Short-range stiffness of slow fibers and twitch fibers in reptilian muscle

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PROSKE, UWE, AND PETER M. H. RACK. Short-range stiffness of slow fibers and twitch fibers in reptilian muscle. Am. J. Physiol. 231(2): 449-453. 1976.—The semitendinous muscle of the lizard *Tiliqua* contains both slow and twitch fibers; by subdivision of its motor nerve, fibers of each type may be stimulated separately. When, during repetitive stimulation of nerve filaments, the muscle was lengthened or shortened, the tension changes included an initial short-range stiffness, followed by a later compliance. With increasing velocities of movement, the short-range stiffness increased toward a limiting value. For slow fibers this limiting value was reached with lower velocities of movement than for the twitch fibers. Provided that the same velocity of movement was used and the movements began from similar initial isometric tensions, the slow fibers resisted the movements with a greater stiffness than the twitch fibers. It is suggested that not all of the observed differences between the two fiber types can be interpreted simply in terms of differences in rates of formation and breakdown of cross-links.

**METHODS**

Experiments were performed on the semitendinosus muscle (9) of the hindlimb of the Australian blue-tongue lizard *Tiliqua scincoides*.

The hindlimb was skinned and the overlying muscles were removed. Fine silk markers were attached to the proximal and distal tendons of the semitendinosus, and the distance between these markers was measured in different limb positions. The muscle lengths used for reference points in this paper were the length of the muscle when both hip and knee joints were at 90° (L90°), and the length when the limb was fully extended. This latter measure is taken to represent the upper limit of the physiological range of muscle lengths.

The muscle and its nerve supply were dissected free from the surrounding connective tissue, and the nerve was traced back to the nerve trunk and spinal roots. The dorsal roots with their ganglion were removed leaving the ventral roots intact. The muscle and nerve were then placed in a bath through which Ringer solution (21) flowed at a constant rate.

One muscle tendon was clamped to an isometric tension transducer (Devices Dynamometer, 2 oz); the other was clamped to an electromagnetic movement generator that consisted of a Ling-type 406 electromagnetic shaker whose displacement was controlled by both position and velocity feedback. Displacement was measured by a Shaevitz-type 300HR transducer. The total compliance of the stretcher, tension transducer, and couplings was less than 1 mm/kg.

The ventral roots were subdivided into a number of fine filaments, each of which was stimulated in turn with a pair of bipolar platinum electrodes while muscle tension was recorded. The criteria used in distinguishing "slow" and "twitch" contractions are described in **RESULTS**. Stimulation of a single filament often produced a contraction comprising a mixture of both fiber types. The filament was then subdivided further until only one type remained.

Muscle tension and length were recorded on a magnetic tape recorder (Amplex FR 1300) employing frequency modulation and later displayed on an oscilloscope for photography and measurement.

**RESULTS**

Nerve filaments supplying either twitch or slow muscle fibers were successfully dissected out in 12 animals. The tension developed on stimulation of a filament varied between experiments and represented up to 20% of the whole-muscle tension. It was generally easier to isolate filaments supplying slow fibers, and these often developed much more tension than corresponding groups of twitch fibers. It did seem as though the major...
ity of fibers in semitendinosus were of the slow type [cf. Barker (2)].

In addition to large differences in speeds of contraction and relaxation, the two types of muscle fiber could be distinguished by the following criteria: first, the slow muscle developed no measurable tension in response to a single supramaximal nerve volley; second, the twitch fibers developed their maximal tension during repetitive stimulation at about 40 pulses/s, whereas the tension in contracting slow fibers increased with increase in stimulation rate up to 100 pulses/s or more.

Figure 1 illustrates the tension developed by slow and by twitch fibers in response to stimulation of the nerve with a single shock and with a train of pulses. The slow fibers (A) developed no measurable tension in response to a single shock. After repetitive stimulation at 40 pulses/s the tension rose, gradually continuing to increase throughout the period of stimulation; at the end of stimulation the muscle relaxed slowly, taking a second or more to return to its initial value. In contrast, the twitch fibers (B) contracted vigorously in response to a single shock. Repetitive stimulation at 40 pulses/s produced a rapid rise in tension up to a steady level that was maintained throughout the tetanus; when stimulation was stopped the tension fell rapidly back to its initial level. All tension changes recorded for slow or twitch fibers resembled those illustrated in Fig. 1.

