

Plasticity in Canine Airway Smooth Muscle

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ABSTRACT The large volume changes of some hollow viscera require a greater length range for the smooth muscle of their walls than can be accommodated by a fixed array of sliding filaments. A possible explanation is that smooth muscles adapt to length changes by forming variable numbers of contractile units in series. To test for such plasticity we examined the muscle length dependence of shortening velocity and compliance, both of which will vary directly with the number of thick filaments in series. Dog tracheal smooth muscle was studied because its cells are arrayed in long, straight, parallel bundles that span the length of the preparation. In experiments where muscle length was changed, both compliance and velocity showed a strong dependence on muscle length, varying by 1.7-fold and 2.2-fold, respectively, over a threefold range of length. The variation in isometric force was substantially less, ranging from a 1.2- to 1.3-fold in two series of experiments where length was varied by twofold to an insignificant 4% variation in a third series where a threefold length range was studied. Tetanic force was below its steady level after both stretches and releases, and increased to a steady level with 5–6 tetani at 5 min intervals. These results suggest strongly that the number of contractile units in series varies directly with the adapted muscle length. Temporary force depression after a length change would occur if the change transiently moved the filaments from their optimum overlap. The relative length independence of the adapted force is explained by the reforming of the filament lattice to produce optimum force development, with commensurate changes of velocity and compliance.

INTRODUCTION

It has been recognized for a long time that smooth muscles can function over a long range of length (Winton, 1926; Evans, 1926 [p. 371]; Uvelius, 1976) as would be required by the large volume changes of some hollow viscera, such as bowel, bladder, and uterus. Uvelius (1976) found a sevenfold length range for the muscles of the urinary bladder, and showed that the overall muscle length change reflected changes of individual cell length. This long working range would be difficult to accommodate by the fixed array of filaments found in skeletal muscle. A possible explanation is that the filament array adapts to different lengths by varying the number of contractile

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units in series. Because the filament arrangement in smooth muscle has been difficult to interpret in structural studies (see Discussion), we investigated the functional effects of length changes on contractile performance to gain some suggestion of the types of structural alterations that might occur. To test for variations in the number of contractile units in series we have compared the muscle length dependence of compliance and shortening velocity with the length dependence of isometric force. Because each contractile unit in series contributes to muscle length changes, both compliance and velocity will vary directly with the number of units in series. On the other hand, the isometric force is experienced by each cross-sectional unit of the muscle, and so should not vary with the number of such units in series. In the absence of a change in the number of active contractile units in the cross-section, velocity and compliance should depend on the number of units in series while force should not be affected. Although it is not known to have a long working length, dog tracheal smooth muscle was studied because of its favorable architecture. It has very little connective tissue and its cells are arrayed in long, straight, parallel bundles that span the length of the preparation (Stephens, Kroeger, and Mehta, 1969). The paucity of connective tissue minimized the corrections for the passive elastic characteristics of the preparation while the long straight bundles minimized the effect of series compliance.

A brief description of this work has been presented to the Biophysical Society (Pratusevich, Seow, and Ford, 1994.)

MATERIALS AND METHODS

Mongrel dogs were killed with an overdose of pentobarbital and the tracheae removed immediately and stored in cold physiological saline for 16 to 64 h before use. Small strips of muscle measuring ~ 4 mm long \times 0.3 mm wide \times 0.1 mm thick were dissected in the morning of each experiment, which lasted ~ 8 –9 h. The muscles were gripped by aluminum foil clips (Ford, Huxley, and Simmons, 1977) used to attach them to the apparatus. Muscles were studied in a covered, heated, horizontal chamber originally designed for cardiac muscle studies (Chiu, Walley, and Ford, 1989). One end of the muscle was attached to a photoelectric force transducer with a resonant frequency of 5 kHz (Chiu, Asayama, and Ford, 1982); the other end was attached to a linear servo motor. Data were recorded digitally using an IBM PC-AT equipped with a Tecmar (Solon, OH) Labmaster computer interface board and the SALT software developed in our laboratory (Fenster and Ford, 1985).

All experiments were done at 37°C in a physiological salt solution at pH 7.4 bubbled with 5% CO₂, 95% O₂ and containing (in millimolar) NaCl 118, KCl 5, NaH₂PO₄ 1.2, NaHCO₃ 22.5, MgSO₄ 2, and CaCl₂ 2. Each muscle was allowed to adapt to the experimental conditions for ~ 1 h while being stimulated to produce 11–12 s tetani at 5-min intervals. During this adaptation period the reference length was determined.

Muscle Length

When muscle force changes the compliant tissue in series with the contractile element will change length. Much of this series elasticity resides in the crushed tissue at the ends of the preparation. To account for these changes in contractile element length, the length of a central segment to the muscle was measured and adjusted. Stripes of carbon granules were painted near each end of the muscle to mark the end of a central segment. An eyepiece reticle in a dissecting microscope was used to measure both the overall, clip-to-clip muscle length and the

central segment length measured as the distance between selected carbon granules in the two stripes. At the outset of the experiment, the length at which steady passive force was maintained at 1–2% of isometric force was determined. Two reference lengths were distinguished. The first, designated L_m , was the overall muscle length at which the 1–2% rest force was maintained. The second, designated L_{ref} , was the central segment length achieved at the plateau of an isometric tetanus when the overall muscle length was set to L_m .

Central segment length at the plateau of the tetanus was the length that was always measured and adjusted when setting the operating length. Wherever possible, the muscle length was normalized to L_{ref} . Because the central segment length was not measured electronically, such normalization was not possible when the muscle length was being changed rapidly by the servo motor during isotonic shortening and oscillations. In those instances, the length change imposed by the servo motor was normalized to L_m .

Protocols

Muscles were stimulated every 5 min to produce sustained tetani with brief (~ 0.5 ms) electrical pulses of alternating polarity at 60 Hz for 11–12 s, until a plateau of force was reached (Fig. 1 *a*). Immediately before stimulation ended, the muscle was subjected either to an isotonic step used to measure the force-velocity properties (Fig. 1 *c*), or to a low amplitude, 500 Hz oscillation used to measure compliance (Fig. 1 *b*). The amplitude of the length oscillation, $\sim \pm 0.2\%$ L_m , was adjusted so that the force oscillation was ± 10 –13% isometric force at the reference length.

After a “running-in” period, the compliance and force-velocity properties were measured in sets at three lengths (Fig. 2). After six contractions used to adapt the muscle to each new length, a full set of measurements included an initial two contractions with oscillations to measure compliance, 9–12 contractions with isotonic steps to measure force-velocity properties, and a final two contractions to measure compliance again. Full sets of measurements were made at the reference length both at the beginning and at the end of the experiments, with full sets made at a short and a long length between these reference measurements. In addition to these full sets, a short set of measurements consisting of two contractions with oscillations was made after six adapting contractions at the reference length, in the middle of the experiment, between the measurements at the long and short lengths. This was done to prorate more accurately the changes of isometric force and compliance without greatly prolonging the experiment.

