Tension in frog single muscle fibers while shortening actively and passively at velocities near V_u

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ABSTRACT Experiments were undertaken to determine the contribution of passive tension to total tension during rapid shortening in a stimulated muscle fiber. Results were obtained by applying shortening movements at constant velocities slightly less than V_u (the velocity of unloaded shortening) to intact twitch fibers isolated from the frog (*Rana temporaria*). The tension maintained by unstimulated fibers during such shortening movements ("dynamic passive tension") from moderately long lengths was greater than zero but much less than the passive tension measured under static conditions ("static passive tension") at the same lengths. Fibers maximally activated by electrical stimulation and then shortened at the same velocity over the same range of average sarcomere lengths maintained tension that was greater than zero but less than the dynamic passive tension. For average sarcomere lengths up to ~3.1 μm, the dynamic passive tension appeared to be substantially abolished by activation. The onset of the apparent disappearance of dynamic passive tension was studied by initiating the stimulation and the shortening movement simultaneously. The resulting tension response exhibited a latency relaxation that was increased in amplitude compared with the isometric case, followed by a brief tension rise, giving way to a steady tension level equal to that expected if stimulation had been initiated well before the release. These changes are qualitatively explained in terms of the establishment of a steady state distribution of deformations of attached cross-bridges.

INTRODUCTION

Many muscle experiments are performed in the presence of passive tension, and interpretation of the results requires some assumption about the effect of activation on passive tension. In most isometric experiments the assumption made is that the passive tension is not affected by activation. This assumption is supported by findings such as those of Gordon et al. (1966), that the difference between the passive and active tensions (the developed tension) is proportional to overlap of thick and thin filaments. In dynamic experiments, two approaches are commonly used. The simplest is to correct the active dynamic tension by subtracting the isometric (static) passive tension at the corresponding length. A more appropriate method may be to subtract the tension developed during the same movements without stimulation, the dynamic passive tension (Brutsaert et al., 1971). This allows for the possibility that passive tension is changed by movement, but ignores the possibility that passive tension is also affected by stimulation. Such a possibility has been apparent at least since D. K. Hill (1968) suggested that part of the passive tension is due to cross-bridges. However, as noted by Woledge et al. (1985), this suggestion has not been clearly proven.

In recent experiments, Claflin et al. (1989) found no increase in the velocity of unloaded shortening (V_u) at long lengths as measured by slack tests. This was so despite the observation that the magnitude of the dynamic passive tension, if acting as a compressive load, was sufficient to produce an observable increase in velocity if the force-velocity relationship can be extrapolated from tension to compression. One interpretation of those results is that the dynamic passive tension is reduced or abolished by tetanic stimulation. The present experiments were designed to test that hypothesis.

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METHODS

Dissection, mounting, and apparatus

Single twitch fibers were isolated from the tibialis anterior muscle of the frog (*Rana temporaria*). Dissections were performed under dark field illumination at room temperature in a Ringer solution with the following composition (in millimolar): NaCl, 115; KCl, 2.5; $CaCl_2$, 1.8; Na_2HPO_4, 2.15; NaH_2PO_4, 0.85; pH 7.2. Experiments were conducted in this solution maintained at 2.5 ± 0.2°C. Fibers were mounted in a chamber by passing the wires protruding from a force transducer and an arm attached to a servomotor, through holes cut in the tendons remaining at

each end of the fiber. The tendons were secured to each wire using loops of 9-0 monofilament nylon suture. After attachment of the tendons, fibers were stretched until taut. Average sarcomere length was then determined from photographs of at least four different areas along the length of the fiber. The fiber length was divided by the average sarcomere length to determine the number of sarcomeres in series. Subsequent sarcomere lengths were set by stretching the fiber to lengths determined by multiplying the desired average sarcomere length by the number of sarcomeres. All length records reported as sarcomere lengths are motor position records calibrated as average sarcomere length by this method. The experimental chamber, stimulation circuitry, force transducer, servomotor and recording system have been described (Julian et al., 1986; Julian and Morgan, 1979).

