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Neuromuscular Organization of Feline Anterior Sartorius: II. Intramuscular Length Changes and Complex Length-Tension Relationships During Stimulation of Individual Nerve Branches

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ABSTRACT The feline anterior sartorius is a long strap-like muscle composed of short muscle fibers. Nerve branches that enter this muscle contain the axons of motor units whose constituent muscle fibers are distributed asymmetrically within the muscle. In the present study, twitch and tetanic isometric contractions were evoked by stimulating individual nerve branches while muscle force was recorded and intramuscular length changes were monitored optically by the movement of reflective markers on the muscle. Contractions elicited by stimulating the parent nerve produced little change in the positions of the surface markers. Contractions elicited by stimulating the proximally or distally directed nerve branches caused the muscle to shorten at the end closest to the nerve branch and lengthen at the opposite end. Some muscles were supplied by a centrally directed nerve branch whose stimulation produced variable effects: in some cases a portion of the muscle shortened whereas the rest lengthened, but in other cases, the positions of the surface markers showed little change. The intramuscular length changes produced by stimulating single nerve branches were greater during isometric contractions at short whole-muscle lengths than at long whole-muscle lengths. The twitch and tetanic length-tension relationships obtained by stimulating the individual nerve branches were not congruent with the length-tension relationship produced when the parent nerve was stimulated. At short whole-muscle lengths, stimulation of a single nerve branch generated only a small fraction of the force that could be generated by the muscle when the parent nerve was stimulated. As whole-muscle length increased, an increased fraction of total muscle force could be generated by stimulating a single nerve branch. The results suggest that a complex relationship between passive and active elements contributes to the total muscle force and depends on the distribution of active and passive muscle units throughout the muscle.

A common presumption in the field of muscle physiology is that the force generated by a whole muscle is a scaled-up version of the force generated by a single motor unit; in turn, the force generated by a single motor unit is a scaled-up version of the force generated by a single muscle fiber. This assumption seems plausible for simple muscles such as cat soleus in which all muscle fibers extend the full length of their fascicles, from the connective tissue of the origin to that of the insertion (Burke et al., '74). However, this assumption may not be true for muscles that have more complex architectural organizations.

One of the specializations that has potentially important implications for the mechanics of muscle is the construction of long muscle fascicles from short muscle fibers. Two modes of construction are used, alone or together, to create long contractile assemblies. In some muscles, fibers are linked in-series by tendinous inscriptions; in others, fibers taper and end intrafascicularly. The former strategy divides the muscle longitudinally into a series of shorter "mini-muscles" as exemplified by cat semitendinosus, splenius, and biventer cervicis (Bodine et al., '82; Richmond et al., '85; Armstrong et al., '88). The mechanics of the individual compartments

might individually follow the relatively simple rules described above. However, control of the overall force of the ensemble of in-series compartments would be complicated, particularly if all compartments were not recruited identically.

In the latter strategy, which is perhaps more common in long muscles, serial linkages between fibers are not clearly demarcated by gross anatomical landmarks such as tendinous inscriptions. Instead, muscles such as cat tenuissimus, biceps femoris, and medial and anterior sartorius have long muscle fascicles composed of short tapering fibers that end intrafascicularly (Adrian, '25; Loeb et al., '87; Lev-Tov et al., '88; Chanaud et al., '91). These fibers do not attach to tendinous tissue that might direct the force generated by the fiber onto the muscle origin and insertion. Rather, the muscle fibers appear to couple their forces into the connective tissue enveloping their tapered (and possibly non-tapered) portions (Trotter, '90; Trotter and Purslow, personal communication). Adjacent fibers generally belong to different muscle units (Smits et al., '91), so that tension must be transmitted for some distance laterally across inactive fibers when only a few motor units have been recruited. Force transmission in the muscle is further complicated by the complex arrangement of muscle-unit territories. Anterior sartorius is supplied by two or three nerve branches that contain largely separate populations of axons and enter the muscle at different rostrocaudal levels. Groups of motor units supplied by a single nerve branch are distributed asymmetrically within anterior sartorius both in the proximodistal and mediolateral axes of the muscle (Thomson et al., '91). Further, the muscle fibers in a single motor unit are distributed over much of the length of the muscle in a circumscribed strip-like territory, but with markedly different numbers of fibers from one end of the muscle to the other (Smits et al., '91). Thus, the cross-sectional area of muscle fibers supplied by an individual motoneuron and even by a whole nerve branch varies markedly at different proximodistal levels along the muscle.

The complex architecture of anterior sartorius provides an anatomically well-characterized and experimentally accessible model in which to examine at least some of the mechanical properties that can emerge in such muscles during different patterns of motor-unit recruitment. In this muscle, the smallest controllable unit of muscle—the motor unit—cannot generate a uniform force from one end of the muscle to the other. However, it is not yet clear how force generation and transmission will be affected by the lateral and longitudinal interposition of passive, relatively compliant muscle units among active muscle fibers. The problem is interesting not merely from a theoretical point of view; the properties of long muscles with short fibers must be understood as a first step in designing appropriate methods to re-animate paralyzed limbs. Implantable electronic devices are being developed to produce functional recovery of paralyzed muscles by stimulating muscle nerves and/or intramuscular branches of motoneurons (Grandjean and Mortimer, '86). It is important to understand the forces that may be produced and transmitted if such stimulation results in the uneven recruitment of muscle fibers along the length of a long muscle.

In this study, we have analyzed the mechanical characteristics of contractions evoked by stimulating nerve branches known to supply asymmetrically distributed muscle territories. The time-course and amplitude of the resulting forces were associated with optically measured changes in intramuscular lengths during whole muscle isometric contractions. These features were compared to the mechanics of contractions produced by synchronous stimulation of all nerve branches supplying anterior sartorius.