Most of the data to be presented were collected in two final series of experiments of nine muscles each. In the first series, the muscles were studied at lengths of L_{ref} , $1.5 L_{ref}$, and $0.75 L_{ref}$, for a twofold range of length. In the second series, the long length was established as the point where rest tension was 10–20% of isometric force and beginning to rise steeply, and the short length was adjusted to be one third of this value, for a threefold range of length. In the second series, the long length was always studied first because this length had to be determined before the short length could be adjusted. In the first series the order of length studied was alternated, with the short length studied first in four muscles and the long length studied first in five.

The purpose of measuring compliance both before and after the isotonic series and of studying the short or long length first in alternate muscles was to look for systematic changes that might have resulted from the order of interventions. None were detected.

In the course of the experiments, it was found that rapid isotonic shortening at low loads reduced the isometric force by a few percent in the subsequent contraction, while the oscillations and slow shortening at high loads had no effect. To minimize the depressant effect of rapid shortening two additions were made to the protocol: (*a*) an additional nonshortening contraction was imposed immediately after a contraction that included rapid shortening and (*b*)

in the second series of experiments on velocity (designated the threefold series) the period of isotonic shortening was ended immediately after the velocity measurement period (116 ms after the release).

Correction for Passive Force

As isometric force develops during a tetanus, the extension of the tissue at the ends of the muscle causes some internal shortening. When the muscle is extended, so that it bears tension at rest, some of the load borne by the passive elements is transferred to the contractile elements

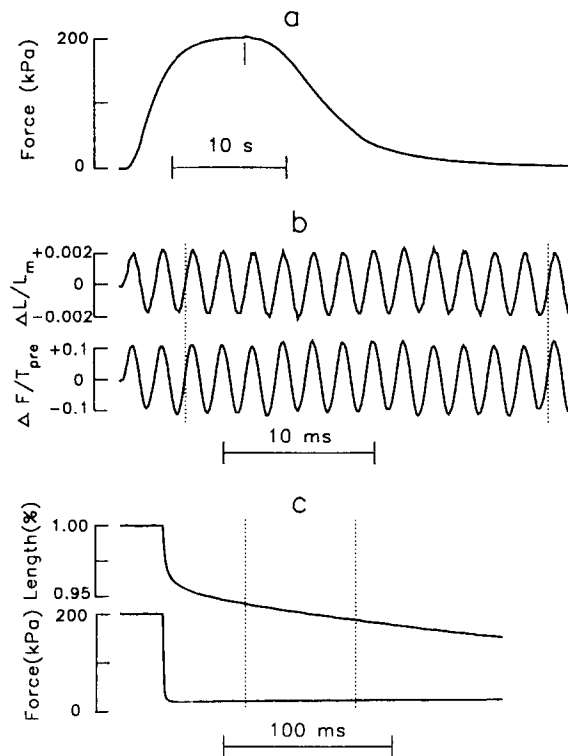


FIGURE 1. Procedures used for dynamic measurements. The muscles were stimulated electrically for 11–12 s until a force plateau was achieved (*a*). Immediately before the end of stimulation (*cursor in a*) either a 500-Hz oscillation was applied for 30 ms (*b*) or the muscle was released to an isotonic load (*c*). The oscillations were timed so that they always began at the same point in the oscillatory cycle, just as the command pulse was passing zero, so that there was not an abrupt step as the oscillations began. Compliance was measured over the last 12 cycles, between the cursors in *b*. Velocity was measured from a tangent fitted to the length record over the period between 50 and 115 ms after the release, between the cursors in *c*. Isotonic force was measured over the same interval.

Data was collected digitally. The digitization rate was 10 Hz for the slow records in *a*, 15 kHz for the oscillatory records in *b*, and 1 kHz for the isotonic records in *c*. A small phase advance of $\sim 70 \mu\text{s}$ can be seen in the tension records in *c*. Of this, $30 \mu\text{s}$ was due to the digitization procedure; length was digitized first and tension second. The remaining phase advance, $40 \mu\text{s}$, equivalent to 2° , is attributed to relaxation in the preparation. Experiment of 7/28/93

during this internal shortening. An additional load is transferred when the muscle shortens isotonicly. Exact estimates of the force generated by the contractile elements requires correction for both types of transferred load. Both corrections were determined from steps applied to the passive muscle. The load transferred during the rise of tetanic force was determined as the force drop produced by a step that would shorten the central segment of the resting muscle to the length achieved during force development. The force drop was measured 9 s after the step was applied so as to approximate time-length integral experienced by the central segment during the 11–12 s rise of tetanic force. An exact estimate of this integral was

not required, however, because force was almost constant over the interval from 3 to 12 s after the onset of the step. The force drop produced by this step was added to all values of active force, both isometric and isotonic.

The load transferred during isotonic shortening was determined in a similar manner and added to the isotonic force. The passive stress-strain characteristics of the muscle were estimated by first shortening the resting muscle to bring the central segment to its length at the plateau of a tetanus. After the muscle had been held at this length for 9 s, a second shortening step was applied. The additional force drop caused by the second step was measured over the interval from 50 to 115 ms after the step, the same interval over which the isotonic force was averaged in force-velocity experiments. The amount of transferred load was estimated by interpolating the length change during the isotonic steps among the data points obtained from a series of such steps. The extent of isotonic shortening was, in turn, estimated as the difference between the length at the end of the series elastic recoil (taken to be 4 ms after the onset of the isotonic step) and the average length over the isotonic measurement interval.

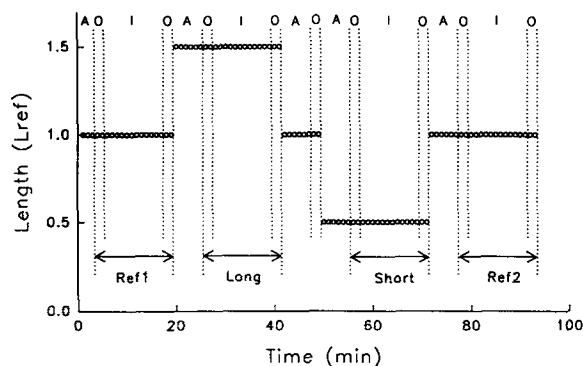


FIGURE 2. Sequence of length changes during experiment. Each point on the line represents one contraction. Muscles were allowed to adapt (*A*) to each new length for five to six contractions before being studied with a sequence of two initial oscillatory contractions (*O*), 9–12 isotonic contractions (*I*), and two final oscillatory contractions. Length was then changed and the sequence repeated.

A short series of five to six adapting contractions and two oscillatory contractions were obtained in the middle of the experiment, between the long and short measurements, to check for deterioration of force and compliance.

