

The Effect of Low-Level Activation on the Mechanical Properties of Isolated Frog Muscle Fibers

J. LÄNNERGREN

From the Nobel Institute for Neurophysiology, Karolinska Institutet, S-104 01 Stockholm 60, Sweden

ABSTRACT The mechanical properties, as revealed by minute length changes, of isolated twitch fibers of the frog have been studied at rest and during low-level activation. Resting tension is 77 ± 23 mN/cm² (mean \pm SD) at 2.2 μ m sarcomere length.¹ The slope of the tension curve ($\Delta P/\Delta L$) recorded during a constant-speed length change of a resting fiber is initially large. At length changes exceeding about 0.18% of the initial length of the fiber $\Delta P/\Delta L$ falls abruptly and remains close to zero during the rest of the length change. The amplitude of the tension response is reduced after a length change and returns to normal in about 3 min. Hypertonic sucrose-Ringer solutions cause a small, maintained rise in tension up to 1.4–1.6 times normal osmotic strength. Higher sucrose concentrations cause relatively large, transient tension responses. The initial $\Delta P/\Delta L$ is increased in moderately hypertonic solutions; it may be reduced in more strongly hypertonic solutions. Elevated $[K]_o$ (range 10–17.5 mM) causes a marked reduction in $\Delta P/\Delta L$. In this range of $[K]_o$ the reduction is not accompanied by changes in resting tension. Addition of 1–1.5 mM caffeine to the Ringer solution affects the resting tension very little but also reduces $\Delta P/\Delta L$. The results suggest that stiffness and tension development are not related in a simple way.

INTRODUCTION

The transition from rest to activity which occurs when a muscle is stimulated is accompanied by several physical changes such as an initial reduction in the resting tension, usually termed "latency relaxation" (Rauh, 1922; Sandow, 1944, 1947), an increase in torsional rigidity (Sten-Knudsen, 1953), and an increase in resistance to passive stretch (Hill, 1950, 1951). The method used to study the change in resistance to stretch has been to extend the muscle by a certain amount at various times after a supramaximal electrical

¹ Force is given in newtons rather than in grams or gram weight. 1 mN very nearly equals 0.1 g wt; 1 μ N corresponds to 0.1 mg wt.

stimulus and record the resulting tension. The mechanical testing has to be performed during a very limited time interval since the activation of frog muscle, even at 0°C, proceeds very rapidly and is complete within 20–40 msec after the stimulus (Jewell and Wilkie, 1958; Edman, 1970).

The aim of the present investigation was to study further the physical changes which occur in striated muscle when the activation process starts. An approach different from that outlined above has been used. The basic experimental technique was the reduction of the membrane potential in a uniform step fashion to a value close to the mechanical threshold followed by a test of the mechanical state of the preparation. The preparation used here was the isolated fiber which can be quickly and homogeneously depolarized by sudden changes in the external potassium concentration (Hodgkin and Horowicz, 1959).

Before assessing the effect of activation, the mechanical properties of the resting fiber were measured. Even in the absence of activation, a muscle or a single fiber exerts a tension which has been called "resting tension." The origin of this is not entirely clear. Various proposals have been made concerning the structure responsible for the resting tension: it could either be due to the sarcolemma (Ramsey and Street, 1940; see also Fields and Faber, 1970) or, it could be due mainly to the fibrillar material as suggested by Buchthal and collaborators (for a summary, see Buchthal, Svensmark and Rosenfalck, 1956). It has recently been suggested that an unstimulated muscle is not entirely inactive but that the process which generates tension in the stimulated muscle operates also in the resting condition albeit at a very low rate (Hill, 1968). This would partly account for the resting tension and also for a peculiar short-range stiffness discovered by Hill in the whole, resting muscle.

The present results, briefly presented elsewhere (Lännergren, 1970), show that the resting, isolated fiber also displays a short-range stiffness. This is increased in a moderately hypertonic solution. The stiffness is considerably reduced as a result of activation. The reduction can take place without changes in the steady tension of the fiber. This suggests that stiffness and tension production are not related in a simple fashion.

METHODS

Fiber Preparation and Mounting Experiments were performed on medium-sized Irish or German frogs (*R. temporaria*) kept at a temperature of 4°–7°C up to the moment of use. Single twitch fibers were dissected from the iliofibularis muscle with the aid of jeweller's forceps and finely pointed scissors. Fibers were regularly selected from the part of the muscle most distant from the nerve entry. After isolation the fiber was mounted in a horizontal channel in the Perspex dissecting trough. A system of stop-cocks was used to introduce different solutions into the channel. A continuous flow

of Ringer solution (about 5 mm/sec) was maintained through the channel to minimize evaporation of the solution and the resultant changes in tonicity which would otherwise have markedly affected the response. It was increased to a rate of 10–15 mm/sec just before a change to a test solution was to be made. The latter was allowed to run in with about the same velocity.

