Adjustable passive length-tension curve in rabbit detrusor smooth muscle

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Speich JE, Dosier C, Borgsmiller L, Quintero K, Koo HP, Ratz PH. Adjustable passive length-tension curve in rabbit detrusor smooth muscle. J Appl Physiol 102: 1746–1755, 2007.—Until the 1990s, the passive and active length-tension (L-T) relationships of smooth muscle were believed to be static, with a single passive force value and a single maximum active force value for each muscle length. However, recent studies have demonstrated that the active L-T relationship in airway smooth muscle is dynamic and adapts to length changes over a period of time. Furthermore, our prior work showed that the passive L-T relationship in rabbit detrusor smooth muscle (DSM) is also dynamic and that in addition to viscoelastic behavior, DSM displays strain-softening behavior characterized by a loss of passive stiffness at shorter lengths following a stretch to a new longer length. This loss of passive stiffness appears to be irreversible when the muscle is not producing active force and during submaximal activation but is reversible on full muscle activation, which indicates that the stiffness component of passive force lost to strain softening is adjustable in DSM. The present study demonstrates that the passive L-T curve for DSM is not static and can shift along the length axis as a function of strain history and activation history. This study also demonstrates that adjustable passive stiffness (APS) can modulate total force (35% increase) for a given muscle length, while active force remains relatively unchanged (4% increase). This finding suggests that the structures responsible for APS act in parallel with the contractile apparatus, and the results are used to further justify the configuration of modeling elements within our previously proposed mechanical model for APS.

MECHANICAL STUDIES in the 1970s and 1980s showed that detrusor smooth muscle (DSM) (59, 61) and vascular smooth muscle (26) each produce a nonlinear length-tension relationship (L-T) curve and a parabolic active L-T curve with ascending and descending limbs very similar to those produced by striated muscles. Until the 1990s, the L-T relationships for smooth muscle were assumed to be static, with a single passive force value and a single maximum active force value for each muscle length. Recent studies on airway smooth muscle challenge the static model, and several dynamic models of the L-T relationships have emerged in which smooth muscle activity can adapt to the ambient muscle length, producing shifts in active (14, 17, 22–24, 29, 30, 39) and passive (38, 45a, 64) L-T curves (reviewed by 2, 13). The active L-T relationship of the carotid artery has also been shown to adapt to changes in muscle length over time, although to a much reduced degree compared with airway smooth muscle (44).

In practice, smooth muscle tissues are typically preconditioned by cyclic stretching to obtain consistent measurements (15), and the active L-T curve is derived by subtraction of the passive curve from the total curve, under the assumption that the passive curve is static following preconditioning. For example, in 1973, Herlihy and Murphy (26) noted that in vascular smooth muscle the “route taken to reach a final length had a profound influence on the ‘passive’ tension recorded, and consequently, on the calculated developed tension” and proposed the breakage of contractile links as one possible cause. Preconditioning is characterized by reductions toward a steady state in stiffness with each subsequent stretching cycle and has been identified in DSM (35). One hypothesis used to explain preconditioning is that the loss of stiffness is due to strain-induced stress softening, or strain softening, which is an essentially irreversible loss of stiffness due to structural changes (9, 20). In this study, the loss of stiffness due to strain softening is characterized by a reduction in the steady-state passive force at a given muscle length following a stretch to a longer length.

Our recent studies show that DSM exhibits both viscoelastic softening (reversible loss of resistance to stretch) and strain softening (54). The latter is irreversible when tissues are either passive (i.e., when cross bridges are not cycling to produce active force because tissues are maintained in a Ca2+–free solution) or submaximally active (i.e., when cross bridges are cycling to permit spontaneous rhythmic tone) (54). Most importantly, our studies show that the decrease in passive stiffness due to strain softening is reversible on full muscle activation with KCl or carbachol, and activation at shorter muscle lengths restores more stiffness than activation at longer muscle lengths (i.e., the level of stiffness restored depends on the muscle length at activation). Thus rabbit DSM displays passive stiffness that is adjustable because it is dependent on both strain history and muscle activation history. To our knowledge, this is the first study to challenge the static passive L-T relationship in DSM and propose a dynamic, adjustable passive L-T relationship for detrusor. The present study reinforces our previous work and presents new data that demonstrate a “dynamic” passive L-T curve for rabbit DSM that shifts to the right as a result of strain softening and to the left following muscle activation at slack length. Furthermore, the present study tests the hypothesis that the structures responsible for adjustable passive stiffness (APS) act in parallel with the contractile apparatus and uses the results to justify the place-
ment of our recently defined variable parallel elastic component (VPEC) within our previously proposed mechanical model for APS (56).

