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Structural Limits on Force Production and Shortening of Smooth Muscle

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Abstract

This study determined the factors that limit force production and shortening in two smooth muscles having very different relationships between active and passive force as a function of muscle length. The rat anococcygeus muscle develops active force over the range of lengths 0.2 – 2.0× the optimum length for force production (L_o). Passive tension due to extension of the resting muscle occurs only at lengths exceeding L_o . In contrast, the rabbit taenia coli develops force in the range of lengths 0.4– 1.1 L_o , and passive force which is detectable at 0.56 L_o , increases to ~0.45 maximum active force at L_o , and increases sharply with further extension. The anococcygeus muscle can shorten to 0.2 L_o and the taenia coli to 0.4 L_o . Dynamic stiffness and energy usage at short muscle lengths suggest that the limit of shortening in the taenia coli, in contrast to the anococcygeus muscle, is not due to a failure of cross bridge interaction. Phosphorylation of the regulatory myosin light chains in intact muscles decreased to a small extent at short lengths compared to the decrease in force production. The differences in force production and the extent of shortening in the two muscles was maintained even when, following permeabilization, the myosin light chains were irreversibly phosphorylated with ATP γ S, indicating that differences in activation played little, if any role. Ultrastructural studies on resting and activated muscles show that the taenia coli, which is rich in connective tissue (unlike the anococcygeus muscle) undergoes marked cellular twisting and contractile filament misalignment at short lengths with compression of the extracellular matrix. As a result, force is not transmitted in the longitudinal axis of the muscle, but is dissipated against an internal load provided by the compressed extracellular matrix. These observations on two very different normal smooth muscles reveal how differences in the relative contribution of active and passive structural elements determine their mechanical behavior, and how this is potentially modified by remodeling that occurs in disease and in response to changes in functional demand.

INTRODUCTION

Studies on the ultrastructure of smooth muscle have shown that the contractile proteins are arranged in filaments (Devine & Somlyo, 1971; Somlyo, 1972) and that these may be organized as contractile units within the smooth muscle cell (Somlyo *et al.*, 1984). The relationships between muscle length and force production have been studied in a variety of vertebrate and invertebrate smooth muscles and, qualitatively, these have proved to be similar to striated muscle (Gordon & Siegman, 1971a; Cornelius & Lowy, 1978; Peterson & Paul, 1974; Murphy, 1976), indicating that contraction in smooth muscle also operates by means of a sliding filament mechanism (Gordon *et al.*, 1966). The relationship between active force and muscle length is approximately bell shaped, with peak force (P_o) occurring at an optimum length designated L_o . The sliding filament hypothesis relies primarily on the

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finding of a proportional relationship between active force production and contractile filament overlap at muscle lengths exceeding L_o and this is traced to the number of myosin cross bridges interacting with actin (Gordon *et al.*, 1966) as filament overlap diminishes. Like striated muscles, all smooth muscles studied exhibit an optimum length for force development. It has been difficult to measure force production at long lengths due to the fact that in most smooth muscles, particularly those from tissues having reservoir or conduit functions, slack length (L_s , the length at which passive force is just detectable) occurs at shorter muscle lengths than L_o . In these muscles there is considerable passive force at L_o , which rises steeply upon further extension of the muscle. Generally, the observation has been that active force diminishes sharply at lengths exceeding $1.1 L_o$ in such muscles (Gordon & Siegman, 1971a; Peterson & Paul, 1974; Herlihy & Murphy, 1973; Lowy & Mulvany, 1973), primarily due to damage resulting from the stretch itself. In certain vascular preparations (mesenteric vein and artery), in which $L_s > L_o$, the steep rise in the passive force curve appears to occur at slightly *longer* lengths than in other preparations, Peterson and Paul (1974) and Mulvany and Warshaw (1979) succeeded in obtaining reliable measurements of active force at lengths longer than $1.1 L_o$, and found that force decreased linearly with increasing length, which extrapolated to zero force at $\sim 1.82 L_o$ and $\sim 2.0 L_o$, respectively. Their work provided strong evidence for a sliding filament mechanism in smooth muscle.

A well-known characteristic of intact smooth muscle is its ability to contract to very short lengths. There are differences among smooth muscles in the apparent minimum length at which force can be developed and the length to which the muscle can shorten; generally based on extrapolated estimates, the values range from $0.2 L_o$ to $0.58 L_o$ (Gordon & Siegman, 1971a; Peterson & Paul, 1974; Herlihy & Murphy, 1973; Mulvany & Warshaw, 1979; Siegman *et al.*, 1976; Uvelius, 1976; Meiss, 1978). Little is known about the basis of the decline in active force production at short lengths in smooth muscle and there probably is not a single explanation that would provide an answer for all muscle types. For example, dynamic stiffness, which is taken as a measure of actin-myosin interaction, has been shown to exceed isometric force at muscle lengths shorter than L_o in rabbit mesotubarium (Meiss, 1978). This led to the suggestion that factors other than cross bridge interaction may determine force output at short lengths. While stiffness may be largely due to actin-myosin interaction, it might also include elements in series with the contractile apparatus, including intercellular connections and the extracellular matrix. A similar hypothesis was proposed earlier (Siegman *et al.*, 1984) for the rabbit taenia coli. More recently, increasing attention has been given to the importance of intercellular interactions of smooth muscle cells in tissues in an attempt to account for mechanical properties that limit shortening (Gunst *et al.*, 1995; Meiss, 1991; Meiss, 1992; Meiss, 1998; Meiss, 1999) but no direct evidence was provided.

Taken together, there is a major lack of information regarding structure that would help explain (1) the very fundamental decrease in force production at short muscle lengths and (2) the range of lengths over which smooth muscles function. From information gained from studies of striated muscles, at least four possible mechanisms could operate: (1) a decrease in the degree of activation at short lengths, (2) the production of opposing forces and the internal dissipation of active force, (3) a structurally-induced decrease in the number of cross bridges able to interact with actin, and (4) misalignment of contractile filaments and/or cells such that force is generated in an axis other than that measured. Each of these factors, alone or in combination, would reduce the force transmitted to and measured at the ends of the muscle, as well as limit the length to which the muscle could shorten under isotonic conditions.