Length Control and Recording An electromechanical transducer was attached to one end of the fiber and a mechanoelectrical transducer connected to the other end. The former, a Goodman vibration generator (model V 47, Goodmans Industries, Ltd., Wembley, England) allowed length changes of desired form and amplitude to be applied and the latter, a force transducer, made it possible to record tension associated with the length changes. In order to minimize the compliance of the connections between the transducers and the fiber itself the following method was adopted. Small stainless steel hooks (*F* and *G* in Fig. 1) of diameter 50 or 80 μm and

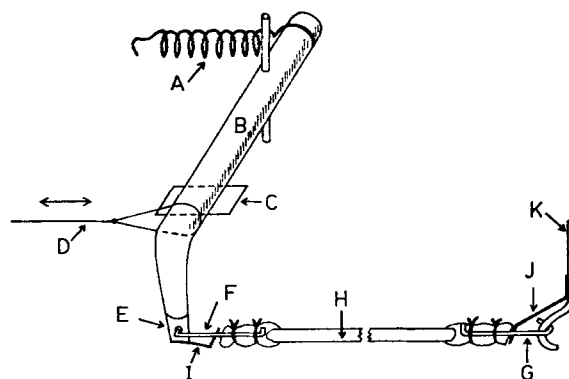


FIGURE 1. Schematic drawing of arrangement used to record tension response of an isolated fiber to imposed small changes in length. *A*, spring; *B*, pivoted glass rod; *C*, vane; *D*, silver wire connected to Goodman vibrator (not shown); *E*, stainless steel foil; *F* and *G*, stainless steel hooks tied to tendons of fiber; *H*, muscle fiber; *I* and *J*, supports for hooks; *K*, extension rod of force transducer (not shown).

length about 2 mm were tied to the tendons of the fiber which had been reduced in size to about 0.5×2 mm. The hooks were secured by means of two separate knots made with 50 μm diameter monofilament nylon thread. The knot closest to the fiber was generally within 100–200 μm from the fiber end. One of the hooks (*F*) was inserted through a hole in a piece of stainless steel 50 μm foil cemented to the short arm of an L-shaped glass rod (*B*). The rod was pivoted on miniature ball bearings to allow movement in the horizontal plane. The Goodman vibrator was connected to the glass rod by a 30 μm annealed silver wire (*D*) which was held taut by a spring situated on the opposite side of the fulcrum. The position of the rod was recorded photoelectrically with a foil (*C*) which partially interrupted a light path. The other hook (*G*) connected the fiber to a 15 mm long, thin glass tube attached to the moveable element of the force transducer.

The force transducer was of variable capacitance type. The moveable plate was made of one-half of a razor blade (0.1 mm in thickness) which was separated from two

fixed brass plates by a 50 μm air gap on each side. The natural frequency was about 300 Hz. A measure of the compliance of the recording system including the connections was obtained in a control experiment with a piece of tendon, 0.5 \times 5 mm, from the iliofibularis muscle. Two hooks of the type used in the fiber experiments were tied so that they were separated by 400 μm of tendon. The preparation was connected to the glass rod and the force transducer rod and was found to have a compliance of 0.07 $\mu\text{m}/\mu\text{N}$. The main contribution to this (0.05 $\mu\text{m}/\mu\text{N}$) came from the force transducer itself.

Microscopy The fiber could be viewed during the experiment, either in a dissecting microscope, or in a high power microscope which rested on a moveable platform. The former was used for the determination of fiber length and of cross-sectional area. The largest diameter ($2 \cdot a$) and the diameter perpendicular to this ($2 \cdot b$) were measured at different places along the fiber with the aid of an ocular scale, and the area (A) calculated according to the formula $A = a \cdot b \cdot \pi$. The high power microscope was fitted with a Leitz UMK 50 objective and an ocular micrometer and was used for the determination of sarcomere spacing by direct microscopy at \times 800. The number of sarcomeres per unit distance (usually 38 or 57 μm) was counted at two or three places in the middle region of the fiber and the mean value for the sarcomere length was calculated.

Stimulation Electrical stimuli could be applied to the fiber through two bright platinum electrodes in the bottom of the perfusion channel. Twitch tension was recorded at the beginning of the experiment and was then measured at intervals. Judged by twitch amplitude and their appearance in the microscope fibers often remained viable up to 2 days.

Measurements of Mechanical Properties In the majority of the experiments a tension change, ΔP , was recorded under the conditions of a constant-speed length change, ΔL , which was about 100 μm . The slope of the tension curve is initially large (see e.g. Fig. 3 A) but after a length change of about 25 μm the fiber "yields" and the tension is more or less constant during the rest of the length change. The point where the yield occurs is termed the "elastic limit." The slope of the initial part of the curve can be used as a measure of the initial stiffness. The value is given either as $\Delta P/\Delta L$ or as E (Young's elastic modulus) calculated as $E = (\Delta P \cdot L_i)/(\Delta L \cdot A)$ where L_i is the starting length and A is the cross-sectional area of the fiber. In both cases it is assumed that the fiber behaves as an undamped elastic body. Experiments with widely varying velocities of the length change showed that this is nearly true as E was only about doubled for a 10-fold increase in the velocity of the length change.

Solutions The Ringer solution had the following composition (mM): NaCl 115, KCl 2.5, CaCl₂ 1.8, Na₂HPO₄ 2.15, NaH₂PO₄ 0.85. When solutions with increased [K] were used, they were made up in accordance with the guidelines given by Hodgkin and Horowitz (1959), i.e. they were isotonic with the Ringer solution and had a constant [K] \times [Cl] product of 300 (mM)². Hypertonic solutions were prepared by the addition of sucrose. Generally, four concentrations were used with 30, 45, 60, or 80 g of sucrose added per liter of Ringer solution giving an estimated osmotic strength of 1.38, 1.57, 1.76, and 2.04 times that of normal Ringer solution, respectively. All experiments were carried out at room temperature (20°–24°C).