MATERIALS AND METHODS

Data acquisition

The experiments were carried out on 11 cats (2.8–4.2 kg; either sex) anesthetized with sodium pentobarbital (initial dose, 35 mg/kg ip; supplemental doses, 5 mg/kg iv to sustain deep anesthesia). An incision was made along the anterior border of the sartorius and its deep surface was exposed and separated from the underlying muscles. The branching pattern of the innervation to sartorius was sketched. Bipolar platinum hook electrodes were placed around the *proximal, distal,* and *central* (when it was present, cf. Thomson et al., '91) nerve branches. Collectively, these branches are defined as the *daughter* nerve branches. The electrodes and surrounding region were covered with low melting-point wax (40°C) to secure the stimulating electrodes and to reduce current spread. The cat was placed on a heating blanket, and muscle temperature was maintained at 37°C using a heating lamp that was controlled by feedback

from a temperature sensor placed on the surface of the muscle. The muscle was moistened with warm saline (37°C) throughout the experiment.

Muscle force was measured using a force transducer (measured linear up to 35 N; compliance 8.9 μm/N) attached to the patella (Fig. 1). A hole was drilled through the patella, the patellar ligament was cut, and all muscles attached to the patella except anterior sartorius were resected. Several loops of surgical suture (Ethicon, 000 Steel) were threaded through the patella to secure the force transducer, which in turn was attached to a motor-driven puller that was used to vary whole-muscle length. A bone screw was fastened to the pelvis and secured to the frame of the puller in order to fix the origin of the muscle. Finally, the insertion of medial sartorius was removed from the tibia. Care was taken to minimize any disruption of the blood vessels that entered the muscle near the insertion of anterior sartorius. At this and later stages of the experiment, data collection was stopped and the results not considered for further analysis if the distal portion of the muscle showed any change from its normal pink coloration, or if it could no longer sustain an isometric contraction (and thus lengthened) during synchronous stimulation of all of the nerves supplying anterior sartorius.

Changes in intramuscular length were measured by monitoring the movement of retroreflective markers, 7 mm in diameter, sutured to the superficial surface of the muscle. These markers represent the position of the superficial surface of the muscle and not necessarily the position at deeper levels. Seven markers divided the muscle into six approximately equal regions from origin to insertion (Fig. 1). An electronically shuttered video camera (NEC Corp., model TI-23A) was placed approximately 1 m above and perpendicular to the line of pull of sartorius. The laboratory lights were dimmed and the reflective markers were illuminated by an array of directional LEDs (Stanley Electric Co., ESRB5701) positioned around the camera lens.

Contractions were elicited by stimulating individual nerve branches while the muscle was held isometrically. The nerve branches were stimulated supramaximally, using a biphasic voltage-regulated pulse with a duration of 0.1 ms/phase and an amplitude of 4 times the threshold voltage required to induce just-visible twitch contractions. Trials

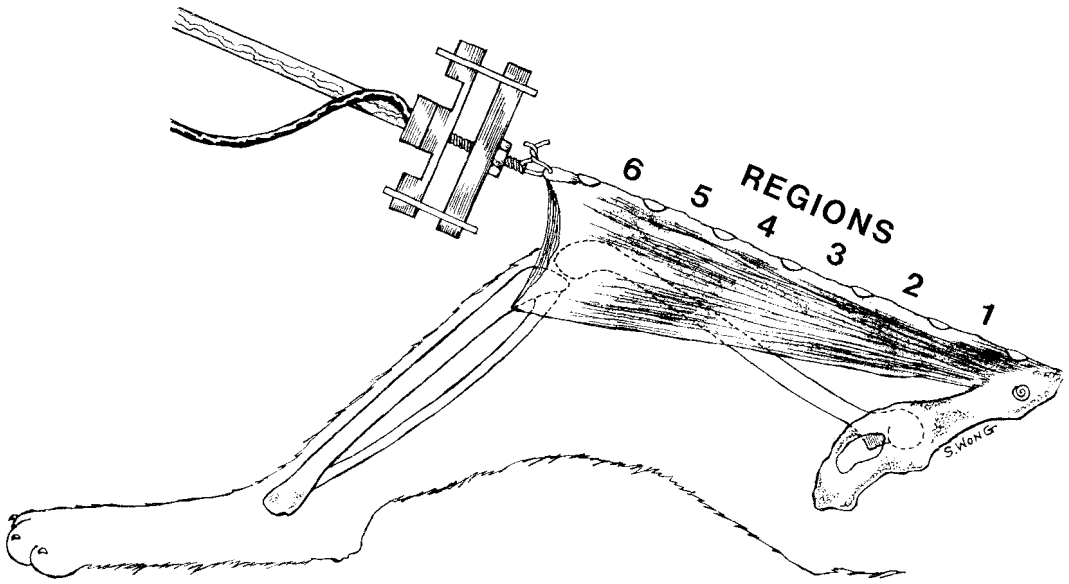

Fig. 1. Line drawing of anterior sartorius of cat (*Felis domestica*) with surface markers sutured to the muscle surface to measure intramuscular length changes. Seven markers divide the muscle into six regions (1–6). A strain gauge was secured to the patella, which in turn, was attached to a motor-driven puller that was used to vary whole-muscle length. A bone screw was fastened to the pelvis and rigidly attached to the muscle puller frame.

were performed throughout the range of physiological muscle lengths (approximately 9.5–14.5 cm) that were permitted physically by the range of motion of the hip and knee joints. Trials were conducted first at a short muscle length, and then at progressively longer lengths at increments of 0.5 cm. Individual nerve branches were stimulated in a randomized sequence at each new length and each nerve branch was stimulated for three successive trials. Results from these trials were compared to those evoked by synchronous stimulation of all nerve branches that entered the whole anterior sartorius (designated *parent*-nerve stimulation). When the twitch characteristics of the contractions had been studied throughout the length range, the testing procedure was repeated for tetanic contractions elicited by a train of stimuli at a frequency of 40 pps, lasting for a duration of 300 ms. A minimum of 10 seconds separated successive tetanic contractions; in repeated trials the muscle showed no reduction in its developed force.

