lattice of active (and rigor) muscle has a positive coefficient of thermal expansion (α) and so expands when heated. Conversely, when tension rises or falls, heat is absorbed or liberated in an amount proportional to the tension change, the proportionality constant being the thermoelastic coefficient ($-\alpha T$). Heat changes from crossbridge biochemical transitions must therefore be detected as deviations from purely thermoelastic behavior. For purposes of the quantitative comparisons described below, the thermoelastic properties of active muscle are assumed to be the same as those of rigor muscle (see Gilbert and Ford, 1986*c*; Goldman et al., 1987), and the thermoelastic coefficient is assumed to be unaffected by the crossbridge transitions that occur in response to release. In other words, we assume that the coefficient of thermal expansion of the myofilament lattice of active muscle is the same as that of rigor muscle and is unaffected by the biochemical transitions responsible for active mechanical responses.

Release and Rapid Tension Recovery

Heat records in Fig. 6 show that heat was produced during release and absorbed during rapid tension recovery, as expected from the thermoelastic effect. However, the fraction of heat reabsorbed was greater than the fraction of tension recovered. After the 0.1-mm release, for example, all the heat was reabsorbed, but tension recovery was not complete. Therefore a process that occurred during rapid tension recovery absorbed heat.

The extra cooling apparent during rapid recovery is not an artifact of the correction for heat diffusion delay, since it appeared in uncorrected records made with a fast thermopile (*lower panel* of Fig. 3, *upper right panel* of Fig. 4) but not in simulated responses of that thermopile to a thermoelastic input (sim of Fig. 3; see Gilbert and Mathias, 1988). The absence of observable cooling in records from the slow thermopile suggests that the immediately preceding transient peak was attenuated by delay. This was confirmed by simulations in which the magnitude of decay of a transient input peak was increased until a transient peak just appeared in the output of the fast thermopile (71% decay of input peak for 25% decay of output peak). No transient peak appeared in the simulated response of the slow thermopile to that input.

Fig. 7 shows heat changes recorded during 4-ms ramps (*A, C*) and isovelocity ramps at 25 mm/s (*B, D*). Heat changes recorded during the initial part of all the releases scale with the coincident tension changes, with a factor approximately equal to the inverse of the thermoelastic coefficient (i.e., -50; see Gilbert and Ford, 1986*c*). As in the records in Fig. 6, the extra heat produced during 4-ms releases was at least partly reabsorbed during rapid tension recovery. Inflections occurred at the same time in both heat and tension records shown in Fig. 7 *D*, indicating that processes responsible for the tension inflection caused heat rate to diminish.

Fig. 8 shows negative heat changes associated with rapid tension recovery after fast releases and with the last part of isovelocity ramps plotted against the size of release before (*A*) and after (*B*) subtraction of thermoelastic heat changes that occurred over the same intervals. The heat changes were measured over an interval that ended at the momentary tension plateau and began either at the end of

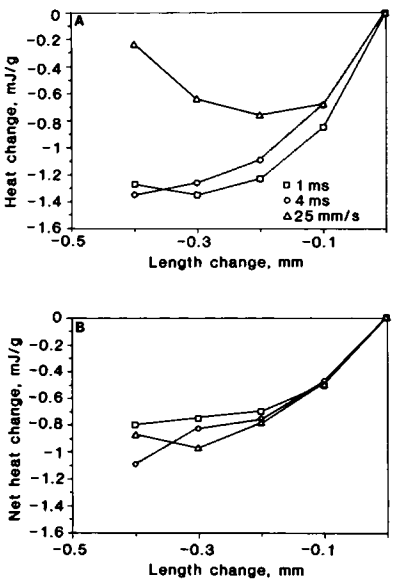

FIGURE 8 Relation between size of release and heat change during rapid tension recovery. (*A*) Heat absorption measured. Measurement intervals all ended at the end of rapid recovery and began at the end of the 1- and 4-ms releases (*squares and diamonds*) or at the tension inflection during releases at 25 mm/s (*triangles;* see Fig. 7 *D*). The graph obtained by averaging measurements of records from individual experiments was similar in all essential features to the one shown here. Variation between experiments ranged from 3 to 30% of the change measured and did not itself vary consistently with release speed. (*B*) Heat change during rapid recovery less thermoelastic heat change calculated by multiplying the tension change measured over the same interval by a thermoelastic coefficient of −0.018 (Gilbert and Ford, 1986*c*).

1- and 4-ms releases or at the tension inflection during isovelocity release (arrows in Fig. 7, *C* and *D*). During rapid recovery after 1- and 4-ms releases, the magnitude of the heat change increased with release size. The relation obtained with isovelocity releases was not monotonic.