RESULTS

Resting Tension

Preliminary measurements indicated that the resting tension of a single fiber was of the order of 5–10 μN at 2.2 μm striation spacing. Measurements of forces of this order of size were made uncertain because of drift in the trans-

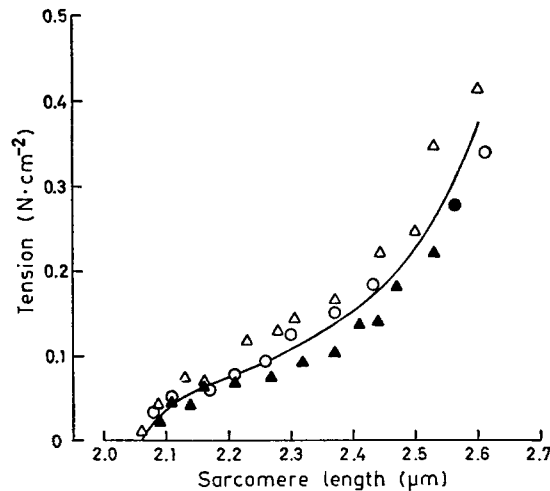


FIGURE 2. Length-tension diagram of three different fibers in Ringer solution 3 min after setting the length. Measurements were first made at the shortest length, then at successively larger extensions. Open triangles, fiber 1, cross-sectional area (A): 8.4×10^{-6} cm^2 , length at 2.2 μm sarcomere spacing: 11.0 mm; solid triangles, fiber 2, A: 7.8×10^{-6} cm^2 , $L_{2.2 \mu\text{m}}$: 14.4 mm; open circles, fiber 3, A: 16.8×10^{-6} cm^2 , $L_{2.2 \mu\text{m}}$: 11.3 mm.

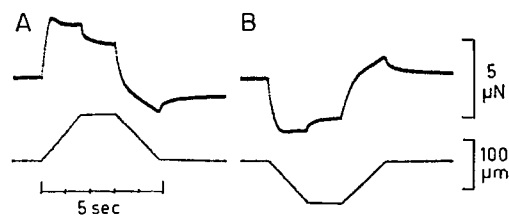


FIGURE 3. Tension response (upper trace) to constant speed extension (A) and release (B) at initial sarcomere length of 2.21 μm . Time course and amplitude of length change indicated on lower trace.

ducer system which for times exceeding 2 min corresponded to about 3 μN . In order to reduce the influence of this drift a base line corresponding to zero tension was recorded for each measurement. This was done by gripping the hook, connecting the fiber to the force transducer with a pair of forceps, and moving it to the right (see Fig. 1), thus taking all load off the strain gauge.

In measurements of this kind on seven fibers at a sarcomere length of 2.2 μm the tension per unit cross-sectional area was found to be 77 ± 23 mN/cm² (SD of an observation).

Additional measurements of the resting tension at different sarcomere lengths (2.0–2.6 μm) were done by the same method on three of the seven fibers (Fig. 2). The form of the length-tension curve in the range considered was found to be neither linear, as would be expected from an elastic body, obeying Hooke's law, nor exponential, as seems to be the case at greater lengths (Buchthal, Kaiser, and Rosenfalck, 1951).

Tension Changes Due to Constant-Speed Length Changes

In the following, results are given from experiments in which tension changes in the fiber were recorded during and after imposed constant-speed length changes. Generally, the length of the fiber was adjusted to give a sarcomere length of 2.2 μm . From this standard, or initial length, L_i , the fiber was extended or released until a new length, L_f , was reached. This was maintained for some period of time and then a change back to L_i was performed.

The typical tension response to a length change of this kind at 2.2 μm initial length is shown in Fig. 3 A and B. During the initial part of an extension (Fig. 3 A) tension was seen to rise rapidly. At an extension of about 25 μm , corresponding to 0.2% of the total length of the fiber, the slope of the tension curve, $\Delta P/\Delta L$, decreased drastically, being quite small or even negative during the rest of the length change. When the length change ended, tension declined rapidly at first, then more slowly. During the return to L_i a change in $\Delta P/\Delta L$ of the tension curve also occurred, but this was less prominent than that seen during extension.

The tension change associated with a release (Fig. 3 B) was found to be very nearly the mirror image of that observed during an extension.

Fig. 4 shows examples of tension changes associated with stretches or releases from different initial lengths. The records are superimposed on a "static" length-tension curve of the type described in the preceding paragraph. When L_i was at 2.10–2.15 μm sarcomere length the tension curve obtained during release did not display a clear-cut change of slope. At shorter lengths below 2.10 μm , i.e. where the resting tension curve approaches zero tension, the amplitude of the release response was much reduced. With increasing initial lengths $\Delta P/\Delta L$ of the initial part of the tension curve became greater, both for releases and stretches. Also, $\Delta P/\Delta L$ of the later part of the tension curve, i.e. beyond the elastic limit, was dependent on L_i , being negative initially for a limited range of initial lengths (2.2–2.4 μm sarcomere spacing) and becoming increasingly positive at greater initial lengths.

When values for $\Delta P/\Delta L$ were converted to E (elastic modulus, see Meth-

ods) and compared, it was found that the values of E obtained from different fibers were quite similar. The mean value for E , in 14 different fibers at a sarcomere length of $2.2 \mu\text{m}$ was calculated to be $22.8 \pm 11.6 \text{ N/cm}^2$ (SD of an observation).