A computer-controlled data-acquisition system initiated nerve stimulation and sampled the force transducer output at time intervals of 1.67 ms for twitch contractions and 3.33 ms for tetanic contractions. The positions of the reflective markers were recorded by the camera onto videotape at 60 fields/s. The video system was synchronized with the computer data-sampling system by a SMPTE time-code generator (FOSTEX, model 4010).

The animal was sacrificed with an overdose of sodium pentobarbital at the end of the experiment. Each sartorius was excised and divided into medial and anterior portions. No fascial boundary exists between these two functionally distinct regions of sartorius. However, it has been shown that a cut made along the border between the thick and thin portions of sartorius introduces only a slight error ($< 5\%$) in the measured muscle cross-sectional area of anterior sartorius (Thomson et al., '91). The anterior portion was weighed and its corresponding mass was used to predict the cross-sectional area (CSA) of the muscle according to the relationship that CSA equals the muscle mass divided by both the optimal muscle length and muscle density. Optimal muscle length, L_o, was defined as the muscle length at which maximal force, F_o, was generated following *parent*-nerve stimulation using a train of stimuli. Muscle density was assumed to be 1.06 gm/ml (Méndez and Keys, '60).

Data analysis

The initial three cats in the study were used to refine the experimental protocol and surgical techniques for the experiment. The results reported in this paper are based on the quantitative analysis of the data from six muscles in five cats. The data from the remaining three cats were excluded either because active force deteriorated in the distal portion of the muscle (see above) or because a full set of data could not be collected for technical reasons.

Changes in intramuscular length were evaluated by measuring changes in the distance between pairs of adjacent markers on the muscle. A computer software package automatically digitized the coordinates of the centroid of the reflective markers on the muscle from digitized video fields (Hu and Loeb, '91). These were scaled relative to two reference markers in each image that were aligned in the same plane as the muscle. The software provides the operator with a display of the marker perimeter that it has chosen from the contrast at each marker and permits the operator to change brightness thresholds or to override manually an obviously incorrect or missing marker location (Hu and Loeb, '91). The calculated and true centroids of the marker could be expected to deviate by no more than the radius of the marker, and then only if the marker reflectance was highly skewed to one outside portion of the marker. Even under the worst-case situation, this would represent only 10% of the average length between adjacent markers; manual adjustment of visibly incorrect centroids further reduced this possible error. The distance between each pair of markers was identified and averaged in the two video fields immediately following nerve stimulation. The distances between markers following individual *daughter*-branch stimulation were compared to the distances following *parent*-nerve stimulation. Only one (usually the second) of the three repeat trials was digitized because the length changes observed were highly consistent from one trial to the next.

The forces generated by the muscle during twitch and tetanic contractions were determined from the digitized voltages recorded from the force transducer. These force profiles were characterized by measuring the peak force, the contraction time from stimulus to peak force, the rise-time from 20 to 80% of peak force, and the half-relaxation time. Peak force was normalized to muscle

cross-sectional area to give a measure of muscle stress. Force-length curves were constructed for twitch and tetanic contractions, as well as for the passive muscle; whole-muscle length was defined by the distance between the most proximal and distal markers on the muscle, which were located on or near the bony attachments. Passive muscle force was obtained from the resting force levels recorded just prior to nerve branch stimulation.

RESULTS

Four of the six anterior sartorii analyzed in this study were supplied by three distinct nerve branches, directed proximally, centrally, and distally. The other two muscles were supplied by only proximally and distally directed nerve branches. In the one cat in which we analyzed both sartorii, two distinct nerve branches supplied the muscle on one side, and three distinct branches supplied the other.

Changes in intramuscular length

Stimulation of the *PARENT* nerve with either a single stimulus or a train of stimuli caused a strong muscular contraction characterized by minimal change in the distances between the intramuscular markers on anterior sartorius (solid circles, Fig. 2). The regions between different pairs of markers remained near pre-stimulus lengths, or shortened slightly when the muscle was shorter than its slack length. In contrast, contractions produced by stimulating individual *daughter* branches induced non-uniform changes in the distances between intramuscular markers, even though the entire muscle was held at a fixed length. In every cat, stimulation of the *proximal* branch caused the proximal regions of the muscle to shorten and the distal regions to lengthen. Conversely, stimulation of the *distal* branch caused the distal regions to shorten and the proximal regions to lengthen. When repetitive stimuli were delivered to a *daughter* branch, the distance between markers changed rapidly until a new equilibrium length was established for each region (Fig. 2). The distance between markers returned to pre-stimulus values at the end of the contraction. Single stimuli applied to a *daughter* branch elicited smaller changes in the distance between markers because the twitch force was smaller and changes in muscle length did not have time to equilibrate before the force began to fall.