In this investigation, we sought to determine how the above factors contribute to the observed decrease in force at lengths shorter than L_0 in smooth muscle. The experimental models chosen are two smooth muscles having strikingly different length-force relationships: the taenia coli of the rabbit and the anococcygeus muscle of the rat. We have measured isometric force output, the degree of myosin light chain phosphorylation, and determined the extent of actin-myosin interaction through measurements of dynamic stiffness and chemical energy usage in these muscles. In addition, we have examined the ultrastructural characteristics of the muscles as a function of initial muscle length and activation state in an effort to understand the differences in the basis of the decline of force on the ascending limb of the length-force relationship. To our knowledge, no comparable systematic study such as this has been published for smooth muscle to date. This work suggests that external force developed is not an adequate measure of cross bridge interaction at short muscle lengths. Rather, structural constraints may limit force output and thereby determine the length-force relation of different smooth muscles. Structural factors may override other processes that limit force production, such as the degree of activation at very short muscle lengths. The influence of structural constraints may be reflected in the degree of passive force at a given muscle length, and this may be traced to the composition and distribution of intercellular elements among smooth muscle cells.

METHODS

1. Smooth muscle preparations

Rabbits and rats were anesthetized and euthanized according to a protocol approved by the Animal Care and Use Committee of Thomas Jefferson University. Strips of taenia coli muscles were excised from the caecum of immature female rabbits (New Zealand strain, white) weighing 1.5–2.5 kg and separated from adhering connective tissue and circular muscle (Gordon & Siegman, 1971a; Siegman *et al.*, 1980). The dimensions of the strips were $\sim 10 \text{ mm} \times 2 \text{ mm} \times 0.22 \text{ mm}$. The anococcygeus muscles were isolated from male rats (Sprague-Dawley) ~ 6 weeks of age in order to optimize the size of the anococcygeus muscles and to avoid possible complexities of the estrus cycle of females. The anococcygeus muscles, which attach to the rectum and to the posterior abdominal wall adjacent to the spinal column by slender tendons, are near-trapezoidal in shape. This is due to the fact that each muscle consists of two overlapping bundles, each with a terminal tendon. They were carefully dissected under transillumination to exclude fibers that ran tangential to the long axis of the muscle; the resulting strips were $\sim 9 \text{ mm} \times 2 \text{ mm} \times 0.14 \text{ mm}$. The muscles were mounted under isometric conditions in Plexiglas chambers and muscles were allowed to equilibrate under 2×10^{-2} newton (N) for two hours prior to the start of the experiment at 20°C . At this temperature, the spontaneous activity of the taenia coli ceases (Gordon and Siegman, 1971a); the anococcygeus muscle shows no spontaneous activity.

2. Solutions

(a) Krebs-bicarbonate solution (mM): NaCl, 118; KCl, 4.7; MgSO_4 , 1.18; KH_2PO_4 , 1.2; CaCl_2 , 1.9; NaHCO_3 , 25.01; glucose, 11. The solution was gassed with a mixture of 95% O_2 -5% CO_2 and the pH was 7.4. (b) Krebs-Hepes solution (mM): NaCl, 141; KCl, 4.7; KH_2PO_4 , 1.18; MgSO_4 , 1.17; CaCl_2 , 1.6; glucose, 5.5; Hepes buffer, 10, pH 7.4.

3. Oxygen consumption and lactate production

The muscles were mounted in a glass chamber designed for measuring isometric force and equipped with a miniature Clark-type polarographic oxygen electrode (Diamond Electro-tech model 731) using methods described in detail previously (Davidheiser *et al.*, 1984). The muscles were bathed in a flowing Krebs-Hepes medium. For the anococcygeus muscle, the bathing medium was equilibrated with 30% oxygen-70% nitrogen at 37°C and, for the taenia

coli, the medium was equilibrated with 21% oxygen in air at 20°C. The latter was necessary to prevent spontaneous contractile activity and to increase the sensitivity of the measurement at the lower temperature. The anococcygeus was stimulated maximally with 5 μ M norepinephrine and the taenia coli with 5 μ M acetylcholine, consistent with their neural activation. Flow of medium through the chamber was stopped for 15 min intervals while J_{O_2} and force were monitored. Aliquots of the medium in the chamber at the end of each 15 min period were removed for the determination of lactate (Hohorst, 1963).

4. Measurement of mechanical properties

The *length-force* relationships were determined according to the method of Gordon and Siegman (Gordon & Siegman, 1971a). This was done in conjunction with measurements of stiffness, in which the muscles were supramaximally stimulated in a transverse electrical field (60Hz, 10V rms) and in conjunction with oxygen consumption measurements during steady-state force maintenance, in which the muscles were maximally stimulated with either 5 μ M norepinephrine (anococcygeus) or 5 μ M acetylcholine (taenia coli). The long sampling time required for oxygen consumption determinations precluded the use of electrical stimulation. There were no significant differences in the length-force relationships with the two methods of stimulation.

For measurements of *stiffness*, the muscles were attached to the arm of a servomotor (Cambridge 300H) for the imposition of rapid changes in length and to a force transducer (Akers AE 801) for the measurement of force. Force and length signals were recorded on a digital storage oscilloscope (Nicolet 4094). Dynamic stiffness was measured according to the method of Bressler and Clinch (Bressler & Clinch, 1974) during the course of length-force determinations. At a given length, muscles were tetanically stimulated to maximum force (25 sec for the taenia coli and 10 sec for the anococcygeus) then quickly released 3–8% L_o at a rate that exceeded their respective V_{max} by a factor of 200. The instantaneous stiffness (S) was taken as the slope of the initial linear portion of the force-extension curve obtained during the release. The stiffness under unstimulated conditions was subtracted from that during contraction, assuming a Maxwell model. For each muscle, the stiffness values obtained at each initial length were normalized to those at L_o (the optimum length for force production). For comparative purposes at muscle lengths shorter than L_o , stiffness was also determined in a limited number of experiments from the changes in active force in response to small rapid step changes in length (stretches or releases of 0.5% L_o to 2% L_o) (Huxley & Simmons, 1971). The results were not significantly different from those obtained by the method of Bressler and Clinch and therefore only the latter are presented.