The main defense against substantial inaccuracies in these corrections was to work at muscle lengths where resting force, and therefore transferred load, was small. At the long length in the threefold study, where the corrections were largest, the rest force averaged 14.6% of isometric force and the greatest rest force encountered in any muscle was 20% of isometric force. In this same series, the correction for load transferred during isometric force development averaged $4.4 \pm 0.8\%$ SE and the correction for load transferred during isotonic shortening at the lowest load averaged $3.2 \pm 0.4\%$ of isometric force. Thus, errors resulting from any inaccuracies in the procedures were likely to have been very small.

Data Analysis

The isometric force generated at each length was taken as the average tetanic force produced by all the contractions at that length after length adaptation had occurred. Changes in velocity at different lengths were determined from changes in the relative force-velocity curves, i.e., plots of velocity against the relative isotonic force (Fig. 3). These changes were quantified as follows: the reference length force-velocity data obtained at the beginning and end of each experiment were pooled and fitted by a Newton-Raphson, least-squares method to the Hill (1938) hyperbola (Chiu, Ballou, and Ford, 1982) (Fig. 3, *solid curve*). This hyperbola was

defined by the equation

$$(T - \alpha)(V - \beta) = c \quad (1)$$

where V is velocity, T is the relative isotonic force (i.e., the isotonic force divided by P_{pre} , the isometric force developed immediately before the step), and α , β and c are constants.¹ A least-squares method was then used to find the factor by which the velocity dimension of these reference curves could be scaled to superimpose them on the force-velocity data obtained at other lengths (interrupted curves in Fig. 3). For each test length the scale factor (R) was determined by the numerical equation

$$R = \Sigma(V_i \bar{V}_i) / \Sigma(\bar{V}_i)^2 \quad (2)$$

where V_i are the individual velocity values at the test length and \bar{V}_i are the values of the fitted hyperbola (Eq. 1) at the same value of relative force. The value R was used as a measure of the change in velocity relative to the reference value.

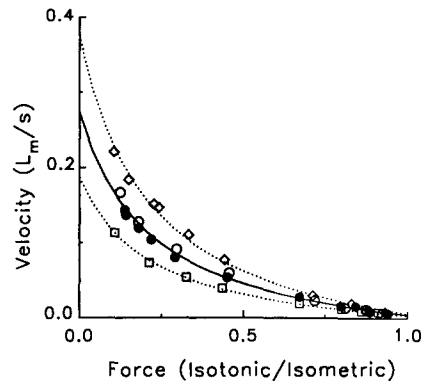


FIGURE 3. Force-velocity data obtained at different lengths. Circles represent data obtained at the reference length, open circles at beginning of experiment and closed circles at end. (Diamonds) Data obtained at 1.69 Lref. (Squares) Data obtained at 0.56 Lref. Solid curve is fitted to the pooled data obtained at the reference length. (Interrupted curves) Reference curve scaled in the velocity dimension to superimpose on the data obtained at the test lengths. Velocity has been normalized to the overall muscle length at the reference length (L_m). Experiment of 7/28/93.

In addition, for some analyses the force velocity data at all lengths were also fitted with Eq. 1 and several physiological parameters were compared. These parameters were derived from the fitted constants by the following equations:

(a) maximum velocity (V_{max}), the zero force intercept of the curves was defined by

$$V_{max} = \beta - c/\alpha; \quad (3)$$

(b) extrapolated isometric force (T_0), the zero velocity intercept of the curve was defined by

$$T_0 = \alpha - c/\beta; \quad (4)$$

¹ The symbols are different from those used by Hill and have a different meaning. This difference arises because the value of T represents relative rather than absolute force and because we distinguish two isometric forces, the developed force immediately before the isotonic step (designated P_{pre}) and the zero velocity intercept of the force-velocity curve (designated T_0). The relative forces, specified as T , are dimensionless. The absolute forces, specified by P , are normalized to the cross-sectional area determined as the muscle weight divided by the reference length, L_m . In addition, the signs of the two constants are negative, indicating that they have negative values in the plots.

(c) velocity at maximum power (V at TV_{\max}), and measure of velocity in the middle of the curve was defined by

$$V \text{ at } TV_{\max} = \beta + \sqrt{\beta c/\alpha} \quad (5)$$

Note that the extrapolated isometric force, T_0 , is different from the isometric force measured as the force developed immediately before the length perturbation and designated σP_{pre} .

Compliance was determined as the average ratio of the maximum length change to the maximum force change during the last 12 full cycles of the oscillatory records (between the vertical cursors in Fig. 1B). For most purposes it was expressed as the length change (percentage of reference length) that would bring isometric force to zero if the stress-strain curves extrapolated linearly to zero force ($\%Lm/Tpre$). For some purposes it was expressed as the absolute length change that would bring isometric force to zero ($\Delta Lm/Tpre$).

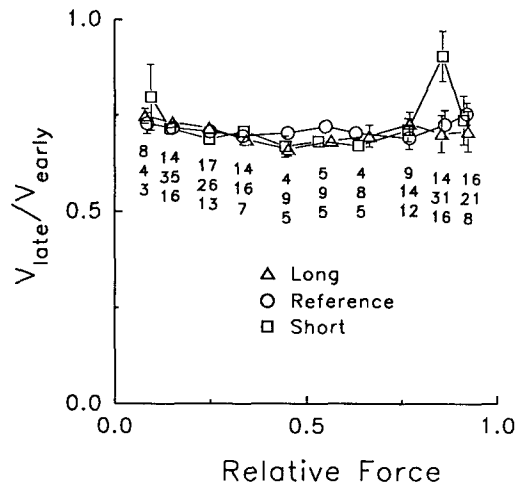


FIGURE 4. Fractional decline of velocity in the twofold series, estimated as the ratio of the velocity over the interval from 170–200 ms after the release to the velocity at the usual time of measurement, 50–115 ms after the release, plotted as function of relative load separately for the three lengths. Error bars indicate SEM. Numbers below symbols indicate the number of points averaged.

Statistics

The values obtained for each muscle were treated as a single observation, even when they were obtained from multiple measurements. These single observations were then averaged and the standard errors of the estimates used to determine significance.

Control Observations

Velocity measurements. Fig. 1c shows that the length records were curved, indicating a progressive velocity decline during the isotonic shortening. To address the question of the proper time for velocity measurements, we estimated the curvature in the records from the ratio of the velocity at the end of the recording period (over the period from 170 to 200 ms after the onset of shortening) to the value usually measured (over the period from 50 to 115 ms.) This ratio is plotted in Fig. 4 for each length and each 10% increment of relative isotonic force for nine muscles in the twofold series (described below). As shown, the plot indicates a ~30% decline in velocity over the 102-ms period between the two measurement intervals, with no dependence on muscle length and almost no dependence on isotonic load. This result suggests that the ratio of velocities measured at different lengths or loads will not vary when the measurements are made at different times, provided that the measurements are made over the same time interval. It also suggests that there was not a substantially greater increase in an internal load

during shortening at short lengths as compared with long lengths, as might be expected of an internal resistance to shortening that increased with shortening at reduced lengths.

