CORRELATION BETWEEN FIBER LENGTH, ULTRASTRUCTURE, AND THE LENGTH-TENSION RELATIONSHIP OF MAMMALIAN SMOOTH MUSCLE

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ABSTRACT

The length-tension relationship was determined for strips of guinea pig taenia coli and correlated with the length and ultrastructural organization of the component fibers. The mean fiber length in "stretched" strips (passive ≥ active tension) was 30% greater than that for fibers in "unstretched" strips (active ≥ passive tension). In stretched fibers the dense bodies and 100 A diameter myofilaments were consolidated into a mass near the center of fibers in cross-sectional profile. The thick myofilaments were segregated into the periphery of the fiber profiles. In unstretched fibers the dense bodies–100 A diameter filaments and the thick myofilaments were uniformly distributed throughout cross-sectional profiles. A tentative model is proposed to account for the change in fiber length and ultrastructural organization that accompanies stretch. The basic features of the model require the dense bodies to be linked together into a network by the 100 A diameter filaments. The functional consequences of stretching the fibers are discussed in relation to the model proposed for this network.

INTRODUCTION

The length-tension relationship of vertebrate smooth muscle is very similar to that observed for striated muscle (1–6), although there is no common structural basis upon which the observed similarities in mechanical behavior can be based. The active force developed by striated muscle fibers is directly proportional to the extent of overlap between arrays of actin- and myosin-containing filaments (7, 8). In contrast, the myofilaments of smooth muscle fibers do not form comparably organized arrays (9, 10), and as yet any relative movements between the actin- and myosin-containing filaments have not been characterized in detail or related in any way to the mechanical properties of smooth muscle. We have correlated changes in the length and tension of smooth muscle strips with changes in the length of the component fibers and the ultrastructural organization of their contractile elements in order to investigate the structural basis of the length-dependent mechanical properties of smooth muscle. Stretching the strips leads to an increase in the length of the fibers and to segregation of the thick myofilaments and dense bodies into peripheral and central zones of the sarcoplasm, respectively. These morphological changes are found over a range of lengths where active tension is a function of length.
FIGURE 1 Length-tension relationship of a strip of taenia coli. Passive tension was measured in the presence of epinephrine and total tension was measured at the peak of a contraction induced by high K\(^+\). Active tension = total tension minus passive tension. Stretched and unstretched strips were fixed at lengths where active tension and passive tension were within the limits shown by the arrows.

METHODS

Guinea pigs weighing between 400 and 500 g were sacrificed by a blow on the head. Strips of taenia coli, having a length of 2 cm in situ, were excised and incubated in Krebs-Ringer bicarbonate saline (143.3 mm Na\(^+\), 6 mm K\(^+\), 1.2 mm Mg\(^{2+}\), 1.3 mm Ca\(^{2+}\), 126.4 mm Cl\(^-\), 24.3 mm HCO\(_3\)\(^-\), 1.2 mm H\(_2\)PO\(_4\)\(^-\), 1.2 mm SO\(_4\)\(^{2-}\), 5.9 mm glucose) equilibrated with 95% oxygen, 5% carbon dioxide at 37°C. Strips were mounted in the organ bath at their in situ length, and changes in isometric tension were monitored with a FT03 force transducer and Grass polygraph (Grass Instrument, Quincy, Mass.). Only those preparations showing spontaneous, phasic contractile activity were utilized in these experiments.