METHODS

Tissue preparation. All experiments involving animals were conducted within the appropriate animal welfare regulations and guidelines and were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee. Tissues were prepared as described previously (41, 47). Whole bladders from adult female New Zealand White rabbits (2–4 kg) were removed immediately after they were euthanized with an overdose of pentobarbital sodium. Bladders were washed, cleaned of adhering tissues, including fat and serosa, and stored in cold (0–4°C) physiological salt solution (PSS). Thin strips (~0.2 mm thick) of longitudinal DSM free of underlying urothelium and overlying serosa were cut from the bladder wall above the trigone and close to the dome (upper detrusor) by following the natural bundling clearly demarcated when bladders were in ice-cold buffer, as described previously (43).

Solutions. PSS is composed of (in mM) 140 NaCl, 4.7 KCl, 1.2 MgSO4, 1.6 CaCl2, 1.2 Na2HPO4, 2.0 morpholinopropanesulfonic acid (adjusted to pH 7.4 at either 0 or 37°C, as appropriate), 0.02 Na2EDTA, and 5.6 dextrose. For clarity, PSS will be referred to as a “Ca2+-free solution,” while PSS with no CaCl2 and the addition of 1 mM EGTA to chelate Ca2+ will be referred to as a “Ca2+-free solution.” Muscle contractions were induced with 110 mM KCl (substituted isomotically for NaCl).

Apparatus. Each tissue was secured by small aluminum foil clips to a micrometer for manual length adjustments and a computer-controlled electronic lever (model 300H with DMC software, Aurora Scientific) to record force and to induce time-controlled muscle length changes. Force and length signals were digitized (PCI-6024E, National Instruments) and stored electronically for analyses.

Determination of slack length. Each tissue was secured such that its initial (0–4°C) zero-preload length was ~3 mm and equilibrated in aerated PSS at 37°C in a water-jacketed tissue bath for 1 h to permit development of spontaneous rhythmic contraction. Tissues were then incubated in a Ca2+-free solution to eliminate spontaneous contractile activity (46), stretched in 0.5-mm step increments, and allowed to stress-relax with each step increase until a stable preload of 0.05 g (the minimum measurable positive force) was established. This length, ~6 mm, was considered slack length (L0) at 37°C. At this length, the tissue thickness and cross-sectional area were estimated to be ~0.2 mm and ~0.9 mm², respectively, on the basis of an average width of ~4.5 mm, a mass of ~0.006 g, and a muscle density of 1.05 g/cm³ (37, 59).

Preconditioning/strain softening. Following the determination of L0, each tissue was incubated in a Ca2+-containing solution for 30 min to obtain spontaneous rhythmic tone, placed in a Ca2+-free solution for 5 min, preconditioned by manually stretching the muscle strip to the maximum strain in the protocol (e.g., 252% L0) using a micrometer, and allowed to stress-relax at this length for 5 min. Tissues were then returned to L0, placed in a Ca2+-containing solution for 1 min, and contracted twice with 110 mM KCl for 3 min to determine the maximum KCl-induced force. Isometric contraction at L0, which permits the restoration of APS lost to strain softening during the stretch (e.g., to 252% L0) (54), was measured as described previously (42, 47).