Velocity changes during the experiment. An analysis similar to that for the different lengths was used to assess the extent to which velocity changed during the course of the experiment. The curves fitted to the pooled reference data were scaled to fit the reference data obtained separately at the beginning and end of the experiments. The ratio of the scale factor for the data at the end of the experiment to that for the beginning of the experiment was then used to quantify changes in velocity. For the two- and threefold series, respectively, these ratios averaged 0.97 ± 0.03 and 0.85 ± 0.3 SE, indicating little decline in velocity over the course of the experiments.

Adaptation period. Five or six nonshortening contractions were used to adapt the muscles to each new length before data was collected. The question arises as to whether this adaptation period was adequate. As explained above, rapid shortening tended to reduce isometric force in the subsequent contraction, so that small changes in isometric force resulting from lengthier adaptation could not be assessed in the main experimental series, when isotonic shortening was studied. We also found, however, that low amplitude oscillations applied at the end of a tetanus did not affect force in the next tetanus, so that increases in isometric forces could be estimated reliably in the first three contractions of each set of measurements. The average relative

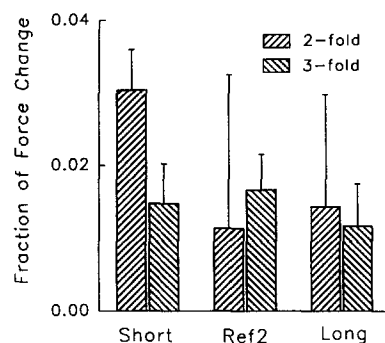


FIGURE 5. Isometric force increase between the first and third contractions at each length in the two series. Only the data for the second reference are included because the muscles usually had a longer period of adaptation before the first reference, while they were "running in." Error bars indicate SEM.

increase of isometric force between the first and third contraction in each set of measurements in both series of muscles is plotted in Fig. 5. As shown, there was a $\sim 1.5\%$ increase in tension with no significant difference in the increases measured at the different lengths. In the results presented below, the isometric force was taken as the average value obtained over 13 to 16 contractions after adaptation. If the increase in tension seen in the first three contractions continued unabated over the others, the results in Fig. 5 suggest that all of the absolute values would have been increased by 4 to 6%, with no variation in this increase at the different lengths. Because the main results are comparisons of the relative changes of force at different lengths, these small increases would have almost no effect on the conclusions.

Amplitude of the oscillations. To be certain that the measured compliance did not depend on the size of the imposed oscillations, the effect of different sizes of oscillations on the measured compliance was investigated specifically in three muscles. The amplitude of the oscillations were varied over a range from 67 to 200% the normal value, corresponding to the inverse of the threefold length changes. The measured compliance varied by $< \pm 2\%$ with no systematic dependence on the amplitude.

RESULTS

If smooth muscles adapt to new lengths by altering the filament array, it seems likely that the alterations might take time. An initial series of experiments was therefore undertaken to define the time course of tetanic force following a length change. Muscles were stimulated to produce isometric tetani every five minutes and the length was changed after every sixth contraction. The results with eight muscles treated nearly identically are shown in Fig. 6. For each length studied, the length was first changed from the reference and then returned, and each length change was made twice in succession (*a* and *b*, *c* and *d*, *e* and *f*, in Fig. 6), with the first changes (*a*, *c*, *e*) being made immediately after relaxation from the last contraction at the old length, and the second change (*b*, *d*, *f*) being made immediately before the first contraction at the new length. As shown, the tetanic force in the first several

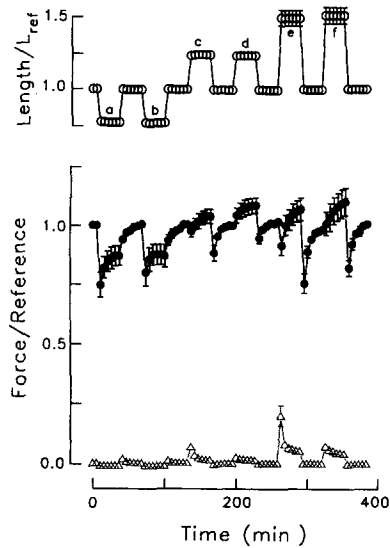


FIGURE 6. Isometric force following length changes of the muscles. The central segment length, normalized to the reference length (L_{ref}), is plotted in the upper panel and the average force, normalized to the force developed at the reference length at the beginning of the experiment is plotted in the lower panel. Circles plot the developed force and triangles the passive force. Eight muscles were treated nearly identically. Average reference central segment length (L_{ref}) was 3.2 mm; average reference force 121 kPa. Error bars indicate SEM.

contractions after a length change was below the steady value for that length, and force rose to its steady value over 4–6 contractions.

The major purpose of these initial experiments was to define the conditions required to bring the muscle to a steady state after a length change. Because of the results shown in Fig. 6, all subsequent steady state measurements were made after the muscles had been allowed to adapt for at least five and usually six tetani at 5-min intervals.

Length adaptation during relaxation. Each length change was studied twice to determine the extent to which the force depression following a length change would be reduced when the length change was made at the beginning of the rest period, rather than immediately before the next contraction. As shown in Fig. 6, the depression was slightly greater when the length change was made immediately before

activation at the new length, but for three of the four smaller length changes, the differences were not significant by paired t tests ($P > 0.05$). The force depression was significantly greater following the largest steps when they were made immediately before stimulation at the new length ($P < 0.02$ for all comparisons), but some of the greater difference following the stretch may have been due to transfer of load from the parallel elastic elements.

The developed force plotted in Fig. 6 has not been corrected for the load transferred during force development, a correction that was applied to all subsequent data described below. This correction was not made because the magnitude of the correction would have been difficult to estimate exactly at the time of the tetanus. Rest force was substantially higher immediately after stretches, and then declined over several minutes. The higher rest force in the contractions immediately following the stretch caused a greater transfer of load during force development and therefore reduced the recorded tetanic force in those contractions. This greater load transfer would explain some of the greater apparent force depression when stretches to long lengths were made immediately before activation at the long length.

The main conclusion from this analysis is that adaptation to a new length requires time and possibly activation at that length. A secondary conclusion is that the adapted force varied in this series of experiments varied by 1.25-fold when length was varied twofold. Correction for load transferred from the parallel elastic elements would reduce this variation to ~ 1.2 -fold.

Length Dependence of Physiological Parameters

The data for shortening velocity and compliance were collected at three lengths in each of two series of nine muscles. The first series is designated the twofold group because data were obtained at 1.0, 1.5, and 0.75 times the reference length. The second is designated the threefold group because that length range was studied. In this second group, the long length was determined as the length where rest force was maintained at 10–20% of developed isometric force and just short of the length where passive force rose very steeply with stretch. The short length was adjusted to be one third of this length. Several lengths were studied to be certain that the observed changes in velocity and compliance occurred progressively over the entire length range and did not occur precipitously over a shorter range, as might happen if the contractile elements met an abrupt and stiff internal obstruction. As shown from the results in Fig. 7, velocity and compliance changed progressively, and almost linearly over the entire range.