Muscle Tension During Movement

Figure 2 shows the tension changes that occurred when the muscle was tetanically stimulated and then forcibly lengthened (A and C) or allowed to shorten (B and D). The muscle length before stretch or release corresponded to the optimum for a twitch. In each part of the figure the tension changes appear above and the length changes below. For both slow and twitch fibers the tension changed in two distinct phases. The first part of the movement was always accompanied by a steep change in tension that later gave way to a more gradual change. This biphasic tension change always occurred, provided an appropriate velocity of movement was selected (14); the initial large change corresponded to the "short-range stiffness" that has been described for frog and cat muscles (5, 16, 23). In Fig. 2, A and B were obtained during stimulation of twitch fibers and C and D during stimulation of slow fibers; in each case the two phases of the tension change can be seen clearly. During lengthening the initial short-range stiffness is separated from the later compliance by a definite corner and in C this corner is further emphasized by a transitory fall in tension that follows it; this fall in tension was often seen during stimulation of the slow fibers and over the whole range of stretch rates used. It was rarely seen during stimulation of twitch fibers. A similar fall in tension has been seen during lengthening movements of cat soleus muscles when low rates of stimulation are used (14). During shortening the transition between the short-range stiffness and later compliance was sometimes quite sudden (Fig. 2B), although it was usually more gradual than during lengthening (Fig. 2D).

Length-Tension Relation

The relation between length and tension could be seen more clearly when tension was displayed as a function of length in a length-tension figure (Fig. 3). Since muscle tension depended on the initial muscle length, a series of length-tension figures was drawn each beginning from a different initial length. The isometric tension developed at each length is shown by a cross. The continuous lines, which represent the length-tension relation during movement, extend from this point upwards and to the right during lengthening or downwards and to the left during shortening. The dashed line joining the crosses represents the length-tension relation (total tension) for the isometrically contracting muscle. The filled circles indicate the passive tension developed at each initial length. The results of Fig. 3 have been taken from the experiment illustrated in Fig. 2 with the same rate and amplitude of stretch employed.
The slope of a length-tension figure indicates the stiffness with which the muscle met the length change. It can be seen in Fig. 3 that the short-range stiffness of the muscle was much larger than the stiffness given by any slope drawn from the isometric length-tension relation. The stiffness during the later part of the movement, on the other hand, was of the same order as the isometric length-tension relation, although at the longer lengths it became somewhat larger. The tension developed by both twitch and slow fibers at the end of a stretch remained well above the corresponding isometric value at that length; after following a release it remained well below the isometric value. This effect was greatest at the longer muscle lengths and seemed to be more pronounced for the slow fibers (14, 19).

**Short-Range Stiffness**

The actual value of the short-range stiffness could be measured precisely in records of either lengthening or shortening movements. During many lengthening movements the short-range stiffness ended sufficiently abruptly to produce a clear transition point or corner where it gave way to the later more gradual tension change. By measuring how far the muscle had to be lengthened to reach this corner, it was possible to determine the range of movement over which the short-range stiffness acted. The short-range stiffness depended on the type of fiber stimulated, the initial resting tension, and the velocity of the movement.

**Velocity of movement.** The short-range stiffness persisted over a larger distance when faster movements were used (Fig. 4). The fastest movements that we could usefully analyze were at about 50 mm/s; at this velocity the short-range stiffness extended over about 180 μm of the lengthening movement, which corresponded to approximately 1% extension of the muscle. With slower movements the short-range stiffness lasted for a shorter distance, and the corner on the tension record occurred after a correspondingly smaller extension. With the slowest movements, however, no clear corner could be seen, and the short-range stiffness merged gradually into the later compliance (Fig. 4A, 7 mm/s). The velocity of movement at which a corner could no longer be distinguished was correspondingly lower for the slow fibers than for the twitch fibers.

The actual resistance to the movement, as measured by the slope of the length-tension figure, increased with increasing velocity of lengthening (Fig. 4A), but there was a limit to the initial resistance that the muscle could develop, and with rapid lengthening movements the early parts of the length-tension figures appeared identical in slope (Fig. 4B). During twitch-fiber stimulation the limiting value for the short range stiffness was reached with higher rates of extension than for the slow fibers; since these rates approached the limits of the equipment used, however, the limiting value of the short-range stiffness of twitch fibers could not be measured.

1 If sarcomere lengths are known, it is possible to form some estimate of the changes in sarcomere length during movements. We therefore made measurements from longitudinal sections of different areas of a muscle that had been fixed and embedded (22) at a length equivalent to L90°. Numerous sarcomere measurements were made from this muscle and all gave values of 2.5-2.6 μm.
fibers could generate during very fast movements. The limiting value was reached during extension of slow values for the short-range stiffness that the two types of greater stiffness than the twitch fibers, and this was condition the slow fibers always resisted extension with a same velocity of movement was used. Under these conditions the slow fibers always resisted extension with a greater stiffness than the twitch fibers, and this was true at all the velocities of movement that were used.

It is of particular interest to compare the limiting values for the short-range stiffness that the two types of fibers could generate during very fast movements. The limiting value was reached during extension of slow fibers at 20–30 mm/s, but for the twitch fibers only when they were extended at 40 mm/s or more (Fig. 4). At these high velocities the short-range stiffness of the slow fibers in our experiments was greater than for the fast fibers by 50–100%.

