Segmental Length Changes in Stimulated Frog Sartorius Muscle during Dynamic Mechanical Responses

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Abstract The nonuniformity of dynamic mechanical responses along the length of stimulated frog sartorius muscle was examined by recording the length changes of muscle segments by means of streak photography. During isometric force development, the central or the pelvic end segment tended to shorten due to the stretching of the other segments. In muscles showing a marked nonuniformity in the segmental length changes occurring during isometric force development, there was also a marked nonuniformity in the segmental length changes occurring during stretches or releases applied at the plateau phase of an isometric tetanus. Marked nonuniform segmental length changes were also observed during afterloaded twitches and tetani. These results indicate that great care should be taken of the muscle segmental nonuniformity in heat and X-ray diffraction studies in which measurements are made using data obtained from a limited region of muscle. During the course of slow stretches or releases applied to tetanized muscles, the tibial and segment exhibited much smaller relative lengthening or shortening than the other segments when it had been appreciably stretched by the other segments during isometric force development. This might be associated with the formation of “locked-on” cross-links in the stretched segment.

Key Words: muscle contraction, muscle segment, mechanical response, cross-bridges.

Although the contractile properties of muscle can be investigated in detail using single fiber preparations, whole muscle preparations are still required for studying X-ray diffraction, heat production and nuclear magnetic resonance occurring during muscle contraction. Recent X-ray diffraction and heat measurement studies on skeletal muscle are frequently coupled with muscle mechanics techniques such as quick or controlled stretch and release. Since measurements are made on a limited region of the preparation in these studies, it is necessary to know the degree of nonuniformity of mechanical response along the entire length of muscle.

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The present experiments were undertaken to examine the segmental length changes of stimulated frog sartorius muscles by recording streak photographs during the course of various dynamic mechanical responses. A preliminary account of this work has been reported (KOBAYASHI et al., 1980).

METHODS

Preparation. All experiments were performed using the sartorius muscle of the frog Rana nigromaculata. The muscle was isolated with the pelvic bone attached, and mounted horizontally at its slack length \( L_0 \) (2.6–3 cm) with its inner surface uppermost in an acrylic plastic experimental chamber (2 × 5 × 1 cm deep) filled with Ringer solution. The tibial end of the muscle was connected to an electromagnetic vibrator (Ling, type 200), while the pelvic end was clamped to the extension of a force transducer (Fig. 1). The Ringer solution had the following composition (mm): NaCl, 115; KCl, 2.5; CaCl\(_2\), 1.8; Na\(_2\)HPO\(_4\), 2.15; NaH\(_2\)PO\(_4\), 0.85 (pH adjusted to 7.2). The temperature of the Ringer solution was maintained at \( 4\pm0.5^\circ\text{C} \) using a thermoelectric device (Yamato Kagaku, Coolnix).

Transducers. The force transducer used was a strain gauge (Shinko, U-gage) having a compliance of 1 \( \mu \text{m/g} \) and a natural frequency of oscillation of 150 Hz. The displacement transducer was a core-type differential transformer with a modulation frequency of 5.5 kHz, and was placed between the preparation and the vibrator. The transducer was linear over a range of 1 cm. The force and length changes of the preparation were simultaneously recorded on an ink-writing oscillograph (Nihon Kohden, Recticorder; frequency response, 100 Hz).

Stimulation. The muscle was stimulated with single or repetitive 1 msec rectangular current pulses through a pair of parallel Pt plates (1 × 4 cm) covering two opposite walls of the chamber, or with a multi-electrode assembly consisting of eight Pt wires serving as alternate anodes and cathodes. The current strength was about 50% above threshold. Twitches were produced at 2 min intervals, while brief tetani were produced with a 0.5–1.5 sec pulse train (20–30 Hz) at 3 min intervals. The maximum tetanic force per unit cross-sectional area was calculated for each preparation as \( P_0L_0/M \), where \( P_0 \) and \( M \) were the maximum isometric force at \( L_0 \) and the blotted weight of the preparation respectively. The values ranged from 2.2 to 2.7 kg/cm\(^2\). The experiments were terminated when the value of \( P_0 \) fell by more than 10% of the initial value.