Passive L-T curve protocols. Following the determination of L0 and preconditioning, DSM strips were subjected to one of two passive L-T curve protocols. The first, protocol P1 (Fig. 1A), was designed to demonstrate that the quasi-steady-state passive force at a particular muscle length depends on the length history of the tissue. Strips of DSM incubated in a Ca2+-free solution were stretched according to the protocol shown in Fig. 1A. Passive force values were measured at 5% increments at progressively longer and then progressively shorter lengths between L0 and 250% L0 (Fig. 1B). Each quasi-steady-state passive force measurement was performed after 1 min of isometric force redevelopment following a release to a shorter length (Fig. 1C) because force typically does not reach steady state following 1 min of force relaxation following a stretch (Fig. 1C) and can take several hours to reach steady state (56). To determine if any of the tissues were significantly damaged during the longest stretches, each strip was stimulated twice with KCl at L0 following the completion of the protocol. If the muscle strip could still contract to a level of force similar to the initial contractions performed at L0, then the muscle would be considered undamaged by the stretch protocol.

The second passive L-T curve protocol, protocol P2, was designed to demonstrate that the passive L-T curve for rabbit DSM can shift to the right as a result of strain softening and to the left following muscle activation at L0. DSM strips were stretched in a Ca2+-free condition according to the protocol shown in Fig. 2A. Long stretches were performed manually using a micrometer, and releases were performed using the electromechanical lever. Passive force values were measured after 1 min of force redevelopment following each quick release to a lower length (gray circles in sequences 1a–6a). After sequences 1a–3a were completed, the tissue was returned to L0, twice stimulated with KCl to restore stiffness lost by the prior strain to 250% L0, incubated in a Ca2+-free solution and subjected to sequences 2b, 3b, 4a, and 4b.

Total force protocols. Following the determination of L0 and preconditioning, DSM strips were subjected to one of two total force protocols. The first, protocol T1 (Fig. 3A), was designed to test the hypothesis that a reduction in passive force due to strain softening is accompanied by a corresponding reduction in total force at a particular muscle length. DSM strips were stretched according to the protocol in Fig. 3A while exposed to three different conditions [Ca2+-containing (+Ca2+), Ca2+-free, and KCl stimulated] for the duration of time indicated along the horizontal axis. Passive and maximum total forces were measured at 215% L0 following a release from 220% L0 (labeled “Before strain softening” in Fig. 3). Tissues were then strain softened with seven 15% stretches to 230% L0 at 1 mm/s using the electromechanical lever, and passive and maximum total forces were again measured at 215% L0, twice stimulated with KCl at 250% L0, incubated in a Ca2+-free solution and subjected to sequences 2b, 3b, 4a, and 4b.

The second total force protocol, protocol T2 (Fig. 4A), was designed to test the hypothesis that an increase in passive force at a particular muscle length due to strain softening reversal is accompanied by a corresponding increase in total force at that muscle length. Tissues were stretched according to the protocol in Fig. 4A, while exposed to three different conditions [Ca2+-containing, Ca2+-free, and KCl stimulated] for the duration of time indicated along the horizontal axis. Each DSM strip was strain softened to 230% L0 in a Ca2+-free condition, released to 215% L0, and then released to 210% L0, where passive and maximum total forces were measured (labeled “Following strain softening” in Fig. 4). Next, strips were returned to L0, twice stimulated with KCl to restore stiffness lost by the prior strain to 230% (i.e., to reverse strain softening), incubated in a Ca2+-free condition, and stimulated directly to 215% L0 (rather than to 230% and then to 215%). In this case, more stiffness should be retained because less strain softening was induced. Finally, tissues were released to 210% L0, where passive and maximum total forces were measured (labeled “Following strain softening reversal” in Fig. 4).

Statistics. ANOVA and the Student-Newman-Keuls test, or the t-test, were used where appropriate to determine significance, and the null hypothesis was rejected at P < 0.05. The population sample size (n value) refers to the number of animals, not the number of tissues.