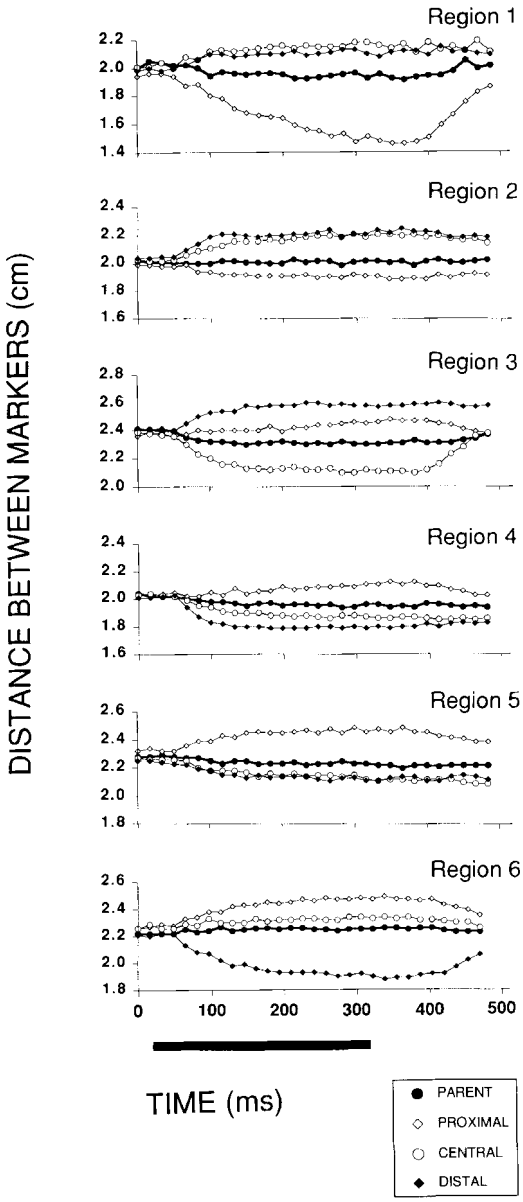

Fig. 2. The distance measured between intramuscular markers during a tetanic contraction of muscle s7 at a whole muscle length of 12.8 cm. Regions 1 through 6 define intramuscular regions in a proximal-to-distal order. Black bar denotes the 300 ms period of stimulation at 40 Hz. Contractions induced by stimulating *parent* anterior sartorius nerve or *proximal, central,* or *distal* branches.

Changes in the distance between markers were more variable when the *central* branch was stimulated. A train of stimuli applied to the *central* branch in cat s7 caused the middle regions to shorten at the expense of both the proximal and distal regions (Fig. 2). However, this pattern was not observed in other experiments; stimulation of this branch caused proximal regions to shorten in one muscle, distal regions to shorten in a second and minimal intramuscular movement in a third.

The amount of length change between markers following individual *daughter*-branch stimulation depended on the length of the whole muscle; length changes were accentuated at short muscle lengths and minimized at long muscle lengths. Figure 3 shows the typical magnitudes of the length changes as a result of *daughter*-branch stimulation in two cats, one with three daughter branches and the other with two. At short muscle lengths, the distance between markers often changed by more than ±20% in the most proximal and distal regions compared to the lengths between markers measured during *parent*-nerve stimulation. At long muscle lengths, the length changes diminished to less than ±5%.

In most instances, the reversal between shortening and lengthening regions usually occurred in the midsection of the muscle. Thus, one half of the muscle shortened at the expense of the other half, which lengthened. However, in some muscles the portion of muscle that shortened was not equal in length to the portion that lengthened. Stimulation of the *proximal* branch in two cats, s7 (Fig. 3) and s8, led to significant shortening in only the most proximal region of the muscle, whereas all other regions usually lengthened. Surprisingly, these length changes were not mirrored during stimulation of the opposing nerve branch(es). Stimulation of these opposing branches caused approximately half of the muscle to shorten and half of the muscle to lengthen.

Force output

Stimulation of the *parent* nerve with a single stimulus resulted in a force profile with an average peak value of 3.7 ± 1.4 N at optimal length and a corresponding contraction time of 52 ± 5 ms. Because larger muscles tended to generate larger forces, the variability between the forces produced by different muscles was reduced when their forces were normalized to muscle cross-sectional area (Table 1). The time from stimulus to peak force, the time from 20 to 80% of peak force, and the half-relaxation time for the twitch contractions were all dependent on muscle length. All three time measures were shorter at short whole-muscle lengths than at longer lengths (Table 1).

The force profiles of twitch contractions produced by *daughter*-branch stimulation were similar in shape, but had a smaller amplitude than the twitch contractions produced by stimulating the *parent* nerve (Fig. 4). Further, the magnitude of the stress (force per cross-sectional area) was more variable than the magnitude produced by stimulating the *parent* nerve. This is shown by the coefficient of variation (CV; see Table 1), defined as the standard deviation (SD) divided by the mean. At optimal length (for tetanic stress), the CV was 31% for the *parent* nerves, with a mean of 59% for the *daughter* branches. There was only a slight increase in the rise time measures and a slight decrease in the half-relaxation time during twitches produced by stimulating a *daughter* branch compared to those measured following *parent*-nerve stimulation at a comparable length (Table 1).

Force profiles recorded following *parent*-nerve stimulation with a train of stimuli at 40 pps were slightly unfused and had an average peak value of 10.1 ± 4.0 N at optimal length (Fig. 4). Stimulation of a *daughter* branch also resulted in an unfused tetanic force profile that was similar in shape, but smaller in magnitude than that produced by *parent*-nerve stimulation (Table 1 and Fig. 4). As with twitch contractions, the CV of contractile stress was larger following *daughter*-branch stimulation; the CV was 25% for the *parent* nerves, with a mean of 37% for the *daughter* branches.

The force-length relationships associated with *parent* nerve stimulation had a broad plateau for both twitch and tetanic contractions. Thus, an almost uniform force was produced over most of the muscle's physiological range. Production of active force was greatest near the middle of the length range and lowest at the extremes (Fig. 5). The ratio of the peak twitch to tetanic force averaged 0.37 at optimal muscle length, but decreased at lengths shorter than slack length.