Electromagnetic length and force control system. The electromagnetic vibrator was used either in the length control mode (for recording isometric force and for applying constant-velocity length changes) or in the force control mode (for recording isotonic length changes). The muscle was first kept isometric by using the signal from the displacement transducer for feedback control. Constant-velocity stretches or releases (0.05–5 \( L_0/\text{sec} \)) were then applied to isometrically contracting muscle (Figs. 8–13) by feeding appropriate command signals (ramp-
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Fig. 1. Diagram of experimental arrangement. The muscle was mounted horizontally between the vibrator and the force transducer with carbon markers on its surface. “Start” refers to a push button to trigger in sequence the film movement of streak camera, stimulator, and command signal for the servo system. The length and force changes of the preparation were simultaneously recorded on an ink-writing oscillograph, while the muscle segmental length changes were recorded as streak photographs on a 35 mm film. For further explanation, see text.

...and-hold voltages) to the feedback amplifier, the current output of which operated the electromagnetic vibrator. Meanwhile, the force generated by the vibrator was also controlled by exchanging the signal from the displacement transducer in the feedback loop for the signal from the force transducer, so that the muscle could be allowed to shorten or lengthen isotonically under various clamped loads to produce afterloaded contractions (Figs. 5–7). An appropriate damping of the vibrator movement was achieved by adjusting the high-frequency response of the feedback loop to an appropriate value. The above electromagnetic servo system is shown diagrammatically in Fig. 1.

Streak photography. Fine carbon particles were firmaly attached to the muscle surface (Fig. 2A) to serve as markers for recording muscle segmental length changes with a streak camera. Immediately before each recording, the muscle was illuminated with a xenon lamp (Nikon) with a heat-absorbing filter, and the whole muscle image was projected through a slit (0.3 mm wide) onto a 35 mm film (Kodak, Tri-X), by means of an optical system (Nikon, Multi-Photograph System). Thus, the streak patterns such as shown in Fig. 2B were obtained by moving the film at 2.5–5 cm/sec during the dynamic mechanical response of the preparation. A light-emitting diode to which rectangular voltages (10–
20 Hz) from an oscillator were fed, was placed near the edge of the film to serve as time marker. The time course of segmental length changes of the preparation was determined by measuring the distance between the carbon markers on enlarged streak photographs (10–12 ×). In most cases, the entire muscle length was divided into three nearly equal segments, i.e., the central segment and the two end segments excluding tendons, and their length changes were compared under various conditions (Figs. 3–13).

For each type of experiment performed, more than ten streak photographs were taken using more than five different preparations.

RESULTS

Segmental length changes during an isometric twitch or tetanus

Figure 3 is a typical result showing length changes of the central and the two end segments during an isometric twitch. In most cases, the central or the pelvic end segment tended to shorten by 2–3% of the initial length to stretch the other segments, especially the tibial one. In a few cases, however, the tibial end segment shortened to stretch the other segments. The shortening or lengthening of the segments was maximum at the peak of twitch force, and did not exceed 3–4% of the initial length.

As shown in Fig. 4, qualitatively similar segmental length changes were observed during an isometric tetanus. Though the segmental length changes were most marked during the rising phase of isometric force, they still tended to proceed slowly during the plateau phase of an isometric tetanus. The maximum relative segmental shortening or lengthening during a 0.5–1.5 sec isometric tetanus differed from preparation to preparation, and did not exceed 5–6% of the initial length.
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Fig. 3. Segmental length changes in frog sartorius muscle during an isometric twitch. In this and subsequent figures, the muscle was divided into three segments of nearly equal lengths, i.e., the tibial end segment (open circles), the central segment (filled circles), and the pelvic end segment (dotted circles) as illustrated in the inset. Bottom trace shows isometric twitch force.

Fig. 4. Segmental length changes during an isometric tetanus. Bottom trace shows isometric tetanic force. Note that the central segment shortens appreciably on stretching the other segments.

Segmental length changes during an afterloaded isotonic twitch or tetanus

Typical examples of the segmental length changes during afterloaded twiches and tetani are shown in Figs. 5 and 6. The amount of afterload ranged from 0.1 to 0.8 $P_0$. Though all the segments were observed to shorten simultaneously,
Fig. 5. Segmental length changes during an afterloaded isotonic twitch. The amount of afterload was 0.25 $P_0$. In this and subsequent figures, force and total muscle length changes are also shown in the lower part.

Fig. 6. Segmental length changes during an afterloaded isotonic tetanus. The amount of afterload was 0.22 $P_0$. Note that the velocity and the extent of isotonic shortening are nonuniform among the segments.