Net heat changes shown in Fig. 8 *B* were obtained from the points in Fig. 8 *A* by subtracting thermoelastic heat, calculated by multiplying tension changes measured over the appropriate intervals by the thermoelastic coefficient. Variation of the net heat changes with release size is independent of whether they occurred during rapid tension recovery after 1- and 4-ms ramps (*squares, circles*) or subsequent to tension inflections seen during isovelocity ramps at 25 mm/s (*triangles*). The graph demonstrates that a heat-absorbing process occurred with all three types of release and that its extent increased with release size but was independent of release speed.

The thermoelastic coefficient used to obtain points plotted in Fig. 8 *B* was −0.018, the highest value measured myothermally from frog muscles in rigor (Gilbert and Ford, 1986*c*). The shape of the graph was not markedly sensitive to the value of the coefficient over a range from −0.013 (mean value, Gilbert and Ford, 1986*c*) to −0.021 (Goldman et al., 1987, thermal expansion of skinned rabbit fibers in rigor). The early heat changes recorded during small stretches shown in Fig. 3 and the theoretical speeds of the thermopiles used (Gilbert and Mathias, 1988) make it unlikely that the coefficient lies outside this range.

Later Stages of Tension Recovery

As shown in Fig. 9, net heat changes measured during the momentary tension plateau (*A*) and subsequent slow recovery (*B*) did not vary consistently with release size. Net changes after 4-ms and isovelocity ramps were near zero, indicating that heat rate was close to the isometric rate. (Isometric heat was subtracted during analysis.) After 1-ms releases, net changes were positive during the plateau and negative during slow recovery, when the heat rate attributable to crossbridge transitions was near zero. For example, the rate of the heat change during slow recovery after the 0.4-mm release was −9 mW/g (−0.6 mJ/g measured over 70 ms). The isometric rate under these conditions was ~20 mW/g. The net heat rate was therefore 11 mW/g, all of which is accounted for by reactions other than the actomyosin ATPase. The labile component of the isometric heat rate after 1.5–2 s of stimulation is ~6–10 mW/g (see Homsher, 1987), and most of it is probably associated with reactions other than the actomyosin ATPase (Curtin and Woledge, 1981). The tension-independent component of the stable maintenance heat rate is ~3 mW/g (Homsher et al., 1972). The total heat rate attributable to processes other than the actomyosin cycle is therefore 9–13 mW/g.

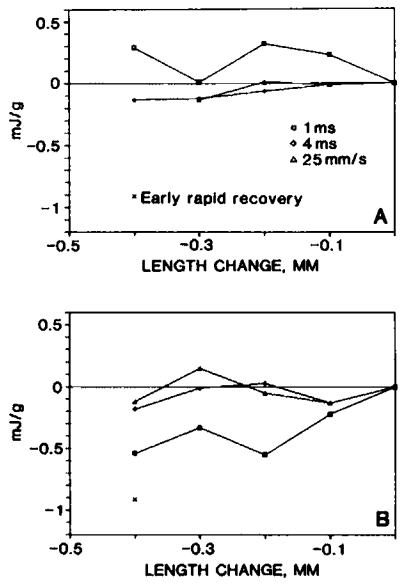

FIGURE 9 Relation between size of release and net heat change during later stages of tension recovery. Each point shows the heat change measured less the thermoelastic heat (coefficient −0.018). Releases in 1 (*squares*) or 4 ms (*diamonds*) or at 25 mm/s (*triangles*). Cross shows average net heat absorbed during rapid recovery after 0.4-mm releases at three speeds. (*A*) tension plateau after tension recovery. No measurement was made after the 0.4-mm release at 25 mm/s because the plateau interval was too short (see tension records in Fig. 7, *B* and *D*). (*B*) Heat changes during late slow recovery from end of tension plateau to end of record.

Interpretation of Heat Absorption During Rapid Recovery

Ford et al. (1977, 499–501) used a Voigt element to model early tension recovery observed in single fibers (see their Fig. 35, p. 500), which Huxley and Simmons (1971, 1973) attributed to movement of attached crossbridges. In the model, the instantaneous tension change during the step to T_1 is attributed to shortening of a linear elastic element V_1. Rapid tension recovery to T_2 occurs when V_1 is re-extended by shortening of a nonlinear viscoelastic element V_2, identified with delayed crossbridge movement. The amount of movement is a unique function of the T_2 tension measured at the end of rapid recovery and is given by the horizontal distance between the T_1 and T_2 length-tension curves.

We compared the amount of heat absorbed during rapid recovery after each size of release with the amount of V_2 shortening predicted by the viscoelastic model. The results are shown in Fig. 10. V_2 shortening (*solid line*) was determined from the V_2 curve (Ford et al., 1977; Fig. 35) for each T_2 tension measured in six preparations in response to releases of 0.05–0.4 mm complete in 1–5 ms. The scaling factor was adjusted to 1 mJ/g = 6.5 nm shortening, to give the best fit to the heat changes. Similarity between the points and the solid curve is consistent with the interpretation that heat is absorbed by the crossbridge transitions responsible for rapid tension recovery.