5. Length-dependence of myosin light chain phosphorylation

Muscles were mounted under isometric conditions and L_o was determined. Muscles were stimulated at L_o and allowed to relax and then recover for 10 min. The muscle length was either kept at L_o or reduced to a specific length and then stimulation was repeated. Taenia coli strips were stimulated supramaximally in a transverse electrical field (60Hz, 10 v rms) and frozen with clamps pre-cooled in liquid N_2 at 25 sec, when peak force was attained. Anococcygeus strips were similarly treated except that they were stimulated with norepinephrine (5 μ M) for 3 min. Resting muscles were stimulated at L_o and allowed to relax and recover for 10 min prior to freezing. The frozen taenia coli and anococcygeus muscles were pulverized with frozen 0.5 M $HClO_4$ and, following thawing and centrifugation, the protein precipitate was either dissolved in a urea-containing solution (taenia coli) or an SDS-containing solution (anococcygeus). Myosin light chain phosphorylation was determined by IEF-SDS two-dimensional gel electrophoresis (Butler *et al.*, 1983) for the taenia coli. The extent of myosin light chain phosphorylation was determined from the densitometric signals from the phosphorylated and unphosphorylated

forms of the light chain in the second dimension gel. The result for each muscle was divided by the average phosphorylation for muscles stimulated at *Lo*. For the anococcygeus, the proteins were subjected to SDS-PAGE. The gels were stained with Pro-Q Diamond phosphoprotein stain (Life Technologies, Grand Island, NY) followed by the use of SYPRO Ruby protein gel stain. A Fluorochem imager (Alpha Innotech, Santa Clara, CA) was used to quantitate the fluorescent signals from the gel. The location of the light chain on the gel was confirmed using an antibody to the regulatory light chain of smooth muscle myosin (Cell Signaling Technology, Danvers, MA). The ratio of the fluorescence from the phosphoprotein stain to the protein stain was determined for each sample in a gel and compared to the average ratio for samples in the same gel that were stimulated at *Lo*. The data are reported as phosphorylation relative to that at *Lo*. Unstimulated anococcygeus muscles at *Lo* had a relative phosphorylation of $0.13 \pm .01$ N=6 (mean $\pm$ SEM).

6. Thiophosphorylation of myosin light chains

Muscles were mounted under isometric conditions and *Lo* was determined as described above. The muscles were permeabilized at *Lo* with 1% Triton-X-100 in rigor solution for 30 min. With this treatment, the plasma membrane is removed but the extracellular matrix and intracellular links remain intact. Force production in these preparations is at least equal to that of the intact, maximally stimulated muscle (data not shown). The constituents of the various solutions used for the permeabilized muscles are similar to those described earlier (Vyas *et al.*, 1994). The regulatory light chain was thiophosphorylated by incubation for 10 min in a solution containing 1 mM ATP γ S, pCa 4.5, 1 μ M calmodulin. The muscle was then subjected to three washes in rigor solution, and then activated in a solution containing 3 mM ATP, 5 mM phosphocreatine with no added calcium. Maximum force was measured at *Lo* and during peak force maintenance following shortening to 0.6 *Lo*, 0.4 *Lo*, 0.3 *Lo*, 0.2 *Lo*, and 0.15 *Lo*.

7. Ultrastructure

The taenia coli and anococcygeus muscles were isolated and mounted under isometric conditions in chambers containing Krebs-bicarbonate solution at 20°C, and *Lo* was determined. Muscle length was set at *Lo* or some shorter length and fixed either at rest or during the plateau of an isometric tetanus while on the myograph. For activation, strips of taenia coli and anococcygeus were stimulated with acetylcholine (5 μ M) or norepinephrine (2 μ M), respectively. When the plateau of force production was reached, the same medium was replaced by one containing fixative; force remained constant during fixation. For muscles fixed at rest, muscles were stimulated at the designated length followed by repeated washout of the agonist and replacement with fresh Krebs solution over a period of 15 min following relaxation. The muscles were carefully removed from the myograph after 15 min and fixation was continued for an additional 105 min, after which the muscles were prepared for electron microscopy according to a protocol reported by us previously (Siegman *et al.*, 1980). Sections were examined in a Hitachi H7000 electron microscope.

8. Statistics

All values are means $\pm$ standard error, and N= number of observations. Comparisons were made using Student's *t* test.

RESULTS

I. Length-force relationships

The length-force relationships for the rabbit taenia coli and rat anococcygeus muscles are shown in Fig. 1. The length at which passive force is detectable is designated as slack

length, or L_s . In the rabbit taenia coli, passive force is detectable at $\sim 0.57 L_o$ and rises monotonically as the muscle is extended. At L_o , passive force is approximately 45% P_o and increases sharply at longer lengths. The high passive force precluded the determination of active force at muscle lengths exceeding $1.2 L_o$; following stimulation at such long lengths, there was a greater than 30% reduction in force when the muscle was subsequently stimulated at L_o , suggesting irreversible damage of the muscle, as has been reported by others (Peterson & Paul, 1974; Mulvany & Warshaw, 1979). The taenia coli can shorten to approximately $0.4 L_o$. This directly measured value exceeds the extrapolated estimate of $0.25 L_o$ previously reported (Gordon & Siegman, 1971a).

In contrast, in the *anococcygeus muscle*, passive force is just detectable at L_o , and rises steeply as muscle length is increased. At approximately $1.45 L_o$, active force was comparable to the passive force of the resting muscle. After stretch to up to $1.4 L_o$, force production on return to L_o is readily reproducible, indicating that stretch did not damage the muscle. However, at lengths of $1.5 L_o$ or greater, the active force upon return to L_o became variable, and any preparation showing less than 80% P_o force under such conditions was discarded. The span of lengths over which the anococcygeus muscle can operate extends from 0.2 to a maximum (extrapolated) of $\sim 1.9 L_o$. The differences in the passive length-force relationships of these two muscles reflect differences in their connective tissue content, noted in early ultrastructural studies by Gillespie and Lullman-Rauch (Gillespie & Lullmann-Rauch, 1974) and Gabella (Gabella, 1983).

2. Force, stiffness and energy usage as a function of muscle length

One of the major differences in the length-force relationships of the taenia coli and anococcygeus muscles is the range of lengths over which the muscles operate. The minimum length at which force can be produced in the anococcygeus is $\sim 0.2 L_o$, but only $\sim 0.4 L_o$ in the taenia coli. Nevertheless, in both the taenia coli and anococcygeus muscles, there is a nearly 10-fold decrease in active force production as muscle length is decreased from L_o to these minimum lengths. In order to assess actin-myosin interaction during force production at various muscle lengths, stiffness and energy usage were measured. Dynamic stiffness was measured by two methods (Bressler & Clinch, 1974; Huxley & Simmons, 1971) for comparative purposes, with similar results. Energy usage was calculated from the oxygen consumption and lactate production during steady-state force maintenance at various muscle lengths. The results are shown in Figs. 2A and B. In the taenia coli, lactate production was nil, and was therefore ignored; energy usage, taken as oxygen consumption, did not change significantly as force decreased. The relative stiffness decreased but exceeded force production at each muscle length (Fig. 2A). In the anococcygeus muscle, both energy usage and stiffness decreased with muscle length as force decreased, but stiffness was higher than both these parameters only at $0.5 L_o$ or shorter lengths (Fig. 2B). If the major energy-consuming process is actin-activated myosin ATPase, then the data for the taenia coli are not consistent with the concept that a decrease in cross bridge interaction is responsible for the force decrease at short muscle lengths. In the anococcygeus, a muscle with no passive force at short lengths, a major portion of the energy usage can be attributed to cross bridge interaction and force production, and these parameters remain tightly coupled until the shortest muscle length is reached at $\sim 0.2 L_o$. This is similar to events in skeletal muscle (Infante *et al.*, 1964; Sandberg & Carlson, 1966), and in vascular smooth muscles (Paul & Peterson, 1975; Gluck & Paul, 1977), which have little or no passive force at short muscle lengths. Stiffness exceeded force at the very short muscle lengths, and may reflect stiffness derived from extracellular elements. It is interesting that in the anococcygeus, force and stiffness were tightly correlated at lengths exceeding L_o . The presence of high passive force in this range of lengths precluded similar determinations on the taenia coli.

