The extensive length-force relationship of porcine airway smooth muscle

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The full functional length range of trachealis muscle was measured to quantify one of its basic properties, its functional length range. The results illustrate the utility of this preparation in studying the physical properties of nonstriated contractile cells.

METHODS

Pig tracheae were obtained from local abattoirs supervised by the State of Indiana. Some muscles were dissected immediately and others after the trachea had been stored in physiological saline at 4°C for up to 24 h. The protocol was approved by the Indiana University School of Medicine Animal Care and Use Committee. Physiological saline for experiments contained (in mM) 112.5 NaCl, 27.5 NaHCO3, 4.0 KCl, 1.2 NaH2PO4, 2.0 MgSO4, 2 CaCl2, and 5 glucose. It was perfused with 95% O2-5% CO2 to achieve pH 7.4. Tracheae were stored and muscles dissected in a solution of similar composition, except that it was not perfused with gas and HEPES buffer was substituted for NaHCO3.

Muscles were studied in a narrow trough, 4 × 4 × 50 mm, with 4 × 10 mm platinum electrodes glued to the sides. Physiological saline equilibrated with gas passed into the trough through a hypodermic needle heated by constant current passing through a wire wrapped around the needle. Trough temperature was 37°C in the unstimulated state and rose by 0.5°C during electrical stimulation. Stimuli consisted of 1-ms duration pulses of alternating polarity at 60 Hz and current amplitude ~10% greater than that needed to elicit full force.

Muscles were held in aluminum foil clips (3), which provided a well-defined edge for measuring muscle length between the clips using a zoom dissecting microscope mounted above the trough. The microscope was equipped with ×10 eyepieces and a reticle having 100 divisions. Muscles were ~0.3 × ~0.8 × ~5 mm long between clips when set to the Lref. To adjust muscle length, the objective magnification of the microscope was set so that the distance between clips was 80 divisions of the reticle ~1.26×, and length changes were made in increments of 1 division of the reticle, equivalent to 0.0125 T ref initial reference length (L 100% ) ~63 μm. Accuracy of the setting to ~0.1 division was enabled by having the edge of the clip nearly parallel to the reticle graduations.

Figure 1 shows complete records from separate experiments that illustrate the two protocols used. Muscles were stimulated to produce 12.5-s tetani at 5-min intervals throughout the experiments until the final measurement of sustained passive tension. Digital recording rate varied, with force sampled at 0.2-s intervals beginning immediately before the onset of stimulation and continuing for 20 s, then at 1-s intervals for 80 s, and then at 10-s intervals for 200 s. For both protocols, muscles were first adapted to the experimental environment for at least 40 min or until spontaneous tone subsided. As shown, spontaneous tone was variable, being almost absent in Fig. 1B and equivalent to ~20% of full tetanic force in Fig. 1A.

Length was next set to the length where rest tension was 10% of total tetanic force (L 10% ) (developed force plus rest tension). They were stimulated at this length for 5–15 tetani. L 10% and total force in the adapted muscles at this length (F ref ) for this muscle and to quantify one of its basic properties, its functional length range. The results illustrate the utility of this preparation in studying the physical properties of nonstriated contractile cells.

U N T I L A D E C A D E A G O, I T W A S widely believed that the filaments and filament lattice of smooth muscle were fixed and that most muscles had a limited length range similar to that of striated muscle. Arterial muscle had been reported (16) to have a reproducible reference length (L ref ) for this muscle and to quantify one of its basic properties, its functional length range. The results illustrate the utility of this preparation in studying the physical properties of nonstriated contractile cells.

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Figure 1A shows the protocol for measuring passive tension. After adaptation at $L_{10\%}$, muscles were shortened to 0.5 $L_{10\%}$, stimulated for one tetanus, and then stretched after a single tetanus at each length in the sequence given in the legend of Fig. 1. This sequence ensured that the shortest length was well below that at which rest tension was zero, minimized the long-term adaptation to new lengths that occurs over hours (24), and minimized the time required to define the length where rest tension was first encountered, while providing greater precision to measurements made over the steep portions of the passive length-tension curve.