Fig. 7 shows that over the entire length range, velocity varied by 2.2-fold and compliance by 1.7-fold while developed force changed substantially less, ~ 1.3 -fold in the first series and negligibly in the second. Over the shorter half of the length range, the slopes of the length-velocity, length-compliance, and length-force relations were 0.82, 0.51, and 0.05, respectively. These findings support the mechanism proposed in the introduction.

The values of the physiological parameters at the reference length are listed in Table I and relative values of these parameters at other lengths are given in Table II.

Comparison of Force-Velocity Data

If the velocity changes were caused by a variation in number of contractile units in series, as postulated, it would be expected that the velocities at each relative isotonic force would scale in proportion to the number of units in series. In fact, this type of scaling was assumed in the method used to compare velocities. The validity of this assumption was tested in two ways. In one, the force-velocity data obtained at each length were fitted with the hyperbola of Eq. 1 and the fitted parameters compared. In the other, the values of the scaled reference curves were subtracted from the individual points obtained at each length, and plots of these residuals against isotonic force were examined for systematic deviations.

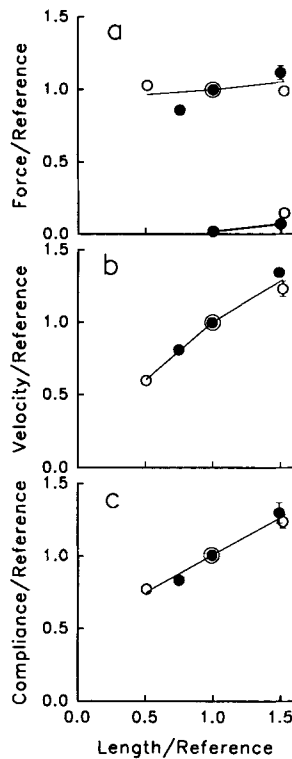


FIGURE 7. Isometric force (a), velocity scale factor (b), and compliance (c) plotted as a function of central segment length. The reference values are given in Table I. (Open circles) Threefold series; (closed circles) two-fold series. The lower set of points in a represents rest force.

Force-Velocity Parameters. The fits to the force-velocity hyperbolae yield several constants that can be used to compare curves obtained under different conditions. These parameters include maximum velocity, velocity at maximum power, and the asymptotes of the curve, α and β . The force asymptote, α , should not change if the curves superimpose by scaling velocity. As shown in Table II, the values of α were not significantly different at the different lengths, as expected, although the standard errors of the means were so large that even a modest difference could not be detected. Another expectation is that the velocities along the curve will scale in the same proportion. Comparison of the relative values of maximum velocity, the value

at the extreme end of the curve, with velocity at maximum power, the value of the middle of the curve, shows that they scaled in almost exact proportion, but the larger standard errors on maximum velocity precluded a precise comparison (Table II). A third expectation is that all of the velocity parameters, including the velocity scale factor, maximum velocity, velocity at maximum power, and β , should scale in the same proportion. Again, this was found but with the same reservation regarding the large standard errors of some parameters.

Table II also shows that velocity at maximum power and the velocity scale factor, which were both determined by interpolation among the data points, have much smaller standard errors than the other two velocity parameters, which are determined by extrapolation of the fitted curves. This is why we chose the velocity scale factor to describe the changes in velocity; as shown in Table II, it varied almost identically with the velocity at maximum power.

TABLE I
Reference Values of Physiological Parameters

	<i>Lm</i>	<i>Lref</i>	<i>Ppre</i>	Compliance	<i>To</i>	<i>V</i> _{max}	<i>V</i> at <i>TV</i> _{max}	β	α
	<i>mm</i>	<i>mm</i>	<i>kPa</i>	% <i>Lm/Tpre</i>		% <i>Lm/s</i>	% <i>Lm/s</i>	% <i>Lm/s</i>	
Twofold	4.03	2.82	131	1.87	1.03	20.0	6.58	-6.81	-0.362
	± 0.49	± 0.31	± 11.2	± 0.22	± 0.02	± 2.4	± 0.75	± 1.99	± 0.038
Threefold	4.74	3.37	176	1.88	1.06	25.8	7.63	-5.74	-0.241
	± 0.19	± 0.23	± 10.0	± 0.12	± 0.01	± 1.6	± 0.42	± 0.49	± 0.024

Ppre is the isometric force measured at the plateau of the tetanus, just before length perturbations were imposed, normalized to the muscle cross-sectional area determined as the muscle weight divided by the reference length, *Lm*; *To* is the zero velocity intercept and *V*_{max} the zero force intercept of the relative force-velocity curves; *V* at *TV*_{max} is the velocity at maximum power; α and β are the force and velocity asymptotes, respectively, of the Hill (1938) hyperbola. *To* and α are relative values, and therefore, dimensionless. Values here and in Table II are means ± SE for nine muscles in each group.

The larger standard errors of the fitted parameters also show that they could not be used to discriminate fine differences in the curves, so that some additional test was needed.

Residuals of the fitted curves. Fig. 8 plots the differences between the individual velocities and the values of the scaled curves at the same isotonic force. Separate plots are made for each length category, and the residuals have been averaged for each 0.1 *Ppre* increment in isotonic force. These plots show clearly that the data points fell very close to the fitted curves except at high loads, in the region of 70–90% of isometric force, where velocity was <10% of maximum. The small size of these residuals suggests that the method of fitting the data gave a good description of the experimental results, and that the method of describing the length dependence of velocity was valid.

The deviation of the data points at high loads was, however, systematic in that the data points fell below the fitted curves at long lengths and above the fitted curves at short lengths. Although the differences were very small, averaging ~0.5% of maximum velocity, they were highly significant ($P < 0.01$ – 0.001). These deviations correlate with the observation that *To*, the isometric force determined by extrapolat-

ing the force-velocity curves to zero velocity, was slightly greater at short lengths and slightly reduced at long lengths (Table II). The question arises as to whether this small deviation occurred because the contractile units did not vary in the manner postulated or because of some other mechanisms. The deviations were so small, and occurred at such low velocities, that it seems unlikely that they indicate inaccurate velocity scaling. It is more likely that they arise from a length dependence of deviations of the data from the Hill (1938) hyperbolae. It is well known that the force-velocity curves deviate from the classical Hill (1938) curves at high loads in all types of tetanized muscle, including skeletal (Edman, Mulieri, and Scubon-Mulieri, 1976), cardiac (Forman, Ford, and Sonnenblick, 1972), and smooth (Wang, Jiang, and Stephens, 1995). A similar small deviation is shown here by the zero velocity intercept of the force-velocity curves (T_0) being slightly greater than the isometric force measured immediately before the isotonic steps (Table I). The cause of these small deviations are not known, but the present experiments show that they vary slightly in a systematic manner with muscle length.