3. Length-dependence of activation processes

It is possible that differences in the degree of activation account for the differences in relationships among force, stiffness and energy usage at the short muscle lengths in the two muscles. To test this, the degree of phosphorylation of the 20kDa light chain of myosin was measured together with force at various muscle lengths. The results are shown in Fig. 3A.

There was a modest, but significant decrease in the degree of phosphorylation at muscle lengths shorter than L_o in both muscles. It is interesting that the decline in force and phosphorylation for both muscles is near-linear when the muscles are shortened to about 0.6 L_o , but further shortening results in a steep decline in force in the face of small changes in myosin light chain phosphorylation. Specifically, when the taenia coli is shortened from 0.64 L_o to 0.52 L_o , force decreases by 30%, but phosphorylation does not change significantly. For the anococcygeus, shortening from 0.6 L_o to 0.4 L_o results in a ~ 30% decrease in force with only a 17% decrease in phosphorylation.

To further test the length-dependence of activation processes, anococcygeus and taenia coli muscle lengths were set at L_o , permeabilized and then thiophosphorylated with ATP γ S to fully phosphorylate the light chains of myosin (Vyas *et al.*, 1994). The thiophosphorylated light chain is not subject to phosphatase activity and therefore the level of thiophosphorylation does not change as muscle length decreased. Force developed when ATP was added, and muscle length was then reduced and measured at each of the various lengths shown in Fig. 3B. Just as in the intact muscles, the relationship between force and muscle length was similar in both muscles at lengths 0.6 L_o – L_o . However, in the taenia coli, force decreased sharply at lengths shorter than 0.6 L_o . These results indicate that the differences in force production at short lengths in these two muscles are not due to differences in the degree of their activation.

4. Ultrastructure and muscle length

The results suggest that in the rabbit taenia coli, the major portion of the decrease in active isometric force at muscles lengths below L_o is probably not due to a decrease in the number of cross bridges interacting with actin. This is unlike the rat anococcygeus muscle, where stiffness and energy usage are tightly coupled except at the very shortest muscle lengths studied, where stiffness exceeded force. The anococcygeus, unlike the taenia coli, has no passive force at short muscle lengths and is not as rich in passive, non-compliant elements. Therefore, it seemed likely that the decrease in force is due to structural complexities, such as the presence of an internal load, or perhaps to filament and/or cell misalignment. These factors may occur to a far greater extent in the taenia coli than in the anococcygeus muscle. To test this hypothesis, the ultrastructure of the rabbit taenia coli and anococcygeus muscles was examined at different muscle lengths under resting and stimulated conditions.

a. Taenia coli—When the length of the rabbit taenia coli muscle is at L_o , there is considerable passive force (Fig. 1). As reported by Gabella (1983) the muscle cells are surrounded by abundant connective and elastic tissue. In both resting and activated conditions (Fig. 4), the contractile filaments and cells are well aligned with the longitudinal axis of the muscle. In transverse section, the cell profiles are somewhat irregular, showing very small evaginations of the plasma membrane (blebs) between plasma membrane dense bodies. One can speculate that such blebbing may be due to contractile elements “pulling” against the extracellular matrix as the large series elasticity of this muscle is extended (~20% muscle length at L_o , (Gordon & Siegman, 1971b)), with significant internal shortening.

At slack length ($0.57 L_o$), where passive force is just detectable (Figs. 5A, B), the resting cell profiles are smooth but in longitudinal section, some irregularities occur that include filament misalignment. When the muscle is stimulated (Figs. 5 C, D) there is greater blebbing of the plasma membrane between dense bodies, apparent in both transverse and longitudinal sections, suggesting some cell shortening. This is not unexpected at L_s since there is no major resistance of the extracellular matrix and because of the cellular series elasticity. In general, the cells at this length remain quite straight and contractile filaments are in alignment with the longitudinal axis of the cells.

At the shorter muscle length, $0.46 L_o$ where there is no passive force, cells at rest have a rather irregular shape Figs. 6 A, B. In longitudinal section it is apparent that the cell profiles follow the contours of the extracellular connective tissue matrix through which they interact mechanically (Fig. 6 B). This is not unexpected considering that the muscle was stimulated and allowed to shorten to this length and then allowed to fully relax and rest prior to fixation. Even allowing for some re-extension of the cells due to intracellular resistive forces, at this length the overall length of the cells is shorter and the extracellular matrix is compressed; on relaxation the cells assume a shape consistent with now floppy extracellular matrix to which they attach. When the muscle is stimulated and only about $0.2 P_o$ active force is produced, marked changes in structure occur (Figs. 6 C, D). The plasma membrane becomes highly irregular, and blebs in regions between dense bodies are larger and more abundant (arrows). Extracellular connective tissue elements are sinusoidal in appearance signifying compression (Fig. 6 D, arrow heads). As can be seen in longitudinal section, some of the shortened cells straighten out somewhat and filaments are better aligned with the longitudinal axis of the cell. These unusual contours and misalignments of filaments, together with the concentration of dense bodies in narrowed areas of the cells, appear to reflect the twisting of cells at the short length. This is also apparent in transverse sections, where, in some cells and in areas within individual cells, the typical cross-sectional view of filaments is, instead, tangential or near-longitudinal (Fig. 7). All of these structural factors would account for the low active force at this length, $\sim 0.5 P_o$.

b. Anococcygeus—In the anococcygeus muscle, L_o , the optimum length for active force production is coincident with L_s , the length at which passive force is just detectable (see Fig. 1). A conspicuous feature of the structure of the anococcygeus muscle is the less dense connective tissue matrix and virtual absence of elastic fibers and laminae that are so prominent in the taenia coli. At L_o , resting cells profiles are smooth in appearance (Figs. 8 A, B) and cells and filaments lie straight and in parallel to one-another (Fig. 8 B). When stimulated, there is modest bleb formation (Figs. 8 C, D, arrows), which is expected, considering that passive force is just detectable at this muscle length.