RESULTS

Passive L-T region. DSM strips subjected to passive L-T curve protocol P1 (see Passive L-T curve protocols) produced the passive L-T loop shown in Fig. 1B (n = 3). The left portion of the L-T loop (Fig. 1B, up arrow) was produced as tissues...
were successively stretched by 7% of $L_s$ and then released by 2% of $L_s$ at 0.06 mm/s, hold isometrically for 1 min, measure force, and repeat until reaching 252% $L_s$. Release sequence: release 5% or 10% of $L_s$ at 0.06 mm/s (5% until reaching 220% $L_s$), hold isometrically for 1 min, measure force, and repeat. B: the pseudo-steady-state passive $L$-$T$ region produced by protocol P1. The left portion of the loop (up arrow) was produced as tissues were successively stretched by 7% of $L_s$ and then released by 2% of $L_s$ at a series of lengths between $L_s$ and 250% $L_s$. The right portion of the loop (down arrow) was produced as tissues were released to successively lower lengths beginning at 250% $L_s$ and ending at $L_s$. Values represent the average passive force ($F_{p}$) $\pm$ SE ($n = 3$), measured after 1 min of force redevelopment at each length, normalized to the passive force at 250% $L_s$ ($F_{pmax}$). C: selected data from one experiment showing that although passive force did not reach a steady state value 1 min after a length increase (see the first 60 s of “220% up”), it did reach a pseudo-steady-state value 1 min after length decreases (see “220% up” and “220% down”). The “up” curves represent 3 typical tracings from the stretch sequence (see A), and the “down” curves represent 2 typical tracings from the release sequence (see A).

were successively stretched by 7% of $L_s$ and then released by 2% of $L_s$ until the maximum strain of 250% $L_s$ was reached. The right portion of the $L$-$T$ loop (Fig. 1B, down arrow) was produced as tissues were incrementally released from 250% $L_s$ to $L_s$. Values were normalized to the passive force at 250% $L_s$. These data demonstrate that the passive $L$-$T$ relationship for DSM is not represented by a single curve but instead by a region that includes a range of possible passive force values for a given muscle length. Furthermore, it is important to note that the $L$-$T$ loop in Fig. 1B is not a dynamic hysteresis loop like those shown in our previous studies (54) because each point on the loop is a pseudo-steady-state force value measured after 1 min of isometric force redevelopment at a particular length (Fig. 1C).

The peak force produced by the final KCl-induced contractions at the end of protocol P1, $55.1 \pm 8.2$ mN, was not significantly weaker than that produced by the contraction performed immediately before the first 7% stretch ($64.8 \pm 2.2$ mN, $n = 3$, $P > 0.05$), which indicated that the tissue had not been significantly damaged during the stretches to 252% $L_s$. Furthermore, based on the estimated cross-sectional area of the DSM strips ($0.9$ mm$^2$; see Determination of slack length), the average peak stress produced during these contractions was $67$ kPa at $L_s$ (on the ascending limb of the active $L$-$T$ curve), which is comparable to the value of $84$ kPa at the optimal length for contraction reported by Hellstrand and Johansson (25) for rabbit DSM and between the maximum and minimum values ($\sim 80$ and $\sim 40$ kPa) on an active $L$-$T$ curve for rat DSM presented by Uvelius (60).

**Shifting of the passive $L$-$T$ curve due to reversible strain softening.** Passive $L$-$T$ curve protocol P2 (see Passive $L$-$T$ curve protocols) produced the $L$-$T$ curves shown in Fig. 2 ($n =$
3). Strain softening caused by protocol sequences 1a, 2a, and 3a produced passive L-T curves that clearly shifted to the right as the maximum previous strain increased (Fig. 2C; curves 2a and 3a). Following protocol sequence 3a, the two KCl contractions at $L_s$ reestablished passive stiffness lost to strain softening during protocol sequences 1a–3a because protocol sequence 2b produced a passive curve shifted far to the left of the previous curve, 3a, and even slightly to the left of the comparable curve, 2a. Likewise, curve 3b was similar to curve 3a (Fig. 2C). Protocol sequences 4a and 4b produced passive
L-T curves that were nearly superimposable (Fig. 2B). This L-T curve produced after muscle stretch to 250% \( L_s \) was relatively flat at short muscle lengths and very steep at longer lengths \( (>230\% \ L_s) \), and we reasoned that this curve represents the “fixed” passive L-T curve determined largely, but not entirely, by components of the extracellular matrix (19, 26, 59).