The length change required to reach the elastic limit was also determined for the same 14 fibers. The mean length change (extension) required was 0.18% of the initial fiber length, which expressed as length change per half-sarcomere equals $2.0 \text{ nm} \pm 0.4$ (mean \pm SD of an observation). A 100-fold increase in the velocity of the length change increased the extension required

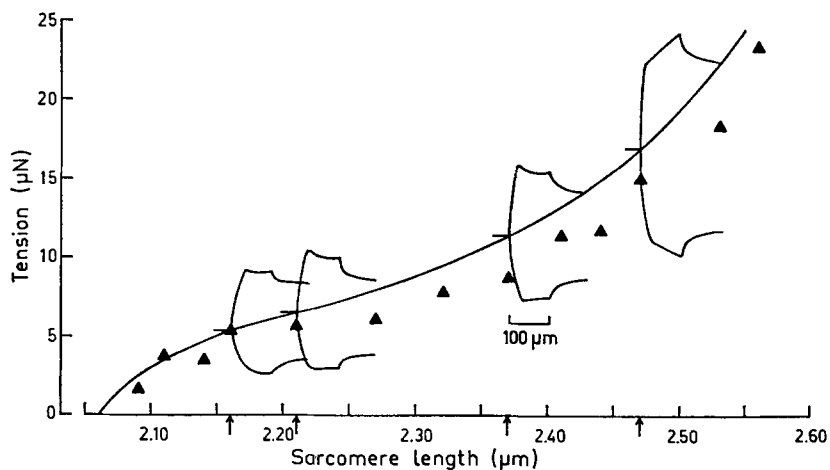


FIGURE 4. Tension response to stretch and to release at four different initial lengths, corresponding to four different sarcomere spacings (indicated by arrows). The abscissa for the tension curves is given by horizontal $100 \mu\text{m}$ bar. The tension curves are shown superimposed on the fitted curve of Fig. 2. Triangles, also from Fig. 2, indicate values obtained in a separate run for the steady-state tension at different lengths. All records are from fiber 2.

to reach the elastic limit by a factor of 2–3. The velocity of the length change affected the elastic modulus in a similar way.

In the experiments just described a period of rest of 3 min was allowed between complete L_i - L_f - L_i cycles. With this precaution identical length changes caused tension changes of constant form and amplitude for very long periods of time, sometimes up to 2 days. Shorter intervals between tests could alter the response (see below).

LONG-LASTING EFFECTS OF LENGTH CHANGES It was noted in preliminary experiments that it took some time for the fiber to “settle” after a preceding length change. The point was studied systematically in four fibers in the following way. Each fiber was stretched to, or just past, the elastic

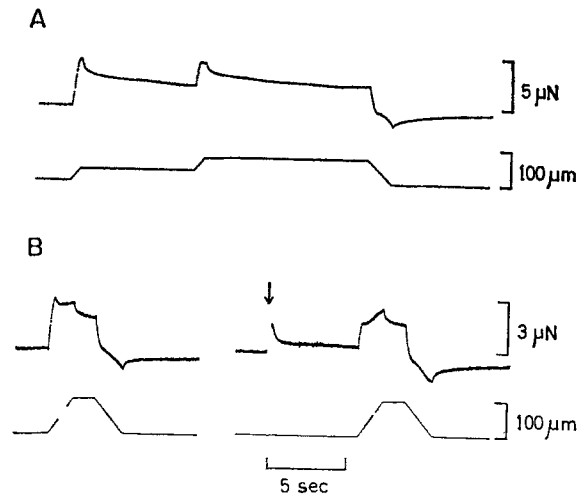


FIGURE 5. A, the effect of extending a fiber in two steps. The first extension was just large enough to exceed the elastic limit. The second (test) extension elicits a tension response of reduced amplitude, Fiber 4, A: $15.5 \times 10^{-5} \text{ cm}^2$ $L_{2,2 \mu\text{m}}$: 17.5 mm. B, the effect of preceding stimulation. Tension response of the unstimulated fiber is shown to the left. In the right-hand part of the record, the fiber was stimulated to give a twitch (at arrow) and extended 5.5 sec later. Note that twitch tension was recorded with the same high gain as the extension response so that only a small part of the whole twitch response is seen. Fiber 1.

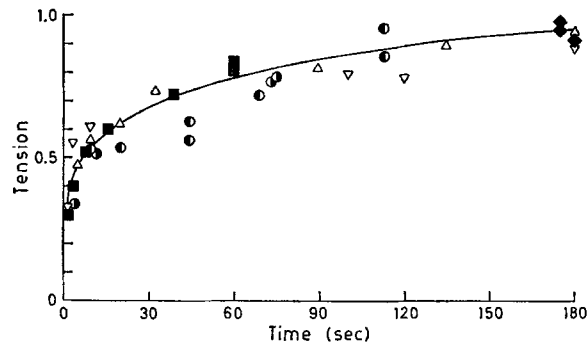


FIGURE 6. Time course of recovery of elastic response. Solid or partly solid symbols refer to data obtained from four different fibers after two extensions as illustrated in Fig. 5 A. Abscissa in this case corresponds to time interval between the first and second (test) extension. Ordinate is amplitude of test response relative to amplitude of response to first extension. Open symbols show the recovery after a conditioning twitch (cf. Fig. 5 B). Abscissa corresponds to time interval between electrical stimulation and test extension. Ordinate is amplitude of test response relative to response of unstimulated fiber. Continuous line fitted by eye.

limit and this length was then maintained. A second extension at the same velocity, applied a short time after the first one caused a second tension response of reduced amplitude (Fig. 5 A). As can be seen, the abrupt fall

in $\Delta P/\Delta L$ of the second tension response occurred at a level very close to that of the first response. The amplitude of the second tension response increased as the time after the first extension was made longer. The time course of the "recovery" of the test response is given in Fig. 6.

In order to compare the effect of a mechanical perturbation of a different kind some fibers were stimulated electrically to give a twitch and were then subjected to a 100 μm extension after various time intervals (Fig. 5 B). The time course of the recovery after a twitch was found to be quite similar to that after an extension to a new length (Fig. 6, open symbols). As with the effects of passive length changes, return towards the normal response occurred quite rapidly at first, then more slowly. Recovery was 50% complete in about 10 sec. The residual recovery took at least another 3 min.