At $0.5 L_o$, the resting cell profiles in transverse section appear slightly irregular in appearance (Figs. 9 A, B) but in longitudinal section the filaments and cells lie straight and parallel to the longitudinal axis of the muscle. On stimulation, however, there is pronounced bleb formation which is readily apparent in transverse and longitudinal sections (Fig. 9 C, D). Compared to the appearance at longer lengths (see Fig. 8A), the extracellular space is smaller, as expected in a constant-volume system, due to the larger diameter of cells in addition to bleb formation.

When anococcygeus muscle length is reduced further and set to $0.3 L_o$, active force is only about 25% P_o (Fig. 1). At this short length, the resting muscle profiles are somewhat irregular (Figs. 10 A, B), and it is apparent from the longitudinal view that the cells are wavy, with filaments aligned in parallel with the plasma membrane. When the muscle is stimulated (Figs. 10 C, D) there is pronounced bleb formation, which is also conspicuous in

longitudinal section. Notable also is the sparse distribution of connective tissue, in marked contrast to the taenia coli. Thin connective tissue strands link neighboring cells (Fig. 10D).

The blebs that form in both muscles at short lengths are rich in caveolae and elements of the sarcoplasmic reticulum (SR), and contractile filaments are notably absent. Dense bodies are conspicuous at inter-bleb regions of the plasma membrane (Fig. 11).

DISCUSSION

Among the hallmark features of vertebrate smooth muscles is the ability to develop as much force as skeletal muscles in the face of far lower myosin content (Tregear & Squire, 1973; Murphy *et al.*, 1974) and the ability to shorten to far less than half their resting length (Peterson & Paul, 1974; Mulvany & Warshaw, 1979; Meiss, 1978). Although the length-force relationships are qualitatively similar, little is known about the basis for the decrease in force at short muscle lengths and the wide and variable range of lengths over which different smooth muscles function. The overall goal of this investigation was to seek an explanation for these characteristics through an analysis of mechanical, energetic and ultrastructural properties of two smooth muscles that have very different relationships between length and force production, the rabbit taenia coli and rat anococcygeus muscles. The taenia coli, which is rich in connective and elastic tissue, has high passive force at short muscle lengths and a limited extent of shortening, whereas the anococcygeus muscle shows passive resistance to stretch only at lengths exceeding L_0 and can shorten to very short length.

At the outset, consideration of what is currently known about some key mechanical and structural features of smooth muscle is warranted. The organization of the contractile apparatus in smooth muscles differs from that of striated muscles. Bundles of 3–5 myosin filaments are arranged in parallel, with each myosin filament surrounded by ~15 actin filaments (Devine & Somlyo, 1971; Ashton *et al.*, 1975). Bundles of actin filaments insert into cytoplasmic dense bodies, and the polarity of actin filaments point away from the dense bodies, analogous to Z lines of striated muscles. Cytoplasmic dense bodies are linked in a scaffold of intermediate filaments that penetrate dense bodies located also at the plasma membrane (Bond & Somlyo, 1982). Taken together this structural arrangement is consistent with a sarcomeric arrangement of contractile filaments. An alternate view is that myosin filaments are side-polar in some smooth muscles and that a non-sarcomeric arrangement of contractile filaments prevails (Craig & Woodhead, 2006), but, importantly this does not call into question the fundamental dependence of force production on muscle length.

The degree of order of thick and thin filaments in smooth muscle at rest and during contraction is controversial and has been so since the structure was first studied by Fisher and Bagby (1977). To our knowledge, there are no published images of intact smooth muscle ultrastructure in which mechanical properties have been measured in muscles fixed under strictly controlled and well-defined isometric conditions and after shortening to known lengths. In resting intact smooth muscle strips, actin and myosin filaments lie parallel to one another, but deviate from this orientation around the nucleus and other cellular organelles, and may run oblique to the long axis of the cell in some vascular muscles (Gabella, 1985). In isolated smooth muscle cells, some rather interesting changes in structure occur. Fay and Delise (1973) found that in resting toad stomach cells, contractile filaments were oriented parallel to the longitudinal axis of the cells, and that the surface membrane was smooth. Upon stimulation, the cells shortened to about 32% of their resting length, evaginations of the plasma membrane (“blebs”) occurred in regions between dense bodies, and contractile filaments were excluded from the blebs and assumed a “more random orientation” (described, but no images shown in the report). They hypothesized that blebs were a consequence of the contractile process; inwardly directed forces at the plasma

membrane dense bodies led to evagination of neighboring regions of the plasma membrane. A later study by Fisher and Bagby (1977) showed marked changes in contractile filament orientation from parallel to oblique with respect to the isolated cell's axis during the transition from rest to activation and shortening, such that the filaments assumed an undulating or helical pattern. Close examination of the images published by both groups suggests that the isolated cells also twisted on shortening, but it was Warshaw and colleagues (1987) who clearly showed that isolated cells tethered at one end and stimulated underwent a corkscrew-like change in shape suggesting that the contractile apparatus was helically oriented. In general these observations were consistent with images of intact guinea pig taenia coli allowed to undergo (but not quantified) isotonic shortening (Gabella, 1976).

Mechanical interactions among smooth muscle cells in tissues may occur directly by cell-cell interaction or indirectly through the extracellular matrix. Mechanical couplings among smooth muscle cells were first described in detail by Gabella (1984). These include (a) intermediate junctions, which are symmetrical cell-to-cell junctions formed by apposing plasma membrane “dense bands” of adjacent cells, and (b) plasma membrane “dense bodies”. According to Gabella, actin filaments, both contractile and cytoskeletal insert into dense bands at intermediate junctions and thereby are considered to be the site of transmission of force *directly* from one cell to another, but exact role of these structures has not been unequivocally defined. Most dense bodies of adjacent cells in the rabbit taenia coli and rat anococcygeus, as in other smooth muscles, are not “coupled”, and appear to link cells through intervening elastin and collagen fibrils. In the intact tissue, torsional forces placed on the cell during actin-myosin interaction resulting in force production and shortening are transmitted to adjoining cells and to the extracellular matrix that links, and thereby constrains, smooth muscle cells. The number and proportion of side-to-side or end-to-end connections (or both) of smooth muscle cells either directly or through connective tissue elements determine the direction and amount of force transmission, the extent of shortening, and the energetic cost of the contractile process. These connections are also important in terms of passive changes in muscle length (or width) in that they would provide a resistance against which active force from the contractile apparatus must operate and would influence the degree of overlap of contractile filaments.