APS modulates total force. Results from the total force protocol T1 (Fig. 3A) show that following the determination of \( L_s \), DSM strips were stretched through a sequence of lengths, calculated as a percentage of \( L_s \), while exposed to 3 different conditions \ ([Ca\(^{2+}\) containing (+Ca\(^{2+}\)), Ca\(^{2+}\) free, and KCl stimulated] for the duration of time indicated along the horizontal axis. Passive force was recorded at points \( P_{N1}, P_1, \) and \( P_2 \) (gray circles), and maximum total force was measured at points \( T_{N1}, T_1, \) and \( T_2 \) (open circles). B: average passive (0.75 ± 0.16 before strain softening and 0.44 ± 0.08 after strain softening), active (0.71 ± 0.14 and 0.65 ± 0.07), and total force (1.46 ± 0.07 and 1.09 ± 0.01) values ± SE. Passive and total forces decreased significantly following strain softening (* \( P < 0.05 \) for paired t-test, \( n = 3 \)), while active force remained relatively unchanged (\( P > 0.05 \) for paired t-test). Data are normalized to the peak active force from the second contraction at \( L_s \) (\( T_{N1} \)). C: estimated average stress values (\( n = 3 \)). D: typical data demonstrating that total force decreased with a corresponding decrease in passive force due to strain softening, whereas active force was relatively unaltered.

L-T curves of protocol T1. In T1, passive and total forces were measured before and after strain softening. In T2, passive and total forces were measured after stretching to a long muscle length (210%) to induce strain softening and then again after release to \( L_s \) and contraction to replace the stiffness lost to strain softening. Results from the total force protocol T2 (Fig. 4A) show that following the restoration of passive stiffness previously lost to strain softening, passive and total forces, but not active force, displayed significant increases above the respective forces produced at 210% \( L_s \) in tissues that had been strain softened (Fig. 4, B and C, \( n = 3 \)). Increases in passive and total force were 53% and 35%, respectively, while the increase in the average active force was only 4%. It is important to note that active force may be somewhat underestimated in Figs. 3 and 4 because active force was calculated by simply subtracting...
passive force from total force as described by Uvelius (60) and was not corrected for load transfer between active and passive elements during activation (36, 37). Together the results from total force protocols T1 and T2 indicate that APS can modulate total force at a given muscle length with relatively little change in active force and suggest that the structures responsible for APS act in parallel with the contractile apparatus because adjustable passive force and active force appear additive.

**DISCUSSION**

APS. The most important contribution provided by our present study is the proposal that structural elements within DSM cells are responsible for passive stiffness that can be adjusted and that as a result the relationship between passive tension and muscle length can best be described by a series of curves over a broad range of muscle lengths (see Fig. 2B) rather than by a unique curve. It is possible that additional shifts in the passive $L$-$T$ curve may occur through the process of adaptation (2, 38, 45a, 64). However, the strain-dependent measurements made in this study were likely not of the durations required to permit adaptation to occur. Thus, whether adaptive changes induced by prolonged periods of strain also can occur in DSM remains to be determined.

The proposal that structural elements within DSM cells are responsible for the physiological phenomenon of APS is based on empirical evidence obtained in this and previous work (54, 56) that application of muscle strain decreases passive force for a given muscle length and that maximum muscle activation using KCl increases passive force for a given muscle length, resulting in shifts in the passive $L$-$T$ curve, as shown in Fig. 2.

We previously introduced a VPEC as an analog element modeling APS (54, 56). What remains to be determined is the
precise structural element(s) within DSM cells represented by our VPEC element that causes APS. There exist several candidate structures that could potentially play a role in causing APS. These include actomyosin cross bridges (8), caldesmon (62), calponin (57), smooth muscle titin (28), a low-molecular-weight titin analogous to twitchin expressed in catch muscle (4), and cytoskeletal proteins that cross-link actin, such as filamin (40, 52, 63), and that cross-link actin with intermediate filaments (32). Such elements may represent a subset of “...link formation and dissolution...” described in the general stochastic model of cytoskeletal dynamics developed by Fredberg and colleagues (50, 51). Because strong muscle activation using KCl or carbachol increases passive stiffness, and the RhoA kinase (ROK) inhibitor Y-27632 prevents these stimuli from inducing passive stiffness, the structure appears to be regulated by RhoA kinase activation (54). ROK causes increases in myosin light chain phosphorylation and activation of actomyosin cross bridges (12, 58). Thus, cross bridges may play a role in APS.