The twitch and tetanic force-length relationships for each *daughter* branch were not congruent in shape with the curves for the *parent* nerve. The force production of the

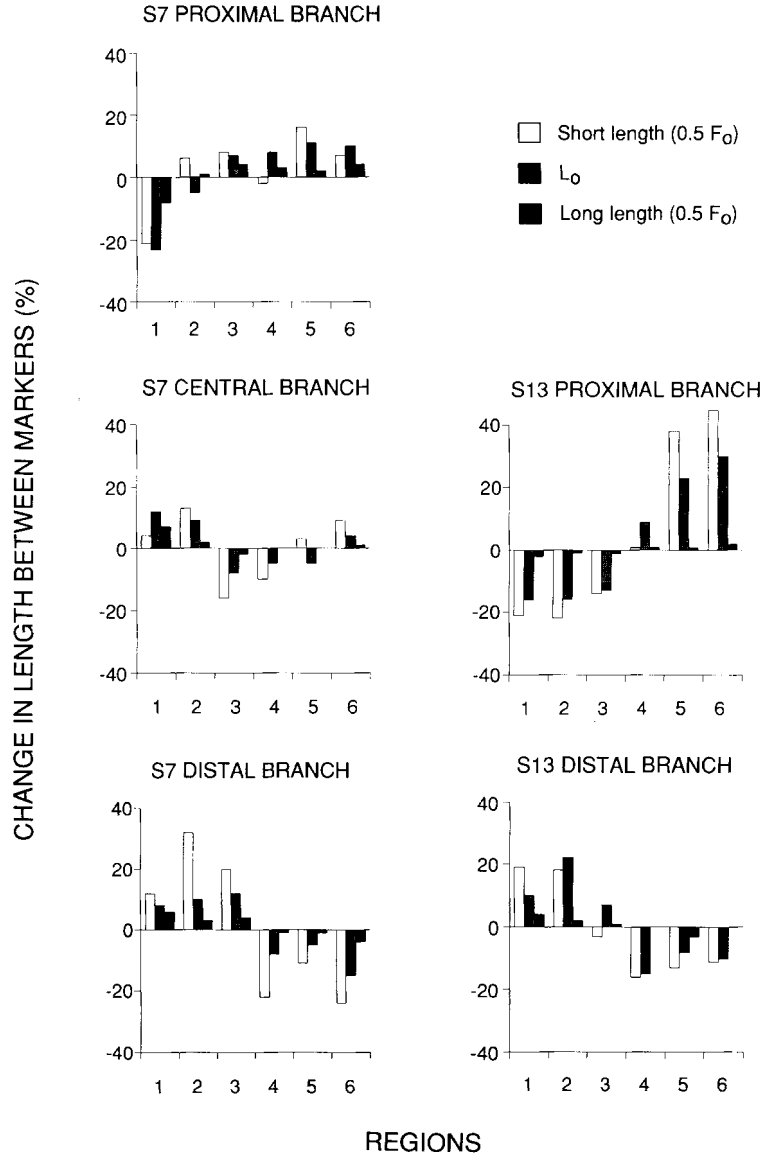

Fig. 3. Change in intramuscular lengths measured immediately following a train of stimuli (40 Hz for 300 ms) to individual daughter nerve branches (*proximal, central, distal*) for two muscles (s7 and s13). Intramuscular lengths are normalized to the lengths measured following a similar train of stimuli to the parent nerve. Changes in each of the 6 regions for three different whole muscle lengths are shown: for the optimal length, defined as the length with maximal force generation due to stimulation of the parent nerve branch (L_0), for a shorter length that produced half-maximal force, and for a longer length that produced half-maximal force.

muscle was always maximal at an equal or longer whole-muscle length when stimulating the *daughter* branches than when stimulating the *PARENT* nerve (Table 1). On average, optimal lengths associated with stimulation of the *proximal* and *distal* branches were 1.2 and 0.5 cm longer, respectively, than the optimal length associated with *parent*-nerve stimulation. At progressively shorter muscle lengths, tetanic forces diminished more when stimulating one *daughter* branch than when stimulating the

TABLE 1. Force characteristics for anterior sartorius when stimulating either individual daughter branches or the parent nerve[1]

	Parent	Proximal	Central	Distal
Number of samples	6	6	4	6
Contraction time (ms ± SD)				
Short length	44 ± 3	48 ± 3	43 ± 6	48 ± 3
Long length	61 ± 5	63 ± 5	59 ± 8	66 ± 5
Rise time: 20–80% F_0 (ms ± SD)				
Short length	14 ± 1	17 ± 1	17 ± 3	19 ± 3
Long length	20 ± 2	24 ± 2	23 ± 2	25 ± 3
Half-relax: 100–50% F_0 (ms ± SD)				
Short length	40 ± 1	42 ± 12	41 ± 4	35 ± 6
Long length	100 ± 13	90 ± 17	82 ± 16	95 ± 12
Twitch (N/cm^2)				
Mean	6.77	2.57	4.00	4.06
CV (%)	31	51	83	44
Tetanic (N/cm^2)				
Mean	18.20	10.37	10.03	14.86
CV (%)	23	33	38	40
L_0 (cm)				
s7	12.80	12.80	12.80	12.80
s8	11.40	12.60	11.40	11.40
s11	12.50	13.60	11.60	12.50
s12	12.50	13.60	12.50	12.70
s13	11.70	14.00		12.90
s15	9.80	11.40		11.40
Mean	11.78	12.99*	12.09	12.28

[1] L_0 is the length at which maximal tetanic force was obtained.
*$P < 0.01$ difference between proximal and parent L_0 (McCall, '86).

parent nerve. Conversely, at progressively longer muscle lengths, tetanic force production increased to a greater extent when stimulating the *daughter* branches as compared to the *parent* nerve. Thus, the force-length curves produced by *daughter*-branch stimulation appeared to be skewed to the left and their peaks were shifted to the right. In Figure 6 the ratio between the force produced by stimulating the individual *daughter* branches is normalized at each length to the force produced by stimulating the *parent* nerve. As muscle length increased, there was a progressive increase in the relative forces that could be generated by stimulation of individual *daughter* branches. There was a wide range in the degree to which forces were enhanced from short to long lengths; stimulation of some *daughter* branches showed an increase from near 0 to 100% of the force produced when stimulating the *parent* nerve, whereas others showed no increase in relative force with increases in muscle length.