Internal mechanical loads combine with external forces to limit muscle shortening (Seow & Stephens, 1986; Harris & Warshaw, 1998). Although “latch” bridges (myosin cross bridges whose light chains have become dephosphorylated while attached and are very slowly or non-cycling) might be considered a source of internal load, our studies on the energetics of shortening in the taenia coli would argue against this explanation (Butler *et al.*, 1983). Rather, as the present investigation shows, structural distortions of cells and the contractile filament array may serve as a compressive load. The influence of intercellular connections were analyzed in detail in mechanics studies by Meiss and colleagues who concluded that mechanical behavior at short muscles lengths is dominated by tissue forces within a tensegrity-like structure made up of connective tissue, other extracellular matrix components and active contractile elements (Meiss, 1999; Meiss & Pidaparti, 2004). This already complex arrangement becomes even further complicated in instances of smooth muscle remodeling in disease or in response to changes in functional demand, where increases in collagen and elastin content, together with or independent of smooth muscle cell hypertrophy and hyperplasia may occur (Gosling & Dixon, 1980; Heagerty *et al.*, 1993; Siegman *et al.*, 1997a; Siegman *et al.*, 1997b).

In addition to mechanical factors, the reduced effectiveness of activation mechanisms may limit force production at short muscle lengths. Activation of contraction in smooth muscle with cross bridge cycling is accomplished primarily by the Ca^{2+} -dependent activation of myosin light chain kinase and the phosphorylation of the 20kDa light chains of myosin

(Sobieszek, 1977). The level of contractile activation and myoplasmic $[Ca^{2+}]$ (Gunst, 1989) and sensitivity to various contractile agonists (Price *et al.*, 1981) may be modulated by tissue length in vascular and airway smooth muscle, respectively. The association of myosin light chain kinase with thick and thin filaments (Sellers & Pato, 1984; Gallagher & Stull, 1997) suggests that myosin light chain phosphorylation levels may be influenced by changes in accessibility to the myosin light chain kinase and phosphatase. In permeabilized vascular smooth muscle the degree, but not the Ca^{2+} sensitivity of myosin light chain phosphorylation decreases at short lengths. The decrease in force exceeded the decrease in myosin light chain phosphorylation, suggesting that changes in force production are independent of the relationship between Ca^{2+} and myosin light chain phosphorylation, and rely primarily on the number of sites of myosin cross bridge interaction with actin (Moreland *et al.*, 1988). As will be discussed, the results of our study are consistent with these findings.

The present study sought to explain the bases of the very fundamental decrease in force production at short muscle lengths and the differences in range of lengths over which smooth muscles function in the rat anococcygeus muscles and rabbit taenia coli. In the anococcygeus muscle, passive force is just detectable at L_0 and increases monotonically thereafter. In contrast, the taenia coli shows considerable passive force at short muscle lengths, such that at L_0 , the optimum length for active force production, passive force is ~45% of active force. Further, the anococcygeus muscle can shorten to ~0.2 L_0 , whereas the taenia coli can shorten only to a length of 0.4 L_0 . These characteristics represent the extremes in length-force relationships of smooth muscles, and, as such, allowed an analysis of the contribution of active (cross bridge) and passive (connective tissue) elements to mechanical behavior.

In addition to active force production, energy usage and dynamic stiffness were taken as indices of cross bridge interaction. In the anococcygeus muscle, force and energy usage are tightly coupled at all muscle lengths studied (0.2 L_0 – L_0). Stiffness decreased with muscle length as force decreased, but stiffness is higher than both energy usage and force only when muscle length falls below 0.5 L_0 (Fig. 2). Although the degree of activation of contraction, measured as the extent of phosphorylation of myosin light chains, diminishes slightly (~20%) with length, it does not parallel the sharp decline in force that occurs at short muscle lengths (Fig. 3). Indeed, when the myosin light chains are maximally thiophosphorylated, force declines with muscle length, with a sharper decline in force at lengths below 0.5 L_0 . This strongly suggests that it is filament overlap and cross bridge interaction and not the degree of activation that is the major determinant of force production at short muscle lengths in this muscle. It is only in this range of short lengths that the cells of the anococcygeus muscle also become increasingly misaligned and undergo shortening (signified by large bleb formation), so that force is dissipated internally and not transmitted to the ends of the muscle. It is likely that the stiffness in this length range may well include a greater fraction of non-contractile stiffness presented by the compressed extracellular matrix.

In the *taenia coli*, stiffness exceeds force at all muscle lengths shorter than L_0 , and energy usage remains high. The degree of myosin light chain phosphorylation remains high although force diminishes with muscle length, as does force in maximally activated (thiophosphorylated) preparations. The high energy usage by phosphorylated cross bridges reflects actin and myosin interaction and occurs at lengths at which structural changes include increasingly complex misalignment of cells and filaments, strongly suggesting that force is dissipated internally and/or not transmitted to the ends of the muscle. A significant portion of the measured stiffness at the short lengths, which exceeds force and energy usage, may be force-independent, and reflect the intercellular stiffness derived from interactions of contractile units transmitted from the cells both to the extracellular matrix and to

neighboring cells. This occurs to a far greater extent in the taenia coli, which is rich in connective and elastic tissue, than in the anococcygeus muscle.

The results suggest that in muscles rich in connective tissue, such as the taenia coli, external force production may not be an adequate measure of cross bridge interaction at short muscle lengths and may also limit shortening. The structural images suggest that this may be largely due to the relative floppiness of intercellular links that allows cell shortening and filament misalignment, so that force transmission is limited. Further, the compression of these links as cells shorten may limit shortening and oppose force production. Therefore, structural factors may override other processes that limit force production, such as the degree of activation at very short muscle lengths. The influence of structural constraints differs among smooth muscles under normal conditions and after remodeling. These structural constraints may be reflected in the degree of passive force at a given length, and be traced to the connective tissue composition and the resistance it presents to contractile elements.

A model for force transmission by intact smooth muscle

A model for force transmission in smooth muscle, based on early observations of Fisher and Bagby (1977) and Fay and Delise (1973) for isolated cells, and adapted to cells in intact tissue based on this study is shown in the following cartoons (Fig. 12 A, B, C). In this model, passive elastic elements largely determine the configuration of the cells both at rest and during stimulation, and, as a consequence, the effectiveness of force transmission. Components are: (a) smooth muscle cells and (b) extracellular matrix links between cells, both lateral and end-to-end. These are arranged in both series and parallel configurations with attachments of links to the plasma membrane at dense bodies but *do not quantitatively represent* the intercellular elements. Lines within the cells indicate the orientation of contractile filaments. The intracellular matrix, consisting of cytoplasmic dense bodies that are linked by intermediate filaments to which actin filaments insert are not shown for the sake of simplicity. Implicit in the model is that as contractile filaments interact they will pull and exert force on the plasma membrane dense bodies, and, if the force exceeds the resistance of the extracellular matrix, the cells will shorten.