TABLE II
Ratios of Physiological Parameters at the Test Lengths to Their Values at the Reference Length

	Length	P_{pre}	Compliance	Velocity scale factor	T_0	V_{max}	V at TV_{max}	α	β
Twofold:									
long	1.50	1.12 ± 0.05	1.28 ± 0.06	1.35 ± 0.04	0.98 ± 0.01	1.36 ± 0.09	1.37 ± 0.05	1.50 ± 0.15	1.14 ± 0.17
short	0.75	0.87 ± 0.02	0.83 ± 0.03	0.81 ± 0.02	1.03 ± 0.02	0.83 ± 0.05	0.81 ± 0.02	0.81 ± 0.09	1.06 ± 0.16
Threefold:									
long	1.53	0.99 ± 0.05	1.23 ± 0.05	1.23 ± 0.06	0.95 ± 0.01	1.17 ± 0.13	1.23 ± 0.07	1.65 ± 0.18	1.56 ± 0.30
short	0.51	1.03 ± 0.02	0.77 ± 0.02	0.60 ± 0.03	1.06 ± 0.02	0.64 ± 0.04	0.59 ± 0.02	0.50 ± 0.03	0.86 ± 0.09

Series Elastic Element Compliance

The finding in Fig. 7 that the compliance varied somewhat less than velocity is expected since the measured compliance contained a fixed component due to the series elastic elements, in addition to the component that varied with muscle length. To the extent that contractile element compliance and velocity varied together, the constant series elastic compliance can be calculated from the difference in the zero length intercepts of the two relationships. This intercept difference in Fig. 7 is 31% of the reference value, equivalent to a compliance value of 0.6 % Lm/T_{pre} ($=0.31 \times 1.9\% Lm/T_{pre}$). Subtracting this value from the overall muscle compliance leaves a value of 1.3% Lm/T_{pre} contractile element compliance at the reference length.

Additional estimates of both contractile element and series element compliances were afforded by a 3.5-fold range of overall muscle lengths in the twofold series. Fig.

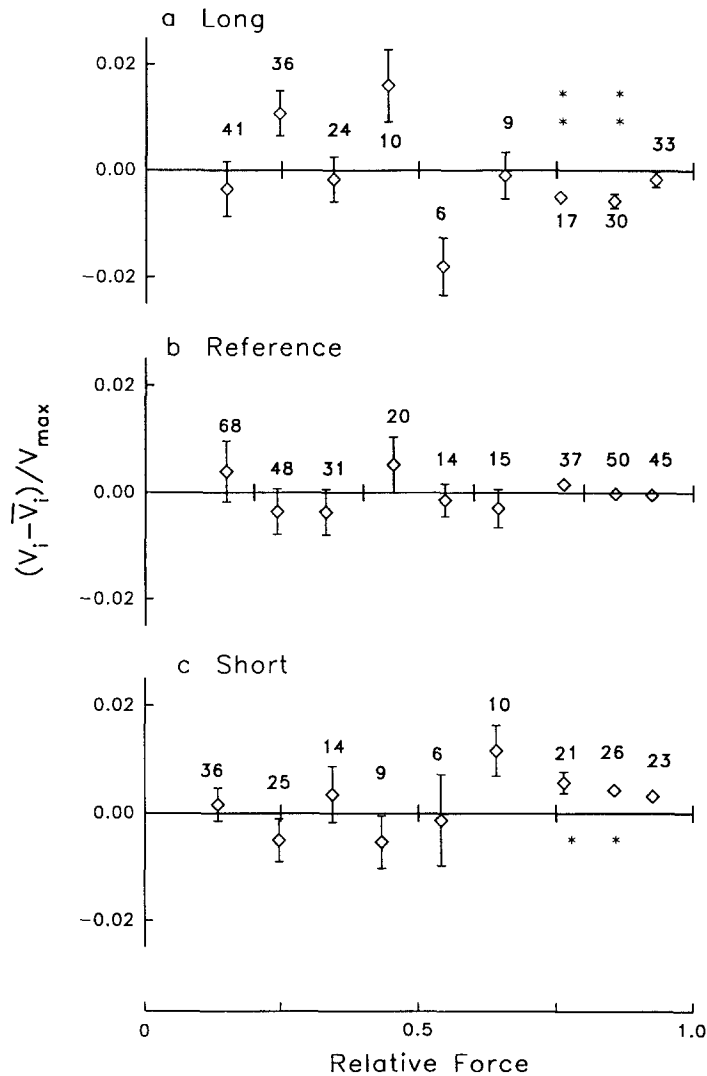


FIGURE 8. Velocity residuals, normalized to maximum velocity, plotted as a function of isotonic force for long lengths (*top*), reference lengths (*middle*), and short lengths (*bottom*). Values plotted are the mean \pm SE of the values in each decade of relative isotonic force. Numbers near each symbol indicate the number of points averaged. Stars indicate values that are significantly different from zero (*one star*: $P < 0.01$; *two stars*: $P < 0.001$).

9 plots the compliance, expressed as the absolute length change that would bring isometric force to zero, against the overall muscle length when the central segment was set to the reference length. As shown, there is a strong positive correlation between this measure of compliance and the overall muscle length, as expected if most of the compliance is in the muscle and not at its ends. A line fitted to the values had a slope of $13.9 \mu\text{m}/T_{pre}\text{-mm}$ of reference length, almost identical to the value of

1.3% L_m/T_{pre} calculated above. Furthermore, the plot had a positive intercept that was 24% of the mean for the group, very similar to the 31% estimate obtained from the difference between the length-compliance and length-velocity plots of Fig. 7. To emphasize the correspondence between the two methods of analysis, the interrupted line in Fig. 9 was calculated as having an intercept that was 31% of the average value of compliance and a slope of 1.3% L_m/T_{pre} . As shown, the lines are very close to each other.

The length variation in the threefold series was much less than in the twofold series, and no reliable estimate of the relationship between compliance and reference length could be attempted. The data are, however, included in Fig. 9 to show that the two series had nearly identical compliances.

The estimated value of the series compliance, 0.6% L_m/T_{pre} , implies a very stiff series elastic element, raising the question of whether this value is reasonable. An independent estimate of the series elastic element compliance can be gained from the measurements of the central segment shortening during isometric force development shown in Fig. 10. As illustrated, the series elastic elements were moderately stiff,

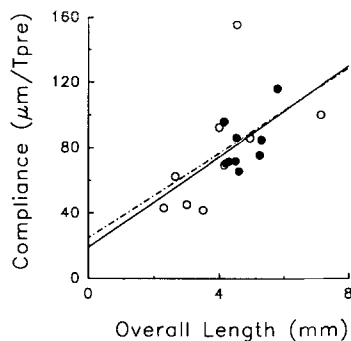


FIGURE 9. Compliance, expressed as the length change that would bring isometric force to zero if the force-extension characteristics were linear, plotted against reference muscle length. (Open circles) twofold series. (Closed circles) threefold series. The solid line is a least-squares linear regression of compliance on length fitted to all the data points. A regression fitted to just the twofold series was almost identical. The interrupted line is derived from the data in Fig. 7 and Table I, as described in the text.

extending by $\sim 4\%$ of overall muscle length during force development at the reference length. More importantly, the amount of internal shortening was reduced to $< 2\%$ at the longest lengths, where the force on the series elastic elements was increased to 15% of the isometric value. This observation indicates both that the series elastic element compliance was nonlinear, and that these elements were very stiff in the region of isometric force, where compliance was measured with oscillations in the activated muscle. In addition, the consideration that viscous elements would damp the series elasticity suggests that the calculated series elastic compliance of 0.6% L_m/P_o is about as expected from this preparation.