Length-Tension Analysis

Changes in passive and active tension were determined as a function of the length of strips according to the methods of Åberg and Axelsson (1965). Passive tension was measured after the addition of 0.01 mg/ml of epinephrine which reversibly abolished spontaneous activity. Strips were activated with Krebs-Ringer's in which the sodium salts were substituted by potassium on a molar basis. After a steady-state tension was reached in response to high potassium, the strips were again relaxed in normal Krebs-Ringer's containing epinephrine and the length of the strips then changed by 2 or 3 mm. When a stable passive tension was obtained (approximately 5 min), the cycle was repeated.
Fixation

Strips were fixed at a given length, after obtaining their length-tension relationship, by rapid injection of 10 ml of 24.5% glutaraldehyde into the incubation bath (70 ml) to give a final concentration of 3%. Strips were routinely fixed during relaxation, although a few were also fixed during the peak of a contraction. Some strips were incubated in Krebs-Ringer's containing ethylenediaminetetraacetate (EDTA) for 1 hr before fixation with glutaraldehyde.

Ultrastructure

Muscle strips were fixed for 24 hr in glutaraldehyde-Krebs-Ringer bicarbonate saline, transected into 2-mm long segments, washed in buffer for 30 min, postfixed in 1.75% osmium tetroxide in 0.1 M collidine (pH 7.3) for 1 hr, and washed briefly in distilled water. Segments were then stained en bloc with 2% uranyl acetate for 1 hr, dehydrated in graded ethanol solutions, and embedded in Epon.

Fiber Lengths

Muscle strips were fixed for 24 hr in glutaraldehyde and then suspended in 25% KOH (w/v) for 16 hr. Single fibers were obtained by gently agitating the suspension. The length of intact single fibers was determined in a phase contrast optical microscope with an ocular micrometer.

RESULTS

Length-Tension Relationship

The length-tension relationship of a strip of taenia coli is shown in Fig. 1. A similar length-tension relationship was observed in all preparations studied and closely resembles the pattern observed by Åberg and Axelsson (1965). The dependence of active and passive tension on length can be divided into two general phases. In the first phase, active tension increases rapidly with only slight changes in passive tension. The second phase is characterized by a decrease in active force and a rapid rise in passive tension. The strips used in the morphological studies were taken from the opposite, extreme ends of each phase. “Unstretched strips” were taken at lengths in the first phase where active tension exceeded passive tension by an order of magnitude. “Stretched strips” were obtained at lengths in the second phase where passive tension equaled or exceeded active force.

Fiber Lengths from Unstretched and Stretched Strips

The length of isolated fibers from muscle strips macerated with potassium hydroxide was measured in order to establish that changes in the length of the entire strip were transmitted to the individual fibers. Typical fibers obtained after

Figure 3  Distribution of length of fibers within strips of unstretched and stretched taenia coli. Lengths were determined on 100 cells in each of four KOH macerated strips, two of which had been stretched and the other two unstretched. The data from the stretched strips were pooled, as were those from the unstretched strips. Mean fiber lengths for unstretched and stretched strips were 319 ± 4 μ (SEM) and 435 ± 6 μ (SEM), respectively.

1 Abbreviation: EDTA, ethylenediaminetetraacetate.
Figure 4  Cross-sectional profile of a smooth muscle fiber from an unstretched strip showing uniformly distributed thick (T) and thin (arrows) filaments, and the widely distributed dense bodies (DB).  × 28,500.
Cross-sectional profile of a smooth muscle fiber from a stretched strip showing the segregation of the dense bodies (DB) into a central region of the profile and the peripheral localization of thick filaments (T). $\times 28,500$. 

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FIGURE 6 Portions of three fiber profiles from an unstretched strip showing thick filaments ($T$), lattice-like arrays of thin filaments (arrows), and 100 A filaments principally around the cross-sectional profiles of dense bodies ($DB$). $\times$ 57,000.