Experimental protocols

The passive tension in a fiber depends in a complex way on the previous history of movement and activation. To accurately determine the difference between the active and passive forces during release, it was important to ensure that the passive tension measurements before the two releases were near equal. Consequently, after any change of length, active and passive releases were repeated while carefully monitoring passive tension before each release, and only when the difference between the passive tension levels of successive records was <1% of the passive tension were records saved for analysis.

For all records from activated fibers, the stimulation rate was 40 pulses per second, a rate sufficiently high to elicit maximum isometric tetanic tension with no stimulus related tension ripples. Initially, occasional minor differences were detected between the latency relaxations due to different polarities of stimulation from the alternating polarity stimulator. To eliminate such effects, the latency relaxation records were obtained using an even number of stimulus pulses, so the responses observed were always initiated by a pulse of the same polarity.

Measurement of stiffness

Stiffness was measured by adding a small amplitude (0.5 nm per half-sarcomere, peak-peak) 2-kHz sinusoidal length oscillation to the length command signal. Muscle tension did not affect the motor response at this frequency. Eight tension signals were averaged and recorded by digital oscilloscope (model 4094; Nicolet Instruments Corp., Madison, WI) and stored on floppy disk. The records were then processed by computer using data analysis software (VU-POINT; Maxwell Laboratories, La Jolla, CA). The tension records were first passed through a digital band-pass filter centered at 2 kHz, with transition frequencies of 1.0 and 3.0 kHz, and a transition width of 1.0 kHz. The resulting signal was a 2-kHz sine wave of amplitude proportional to the stiffness of the fiber. The second processing step was a full-wave rectification. In the third step, the signal was passed through a digital low-pass filter with a transition frequency of 1.0 kHz and a transition width of 1.0 kHz. Finally the signals were divided by the mean rectified component of movement to give stiffness. The procedure was tested by processing a record consisting of a step change in the amplitude of a 2-kHz sine wave. Further details were given in Claflin et al. (1990).

Measurement of stiffness by sinusoidal length changes applied to a fiber is subject to errors arising from several sources. These have been discussed by Claflin et al. (1990). The arguments presented there, to show that the errors are not significant to the conclusions drawn, also apply here.

RESULTS

Active and passive shortening

All the shortening velocities used were near V_u, producing tensions between zero and 3% of maximum isometric tetanic tension. This required that the zero tension voltage be very accurately known. The basic experiment is shown in Fig. 1. The fiber was tetanized, then shortened at a constant velocity ("ramp" release) for a distance corresponding to 0.45 μm per sarcomere. At the end of the ramp, a shortening step was imposed to make the fiber go slack, allowing a check on the zero of tension. After a period of unloaded shortening to take up the slack, the fiber redeveloped tension.

A typical series of records on expanded amplitude and time scales are shown in Fig. 2. Each panel shows the tension responses to a pair of successive identical releases, one with the fiber tetanized, and one without stimulation. In the left-hand column the ramp extends from an average sarcomere length of 2.46 μm to an average sarcomere length of 2.01 μm, a length range where the passive tension is small. In the right-hand column, the ramp began at an average sarcomere length of 3.08 μm. The effect of increasing the velocity can be seen by moving from top to bottom in each column of Fig. 2. The greatest velocity was very close to, but clearly not greater than V_u. This is evident from the tension response that did not fall to zero when the ramp was applied to the active

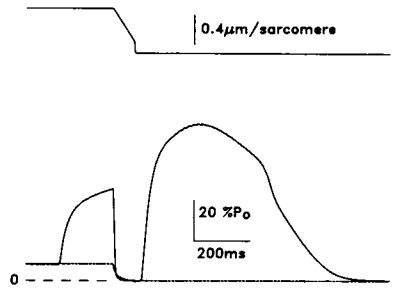

FIGURE 1 A typical record of ramp shortening and the resulting tension response. The upper trace shows the length changes applied, consisting of a ramp of amplitude sufficient to change the average sarcomere length by 0.45 μm, followed by a step to reduce the tension to zero, providing an accurate determination of zero tension adjacent to the tension maintained during shortening. The two superimposed tension responses shown below resulted when this movement was applied to an active (*solid trace*) and a passive (*broken trace*) fiber from a mean initial sarcomere length of 3.08 μm. Stimulation caused tension generation at the initial length, the ramp shortening dropped the tension to <3% of P_o (isometric tetanic tension measured at a sarcomere length of 2.1 μm), the step introduced slack into the fiber, which took up the slack, and then developed tension again at the new length. The zero tension level is indicated.