Relationship of Passive Elastic Elements to Reference Length

In whole striated muscle studies where sarcomere length cannot be measured easily, it is customary to use a physiologically defined length as a reference. The usual reference is the length at which maximum force is developed (e.g., Hill, 1938). This practice is often followed in smooth muscle as well (e.g., Murphy, 1980), and maximum force is frequently found at lengths where rest force begins to increase with stretch. In the present experiments no peak was found in the force-length relation

when the developed force was corrected for the load transferred from the parallel elements (Fig. 4). A peak would have been found, however, if the correction for passive force had not been made. This consideration suggests that the position of the force peak may depend on passive structures in the preparation. In addition, if the muscle structure is plastic, as proposed in the Introduction, there might be no easily recognized reference related to contractile performance. These considerations suggest that the use of a passive reference length deserves further examination.

Although a systematic study of rest tension in the muscles was not performed, it was noted that the steady passive tension remained below 10–20% isometric force as the muscles were stretched to some critical point. (The term “steady” is used here because the rest tension rose substantially above the steady level during stretches and then declined to this level over several minutes.) Stretches by as little as 5% beyond the critical point would cause rest force to rise very steeply, sometimes by more than 50–100% of isometric force. We also noted that the slope of the passive force-length relation at the shorter lengths became less steep as our experience increased. This

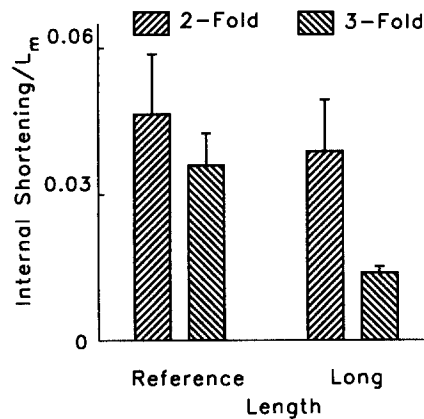


FIGURE 10. Central segment shortening during tetanic force development at the reference and long lengths in the two series of muscles. Internal shortening due to extension of the ends of the muscle have been normalized to the overall muscle length to make it comparable to the dynamic compliance measurements (Table I).

less steep relation at shorter lengths made the abrupt transition to the very stiff part of the passive stress-strain relationship all the more apparent in the later experiments. In addition, as our dissecting experience increased, the length where the transition from low to high stiffness appeared to decrease relative to the reference length. All of the muscles in the twofold group, which were studied first, could be stretched to 1.5 times the reference length without meeting the transition point, and some of these could be stretched by as much as 1.8 times without developing high rest tension. Three of the nine muscles in the threefold group, that was studied later, could not be stretched to 1.5 times the reference length without developing very high rest forces. These observations suggest that the low rest force at shorter lengths was due to connective tissue, which was more completely removed in later experiments. A consequence of this more thorough dissection was an increase in the physiological length where rest force became finite at 1–2% of isometric force, the length used as a reference.

The abrupt transition to a steep stress-strain relation at a long length in the resting muscle was undoubtedly due to some parallel element reaching its elastic limit. The

nature of this parallel element is not known, but the abruptness of the transition makes it likely that it was a structure separate from the elements that cause the low resistance to stretch at shorter lengths. It is possible, for example, that these very stiff elements were intracellular structures that protect the cells from overstretch. The steep rise of force was the factor that limited the stretch in the threefold series. Although the same reference length was used in that experiment, the long and short lengths were adjusted according to the length at which rest tension began to rise very steeply. To the extent that this length is not determined by the dissection procedure, it might be a more reliable reference, particularly if it is determined by intracellular structures.

DISCUSSION

It seems very likely that smooth muscle, like skeletal muscle, is a series arrangement of identical contractile units whose small, individual length changes sum to produce the large length changes of the overall muscle (Huxley, 1980, p 41). Since each unit in series contributes incrementally to the length changes of the whole, a change in the number of units in series will directly alter muscle velocity and compliance. The findings of a direct and nearly linear dependence of these two parameters on adapted muscle length is therefore highly suggestive that the muscle accommodates to each new length by varying the number of contractile units in series.

The simplest expectation of such plasticity is that both compliance and velocity will be exactly proportional to length, and extrapolate to zero at zero length. There are, however, reasons why such an exact proportionality would not exist. As mentioned above, the less steep relationship between compliance and length is expected because total compliance contains a fixed component due to elastic elements in series with the contractile units. The observation that the slope of the velocity-length relation was less than unity, ~ 0.8 , can be explained by at least two reasonable mechanisms. The first is that $\sim 20\%$ of the filament structures are fixed and do not change with length, as might be expected of the filaments that anchor the lattice to the membranes. The second is that there is a feedback mechanism which reduces the number of filaments at short length and that this feedback has a relatively low gain, i.e., that the mechanism responsible for altering the filament structure requires a large length change to function.

The relative length independence of the steady state isometric force would be explained by reformation of the filament lattice to produce optimum force development at different lengths. The rise of force after length changes in either direction (Fig. 3) would be obtained if the reformation took many minutes and possible several contractions for completion.

The question arises as to why the length-force relationship does not have a negative slope, as expected if the reduced number of contractile units in series at short lengths made more units available to pull in parallel. A possible explanation in terms of the mechanism of thick filament evanescence, discussed below, is that the filaments in parallel do not increase because their number is fixed by some other constraint, such as a constant number of anchor points to the surface membrane. Another possibility is that activation at short lengths is decreased in a way that offsets the increased number of contractile units in parallel. Such a mechanism assumes the

plastic changes postulated here but leaves open the question of the degree to which changes of velocity reflect the number of units in series and the degree to which they are caused by changes of activation. Whatever the mechanism, it is known that the length-force relation of striated muscle must have a positive slope to maintain homogeneity of sarcomere spacing (Hill, 1970, pp 125–127), and that under physiological conditions, such a positive slope is maintained even where lengthening diminishes filament overlap (Rack and Westbury, 1969). Thus, adaptive processes are expected to produce a positive slope of the length-tension relationship under physiological conditions.