DISCUSSION

Huxley and Simmons (1971, 1973) proposed that muscle force is generated by discrete transitions or stepping of crossbridges from one attached state to another. Ford et al. (1977) were able to simulate many features of ramp responses observed in fibers with equations describing their step responses, which suggests that rapid tension recovery after quick release and diminution of the rate of tension drop during slow release both result from crossbridge stepping. In the experiments reported here, heat changes were measured in whole muscles under these two mechanical conditions, in which crossbridge stepping is expected to

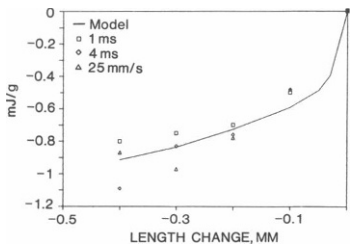

FIGURE 10 Relation between release size and net heat absorption (*symbols*) or crossbridge movement (*solid line*) represented as shortening of a nonlinear viscoelastic element (Ford et al., 1977; see text). The ordinate scale for the solid line was chosen so that it passed through the average heat absorbed during rapid tension recovery following 0.4-mm releases at three speeds. The scaling factor is 1 mJ/g = 6.5 nm of crossbridge movement.

be prominent. The muscles' tension responses were like those of fibers, indicating that similar processes occurred in response to release. Heat was absorbed during rapid tension recovery after quick releases and during the last part of slow ones. The amount of heat absorbed varied with the size of release in the same way as predicted for the stepping process, which suggests that it absorbs heat.

The quantitative similarity of heat absorption during the last part of slower isovelocity releases and rapid tension recovery after quick ones is somewhat surprising. Despite the fact that the plateau tension levels were similar, processes in addition to rapid stepping may have occurred during the longer intervals required for measuring responses to isovelocity release. The fact that the same amount of heat was absorbed does not rule out that possibility, but it does show that whatever additional processes occurred produced no tension and were thermally neutral.

During later stages of the tension responses, net heat changes (measured as departures from isometric heat) were only observed after 1-ms releases. They were positive during the momentary plateau and negative during slow tension recovery. Thus the isometric steady state was restored after rapid release by a heat-absorbing process that developed force rapidly, followed by a heat-producing transition associated with the momentary plateau and a final slow force recovery by processes that produced little or no heat. The heat changes provide information about the thermodynamic characteristics of transitions within the crossbridge cycle that prevailed during these stages of steady-state restoration.

The entropy change of the heat-absorbing process during rapid recovery must be positive. While heat can be produced by any spontaneous process whose free energy is all dissipated as heat and/or whose entropy change is negative, heat can only be absorbed by a spontaneous process whose entropy change is positive and larger than the absolute value of the free energy dissipated as heat. Rapid tension recovery was spontaneous and produced work in the form of mechanical energy stored in elastic structures. Its free energy change was therefore negative, and it may correspond to the crossbridge power stroke (Goldman, 1987). Its enthalpy change is probably small, with a sign determined by the relative sizes of the entropy and free energy terms.

Energy transduction by the actomyosin ATPase is a cyclic process, so that an increase in the entropy of the contractile-protein system in one part of the cycle requires a decrease in entropy in another part. The positive heat change that occurred during the momentary tension plateau may therefore be produced by transitions in which the positive entropy change of the power stroke is reversed. The low heat rate during slow tension recovery may reflect a further shift in crossbridge distribution so that the heat-absorbing power stroke was again favored. By that time, however, the momentary synchronization of cross-

bridges in the heat-absorbing transition observed immediately after release would have diminished, so that heat absorption was partly masked by heat-producing transitions in other parts of the cycle. The failure of slower releases to elicit a sustained departure of heat rate from the isometric value may be explained by their weaker effectiveness in producing the sustained synchronization required.

The myothermic experiments described here are complementary to some aspectes of the temperature-jump experiments with skinned rabbit psoas fibers reported recently by Goldman et al. (1987). A sudden increase in the temperature of isometric fibers produced an instantaneous drop in active tension due to lengthening of the myofilament lattice by thermal expansion (a manifestation of the thermoelastic effect). Tension recovered rapidly to a level slightly higher than the pre-jump isometric tension, suggesting that the process responsible for rapid recovery has a small positive enthalpy change. This conclusion is consistent with our own conclusion that the crossbridge power stroke has a positive entropy change.

Although these observations have been interpreted within the framework of the Huxley-Simmons hypothesis, their thermodynamic consequences are independent of any particular model. The close association of rapid force recovery with a process having a positive entropy change places some restrictions on the kind of process that could be responsible. Reasonable candidates are contractile-protein isomerizations involving a helix-coil transition (Harrington, 1979) and/or loss of crystallized water produced by increased hydrophobic or charge interactions (see Kodama, 1985).

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