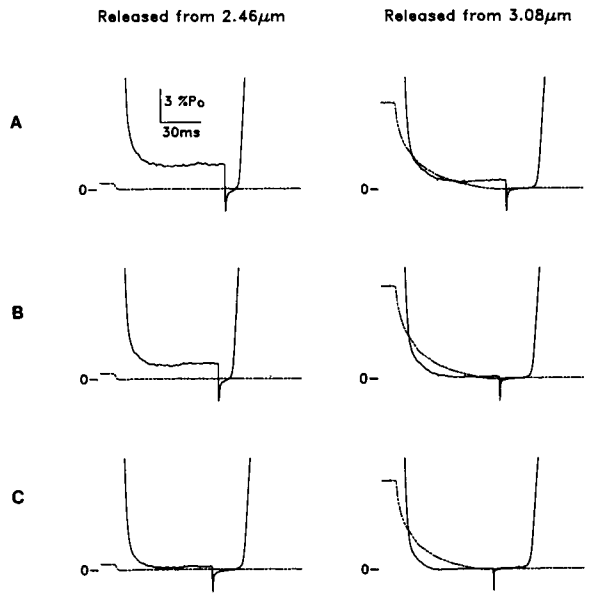

FIGURE 2 Expanded records of tension during active (*solid traces*) and passive (*broken traces*) shortening. Zero tension is indicated. In the left-hand panels the ramp shortening was from an average sarcomere length of 2.46 μm to an average sarcomere length of 2.01 μm. In the right-hand panels the ramp was from 3.08 μm to 2.63 μm. The velocity of shortening was increased from top to bottom, taking values equivalent to: (*A*) 2.85 μm/s per half-sarcomere; (*B*) 3.05 μm/s per half-sarcomere; and (*C*) 3.25 μm/s per half-sarcomere. Note that all three velocities were less than V_u, as shown by the nonzero active tension at the short length, but all three caused the active tension to drop below the corresponding dynamic passive tension at long length. P_o is defined as the isometric tetanic tension measured at a sarcomere length of 2.1 μm. The step releases applied following the ramps from 2.46 μm in *A* and *B* were half the size of the remaining step releases. The resulting reductions in slack times are evident in the corresponding active tension traces.

fiber at lengths near and on the plateau of the length-tension relationship. If the dynamic passive tension was unchanged by the activation, the tension during ramp releases of the stimulated fiber at the longer length would be expected to follow slightly above the tension for the corresponding passive release. If the dynamic passive tension was abolished by activation, the tension during an active release would be constant during most of the release, as occurs at the shorter lengths shown in the left-hand column of Fig. 2. The records shown in the right-hand column of Fig. 2 clearly fall closer to the second prediction than the first. That is, the dynamic passive tension over this length range is more nearly abolished than preserved unchanged during stimulation. The active tension was less than the dynamic passive tension for a significant range of shortening velocities less than V_u.

Similar records from another fiber are shown in Fig. 3 as plots of tension against length. In Fig. 3, *A* and *B*, the releases were all at the same velocity, but began at a series of initial average sarcomere lengths up to 3.4 μm. Fig. 3 *A* shows only active releases with their zeros aligned. Fig. 3 *B* shows the same records with the corresponding passive records, but with the zero levels (indicated by the small horizontal lines) offset for clarity. The releases were performed in order from the shortest to the longest average sarcomere lengths on a fiber that had been carefully protected from being stretched previously to long lengths. Responses to releases from initial average sarcomere lengths of up to 3.1 μm had the same features as those shown in Fig. 2, with active tension falling below passive tension, and with the passive tension appearing to