At muscle lengths longer than slack length (L_s) (Fig. 12 A), the intra- and extracellular matrices are strained, and provide a resistance against which cross bridges must pull when shortening and developing force. The constraints of the matrix at least match the force of contraction, cells are aligned parallel to the longitudinal axis of the muscle, and force is transmitted to the ends of the muscle. In terms of structure, there is very little, if any, difference in appearance of the resting and stimulated muscle. At slack length, by definition, passive intra- and extracellular matrix are unstrained and there is no measurable force at rest. When the muscle is stimulated, contractile filaments slide and the cells shorten to the extent allowed by the elasticity of series and parallel links, and force develops. This is manifest structurally as some cell shortening, with slight blebbing of the plasma membrane between surface dense bodies.

As muscle length is decreased, the constraining force of these links diminishes, and is less than the active force (Fig. 12 B). The cells shorten, exerting force against the now floppy extracellular matrix links, with increased blebbing of the plasma membrane and dissipation of force internally. At shorter muscle lengths, the extracellular matrix links are sufficiently floppy to allow the twisting of muscle cells, with marked misalignment of cells and filaments relative to the longitudinal axis of the tissue, with a further decrease in force transmission (Fig. 12 C). A critical factor is the resistive force to compression experienced by the extracellular matrix as the muscle shortens. As this study shows, this is highly dependent on the quantity, quality, and distribution of the matrix in a particular tissue. This was obvious in the rabbit taenia coli, which showed evidence of compression of connective

tissue even at rest (Fig. 6 B), and by its inability to shorten extensively even in the permeabilized, fully activated preparation (Fig. 3 B).

The results of this study impact our understanding of the functional differences among various smooth muscles and how they remodel in response to changes in functional demand. The rabbit taenia coli, as a muscle with high elasticity, is well suited in design for its physiological function. The taenia coli are slender bands of longitudinal muscle of the caecum. They are slightly shorter than the segment of caecum on which they occur, and when the underlying circular muscle constricts, circular furrows, or haustra form. The range of active force production and stiffness of the taenia coli limit the extension and control the length of the caecum segment on which they lie. The rat anococcygeus muscles are anatomically unusual smooth muscles, in that they are strap muscles that attach to the ventral surface of the distal rectum and, through slender true tendinous ends are tethered to the posterior abdominal wall. They function to maintain the position of the distal rectum primarily through active contractile behavior.

Equally interesting are the differences in mechanical properties of vascular smooth muscle, as shown in intrapulmonary arteries of the cat. We found that small vessels ($<400\ \mu\text{m}$ effective luminal diameter) at rest showed the highest ratios of L_s/L_o and low passive tension at short lengths, and the larger vessels ($500\text{--}900\ \mu\text{m}$ luminal diameter) had low ratios of L_s/L_o and high passive tension at short lengths (Siegman & Butler, 1986). In other words, in the small vessels, passive tension was detectable at diameters close to the optimum length for active force production whereas in large vessels this occurred at much shorter lengths. These two patterns have important functional consequences. L_s defines the length (or diameter) that the resting vessel will seek, and, as such, defines the upper limit of the range of lengths at which the undistended muscle operates. The length at which zero active force is developed designates the minimum length that the muscle can attain when freely shortening on activation. Although the small and large vessels can shorten to about the same length ($10\% L_o$), those showing low passive force can actively shorten and do more work, for they will have started at longer lengths; that is, the ratio L_s/L_o was higher. Furthermore, in small vessels, active force production was greater at L_s than in the large vessels. Thus in small vessels, in which passive tension is low, considerable force could be generated by active contractile processes, but in the large vessels, most of the total force originated in connective tissue elements. Therefore, the potential for active regulation of small vessels is much greater than in large vessels, and the latter rely mainly on passive elements to resist pressure (Siegman & Butler, 1986).

Another property of smooth muscle behavior is its remarkable ability to adapt to changes in functional demand by remodeling. For example, in response to obstruction or strain the muscle undergoes hypertrophy with or without hyperplasia and this may occur with or without concomitant changes in extracellular matrix. These events have been studied in many visceral and vascular smooth muscle models with focus on resulting changes in force production at a single muscle length with little, if any attention given to changes in chemical composition and regulation, the characteristics of which are modified dramatically as a result of remodeling. The pattern of remodeling of visceral and vascular smooth muscle may vary (see review by Mulvany, 2002). In essential hypertension the remodeling is eutrophic, that is, a rearrangement of the same number and size smooth muscle cells around a smaller lumen. High pressure and high flow lead to hypertrophic remodeling, in which an increased number of muscle cells occurs. In arterial resistance vessels from spontaneously hypertensive (SHR) rats there is an increase of force due to growth of smooth muscle cells with no change in passive wall stress (Mulvany & Halpern, 1977), yet in pulmonary hypertension (monocrotaline induced), feline intrapulmonary resistance vessels undergo a marked increase in passive force at all muscle lengths, reflecting an increase in collagen

concentration. The remodeling was such that passive elements, *e.g.*, connective tissue, were the primary means of resisting pressure, not the contractile apparatus (Siegman & Butler, 1986) and Siegman (unpublished results).

In a detailed study of megacolon in a mouse model of Hirschsprung's disease in which mechanical properties, composition and regulation of contraction were examined (Siegman *et al.*, 1997b), hypertrophy and hyperplasia of both circular and longitudinal muscle layers occurred with no change in force per cross-sectional area. Structural remodeling was expressed as an amplification of active force production, with no change in the range of lengths for force production. There was also a high resting compliance in megacolon, consistent with no disproportionate change in collagen or elastin composition. As a result, there was no change in the extent of shortening the muscle could undergo.

All of the above examples demonstrate some of the differences among various normal smooth muscles and the broad range of changes that may occur during remodeling. In this regard, the taenia coli and anococcygeus muscles, the two normal muscle models examined in the current study, represent the extreme limits that exist in terms of whole smooth muscle composition and function, and, as such, demonstrate explicitly how the interplay of active, contractile elements within a complex passive extracellular matrix and the relative contribution of each ultimately determine functional behavior, expressed as force production and shortening.

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Figure 1. Length-force relationships

Panel A. Rabbit taenia coli, **Panel B.** Rat anococcygeus. Passive, total and active force are plotted as a function of muscle length. Values are mean $\pm$ SEM, $n > 8$ for each length. Length is normalized to the optimum length for active force production, (L_o) and force is normalized to the maximum active force produced at L_o on stimulation (P_o). Passive force is just detectable at $\sim 0.56 L_o$ in the taenia coli and is coincident with L_o in the anococcygeus. The taenia coli can actively shorten to $\sim 0.4 L_o$, but the anococcygeus can shorten to $\sim 0.2 L_o$.