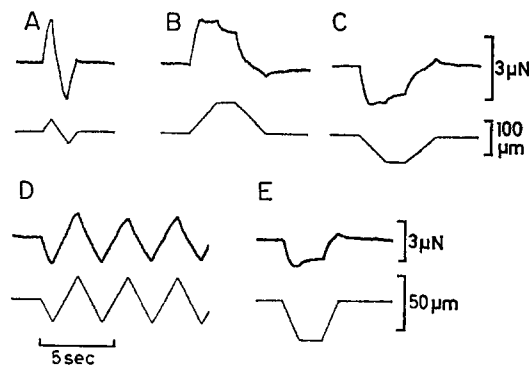


FIGURE 7. A and D, tension response to sawtooth, symmetrical length changes. Amplitude of length change was just large enough to reach the elastic limit in both directions. B, C, and E are controls showing more clearly the length change required to reach the elastic limit. Upper set of traces corresponds to fiber 5, A: $11.3 \times 10^{-5} \text{ cm}^2$, $L_{2,2 \mu\text{m}}$: 13.5 mm and the lower set to fiber 6, A: $12.9 \times 10^{-5} \text{ cm}^2$, $L_{2,2 \mu\text{m}}$: 11.9 mm.

If a change was made to a new length and this was maintained for a short while only before returning the fiber to the starting length, the aftereffects were less long lasting. Tests of this kind could be repeated with $1\frac{1}{2}$ -2 min intervals without any change in the response. In most instances in which the effect of altered external environment was studied (see below) an interval of 3 min was allowed between length changes.

SMALL REPEATED LENGTH CHANGES When sawtooth length changes, symmetrical about the starting length, were applied the form of the tension changes closely followed that of the length changes. This was found to be true for length change amplitudes up to that required to reach the elastic limit (Fig. 7 A and D). Hence the range for the elastic response was twice the distance from the starting position to the elastic limit in one direction.

Effects of Hypertonic Solutions

Measurements of tension and elastic modulus were also made on fibers in hypertonic media. The solutions used in these experiments were made hypertonic by the addition of appropriate amounts of sucrose to the ordinary Ringer fluid. The osmotic strength of these solutions is given relative to that of ordinary Ringer solution. In most experiments solutions with an osmotic strength of 1.4, 1.6, 1.8, and 2.0 times normal were used. They correspond to Hill's (1968) "R3S," "R4.5S," "R6S," and "R8S" solutions, respectively.

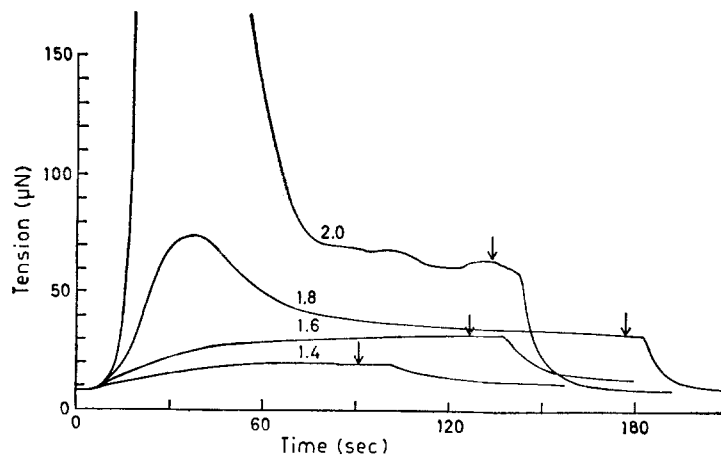


FIGURE 8. Tension changes associated with changes in osmotic strength of the bathing solution. Superimposed tracings from original records. Osmotic strength indicated above traces. Solutions applied in order 1.4, 1.6, 1.8, and 2.0. Arrows indicate change back to Ringer solution. Fiber 9, A: 11.7×10^{-6} cm², $L_{2.2 \mu\text{m}}$: 13.4 mm, resting tension (2.2 μm): 8.3 μN.

A change to a hypertonic medium was associated with an increase in tension in all eight fibers tested (Figs. 8–10). The tension rose slowly and reached a steady level in about 60 sec at an osmotic strength of 1.4–1.6 times normal. At a critical concentration, which varied somewhat from fiber to fiber, a large, mainly transient increase in tension was seen which reached a peak in about 30 sec. After this a plateau followed which was 10–40% of the peak tension (Fig. 8). The values of peak tension at various osmotic strengths are given in Fig. 10.

The elastic modulus of the fiber increased with increasing osmotic strength but only up to a sucrose concentration which was below that which was associated with a large transient tension rise (Fig. 9 A). At these osmotic strengths (1.4–1.6 times normal) the length change required to reach the

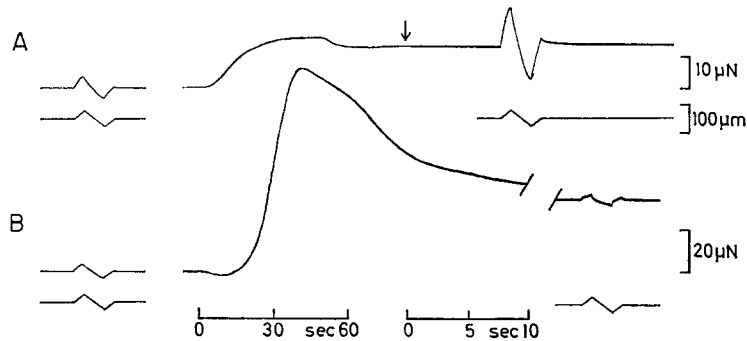


FIGURE 9. A, tension response to sawtooth length change in Ringer solution (left-hand part) and at 1.4 times normal osmotic strength (right-hand part). The solution change (at zero time) was associated with a small increase in resting tension. Arrow indicates where paper speed was changed from 1 mm/sec to 5 mm/sec. B, corresponding records (at lower gain) before and 3 min after increasing the osmotic strength 1.8 times. Note the large, mainly transient rise in tension. 60 sec time bar refers to the first part of the right-hand records; 10 sec time bar refers to parts where tension responses to length changes are shown. Fiber 4.