**DISCUSSION**

The short-range stiffness of the lizard semitendinosus muscle resembles the tension changes observed during extension of frog (6, 8, 16) and cat muscles (23). In their description of tension changes in the cat soleus and gastrocnemius muscles, Rack and Westbury (23) suggested that the short-range stiffness represented the summed resistances to distortion of the cross-links between thick and thin filaments within the myofibrils. They argued that if sufficiently rapid movements were used, there would be negligible breakdown of cross-links during the initial steep tension change.

The recent experiments of Flitney and Hirst (5, 6) using the frog sartorius muscle suggest that the short-range stiffness is made up of two components. They interpret the initial tension change as due to extension of crossbridges, and the subsequent less steep region as representing backward rotation of the myosin head. At the end of the second discontinuity, which corresponds to the corner referred to by us, the muscle suddenly yields, representing detachment of cross-bridges. Although in the experiments on lizard muscle two discontinuities could not be distinguished (presumably because of the presence of elastic elements in series with the muscle fibers), a similar interpretation to that proposed for frog muscle is likely to apply.

When rapid extensions were used, the short-range stiffness of the lizard muscle lasted for about 1% of the muscle length, and this was true whichever group of fibers was stimulated. If extension occurred entirely within the sarcomeres and was evenly distributed over the whole length of the muscle fibers, it would amount to about 13 nm per half sarcomere (measured at L90°). This compares well with estimates made from the results of Huxley and Simmons (12) of the distance that frog muscle fibers can shorten from an isometric contraction without breakage of cross-links. With slower movements, more time would be available for breakdown and reformation of cross-links. Links formed during the preceding isometric contraction therefore would be likely to last for less of the movement, so that the short-range stiffness could be expected to have a lower value and to last for a shorter distance (Fig. 4).

When muscle length or rate of stimulation was altered in such a way as to increase the isometric tension, this increase was presumably a result of the formation of a larger number of cross-links. If these were then distorted by movement, they would resist with a correspondingly larger tension change, so that the initial tension and the short-range stiffness could be expected to change together (Figs. 3 and 5).

On the assumption that the initial extension occurred entirely by distortion of cross-links, and ignoring other sources of compliance, one would expect the short-range stiffness to increase in direct proportion to the isometric
tension, since both would depend on the number of cross-links made. An increase in activity of the muscle fibers that doubled the isometric tension would then double the short-range stiffness. The increase in short-range stiffness actually observed with each increment in force was smaller than one would have expected from such a simple model. This merely confirms that there are within the muscle (and within the recording system) other elastic elements in series with the muscle fibers. Similarly the stiffness would be expected to change in direct proportion to the amount of overlap between myofilaments. Such a result has been achieved by Flitney and Hirsh (4), who have succeeded in showing that for muscle lengths greater than L0 the fall in twitch tension L is accompanied by a similar fall in the elastic modulus of the short-range stiffness.

Comparison of Fast and Slow Fibers

The responses to movements of the two types of fiber were surprisingly similar. They each exhibited a short-range stiffness, and this lasted through a similar distance of movement. This similarity suggests that in the two fiber types the cross-links and perhaps also the thick and thin filaments have similar (though perhaps not identical) mechanical properties.

The most striking difference was the speed of tension development. Provided the rate of activation is not limiting (see ref. 4), the rate of rise of tension during a tetanic contraction and thus the rate of formation of cross-links is thought to be largely dependent on the activity of the actomyosin ATPase (1). Can the differences between lizard slow and twitch fibers be explained simply in terms of the kinetics of formation and breakdown of cross-links? Joyce et al. (14) concluded that the properties of cat soleus muscle stimulated at different rates could be explained in terms of rates of formation of cross-links. It is tempting to apply similar reasoning to the lizard muscle and to suggest that the slow fibers form cross-links at a relatively lower rate than the twitch fibers (see also Brown (3)).

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REFERENCES


2 Assuming that the two types of fiber within the muscle have similar tendinous attachments and that the stimulus activates the fibers along their entire length, then any differences in short-range stiffness between the two types of fiber must reflect the properties of the fibers themselves. If in a rapid movement the short-range stiffness gives a measure of the elastic resistance of the cross-links and the filaments that they unite, then comparison of the short-range stiffness during stimulation of the two types of fiber should allow one to draw some conclusions about the mechanical properties of these filaments as distinct from the rates of formation and breakdown of cross-links. If their mechanical properties were the same, then groups of fast and slow fibers, each of which generated the same isometric tension, would meet a rapid movement with the same short-range stiffness. In fact this was not so; the slow fibers were always relatively stiffer than the fast fibers, which suggests that the myosin side chains in the two different types of fiber may well have different mechanical properties. Unfortunately the rates of movement necessary to achieve the limiting conditions in which there was a maximal short-range stiffness approached the limits of our apparatus, so that some uncertainty remains. If some of the slow fibers were only activated for a part of their length, then the difference between the two fiber types would be even greater than our results suggest.