Thick Filament Evanescence

The present experiments were undertaken, in part, because of the recognition that the long functioning range of smooth muscle might be accommodated by the controversial mechanism of thick filament evanescence. This evanescence was first suggested by Schoenberg (1969) and by Rice, Moses, McManus, Brady, and Blasik (1970) who proposed that myosin filaments might form during contraction and dissolve during relaxation. This proposal was based on the findings that low pH (Kelly and Rice, 1968) or high divalent cations (Schoenberg, 1969) in the preparative solutions produced more abundant thick filaments. Because activation produces both a low pH and an increased calcium, these findings were consistent with the proposal that thick filaments form during activation and dissolve during relaxation. Further support for the lability of the thick filaments might have been provided by the variable appearance of thick filaments in different studies (see review by Schoenberg and Needham, 1976). In addition, several recent studies have indicated that there is more myosin incorporated into thick filaments during activation than at rest (Gillis, Cao, and Godfrain-De Becker, 1988; Godfrain-De Becker and Gillis, 1988; Watanabe et al., 1993). Finally, a molecular mechanism for this evanescence has been indicated by the work of Trybus, Huiatt, and Lowy (1982), Onishi and Wakabayashi (1989), and Craig, Smith, and Kendrick-Jones (1983). They found that the tail of unphosphorylated myosin is folded in a way that prevents its incorporation into thick filaments, and that phosphorylation of the light chain, which is required for activation of the ATPase (Sobieszek, 1977; Adelstein and Eisenberg, 1980; Hartshorn and Siemankowski, 1980), also permits filament formation, apparently by unfolding the tail. These more recent studies suggest that thick filaments form and dissolve, at least partially, during the contraction-relaxation cycle.

In spite of the abundance of evidence suggesting thick filament evanescence, attention has focused mainly on the question of whether the presence or absence of thick filaments was due to different preparative techniques. With increasing experience, and the greater attention to preparative conditions, thick filaments similar to those of skeletal muscle were seen regularly both during contraction and relaxation (Cooke and Fay, 1972; Garamvolgyi, Vizi, and Knoll, 1971, 1973; Somlyo, Devine, Somlyo, and Rice, 1973; Somlyo, Butler, Bond, and Somlyo, 1981). It has thus been concluded by some that thick filaments are a constant presence in smooth muscle (Somlyo and Somlyo, 1992). But the fact remains that reliable workers have shown that the thick filaments are not as robust as those of skeletal muscle, and that they dissolve, at least partially, during relaxation. These disparate descriptions of the

filament structure all make sense in the context of the plasticity proposed here; the filament lattice undergoes adaptation to different lengths by forming variable numbers of contractile units in series. In our view, the issue to be addressed in the structural studies is not which of them is correct, but how they can be reconciled with each other and with the physiological behavior of the muscle.

It should also be mentioned that the complete reformation of the lattice may not occur over a single contraction-relaxation cycle, particularly in view of our finding that adaptation to a new length required five to six such cycles. A mechanism where complete reformation of the lattice required several cycles would explain the finding of aggregated thick filaments in resting muscle (Somlyo et al., 1981), while the finding of a decreased but finite fraction of the myosin in thick filaments during relaxation (Godfraind-De Becker and Gillis, 1988; Gillis et al., 1988; Watanabe et al., 1993), suggests that these resting filaments dissolve only partially. It is also possible that the plastic changes result from a rearrangement of partially formed thick filaments, rather than from a complete dissolution and reformation of these filaments. If such rearrangements of existing filaments do occur, it seems likely that they would be facilitated by the partial dissolution and reformation of the thick filaments. Also, such partial dissolution might go unrecognized in anatomical studies that were not designed to look for it.

The exact structural alterations that produce the plastic rearrangements proposed here will ultimately require a structural study for its discovery. However, the lack of unanimity in the interpretation of previous structural studies further suggests that different types of experiments are needed to help resolve this issue. The value of functional studies, such as the present one, therefore, is that they provide a clue to the types of structural changes that might be sought and the conditions under which they are most likely to be revealed.

Relationship to Earlier Work

It has been known for a long time that smooth muscle has a substantially longer working range than skeletal muscle, but exact limits of this range are difficult to find in the literature, mainly because measurements are not made at the extreme ends of the range. Evans (1926, p 371), for example, mentions in passing that a guinea pig uterine muscle length may vary five- to sixfold but does not quote the values of developed force. Winton (1926) estimated the working range of the muscle by measuring the area under the length-tension curves below the length (L_m) where maximum force (T_m) was developed. He found that this integral was fairly large, $\sim 0.4 T_m \cdot L_m$, and substantially larger than the similar integral for skeletal muscle.

If the extremes of the working range are taken as being the two lengths where the length-tension curves extrapolate to zero force, skeletal muscle has a total length range of \sim threefold (Gordon, Huxley, and Julian, 1966). The comparable value for dog retractor penis muscle (Winton, 1926) is \sim fivefold, and for rabbit urinary bladder muscle (Uvelius, 1976) \sim sevenfold. For the most part, no effort was made in these studies to adapt the muscles to each length, so that total range of length could be even larger.

Equally important are the observations that smooth muscle is able to shorten for a long distance without being damaged. Seow and Stephens (1988), for example,

showed that during a single tetanus dog tracheal smooth muscle could shorten to $\frac{1}{3}$ to $\frac{1}{4}$ of the length at which maximum force was developed, which is substantially shorter than the comparable value for skeletal muscle. Furthermore, the muscles were not harmed by this extensive shortening and could undergo multiple contractions in which they shortened to lengths where zero force was developed. When similar experiments are done in skeletal muscle, they develop irreversibly contracted regions and are permanently damaged (Ramsey and Street, 1941). These observations indicate that smooth muscle possesses mechanisms that allow it to undergo the large length changes required to for the extensive volume range of hollow viscera. The question remaining, therefore, is not whether a long length range occurs, but how it is accommodated by a sliding filament mechanism.

Concepts of structural plasticity have been invoked before to explain the working range of smooth muscle. In a single cell preparation that could give only one contraction per cell, Harris and Warshaw (1991) found a steep length dependence of redeveloped isometric force when the muscle was allowed to shorten from its initial isometric length to different test lengths, but a length independence of initial isometric force developed at different stretched lengths. In addition, the steep parts of their length-force relationships had similar slopes but were displaced along the length axis according to the starting length. They interpreted their findings as suggesting a form of plasticity in which a passive element in series with the contractile elements was distensible at rest but became rigid on activation. Although they did not measure velocity, their mechanism would predict a length independence of velocity, different from that described here. The importance of their single fiber work is that it shows that the plasticity results from intracellular structures. Other proposals have suggested that plasticity might result from shifting whole cells relative to one another, e.g., Rasmussen, Takuwa, and Park (1987).

Conclusion

These experiments strongly suggest that the long functioning range of smooth muscle is facilitated by structural changes which vary the number of contractile units in series. These structural changes may explain why smooth muscles are smooth, i.e., why they have no sarcomeres. The absence of regular sarcomeres in both smooth muscle and nonmuscle motile cells may result from the dynamic changes of structure.

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