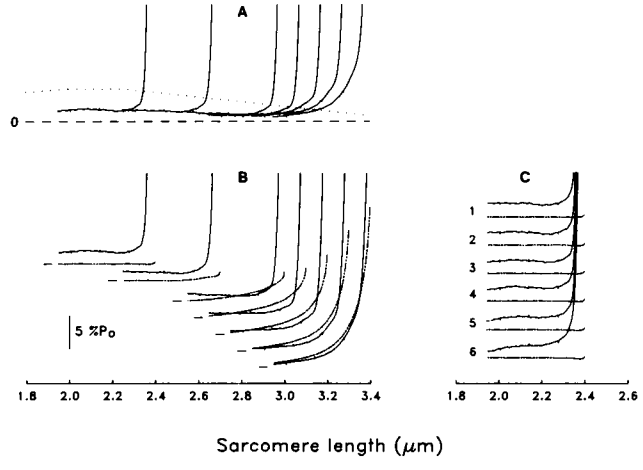

FIGURE 3 Plots of tension as a function of sarcomere length during active and passive shortening at various lengths. All releases were at the same velocity, sufficient to produce ~2% of P_o (isometric tetanic tension at a sarcomere length of 2.1 μm) over the plateau of the length-tension relationship. For clarity, all records have been truncated at the end of the ramp movement. Consequently, the fall to zero tension caused by the step release and the subsequent tension redevelopment do not appear. (*A*) A composite plot of seven releases, all performed on an active fiber, with zero tension shown by the dashed line. The dotted line is a scaled version of the static sarcomere length-tension relationship (Gordon et al., 1966), indicating that the tension during shortening shows a similar rise as sarcomere length is decreased. The horizontal axis and tension calibration bar under *B* apply to both *A* and *B*. (*B*) The same tension records as in *A*, vertically offset for clarity, and paired with tension responses from the same fiber released without stimulation (*broken traces*) at the same velocity and over the same length range. (*C*) Control releases, all at the same velocity, all from a sarcomere length of 2.4 μm to a sarcomere length of 1.95 μm, to monitor the condition of the fiber. Solid traces are active, broken traces are passive, and vertical calibration is as shown in *B*. Records are vertically offset for clarity and were taken: (1) at the beginning of the experiment; (2) after the releases from 3.0 μm; (3) after 3.1 μm; (4) after 3.2 μm; (5) after 3.3 μm; and (6) after 3.4 μm. Note the change from constant tension to declining tension during the ramp release after stretching to long sarcomere lengths.

have been abolished. At longer lengths, the active tension still fell below the passive tension, but was not a constant value above zero, suggesting that a passive tension component was still present, and declining throughout the release.

Stretching to long lengths produced subtle irreversible changes in the fiber, as documented in Fig. 3 *C*. These records show releases across the plateau of the length-tension relationship performed after each of the releases shown in Fig. 3, *A* and *B* (see figure legend for details). After being stretched to longer lengths, the fiber became progressively unable to maintain a constant tension at a constant shortening velocity at any length. In other respects the fiber was not damaged. In particular, fibers that had been stretched continued to contract many times and showed no significant decrease in tension generating capability nor any decline in shortening velocity. The cause of the changes that occur with stretch is not clear. We would suggest that the explanation involves sarcomere nonuniformities, possibly contributed to by breakage of titin or nebulin strands in some half-sarcomeres (Horowits et al., 1986). However, no detailed examination of this phenomenon has yet been made.