Two interesting results occurred at very long (but still physiological) muscle lengths. First, there was a decrease in the active force produced by stimulating a *daughter* branch compared to the force produced by stimulating the *parent* nerve (Fig. 6). At these lengths, minimal changes were observed in the distances between intramuscular markers (Fig. 3). Second, there was a considerable amount of passive tension generated by the muscle at these long muscle lengths. Passive forces averaged 20 N when the muscle was at a length at which it could still generate half-maximal active force by stimulating the *parent* nerve (Fig. 7).

DISCUSSION

In the present study we analyzed the mechanical characteristics of contractions evoked by stimulation of individual nerve branches supplying anterior sartorius. There are two main findings: 1) stimulation of a daughter branch generally caused one portion of the muscle to shorten at the expense of another portion, which lengthened; and 2) the muscle force-length relationship associated with stimulation of a single daughter branch was not congruent in shape with that associated with simultaneous stimulation of all branches that entered anterior sartorius.

What determines muscle force?

The force produced by an actively contracting muscle is commonly assumed to reflect the summed actions of large numbers of sarcomeres in-parallel, all with similar lengths. In a passive situation, the assumption that all sarcomeres have similar lengths would seem reasonable. It should be dictated by the

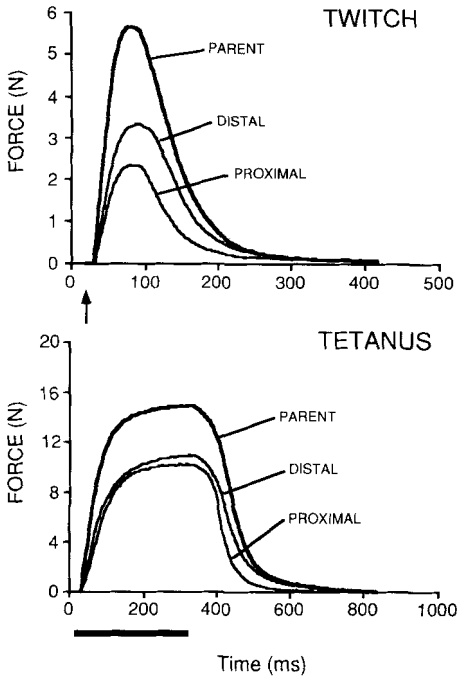

Fig. 4. The force generated by twitch and tetanic contractions in anterior sartorius upon stimulation of individual daughter branches and the parent nerve (muscle s15). Muscle lengths were 12.4 and 12.0 cm for the twitch and tetanus trials, respectively. Stimuli began 20 ms after the beginning of data collection. The arrow denotes the time of stimulation for the twitch contractions while the black bar denotes the 300 ms period of 40 Hz stimulation for the tetanic contraction.

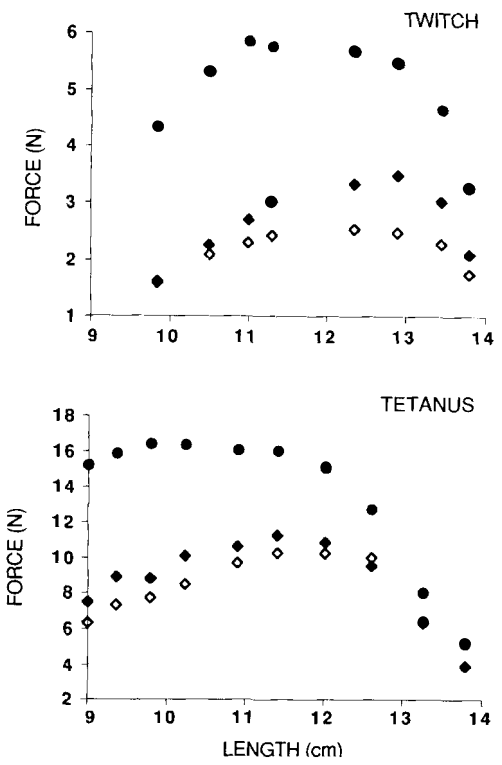

Fig. 5. Twitch and tetanic length-tension relations for anterior sartorius created by stimulation of the proximally and distally directed branches and the parent nerve (muscle s15). Each point represents the average peak response from three successive trials. Filled circles, filled diamonds, and unfilled diamonds denote muscle force produced at various muscle lengths when stimulating the parent nerve, or the distal and proximal branch nerves, respectively.

passive tension alone, which presumably should be uniform throughout the length of the muscle. Further, the assumption seems tenable during a synchronous maximal muscle contraction, because all sarcomeres are activated and there should not be large differences in sarcomere lengths. The present findings that distances between surface markers changed little during whole-muscle contraction would appear to support this view.

The intramuscular length changes observed following the stimulation of a single nerve branch can be explained by the physical requirement that the tension must be uniform along the proximodistal extent of the muscle. Regions of the muscle that contain the greatest number of recruited fibers will be capable of generating more isometric force than regions with few active fibers; for purposes of discussion, these regions can be called dominant and subordinate regions, respectively. The dominant region will immediately begin to stretch out the subordinate region, and this internal movement will affect the force-generating properties of the sarcomeres in these regions in two ways: 1) the force-velocity relationship predicts that the dominant (shortening) region will decrease its active force output while the subordinate (lengthening) region increases its force output (Joyce et al., '69); 2) if the muscle fibers are on the ascending limb of the force-length relationship, then the dominant region will descend the curve to produce lower active force while the subordinate region ascends to produce more active force (Gordon et al., '66). However, the resultant motion depends on the total force, not just the active force. Structural elements both inside (Magid and Law, '85) and outside of the muscle fibers (Purslow, '89) generate passive forces.