Figure 2. Length-dependence of steady-state active force production, dynamic stiffness, and steady-state energy usage

Panel A. Rabbit taenia coli, **Panel B.** Rat anococcygeus. For each muscle, active force (normalized to P_o), dynamic stiffness (normalized to the stiffness at P_o), and corresponding energy usage ($\mu\text{mol/g/min}$ derived from oxygen consumption and lactate production,) were determined at each muscle length. Values are means $\pm$ SEM, $N > 10$ for each muscle.

Figure 3. Length-dependence of activation by myosin light chain phosphorylation (MyLCP) and force production in intact and permeabilized taenia coli and anococcygeus muscles
Panel A, Intact muscles. Very small changes in the degree of myosin light chain phosphorylation accompany the large decreases in force as muscle length decreases. **Panel B**, Permeabilized muscles with myosin light chains thiophosphorylated with ATP γ S. The patterns of the decline in force with muscle length observed in intact muscles persist even when myosin light chains are maximally thiophosphorylated.

Figure 4. Ultrastructure of taenia coli at optimum length for active force production, *Lo*
Panel A. Resting muscle, transverse section. Cells are surrounded by abundant connective tissue. **Panel B.** Resting muscle, longitudinal section. **Panel C.** Stimulated muscle, transverse section. Small evaginations of the plasma membrane (“blebs”) are noted between plasma membrane dense bodies (arrows). **Panel D.** Stimulated muscle, longitudinal section. Contractile filaments run parallel to longitudinal axis of muscle. Very small blebs (arrows) occur on the plasma membrane.

Taenia coli, Ls (0.57 Lo)

Figure 5. Ultrastructure of taenia coli at slack length (*Ls*) 0.57 *Lo*

Panel A. Resting muscle, transverse section. **Panel B.** Resting muscle, longitudinal section. Note waviness of some cells, and that contractile filaments follow the contour of the cells (arrows). **Panel C.** Stimulated muscle, transverse section. Note larger and more abundant bleb formation between dense bodies on the plasma membrane (arrows) than at *Lo* (Fig.4C). **Panel D.** Stimulated muscle, longitudinal section. Note extensive bleb formation at plasma membrane (arrows). Cells shorten and contractile filaments within run parallel to the longitudinal axis of the muscle.

Figure 6. Ultrastructure of taenia coli at length 0.46 Lo

Panel A. Resting muscle, transverse section. Note marked irregularity of cell profiles and abundant connective tissue. **Panel B.** Resting muscle, longitudinal section. Note extreme waviness of cell profiles, and clustering of cytoplasmic dense bodies in narrow areas (arrows) of cells. **Panel C.** Stimulated muscle, transverse section. Note abundant formation of very large blebs at plasma membrane (arrows). **Panel D.** Stimulated muscle, longitudinal section. Contour of cells is irregular, with conspicuous compression of elements of extracellular matrix (arrowheads). Very large, bulbous dense bodies have formed (arrows) extending around the perimeter of the cells.

Taenia coli twisted cell

Figure 7. Cells appear to twist in the taenia coli at length 0.46 *L*_o
Stimulated muscle, transverse section. Note that within cells there are filaments in cross section (arrows) and in tangential or near-longitudinal view (arrowheads), suggesting twisting of activated cells.

ANOCOCCYGEUS, L_0

Figure 8. Anococcygeus muscle at length L_0 , coincident with slack length, L_s

Panel A. Resting muscle, transverse section. **Panel B.** Resting muscle, longitudinal section. Cell margins are smooth, cells are straight with filaments running parallel to the long axis of the muscle. **Panel C.** Stimulated muscle, transverse section. **Panel D.** Stimulated muscle, longitudinal section. Bleb formation is evident in both transverse and longitudinal sections in stimulated muscles, indicating some cell shortening. Cells are straight and filaments run parallel to the long axis of the tissue. At this length, passive force is zero, and cell shortening is opposed by little resistance of the extracellular matrix.

ANOCOCCYGEUS, 0.5 Lo

Figure 9. Anococcygeus muscle at length 0.5 Lo

Panel A. Resting muscle, transverse section. **Panel B.** Resting muscle, longitudinal section. Cell profiles are highly irregular in both transverse and longitudinal views. **Panel C.** Stimulated muscle, transverse section. **Panel D.** Stimulated muscle, longitudinal section. Blebs are abundant and larger (arrows) than at longer muscle lengths. Cytoplasm is dense with abundant filaments in shortened cells, and these lie parallel to longitudinal axis of the tissue.

Figure 10. Anococcygeus muscle at length 0.3 Lo

Panel A. Resting muscle, transverse section. **Panel B.** Resting muscle, longitudinal section. Cells are wavy, with contractile filaments within following the cell contour (arrows). **Panel C.** Stimulated muscle, transverse section. **Panel D.** Stimulated muscle, longitudinal section. In the stimulated muscle, blebs are abundant and much larger (arrows) than at longer muscle lengths. Cells are straight and contractile filaments within run parallel to the longitudinal axis of the cell. Contractile filaments and dense bodies are in very close proximity in some areas of the cells (arrowhead).

Figure 11. Details of blebs at the plasma membrane of the anococcygeus muscle

Note large dense bodies at areas of plasma membrane between dense bodies (white arrows). Blebs are rich in caveolae (black arrowheads) and tubular elements of the sarcoplasmic reticulum can be seen (black arrow), extending close to caveolae at the plasma membrane. The surface of a bleb full of caveolae is indicated by the white arrowhead.

Figure 12. Model of the length-dependent interaction of contractile and extracellular matrix elements of smooth muscle under isometric conditions

This cartoon shows the changes in smooth muscle cells in relation to the extracellular matrix at various muscle lengths.

A. Muscle length at lengths greater than L_s , where passive elements of the extracellular matrix exert a resistive force to cell shortening on stimulation.

B. At muscle lengths $<L_s$, the extracellular matrix is floppy and provides moderate resistance to shortening of the cell on stimulation. As filaments slide and the cell shortens, force is transmitted to dense bodies at the plasma membrane to which the cytoskeleton attaches. As the cell shortens, intra-dense body regions of the plasma membrane bulge, producing “blebs.” The contractile force is transmitted to and opposed by the now partially floppy extracellular matrix.

C. At very short muscle lengths, the extracellular matrix is sufficiently floppy to allow greater internal shortening and twisting of cells. As a result, many filaments and cells are no longer aligned with the longitudinal axis of the tissue, and much of the force generated by interacting contractile filaments is dissipated internally.