FIGURE 7 Portion of a fiber profile from a stretched strip showing the central region of the fiber containing consolidated and single dense bodies ($DB$) with attendant 100 A filaments. Thick filaments ($T$) and lattice-like arrays of thin filaments (arrows) occupy the surrounding area. $\times$ 57,000.
KOH maceration of stretched and unstretched strips are shown in Fig. 2. The distribution of fiber lengths in unstretched and stretched strips is plotted in Fig. 3. The mean length of fibers from two unstretched strips is $319 \pm 4 \mu$ (SEM), and that obtained from two stretched strips is $435 \pm 5 \mu$ (SEM). Despite some overlap of fiber lengths observed within the two population samples, the difference in mean fiber length is highly significant ($P < 0.001$). Fiber length determinations on paired strips fixed in either the stretched or the unstretched state yield similar distributions. Mean fiber lengths of $372 \pm 9 \mu$ (SEM) and $390 \pm 10 \mu$ were obtained for two strips fixed at the point in their length-tension curve where active tension and passive tension were equal.

Ultrastructure of Unstretched and Stretched Fibers

The ultrastructure of fibers in unstretched and stretched strips was examined in order to relate the changes in fiber length to changes in the organization of the contractile apparatus. Unstretched fibers in cross-sectional profile show uniformly distributed thick and thin myofilaments, and randomly distributed dense bodies and 100 A diameter myofilaments (Figs. 4 and 6). In contrast, the myofilaments and dense bodies of stretched fibers are segregated into different regions of the cross-sectional profile. The thick myofilaments are limited to an outer ring surrounding a central area containing closely packed dense bodies. The dense bodies in the central area are consolidated into large amorphous groups. The 100 A filaments are also localized within this region (Figs. 5 and 7). The thin myofilaments are found in both central and peripheral areas. Although in some stretched fibers the thick myofilaments are not observed, the segregation of dense bodies and 100 A filaments into the central area is nevertheless apparent. The peripheral area in these cases is occupied by thin myofilaments alone.

The segregation of dense bodies and filaments observed in stretched strips is unrelated to the contractile activity. It is found in fibers of strips fixed during relaxation as well as at the peak of a contraction. The segregation of dense bodies and 100 A filaments is found only in fibers of stretched strips. A small fraction of the fiber profiles (around 10%) in stretched strips do not, however, show the characteristic segregation of these components.

To clarify the relationship between dense bodies and 100 A filaments, unstretched and stretched strips were incubated in the presence of 5 mM EDTA. The thick and thin myofilaments are selectively labile under these conditions, leaving the 100 A filaments and dense bodies as the major filamentous component in the sarcoplasm. Under these conditions, the difference in the distribution of dense bodies and 100 A filaments in unstretched and stretched fibers is very clearly demonstrated. In unstretched fibers the dense bodies and 100 A filaments are widely distributed within the profile of the fiber (Fig. 8), while in stretched fibers the dense bodies and the 100 A filaments are consolidated in the central region (Figs. 9 and 10); this segregation is also seen on the longitudinal axis of fibers in appropriately oriented sections (Figs. 11 and 12). The difference in the pattern of distribution with EDTA is observed in the absence of both thick and thin myofilaments.

Discussion

This study indicates that alterations in the length of a strip of smooth muscle lead to changes in the length of the component fibers and to specific changes in the distribution of the components of the contractile apparatus within the individual fibers. Differences in the pattern of distribution of dense bodies and filaments in fibers of unstretched and stretched muscle strips appear to result from changes in fiber length. The segregation of these components could be correlated only with stretching of the strip and was unrelated to contractile activity at the time of fixation.

virtually all the fiber profiles in unstretched strips contain uniformly distributed myofilaments and dense bodies. Segregation of dense bodies and filaments was consistently observed in fiber profiles in stretched strips; however, a small number of profiles did not show the characteristic segregation and, in this way, they resembled profiles from unstretched fibers. This variation may be due to the presence of unstretched fibers within stretched strips; this interpretation is consistent with the overlap in fiber lengths observed in the two population samples (Fig. 3).

Regardless of the pattern of distribution of dense bodies and 100 A filaments within a fiber profile, these two elements are always found together. This observation could be explained if the dense bodies and 100 A filaments were structurally
interconnected. Direct structural connections between dense bodies and 100 A filaments have been observed in preparations of isolated dense bodies (11), and these connections are also implied by numerous studies in which thin sections of embedded smooth muscle have been used (12-15).