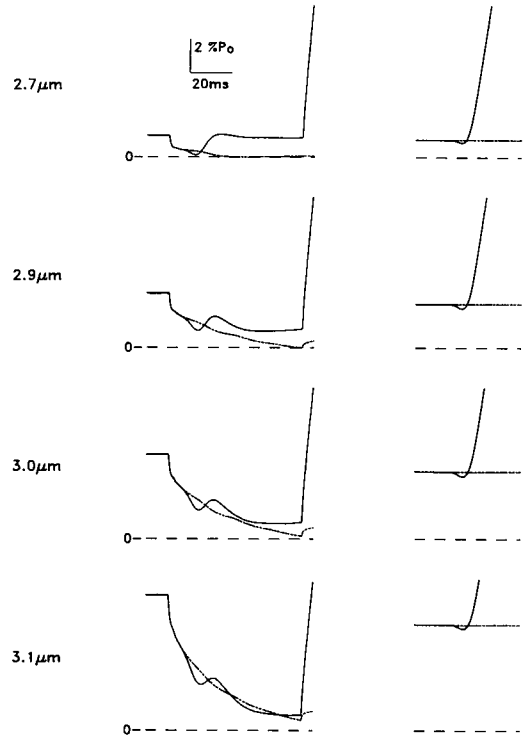

FIGURE 4 Commencing stimulation during releases from different sarcomere lengths. The left-hand records are tension responses to ramp releases, the right-hand records are isometric tension responses. For each pair of superimposed records, the broken trace is the passive tension, whereas the solid trace results from giving the first stimulus pulse at the same time as the beginning of the ramp release in the left-hand panels (10 ms after the beginning of the trace), and at the corresponding time point in the right-hand panels. Initial lengths were adjusted so that the latency relaxation began at the same average sarcomere length (indicated at the left) in the dynamic and the isometric records. Ramp amplitude in each case was sufficient to reduce mean sarcomere length by 0.3 μm. Step releases were not applied following the ramps. The zero tension level is shown with a dashed line.

Activation during shortening

These observations raised questions about the transition of the fiber from the passive to the active state. A series of experiments was undertaken to determine whether the apparent disappearance of dynamic passive tension occurred immediately on stimulation or after some minimum required shortening distance. The left-hand side of Fig. 4 shows records of releases from various lengths at one velocity. Each superimposed pair consists of one record for which the fiber was passive throughout, and one record where the fiber was stimulated coincident with the beginning of the release. The sarcomere length specified for each record is the average sarcomere length at the beginning of latency relaxation, for comparison with isometric latency relaxation records shown on the right. The effect of stimulation during release had three phases. In the first, the tension fell at the time after stimulation that latency relaxation would have begun in the isometric case. However, the "dynamic latency relaxation" was much greater than the normal isometric latency relaxation. The second phase consisted of a rise in tension, which often caused the tension to re-cross the dynamic passive record, depending on the average sarcomere length and velocity of shortening. The third phase consisted of another fall in tension toward the value that would have been obtained if the fiber had been stimulated well before the release began. Note that the passive fiber fell slack at the shortest average sarcomere length, and that at intermediate lengths the active tensions fell to steady values despite the declining dynamic passive tension.

The increased amplitude of the latency relaxation is shown as a function of average sarcomere length in Fig. 5. For each pair of records like those shown in Fig. 4, the response of the passive fiber was subtracted from that of the stimulated fiber to produce a record of the changes in tension produced by the stimulus. (In the isometric case, this amounted to a shift in the base line.) The amplitude of the latency relaxation was then measured and plotted against the average sarcomere length. Note that for the dynamic records, the amplitude is plotted against the average sarcomere length at the beginning of latency relaxation.

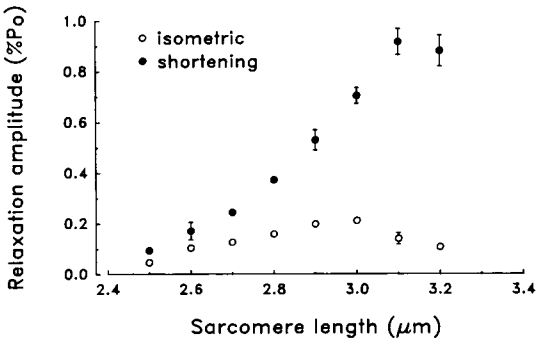

FIGURE 5 Comparative amplitudes of the latency relaxation for isometric and shortening fibers. This plot was prepared from responses such as those shown in Fig. 4 by subtracting the response of the passive fiber from the response of the stimulated fiber in each pair of records, and measuring the maximum fall during the latent period from the resulting difference record. Note that for the dynamic case, points are plotted against the length at the time of onset of latency relaxation. Symbols show mean ± SEM for two fibers and error bars are omitted where they fall within the symbol.

Stiffness during dynamic latency

In view of recent measurements showing that the stiffness of an isometric fiber begins to rise at the same time as the tension begins to fall at the beginning of latency relaxation (Claflin et al., 1990), the behavior of stiffness during this "shortening-enhanced latency relaxation" was of interest. Stiffness was obtained using small sinusoidal length oscillations as outlined in Methods. Typical stiffness records are shown in Fig. 6. The solid lines are the tension changes introduced by stimulation during shortening and the broken lines are the stiffness changes produced under the same circumstances. The tension records were produced by subtracting the tension recorded with shortening but no stimulation from the tension recorded during shortening with stimulation. The stiffness records were produced by processing a tension record obtained with ramp release and length oscillation but no stimulation, and a record with ramp release, length oscillation, and stimulation, to produce records of stiffness. Subtracting one stiffness trace from the other produced the record of the changes in stiffness due to stimulation. The left- and right-hand panels are the same records on different vertical scales.