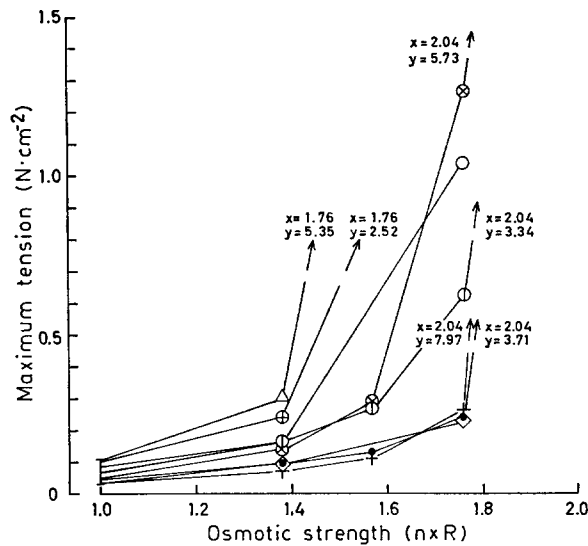


FIGURE 10. Peak tension at various osmotic strengths. All records were made in order of increasing osmotic strength. Coordinates at arrows and direction of arrows indicate position of values above $1.5 \text{ N} \cdot \text{cm}^{-2}$.

elastic limit was approximately the same as in normal Ringer solution. The tension response was “symmetrical” also in the hypertonic solutions. The elastic modulus could not be measured during the large transient tension changes in the more concentrated solutions. During the plateau after the

peak, however, the tension response to a length change was found to be markedly altered in its form (Fig. 9 B). The change in slope was less prominent and occurred at a smaller length change. The E value of the initial part of the tension response was often quite low.

Effects of Increased External Potassium Concentration

The aim of the experiments to be described in this section was to determine the effect on the elastic modulus and the elastic limit produced by a reduction in membrane potential to values close to the mechanical threshold. In order to obtain meaningful measurements of the mechanical properties it was necessary to have the fiber membrane homogeneously depolarized. This was achieved by rapid changes of the Ringer fluid surrounding the fiber to a solution with increased potassium concentration (Hodgkin and Horowicz, 1959). The membrane potential was not measured in these experiments but the values obtained at different $[K]_o$ by Hodgkin and Horowicz are assumed to apply since both the preparation and the solutions were similar. Measurements were made in solutions with 10, 15, 17.5, and 20 mM K, usually in the order of increasing concentration and with control measurements in Ringer between each run in the test solution.

The threshold concentration for active tension production varied somewhat from fiber to fiber but was usually between 17.5 and 20 mM K. The contracture in 20 mM K was as a rule small (less than 5% of the estimated tetanic tension) and short lasting.

Data collected from five fibers, showing the effect of increased $[K]_o$ on the elastic modulus are presented in Fig. 11. It is seen that increased $[K]_o$ decreased the E value in all fibers. The threshold for the effect showed some interfiber variation but for each fiber the effect was more marked at higher $[K]_o$. In many cases the fibers were seen to recover some stiffness while being kept in the test solution. This tendency was more pronounced at higher $[K]_o$. The increase in $[K]_o$ not only caused a reduction in E , but also affected the form of the tension response to extension, as can be seen from the records on the right-hand side in Fig. 11. The bend of the curve at the elastic limit became less distinct and the slope of the curve beyond the elastic limit, which was close to zero in Ringer solution, attained a positive value in the increased $[K]$ solutions. Quite often the slope was seen to change continuously. All effects of increased $[K]_o$ were well reversible with a return to the control response usually taking less than 5 min but occasionally up to 10 min.

Increased $[K]_o$ was thus found to have two effects: an increase in tension and a decrease in elastic modulus. The question now arose whether or not the threshold concentration was the same for the two effects. This was investigated in three consecutive experiments in which the resting tension was measured from the deflection caused by unloading the force transducer (see