The segregation that occurs upon stretching suggests that the dense bodies–100 A filaments form relatively inelastic three-dimensional network(s). A model for this network that would explain the segregation of dense bodies–100 A filaments upon stretching the fiber is illustrated in Fig. 13. An important feature of this model is that the dense bodies are interconnected by relatively inelastic 100 A filaments into networks whose components have a common point of attachment at the plasma membrane. Using this model, the peripheral distribution of thick myofilaments obtained upon stretching the fibers could result from their exclusion from areas occupied by dense bodies–100 A filaments. Alternatively, the peripheral localization of thick myofilaments could be obtained upon stretch if they were arranged with attachments localized evenly along the plasma membrane and excluded specifically from the vertices of the fiber. In this case consolidation of dense bodies–100 A filaments could occur by exclusion from the peripheral areas. Another possibility is that the movements are directed by networks of both segregating components. The last two mechanisms are not favored, however, because the consolidation of dense bodies–100 A filaments exists even in profiles of fibers where both thick myofilaments and thin myofilaments (EDTA) are absent. Moreover, there is no evidence that thick myofilaments are either directly or indirectly inserted upon the plasma membrane. There are a number of other models that could result in the observed segregation of components that occurs upon stretching, but they require assumptions for which there are no bases at present.

According to the model proposed for the network of dense bodies–100 A filaments, the consolidation of dense bodies upon stretching the fiber occurs through a reorientation of inelastic links connecting these elements. Once the links are oriented parallel to the long axis of the network, further extension would be resisted. Hence, passive tension will rise rapidly as the network approaches full extension. This model could explain, at least in part, the rise in passive tension observed in smooth muscle strips that are highly stretched. Finally, in view of the relationship of thin myofilaments with dense bodies (16, 17), it is possible that the consolidation of dense bodies and the peripheral localization of thick myofilaments that occur upon stretching may influence the ability of the contractile filaments to effectively interact within the stretched fiber. Further analysis of the possible role of consolidation and segregation of dense bodies and myofilaments on the length-dependence of active force development must await a detailed description of thick and thin filament polarity and the extent of filament overlap at different degrees of stretch.

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**Figure 8** Cross-sectional profile of a fiber from an unstretched strip after treatment with EDTA. The dense bodies (DB) are widely distributed. Thick filaments and lattice-like arrays of thin filaments are not present. The 100 A filaments are principally found around the dense bodies, but single and small groups are widely distributed. X 28,000.

**Figure 9** Cross-sectional profile of a fiber from a stretched strip after treatment with EDTA. The dense bodies (DB) and most of the 100 A filaments (arrows) are segregated into a central area. Thick filaments and lattice-like arrays of thin filaments are not present in the peripheral areas. Instead, these areas contain a variable amount of flocculent-appearing material. X 28,000.
FIGURE 10 Small area of Fig. 9 enlarged to show the presence of amorphous density and profiles of 100 A filaments in the central area (arrows), and the flocculent-appearing material in the peripheral area inside the plasma membrane (pm). × 100,000.
Figure 11. Longitudinal section of a portion of an unstretched EDTA-treated fiber showing the widely distributed dense bodies (DB) and 100 Å filaments. × 15,800.

Figure 12. Longitudinal section of stretched, EDTA-treated fibers showing a portion of the longitudinal extent of the segregated dense bodies (DB) and 100 Å filaments. × 9000.
Figure 13. Schematic representation of a model for the arrangement of dense bodies (dark circles) within smooth muscle fibers of taenia coli. The dense bodies in A and B are shown connected by inelastic 100 Å filaments (dark straight lines) into a network anchored at opposite ends of the fibers. Stretching causes an increase in fiber length at C and D and it leads to consolidation of the network as a result of reorientation of the filaments linking the dense bodies.

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