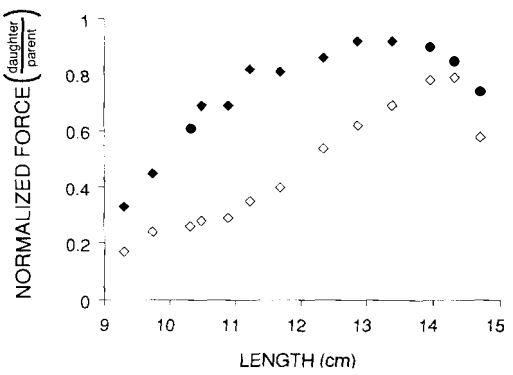

Fig. 6. Tetanic force produced by anterior sartorius upon stimulation of the proximally and distally directed nerve branches (muscle s13). Values are the average of three successive trials and are normalized to the tetanic force produced by stimulating the parent nerve at each length. Note that as muscle length increases, the relative force generated by stimulating a daughter branch also increases. Filled diamonds and unfilled diamonds denote normalized muscle force produced when stimulating the distal and proximal branch, respectively.

At longer muscle lengths, the slope of the passive stiffness curve increases sharply, so that there is increasing resistance to applied length changes. The differences in the passive and active forces in the dominant and subordinate ends compensate for the non-uniform distribution of active fibers within the muscle. Fibers in the shortened dominant regions will exert less total active and passive force than they would if they were clamped at their precontraction length. Conversely, fibers in the lengthened subordinate regions will tend to increase their force over values that would be expected if sarcomere lengths had not changed. *In all cases, a steady-state situation will develop in which equivalent forces under isometric conditions are present at all levels of the muscle, but this steady state can only be achieved as the result of heterogeneity in the sarcomere lengths from one end of the muscle to the other.*

Consideration of sarcomere heterogeneity during contractions is not new (Morgan, '85). Several experiments on single muscle fibers have demonstrated that in order for a portion of the fiber to remain isometric during a contraction, the overall length of the fiber had to be altered (Gordon et al., '66; Julian and Morgan, '79). Thus, although the portion of the fiber being monitored remained isometric throughout the contraction, other portions must have changed their lengths when whole-fiber length was altered. Morgan ('85) postulated that intersarcomere dynamics during isometric contractions are dictated by the same principles as the intramuscular length changes obtained in the present study. The force-velocity relationship compensates initially between stronger and weaker sarcomeres, but ultimately the force-length relationship of active and passive forces must equalize force production.

Intramuscular length changes develop because of non-uniform force capabilities between muscle regions in-series, but the magnitude of these changes and the force generated by the muscle are related in a complex way to several different conditions.

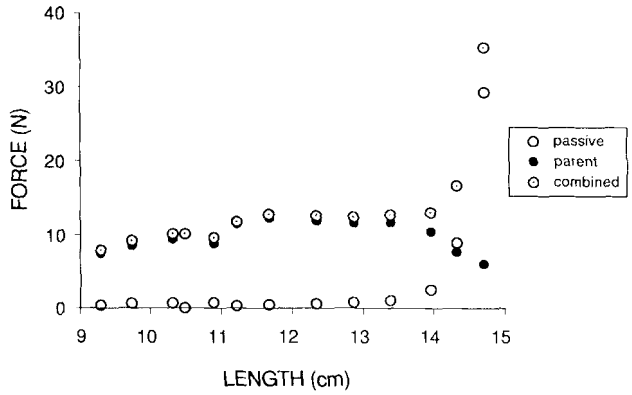

Fig. 7. Active, passive, and combined active and passive length-tension relations for muscle s13. Active force is produced by stimulation of the parent nerve by a train of stimuli for 300 ms at a rate of 40 Hz. The passive force generated by the muscle increases rapidly at long muscle lengths. The values are the average of 3 successive trials.

First, the magnitude of length changes is dependent on the initial length of the muscle. At short initial lengths, intramuscular motion is larger than at longer lengths (Fig. 3). The magnitude of the length change is dictated by the force-length relationship of the active and passive elements of muscle. At short lengths, large length changes occur in the subordinate region because the passive forces do not increase substantially in magnitude until very long lengths. Concomitantly, the dominant region shortens significantly to a length at which little force can be generated. Peak force generated by the muscle occurs at a length at which the dominant region can shorten to its optimal length. At long whole-muscle lengths, the intramuscular length changes are smaller because passive tension in the subordinate regions increases rapidly with small increments in length. If the muscle is very long (approximately 14.5 cm in Fig. 6), the negligible intramuscular length changes do not allow the dominant regions to shorten to optimal muscle length and the force generated by the muscle decreases. *The skewed force-length characteristics associated with stimulation of a single nerve branch demonstrate that the force generated by a selected group of muscle units distributed asymmetrically within the muscle is not a scaled version of the force generated by all of the motor units at that whole-muscle length.*

Initial muscle length is not the only factor that will modify both the intramuscular length changes and the consequent force produced when stimulating a single nerve branch of anterior sartorius. The number of motor units and the degree to which they are asymmetrically distributed will also be important. Thomson et al., ('91) demonstrated that both of these factors varied significantly between cats, and even between sartorii of the same cat. The smaller the asymmetry in fiber distribution along the length of the muscle, the smaller the intramuscular length changes that will be required to balance the isometric force in each region. The length changes will also be smaller if the asymmetry in motor-unit distribution remains the same, but fewer motor units are active. Fewer active fibers in the dominant region decreases its force, while the passive tension generated by both active and passive fibers remains constant in the subordinate region.