Several features can be noted from these records. At longer lengths the tension difference is negative during most or even all of the release, again showing that, while shortening at a velocity slightly less than V_u, stimulation reduces the tension to levels below those maintained by the passive fiber shortening at the same velocity. The three phases of the response to stimulation noted in Fig. 4 are very clear in these difference records. The final rise in the tension difference record corresponds to the fall in

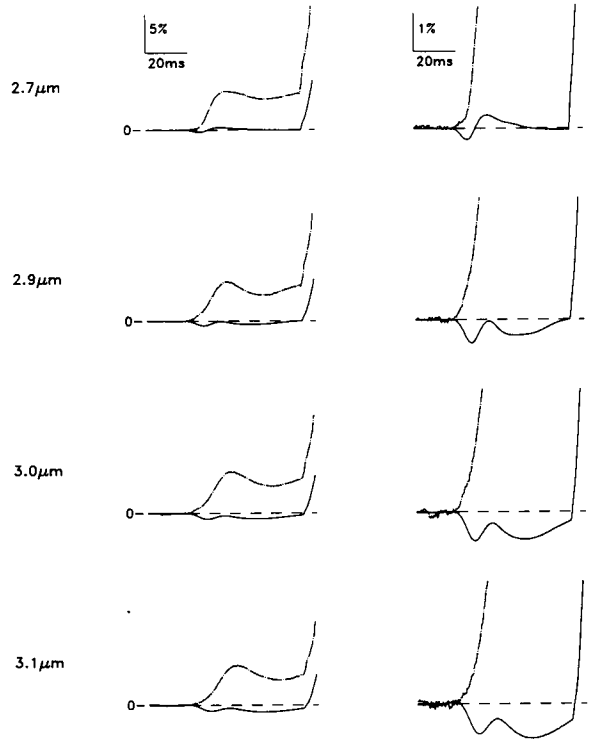

FIGURE 6 Tension and stiffness changes due to stimulation during shortening from a series of lengths. Both tension (*solid*) and stiffness (*broken*) records were produced by subtracting a record obtained during shortening of the passive fiber from a record obtained during shortening of the stimulated fiber. Consequently, the zero levels indicated correspond to no difference between stimulated and passive responses. The records shown in the right-hand column are the same as those shown in the left-hand column with an additional five-fold vertical expansion. The vertical calibrations are in units of percent maximum tension or stiffness as measured during a tetanic contraction at a sarcomere length of 2.1 μm. The average sarcomere lengths at the time of onset of latency relaxation are indicated. The first stimulus pulse was delivered simultaneous with the onset of the shortening release, 10 ms after the beginning of the trace in each case. Ramp amplitude in each case was sufficient to reduce mean sarcomere length by 0.3 μm. Step releases were not applied following the ramps. Note that, at long lengths, the difference between stimulated and passive fiber is a sustained reduction in tension.

passive tension and near constant active tension pointed out in Figs. 2, 3, and 4. There was no detectable separation in time of the stiffness increase and the tension fall, despite the clear definition of these times afforded by the increased amplitude of the latency relaxation.

DISCUSSION

Active and passive shortening

For releases from moderately long lengths, the dynamic passive tension seems to disappear on activation, as shown in Figs. 2 and 3. For very long lengths, irreversible

changes in the fiber make the situation more complicated, but a decrease with activation is still consistently seen. These observations suggest that a component of dynamic passive tension, which at some lengths is the dominant component, is abolished by activation. One possibility is that this component is of cross-bridge origin as suggested by D. K. Hill (1968).