Active force at lengths $\leq L_{10\%}$ was measured in a separate protocol (Fig. 1B) as the force produced in the last of six tetani at each length, as muscles were shortened in the sequence given in the legend of Fig. 1. This sequence allowed the muscle to adapt gradually to shorter lengths as muscles were shortened in the sequence given in the legend of Fig. 1. A plot of the difference between this total force and the passive force at each length (open circles in Fig. 2) was linear, extrapolating to zero at 1.175 ± 0.004 $L_{10\%}$.

Mean values of total force for the single contraction at each length $>1.03 L_{10\%}$ (diamonds in Fig. 2) show that substantial force can be generated above the passive level at the longest lengths. A plot of the difference between this total force and the passive force at each length (open circles in Fig. 2) was linear, extrapolating to zero at 1.175 ± 0.004 $L_{10\%}$.

DISCUSSION

The main purpose of this study was to define the full functional range of airway smooth muscle and thus the full length range that must be explained by filament lattice accommodation. Substantial active force generation at the extremes of the 11-fold length range, 0.1–1.1 $L_{10\%}$, indicates that force can be generated over an even greater range. If the shortest and longest lengths for force generation are taken as 0.076 and 1.175 $L_{10\%}$, where active force extrapolated to zero, the full functional length is >15-fold.
The length where the muscle strongly resisted stretch and where active force began to decline probably marked the upper boundary of filament lattice plasticity, although it is possible that, if the passive structures resisting stretch were removed, further length adaptation at longer lengths might have occurred. The lower boundary was indicated by the muscle reextension seen during relaxation, suggesting deformation of structures that restore length during relaxation from contractions to lengths <0.25 \( L_{10\%} \). Thus filament lattice plasticity was limited to a three- to fourfold length range.

Reproducible \( L_{ref} \). The absence in these muscles of a distinct peak in the length-force curve precluded using peak force as a reference, as is done in striated muscle. A recent paper (1), coauthored by many workers in the field of airway smooth muscle physiology who sought to arrive at uniform terminology for the field, underscored the need for a reliable \( L_{ref} \). A more recent study using a similar reference, as is done in striated muscle. The peak in the length-force curve precluded using peak force as a reference, but the necessity of choosing a reference, as is done in striated muscle. The present observations that rest tension rises very steeply when some limit is reached and that this rest tension is stable suggest that a \( L_{ref} \) based on this steep rise might be used as a reference. For example, if the reference is chosen as the length at which rest tension is 50% of developed force, misjudging the tension level by 20%, so that it is either 60% or 40% of reference force, would cause <1% error in the \( L_{ref} \). We did not use this reference in the present experiments because we only discovered its advantages as the experiments progressed. The reference we chose was nearly as accurate, having only a 0.013 \( L_{10\%} \) SE with respect to the variation between the two. Another possible reference is the zero-force intercept of the linear part of the curve. An advantage of this reference is that it is determined from the linear plot of the passive length-force data and thus uses all of the data, so that it may be more robust than a single point on the curve. Again, the small SE in this value relative to \( L_{10\%} \) suggests that it correlates well with the reference used here.

However, the important issue here is not the parameter of the steeply rising curve chosen as a reference, but the necessity of ensuring that the steeply rising curve has been reached, because this appears to be a stable characteristic of the muscle.

Importance of a reproducible \( L_{ref} \). Most studies of smooth muscle use a parameter of passive length tension to define a \( L_{ref} \). As explained below, there are likely to be two sets of parallel elastic elements in this muscle, and the more compliant one, not seen in these experiments, appears to depend on the way the muscles are treated early in the experiments. Using a parameter of the more compliant curve as a reference can produce very different results. For example, our first study of airway muscle plasticity (17) was done in two groups of muscles, both using as a reference the length at which rest tension was 2% of developed force. All nine muscles in the first group could be stretched by 50% from this reference, and some could be stretched by 80%, with rest tension remaining <20% developed force. Three muscles in the second group of nine could not be stretched by 50% without a steep rise in rest tension. A more recent study using a similar \( L_{ref} \), that where rest tension was just detectable, found that muscles could be stretched twofold without encountering stiff resistance (13). By contrast, the muscles used for the present experiments, first adapted near \( L_{10\%} \), could not be stretched by >18% from the length at which tension was just detectable, 0.9 \( L_{10\%} \), without reaching the length at which rest tension was >50% \( F_{ref} \) (Fig. 2). Thus it appears that the length at which small amounts of rest tension are encountered is highly variable and seems to depend on the length at which the muscles are initially adapted. Recent work (22, 23) describing variable rest tension in smooth muscle is likely to explain both the different rest tension curves in our experiments and the much greater differences in results from different laboratories (c.f. Refs. 15, 17, 20).