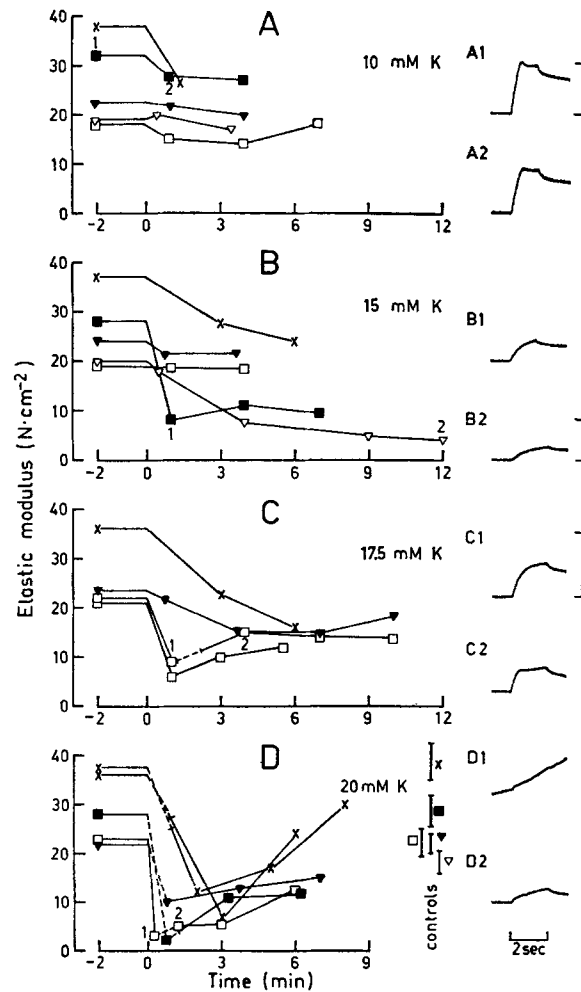


FIGURE 11. Time course of change in elastic modulus after application of solution with increased $[K]$. Five different fibers are each represented by a symbol. Interrupted line indicates period of time during which fiber was in contracture (C and D). Controls were taken in Ringer solution before and after the application of each test solution. The range of E values obtained for the controls is given in D. Records in right-hand part of figure are examples of tension responses to the $100 \mu\text{m}$ constant-speed extension used as test. Letter and number for each response correspond to those in the left-hand part of the figure. Vertical bars on the right-hand side equal 50 mN/cm^2 . Upper vertical bar refers to A1, A2, and B1, middle bar to B2, and lower bar to the rest of the responses.

p.149). The measurements were made in Ringer solution and at a $[K]_o$ which caused a reduction in E . The results indicated that a definite change in elastic modulus could be obtained without a concomitant increase in tension (no clear change in two fibers and less than 10% increase in one fiber).

Effects of Applied Caffeine

The effects of the application of a low, subthreshold, concentration of caffeine (1–1.5 mM) were studied to determine whether the results obtained at increased potassium concentration were due to an effect on the membrane

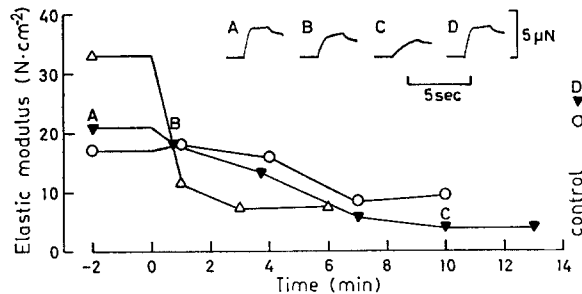


FIGURE 12. Time course of change in elastic modulus after application of Ringer solution with 1 mM caffeine. A, B, C, and D are examples of tension responses of one fiber at various times after the change of solution. Open triangles, fiber 1; open circles, fiber 3; solid inverted triangles, fiber 8, A: $17.3 \times 10^{-6} \text{ cm}^2$, $L_{2.2} \mu\text{m}$: 17.5 mm.

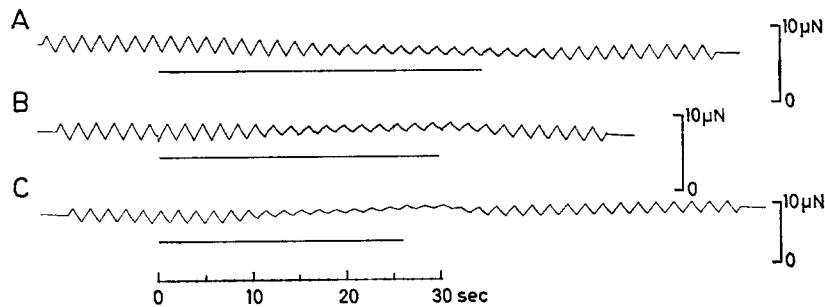


FIGURE 13. Effect of caffeine on the elastic modulus. The fiber was subjected to continuous sawtooth length changes of peak-to-peak amplitude $14 \mu\text{m}$. Horizontal line below each record indicates period of caffeine application. Caffeine concentration in A: 1.1 mM; in B: 1.2 mM and in C: 1.4 mM. The records were made in order C, A, and B. Note that the amplifier gain was somewhat lower in C. Tension calibration bars indicate absolute tension. Fiber 9.

potential itself or to some kind of activation of the contractile system induced by depolarization. Caffeine in higher concentrations (2–10 mM) is known to cause a near maximal or maximal contracture (Axelsson and Thesleff, 1958; Lüttgau and Oetliker, 1968) presumably by a direct action on the sarcoplasmic reticulum, raising the intracellular concentration of activator. The direct effect of caffeine on the activator of the contractile system could thus serve as a basis for comparison of the increased $[\text{K}]_o$ results.

The effects of applied caffeine were studied in two ways. In three fibers the change in elastic modulus was determined from the form of the tension response to single stretch-release cycles, performed with 3 min intervals, as was done in the experiments with increased $[K]_o$. The results are given in Fig. 12. With a 1 mM caffeine test solution the elastic modulus was seen to decrease continuously during 6–10 min and then remain constant. No change in resting tension was observed. The original stiffness was regained after 5–10 min washing in normal Ringer solution.

In four other experiments the elastic modulus was determined by recording the tension oscillation associated with continuously running sawtooth length changes of amplitude 10–14 μm (cf. Hill, 1968). This method of study had the advantage that it allowed a more accurate determination of the time course of changes in E since the length oscillations could be applied continuously during the solution changes. An example of the effect of applying different concentrations of caffeine (1.1–1.4 mM) to a fiber is given in Fig. 13. It is seen that at these concentrations there was very little change in resting tension whereas the stiffness markedly decreased. It is also seen that the effect sets in quite rapidly, i.e. within 10–20 sec. The recovery in normal Ringer solution was also very rapid.