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the velocity and the extent of isotonic shortening differed from segment to segment. The nonuniformity in the extent of isotonic shortening among the segments was more marked during isotonic tetani than during isotonic twitches. As with isometric contraction, the extent of isotonic shortening tended to be largest in the central or the pelvic end segment and smallest in the tibial end segment; in the case of afterloaded tetani under a relatively small load of 0.2-0.25 P₀, the central or the pelvic end segment shortened by about 35-40% while the tibial end segment shortened by 30% or less. Under a large load above 0.6 P₀, the segmental shortening was extremely nonuniform in some preparations; as shown in Fig. 7, the tibial end segment did not shorten appreciably while the pelvic end segment shortened by 17%.

Segmental length changes during controlled stretches and releases applied at the plateau of an isometric tetanus

The muscles were first tetanized isometrically, and when the steady isometric force was reached, they were subjected to constant-velocity stretches or releases at 0.1-0.2 L₀/sec. It was found that the degree of nonuniformity of the segmental length changes during the constant-velocity stretches or releases were closely associated with those during the preceding isometric tetanus. In preparations with a small degree of nonuniformity in the segmental length changes occurring during the preceding isometric tetanus, the segments showed fairly uniform length changes in response to the subsequent constant-velocity stretches or releases (Figs. 8 and 9).

In preparations with a marked nonuniformity of segmental length changes
Fig. 8. Fairly uniform segmental length changes during a slow stretch applied at the plateau of an isometric tetanus. The velocity and the extent of stretch were 0.2 $L_0$/sec and 4%, respectively. Note a fairly uniform segmental length changes during the preceding isometric tetanus.

Fig. 9. Fairly uniform segmental length changes during a slow release applied at the plateau of an isometric tetanus. The velocity and the extent of release were 0.2 $L_0$/sec and 4%, respectively. Note a fairly uniform segmental length change during the preceding isometric tetanus.
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Fig. 10. Nonuniform segmental length changes during a slow stretch applied at the plateau of an isometric tetanus. The velocity and the extent of stretch were 0.13 $L_0$/sec and 3%, respectively. Note that the tibial end segment, which has been stretched by the other segments during the preceding isometric tetanus, exhibits much smaller relative lengthening than that of the other segments.

Fig. 11. Nonuniform segmental length changes during a slow release applied during the plateau of an isometric tetanus. The velocity and the extent of release were 0.13 $L_0$/sec and 3%, respectively. Note that the tibial end segment, which has been stretched by the other segments during the preceding isometric tetanus, shows much smaller relative shortening than the other segments.

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during the preceding isometric contraction the segmental length changes during the subsequent constant-velocity stretches and releases also showed a marked nonuniformity (Figs. 10, 11). It can be seen that the tibial end segment, which had been stretched appreciably by the other two segments during the preceding isometric tetanus, exhibited much smaller length changes than the other two segments during the course of the constant-velocity stretch (Fig. 10) or release (Fig. 11). Similar results were always observed on the four other preparations examined.

**Segmental length changes following quick stretches and releases applied at the plateau of an isometric tetanus**

Examples of the experiments, in which the muscles were stretched or released quickly at 2–4 L0/sec at the plateau of isometric tetanus, are shown in Figs. 12 and 13. The relative amount of quick lengthening or quick shortening at the time of a quick stretch or a quick release (1.5–3%) appeared to be fairly uniform in all the segments (STIENEN and BLANGE, 1981), even in preparations with an appreciable segmental nonuniformity during the preceding isometric tetanus. On the other hand, the segmental length changes after a quick stretch or a quick release were definitely nonuniform in preparations with an appreciable segmental nonuniformity during the preceding isometric tetanus; the degree of nonuniformity was more marked during the redevelopment of isometric force following a quick release (Fig. 13) than during the decay of isometric force following a quick stretch.

![Fig. 12. Segmental length changes following a quick stretch applied at the plateau of an isometric tetanus. The velocity and the extent of stretch were 3 L0/sec and 3%, respectively.](image-url)
Fig. 13. Segmental length changes following a quick release applied at the plateau of an isometric tetanus. The velocity and the extent of release were 3 L0/sec and 3%, respectively. Note that the central segment, which has shortened by stretching the other segments during the preceding isometric tetanus, also shortens by stretching the other segments following the quick release.

(Fig. 12). In both cases, the segment which had shortened by the stretching of the other segments during the preceding isometric tetanus, also shortened by stretching the other segments after the completion of a quick stretch or a quick release.

DISCUSSION

Nonuniformity of mechanical response in stimulated muscle. Local movements in frog sartorius muscle during an isometric tetanus were first examined by Jewell and Wilkie (1958) by microscopic observation of carbon markers on the muscle surface; these workers found a considerable variation of the global movements from one muscle to another when the muscles were tetanized at L0. Recently, the local movements in tetanized frog sartorius muscle have been studied by Stienen and Blangé (1981) using an electronic marker-follower system to examine the nonuniform distribution of muscle compliance along its entire length. In the present study, the segmental length changes in stimulated frog sartorius muscle were investigated by use of streak photography not only during isometric contraction but also during various dynamic mechanical responses.