Variable passive tension in smooth muscle. In two recent papers, Speich et al. (22, 23) present evidence that the passive compliance of urinary bladder smooth muscle can be highly dynamic. In the first (22), they showed that a component of passive tension was eliminated by “strain softening”, i.e., stretching the unstimulated muscle significantly reduced passive tension at lengths shorter than the maximum reached during the stretch. Such strain softening could explain why we now see very little passive tension until the muscle is stretched to near its elastic limit; our muscles might have been maximally strain softened when we first identified the length at which passive tension begins to rise steeply. More recently (23), they show that higher passive rest tensions can be restored by KCl or carbachol contractures. This is a very timely discovery, because the large differences between the results presented here and those described earlier might be explained by sustained contractures, but not brief tetani, causing substantial increases in rest tension.

Model to explain results. Figure 3 is a schematic diagram of the elements required to explain the results. Active force is shown as being generated by actin thin filaments overlapping row-polar myosin thick filaments. As drawn, the free ends of the thin filaments extend beyond the ends of the thick filaments. This arrangement allows filament sliding that alters the distance between the anchors of opposing thin filaments while maintaining maximum overlap of each thin filament with the adjacent row of cross bridges. Thus it avoids force inequalities.
between thin filaments pulling from opposite ends of the thick filament. It could also lessen or even eliminate force changes associated with length changes. By themselves, the present results are consistent with such an arrangement being responsible for the length independence of force over the range 0.33 and 1.0 $L_{10\%}$. Other experiments have shown, however, that both thick filament mass (13, 21) and shortening velocity (17) double when adapted muscle length increases threefold. This doubling is illustrated as muscle length triples between Fig. 3, B and C. The increase in lattice length not produced by filament sliding or increases in thick filaments in series must be accommodated by length increases in passive structures in series with the force-generating filaments. For simplicity, Fig. 3 shows this additional lattice lengthening as an increase in thin filament length.

**Stiff parallel elastic elements.** The observation that total force at the longest lengths (diamonds in Fig. 2) was substantially greater than maximum developed force at shorter lengths suggests that most of the rest tension in unstimulated muscle was due to passive elements in parallel with the contractile filaments. If this were not so, the contractile elements at long length would have to generate substantially more force at stretched lengths than the maximum they generate over the plateau of the length-force curve at shorter lengths. The observation that force remained nearly constant as the muscles were shortened to $<0.33 L_{10\%}$ suggests that these parallel structures became slack and did not impede shortening when muscle length was reduced. In Fig. 3, these parallel structures are drawn as intermediate filaments. Finally, the shape of the passive length-tension plots suggests that these filaments may be composed of elements having a microstructure of the sort illustrated in Fig. 3D.

The compound filaments are composed of segments of nonextensible but flexible strands cross-linked by compliant fibers, which become taut at different extents of sliding between the strands. These cross-linking fibers obey Hooke’s Law when taut, and flexibility in their connections to the strands confers flexibility on the filaments when slack. At long lengths, where all of the fibers are taut, the compound filaments obey Hooke’s Law, with stiffness equal to the sum of the stiffnesses of the individual fibers in parallel. At intermediate lengths, the slope of the stress-strain curves increases with stretch, as more fibers are drawn taut and thus recruited to resist further stretch. Stress-strain curves of such filaments resemble those of the passive muscle.

**Compliant parallel elastic elements.** As discussed above, prior experiments have shown airway smooth muscle to bear passive tension over a much more extensive range than observed here. The stiff parallel filaments described immediately above could not, by themselves, produce this greater compliance, because the fibers and filaments are very stiff when taut and flexible when not taut. Additional structures are needed to account for the more compliant stress-strain relationships seen in some experiments. In Fig. 3, these are drawn as compliant springs that can form transient attachments between loops in the stiff compound filaments.