In one of these experiments, as well as in one of the experiments with increased $[K]_o$, the fiber was photographed before, during, and after a change to a solution which caused a marked reduction in stiffness. Measurements from the film indicated in both cases that the change in stiffness was not associated with a measurable change in fiber diameter.

DISCUSSION

The present results show that tension changes associated with very small length changes, below 1% of the reference length, can be measured with satisfactory resolution in isolated muscle fibers. Reproducible measurements could also be obtained of the resting tension at lengths approximately equivalent to those of the fiber in the body. Records were made of the tension response to small extensions or releases under various conditions. The experiments were designed to study mainly the effects of alterations in tonicity or potassium concentration of the bathing solution.

The length at which the length-tension curve approached zero tension was found to correspond to a sarcomere length of 2.05–2.10 μm . This agrees well with the statement of Gordon, Huxley, and Julian (1966) that the slack length of the isolated fiber is at 2.1 μm sarcomere spacing. The form of the curves presented here agrees with that of Ramsey and Street (1940) for isolated semitendinosus fibers provided that a sarcomere length of 2.1 μm is taken to correspond with the starting point of their length-tension curves.

There are discrepancies in the length-tension characteristics for single fibers and for whole muscle preparations. A resting sartorius muscle exerts tension down to 60–75% of its standard length in the body, corresponding to a sarcomere length of 1.5–1.8 μm (A. V. Hill, 1949; D. K. Hill, 1968). The reason for this difference is not clear. There is no evidence to suggest that the muscle fibers themselves differ in this respect whether taken from the semitendinosus, iliofibularis, or sartorius muscle. The isolated fibers in question have given consistent tension responses for long periods of time. Thus “overstretch” or other forms of damage during the dissection also do not seem plausible. The most likely explanation would seem to be that structures which exert tension even at short lengths are cut during the dissection. Connective tissue strands or other connections between the tendons could be the structures involved.

The tension response of a single fiber to small length changes was found to be strikingly similar to that of the whole muscle. This indicates that the main mechanism responsible for the characteristic response is indeed located within the fiber.

With the single fiber preparation it was possible to demonstrate that the elastic limit was reached at a corresponding tension change in each direction when the fiber was subjected to a symmetrical sawtooth length change (Fig. 7). This result suggests that the component in the fiber responsible for the elastic effect is initially in an intermediate position and that the whole range for the elastic effect is about 4 nm.

When the fiber had been perturbed mechanically it was found that the tension response did not return to its original amplitude until the fiber had been allowed to rest for about 3 min. Hill (1968, p. 645) when using the whole muscle found that in order to obtain a “clear-cut” tension response the muscle had to be extended (or released) some tenths of a millimeter and allowed to rest for about 30 sec before subjecting it to the test stretch (release). If the stiffness recovers with a similar time course in the whole muscle as in the isolated fiber, this would mean, for example, that measurements of the length change needed to reach the elastic limit taken 30 sec after a prestretch would be spuriously low by about 30%.

Hill found that solutions made hypertonic by the addition of sucrose caused a rise in the “resting” tension of the whole sartorius which increased with increasing tonicity and which was maintained “indefinitely.” An isolated fiber was seen to respond in a different way. Up to a certain critical osmotic strength there was a small and well-maintained rise in tension which was greater at greater osmotic strengths. Above this “threshold” concentration which was found to vary somewhat from fiber to fiber, a phasic tension response was obtained with peak values of several hundred micronewtons.

Gordon and Godt (1970) using small bundles of fibers also recorded transient tension responses in media twice normal osmotic strength. This type of tension response may adequately be termed a contracture. The form of the tension response to an imposed small length change indicated that the increased tension level was actively generated since the response was quite similar to that seen during submaximal potassium contractures (cf. Fig. 9 B and Fig. 11 D2). A similar suggestion was made by Isaacson (1969). He found that a solution made hypertonic by the addition of sucrose caused a rise in tension as well as an increased calcium flux. Thus, the activation might be caused by a rise in intracellular calcium concentration induced by hypertonicity. The difference in response between the whole muscle and a small preparation such as a fiber or a small bundle of fibers is not easily explained. It is probable that the difference in diffusion plays a role as has already been suggested by Gordon and Godt. The relatively slow diffusion through the whole muscle might allow some fibers to start to develop tension while others are relaxing and thus partly conceal the transient nature of the response.

It is at present not clear which component of the fiber accounts for the elastic effects seen when minute length changes are performed. It has been suggested (Hill, 1968) that these elastic effects arise in a structure located between, and hence in series with, the actin and myosin filaments. Hill tentatively identifies it with a small number of cross-bridges which are thought to form long-lived connections between the filaments in resting muscle. He furthermore proposes that in the resting fiber this interfilamentary component is somewhat extended by an "active process" and this contributes to the resting tension.

If the elastic component that is measured is arranged in series with the tension-generating sites, as Hill suggests, then its stiffness would be expected to increase as the tension increases during low-level activation. In the present experiments the opposite effect was seen, i.e. the elastic modulus decreased both when raised $[K]_o$ and caffeine were used to slightly increase the activity of the fiber. The finding cannot be explained at present. The effect could be seen within 10–15 sec after the solution change (Fig. 13) which seems to exclude the possibility that it is due to concentration changes inside the fiber. Also, measurements indicated that no change in fiber diameter took place when the E value was lowered. A possible explanation is that the elastic modulus, measured in the present manner, reflects the mechanical properties of some other component of the fiber than the filaments or the cross-bridges.

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