At the whole-muscle level, Bodine et al. ('82) have suggested that muscle force is proportional to the smallest total cross-sectional area of active muscle fibers. This contradicts their own data showing that the cat semitendinosus muscle was still able to generate maximum isometric tension even though only one of the two in-series compartments was stimulated. In fact, the maximal active force generated by the muscle should be dictated by the largest cross-sectional area of active muscle fibers, but it can be produced only when this region is at its optimal length. Achieving this condition during a contraction may require substantial prestretching of a muscle in which the recruited motor units constitute a variable percentage of the cross-sectional area along the length of the muscle.

What determines time-course of force?

The present study analyzed the isometric characteristic of anterior sartorius when selected populations of motor units were stimulated. Much of the data was obtained from the end of tetanic contractions, where intramuscular length changes and muscle force remained relatively constant. Although twitch contractions become isometric briefly as muscle force peaks, the magnitude and time of that peak force are dependent on many conflicting factors, including the amount of in-series compliance (Hill, '51), the rate that intracellular calcium is re-sequestrated during the twitch (Blinks et al., '78), the hypothesized dependence on muscle length on the uptake of intracellular calcium (Bahler, '67), and the rate constant for cross-bridge formation (Julian, '69; Stein et al., '88). As a result, seemingly counter-intuitive phenomena occur during twitch contractions at different muscle lengths. Despite the fact that the muscle fibers remain closer to isometric at long muscle-lengths, contraction times increase with muscle length (Table 1; Bahler et al., '67; Hartree and Hill, '21). In contrast, the asymmetric recruitment of muscle fibers within anterior sartorius only increased marginally the twitch rise-time (Table 1). These phenomena appear to suggest that the length-dependent dynamics of calcium release and re-uptake may dominate twitch dynamics. However, new length- and time-dependent processes continue to be discovered that affect the force produced by the complex ensembles of muscle fibers and endomysial connective tissues that constitute whole mammalian muscles (e.g., Heckman, Weytjens and Loeb, personal communication).

The natural use of in-series fibered muscles

One could argue that stimulation of a single nerve branch entering anterior sartorius does not represent the normal recruitment of motor units within the muscle and that the observed imbalances in the force produced in this muscle do not occur naturally. Motoneurons with axons in the proximally and distally directed nerve branches are intermingled extensively within the sartorius motor nucleus (Gordon et al., '91). Command signals would have to be distributed in a specialized manner unrelated to the apparent topography of motoneurons if they were to produce a specialized contraction of one muscle part. Thus, it is intriguing that specialized patterns of recruitment have been reported in anterior sartorius. For example, Hoffer et al. ('87) recorded the activity of single motoneurons during locomotion. Motoneurons supplying anterior sartorius were shown to have one of two patterns of activity. They were active either during the stance phase or during the swing phase but not during both phases. Further, early studies by Sherrington ('10) identified contractions in anterior sartorius that were isolated to the proximal portion during the flexion reflex and to the distal portion during crossed extension. These observations were performed on decerebrated cats with their hindlimbs free to move from a gravitationally defined vertical position, which would accentuate the kinematic effect of even slight kinetic imbalances. However, Cooper ('29) could not reproduce the results of Sherrington under similar conditions. It remains to be explored whether motor units can be recruited in a non-uniform manner (proximodistally) during normal activity in alert animals. No differences in electromyographic (EMG) activity at different muscle levels have yet been recorded during normal activity, but recording sites used to monitor the EMG activity have been distal to the entry regions for the various nerve branches and thus not placed optimally to detect such differences (Pratt and Loeb, '91).

Experimental and clinical significance of anterior sartorius

Anterior sartorius is probably not unique with regard to its motor unit organization. Long fascicles with short in-series fibers constitute a necessary but not sufficient condition for proximodistal gradients of recruitment. Nerve branches that enter at different rostrocaudal levels are not necessary, but they may suggest the likelihood of muscle-unit asymmetry. Other feline muscles such as medial sartorius and medial splenius share these traits with anterior sartorius (Richmond et al., '85; Loeb et al., '87). Analysis of human sartorius has demonstrated that single muscle fibers often end intrafascicularly (Barrett, '62; Smits et al., '91). However, many feline and human muscles remain to be analyzed to determine if they have both in-series fibers and asymmetric distributions of muscle units. Much more systematic work will be required to determine the prevalence and functional significance of this architecture.

Under normal physiological conditions, muscles with in-series fibers presumably are recruited and controlled in a stable manner, although the extent of the neural control strategies to achieve this end result are still unclear. However, problems might be anticipated under pathological conditions. Damage of a nerve branch or of fibers at one end of the muscle would presumably modify the force-generating properties of the muscle in much the same way as that shown here. Many different processes of adaptation and repair could modify this situation over time, including the loss or addition of sarcomeres, the hypertrophy of remaining muscle fibers and/or connective tissue, and sprouting and reinnervation from remaining motor axons.

The asymmetric distribution of motor units supplied by a nerve branch poses a significant problem for the use of functional neuromuscular stimulation, whereby paralyzed muscles are re-animated by electrically stimulating their terminal nerve branches (Grandjean and Mortimer, '86). If such stimuli were applied at only one intramuscular site, asymmetric recruitment of muscle fibers would cause intramuscular length changes, thus affecting the force output of the muscle as a whole and perhaps even the mechanical integrity of the largely unstimulated muscle regions.

The physiology of individual muscle fibers and motor units has been studied extensively, but such studies have been conducted almost exclusively in a few muscles with relatively simple patterns of neuromuscular architecture. Little information exists on the mechanics of the large number of muscles that have more complex architectural features. In the present study, the in-series arrangement of motor units in anterior sartorius has been shown to confer specialized

mechanical properties during submaximal recruitment that may affect the force-generating capabilities of the muscle under physiological and/or pathological conditions. Much more work is required to determine the range, prevalence, and importance of complexity in neuromuscular architecture and its implications for the motor control of complete musculoskeletal systems.

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