**Series elastic elements.** All isolated muscles have compliant elements in series. At a minimum, these elements include the equipment (force transducer, hooks, clips), as well as tendons or crushed tissue at the junctions of muscle and clips. These elements are important to the interpretation of the present experiments for two reasons. First, because they lengthen as passive tension increases, they increase the measured compliance of the unstimulated muscle. Thus the stiffness of passive elastic elements is greater than that inferred from the slope of the passive length-force curves. Second, they reduce the measured increase in force when the muscle is stimulated at long lengths, because lengthening of the series elements during force development shortens the parallel elements, thereby transferring some of their load to the contractile elements.

**Length-restoring elements.** The observation that muscles relengthened after being stimulated to shorten to lengths $<0.25 L_{10\%}$ suggests distortion of elastic elements that restore length to a minimum resting value. The identity of these length-restoring elements is not known, but Fig. 3 shows a possible contributor to the restoring force. The individual muscle cells must have a constant volume at different lengths, if they do not gain or lose water, and electron micrographic measurements have confirmed this constant-volume behavior (13). Thus cell width must increase as the muscle shortens. Passive elastic restraints on widening will provide length-restoring elements. These are drawn in Fig. 3 as transverse intermediate filaments similar to the longitudinal filaments that resist passive stretch at long lengths. These transverse filaments are slack at longer muscle lengths and become taut only at very short lengths, where the cell diameter exceeds some limit.

**Comparison with earlier studies.** All of our studies have been done using brief tetani at lengths where rest tension was low, and they all showed the muscles to have nearly flat plots of active force over a two- to threefold length range (13, 17). By contrast, other studies using sustained contractures to activate the muscle have reported the active length-force curves in trachealis to be moderately steep. Experiments using bovine trachealis and 10-min contractures induced either by carbachol or KCl found that force declined by 50% from its initial value when the muscle was shortened by $\sim60\%$ (15). Experiments using 10-min acetylcholine contractures in dog trachealis found that force declined by 50% when the muscles were shortened by 75%. In both experiments, the slopes of the length-force relationships are comparable to those reported here at lengths $<0.25 L_{ref}$, where 40% shortening decreased force by 50%. This comparison suggests that the earlier experiments were done under conditions in which there was little filament lattice plasticity. Unlike the dog experiments, which did not test longer lengths, the bovine trachealis experiments were intended to test for length plasticity and, therefore, examined longer lengths. When these muscles were extended to 150% of their initial length, force was increased by a further $\sim30\%$. The less steep slope over these longer lengths was taken as a suggestion of possible length plasticity, but force was substantially reduced when the muscles were returned to $L_{ref}$ after eight contractures at the longest length and further still when the muscles were tested again at 1.5 $L_{ref}$. Thus it is questionable whether the muscles demonstrated any lattice plasticity at all. An explanation for the lack of extensibility suggested by the report of variable passive elasticity is that the sustained contractures induced the formation of structures that increase the muscle’s resistance to length changes (23).

An earlier study of porcine tracheal muscle (10) measured force over an $\sim5$-fold length range, from 0.3 to 1.45 $L_{ref}$, and extrapolating the active force to zero at both ends of the curve.
yielded an ~7.5-fold range, from 0.2 to 1.45 $L_{\text{ref}}$. Both of these ranges are about one-half of those described here, but, instead of a flat central plateau, this study found a sharp peak. This study did not use contractures but electric field stimulation similar to that used here. Thus the possible reasons for the disparities between this carefully done study and the present results are not as easily explained.