An alternative explanation of these results is that the dynamic passive tension is not affected by stimulation, but that the ramps used here were in excess of the truly unloaded average shortening velocities of the sarcomeres at these lengths. This would require that the unloaded shortening velocity of the sarcomeres decrease with increasing length. The decrease required would be quite significant, as can be seen in the right-hand side of Fig. 2. The velocity in the top panel was <90% of the unloaded velocity at optimum length, yet it caused the tension to drop below the passive tension response at 3.0 μm, so that under this alternative explanation, the unloaded shortening velocity at 3.0 μm must have been <90% of that at 2.1 μm. Furthermore, the unloaded velocity of shortening would be required to change in a most unexpected way with sarcomere length, closely paralleling the nonlinear changes in passive tension.

Activation during shortening

Attempts to monitor the onset of the activation-induced decline in dynamic passive tension by stimulating fibers during shortening led to several conclusions. Figs. 4 and 6 show that the decline was well established within 30 ms of the first response to the stimulation. The tension maintained after this time was that expected if stimulation had been initiated well before the release. Any earlier changes in dynamic passive tension occurred over the same time as, and hence were inseparable from, the modified latency relaxation and the subsequent brief rise of tension. This suggests, but does not prove, that the loss of passive tension and the latency relaxation have a common basis, probably in cross-bridge dynamics.

The enhancement of latency relaxation provides further information on its cause. If, as suggested by Haugen and Sten-Knudsen (1976), the onset of latency relaxation precedes any cross-bridge activity, and is caused by elongation of thin filaments transmitted through the short range elastic component (SREC) described by D. K. Hill (1968), it should be decreased during shortening because of the disruption of the SREC by the movement before activation. If latency relaxation is relaxation of resting tension, then again it would be expected to be diminished by the ramp-induced reduction of the resting tension, unless the component of resting tension reduced by activation is somehow enhanced by shortening, despite the dimunition of the total passive tension. A possible explanation is provided by the observation that the stiffness increase and the beginning of latency relaxation are simultaneous (see also Claflin et al., 1990). The increased stiffness of the shortening fiber could be responsible for the extra fall in tension during shortening of the fiber simply because the stiffer sarcomeres resist shortening, causing the end connections to absorb more of the movement, and so cause the tension to drop faster than in a shortening passive fiber.

The rising phase of the tension response is proposed to be due to the attachment of force generating cross-bridges, that is bridges with deformations between 0 and +h in the model of A. F. Huxley (1957). The subsequent fall would then be due to the development of a "shortening tail" in the distribution, a significant number of cross-bridges swept into the region where they oppose shortening. This sort of transient could be seen as support for the idea that V_u is determined by a balance of forces aiding and opposing shortening.

Stiffness during dynamic latency

The finding of Claflin et al. (1990), that the stiffness of a fiber begins to rise at the same time that tension begins to fall at the beginning of isometric latency relaxation, is confirmed here for latency relaxation during shortening. Furthermore, the enhanced amplitude of the dynamic latency relaxation made the time comparison very clear. This finding further supports the idea that latency relaxation is closely connected with attachment of cross-bridges.

The sources of passive tension

Attempts to define the dominant source of the passive tension in single fibers have not been conclusive (for a review, see Woledge et al., 1985). D.K. Hill (1968) suggested that the SREC and at least part of the passive tension were due to interaction between thick and thin filaments. There are indications that the viscosity seen in passive fibers is not present in active fibers (Ford et al., 1977, p 484). If a component of the passive tension is associated with the SREC, the present finding that it disappears during stimulation would be consistent with the finding that viscosity disappears during activation. If the tension seen during shortening of a passive fiber at velocities near V_u is due to cross-bridges, then they are cross-bridges that are able to develop tension at higher velocities than can normal active cross-bridges. In fact, the dynamic passive tension at these lengths is notably insensitive to velocity in comparison with the active tension, as can be seen from Fig. 2.

As noted in the Introduction, previous observations have made it clear that not all of the static passive tension

disappears on activation. However, passive tension changes due to stretch do show long time constant viscous effects, consistent with some of the structures that provide isometric passive tension being sufficiently viscous to fall slack during shortening at velocities near V_u. One possibility is that only a small fraction of the passive tension observed under isometric conditions is of cross-bridge origin, the remainder being due to visco-elastic structures in parallel with the contractile machinery. The visco-elastic component could fall slack during relatively fast releases, leaving only the small cross-bridge component, which then substantially disappears with activation.

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