In the case of isometric twitch and tetanus, the present results confirmed the previous reports on frog sartorius muscle that, during the development of isometric force, the length of each muscle or muscle fiber segment is not kept isometric; some segments shorten on the stretching of others (Jewell and Wilkie,
1958; STIENEN and BLANGÉ, 1981). Similar results have been reported on dog papillary muscle (SUGI and KAMIYAMA, 1977; KRUEGER and POLLACK, 1975) and Ca-activated skinned frog muscle fibers (JULIAN and MOSS, 1980). When a tetanized muscle excluding tendons was divided into three segments of nearly equal length, the central or the pelvic end segment tended to shorten on stretching the other segments, and the resulting nonuniform segmental length changes were more marked during an isometric tetanus than during an isometric twitch (Figs. 3, 4). The maximum relative segmental length changes during an isometric tetanus were within 5-6%, in agreement with the local length changes of up to 6% observed by STIENEN and BLANGÉ (1981).

During the course of an afterloaded twitch or tetanus, the velocity and the extent of isotonic shortening differed from segment to segment, though all the segments were observed to shorten simultaneously (Figs. 5, 6). Such a nonuniform segmental shortening was sometimes extremely marked when the amount of afterload was more than 0.6 P0 (Fig. 7). Similarly, the segmental length changes could be markedly nonuniform during the course of controlled slow length changes (Figs. 10, 11) or following the quick length changes (Figs. 12, 13), depending on the degree of nonuniformity of segmental length changes occurring during the preceding tetanus. These results should be taken into consideration when X-ray diffraction or heat measurement studies are performed during the dynamic mechanical responses of the whole muscles.

In the case of intact single fiber preparations, on the other hand, the segmental nonuniformity during isometric contraction can be made very small if the fibers are uniformly stimulated at L0 along the entire length (CLEWORTH and EDMAN, 1972). At lengths above L0, however, a marked segmental nonuniformity takes place during both isometric contraction and isotonic shortening due to nonuniform sarcomere length distribution along the fiber length (HUXLEY and PEACHEY, 1961). Thus, even in single fiber preparations, the segmental nonuniformity in the mechanical response should be seriously considered to obtain more precise results on muscle mechanics.

Segmental length changes in tetanized muscle during slow stretches and releases. It has long been known that, when a tetanized muscle is stretched, the steady isometric force attained after the completion of stretch is higher than the normal isometric force at the same muscle length, though the amount of overlap between the myofilaments decreases on stretching (ABBOTT and AUBERT, 1952; SUGI, 1972). This phenomenon is effective in preventing an extreme inhomogeneity of segmental lengths during isometric force development, since the stretched sarcomeres can exert more force to resist the pull from the shorter sarcomeres. Heat measurement studies indicate that the net energy output of a contracting muscle (heat+work) can be made negative during a slow stretch (FENN, 1924; ABBOTT and AUBERT, 1951; HILL and HOWARTH, 1959). According to CURTIN and DAVIES (1972), the rate of ATP splitting is considerably reduced with slow stretches.

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Thus, it has been suggested that, when the cross-bridges are displaced in a direction opposite to that of muscle shortening, they tend to be “locked-on” so that they remain attached to the thin filaments in the displaced configuration, only detaching if the stress exceeds a certain value (H. E. Huxley, 1960).

In this connection, it is of interest that, during controlled slow stretches or releases applied to a tetanized muscle, the tibial end segment exhibited much smaller relative lengthening (Fig. 10) or shortening (Fig. 11) than the other two segments, when it had been appreciably stretched by the two segments during the preceding isometric tetanus. The smaller amount of relative lengthening in the tibial end segment than in the other two segments may be taken to indicate that the tibial end segment can be lengthened less readily than the other segments when it has been stretched by the other segments during the period of isometric force development. Meanwhile, the smaller amount of relative shortening in the tibial end segment may result from the fact that the tibial segment cannot shorten as fast as the other segments when it has been stretched by the other segments. Thus, these phenomena may be accounted for in terms of the locked-on cross-links in the stretched segment, since they are expected to increase the resistance of the segment to stretch and to decrease the velocity of shortening of the segment.

The presence of the locked-on cross-links can be ascertained in the future by comparing the rate of heat production and the X-ray diffraction among the segments of tetanized muscles.

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