In these earlier experiments, the force peak ($F_{\text{ref}}$) occurred at a $L_{\text{ref}}$ at which rest tension was ~10% of developed force, similar to that used here. At lengths shorter than this, force declined nearly linearly and extrapolated to zero at 20% $L_{\text{ref}}$. In the present experiments, an 80% length reduction from 35 to 7% $L_{\text{ref}}$ would have caused a similar reduction in force from ~100% $F_{\text{ref}}$ to zero. Force at longer lengths in the earlier experiments was not constant but declined quasi-linearly to reach ~40% $F_{\text{ref}}$ at the longest length studies, ~145% $L_{\text{ref}}$ (equivalent to ~0.5 $L_{\text{ref}}$) in the present experiments if their $L_{\text{ref}}$ is equivalent to 0.35 $L_{\text{tort}}$ here), but total force (passive tension plus developed force) rose slightly, by ~12%, as muscles were extended to the maximum length. Thus these results could be reconciled almost exactly with the present experiments, if a shorter, stiffer parallel elastic element in the earlier experiments caused the experiments to be done at a shorter length, and if the decline in force at the longer lengths were due entirely to internal shortening that transferred load from the passive elastic elements to the contractile elements.

This explanation for the differences in the two studies relies heavily on there being a substantial compliance of the series elastic elements, and this compliance is not stated. The method of attaching the muscle to the apparatus is not described, except to say that it was accomplished “using clips and silk thread.” Thus no firm statement can be made about the active force developed by the muscle at the longer lengths.

It seems unlikely that differences in the parallel elastic elements and load transfer are the complete explanation of the apparent differences in the results, but it might also be pointed out that later experiments from the same laboratory (13) found a nearly flat force plateau over a twofold length range. This difference in results acquired in experiments done for different reasons and separated by more than a decade may be the most salient observation to be made from these comparisons of different studies. Experiments are almost always optimized to examine a specific issue, and the techniques employed may obscure mechanisms discovered later.

Comparison with skeletal muscle. The classic work of Gordon et al. (6) correlating isometric force decline at long muscle lengths with reduced filament overlap was so persuasive in gaining acceptance for the sliding filament hypothesis and is so well known that a similar correlation might be expected in the present study. Such an analysis is not possible here, because of the difference between total force and passive force (open circles in Fig. 2) extrapolated to zero over a much smaller range then would be expected on the basis of declining filament overlap. This steeper decline is likely to have resulted from at least three factors known to diminish the force increase during stimulation at stretched lengths. 1) Muscles were stimulated to produce only one contraction at each length, and, as shown in Fig. 1B as well as in previous work (14), tetanic force increases by 10–20% when muscles are allowed to adapt at each new length for six isometric tetani. 2) Force development stretches elastic elements in series with the muscle, thereby shortening the parallel elastic elements that bear tension at rest, and this shortening transfers load borne by the passive elements to the contractile elements. 3) The measured passive tension is likely to have been increased by a component of active force in the stretched, unstimulated muscle, and a complete accounting would require that this component be subtracted from passive tension and added to active force. Active force generation in unstimulated, stretched muscle has been well known since the description of the Feng effect (3) (increased heat production with passive muscle stretch) and likely helps relaxed muscle straighten when released from a stretched length.

Airway smooth muscle as a model for nonstriated contractile tissue. Smooth muscle is more similar to nonmuscle motile cells than to striated muscle in several ways, of which its plasticity may be the most functionally relevant. Three important and unusual properties of trachealis make it ideal for physical studies: it possesses very little connective tissue; it relaxes completely over much of its length range; and its cells are arranged in straight, parallel bundles. By contrast, most smooth muscles enclose hollow vessels or visera that require restraints on stretch and have a branching architecture to prevent lateral separation of the muscle bundles. Some smooth muscles, such as vascular muscle, are so heavily invested with connective tissue that the muscle bundles cannot be isolated from the extracellular matrix, and this sturdy extracellular matrix could explain the much shorter length-force relationship in that preparation (16). Other smooth muscles, e.g., taenia coli, have been shown to have spontaneous contractile activity that makes it impossible to demonstrate the thick filament evanescence observed in rat anococcygeus muscle (24). Finally, the branching architecture of most smooth muscles introduces substantial complexity into such physical measurements as force, shortening, and birefringence, that usually depend on an assumption of parallel filaments for straightforward interpretation. These attributes are described to suggest that trachealis may be one of the most suitable muscles for defining the basic mechanisms of smooth muscle and, because the tissue is of a convenient size for physical measurements, such as force, velocity, and birefringence, possibly studying nonmuscle motile cells as well.

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