Studies performed several decades ago show that smooth muscle at rest (i.e., not stimulated by any contractile agent) does display high passive stiffness that is attributable to cross bridges (5, 48, 49). Moreover, a component of diastolic stiffness of cardiac muscle is caused by cross bridges (6). However, what is unique about our studies compared with others is that we find that the passive stiffness induced on stimulation of DSM with KCl or carbachol is retained when tissues are incubated for long periods of time in a Ca\(^{2+}\)-free solution (54). This is not the case for the passive stiffness found earlier in taenia coli, vascular, and myometrial smooth muscles (5, 48, 49). Moreover, passive stiffness in strain-softened DSM is not induced by the spontaneous rhythmic contractions that occur when tissues are exposed to normal extracellular levels of Ca\(^{2+}\) (54). Last, results from the present study indicate that the appropriate location for the VPEC in our analog APS model is in parallel with the CC (see An APS model for a discussion of the model). Thus, if the cellular structure responsible for APS is a cross bridge, then this cross bridge appears to be distinct from those responsible for establishment of active contraction.

The passive L-T loop in Fig. 1 and the shifting passive L-T curves in Fig. 2 demonstrate that for a given muscle length, a range of passive force levels can be achieved, depending on the strain and activation history of the tissue. The material properties of the various structures that compose the tissue must impose limitations on the maximum and minimum passive forces that can be achieved at a particular muscle length and thus provide boundaries for a passive L-T region. The region contained within the passive L-T loop in Fig. 1 likely does not reflect the absolute boundary of the passive L-T region for the tissue because releasing the tissue by less than 2% at each length should shift the upward curve further to the left, and strain softening beyond 250% \(L_s\) should shift the downward curve further to the right, effectively increasing the size of the region by a small amount. We hypothesize that the lower boundary of this region (when passive force is minimum for a given length) is reached when the tissue has been completely strain softened and that forces along this boundary are produced by static structural elements (primarily extracellular collagen at long muscle lengths). We further hypothesize that the upper boundary of this region (when passive force is maximum for a given length) is reached when the maximum amount of strain-softenable stiffness has been induced in the tissue and that passive force along this boundary is the sum of forces produced by both static structural elements (including collagen and elastin) and variable (dynamic) structural elements. Although these dynamic elements remain to be identified, we have previously shown that they are adjusted by a ROK-dependent mechanism that is activated on muscle stimulation with KCl or the muscarinic receptor agonist carbachol (54). Therefore, the dynamic structures are intracellular and distinct from viscoelastic structures in the extracellular matrix. Furthermore, because the mechanism is ROK dependent, the adjustable behavior is likely not due to pressure-induced fluid transfer between tissue compartments. The role of APS in detrusor physiology remains to be determined; however, one likely function is to maintain suitable spacing between actin and myosin filaments to enable efficient force generation throughout the broad range of muscle lengths over which detrusor operates.

**Passive force measurement guidelines.** The identification of APS implies that a single passive L-T curve is insufficient to define passive force in DSM and creates the need for a new, well-defined, and uniform procedure for measuring passive force in DSM. We propose the following general guidelines for measuring passive force at a given muscle length. First, the measurement should be performed while in a Ca\(^{2+}\)-free solution after the elimination of spontaneous rhythmic tone; however, because isometric passive force in a Ca\(^{2+}\)-free condition can slowly decline over a period of minutes to hours (56), the time in Ca\(^{2+}\)-free solution may be significant and should be minimized. Second, quasi-steady-state force should be measured following a release (>1 mm/s) from a slightly longer length (~2% of \(L_s\)) to minimize strain softening. After the release, isometric passive force may quickly redevelop (<30 s) to a quasi-steady-state value and then decline relatively slowly (see Fig. 4 in Ref. 56). Passive force should be measured during the quasi-steady-state phase, and then the muscle should be activated to measure total force at that length. This pair of passive and total force measurements can then be used to calculate the active force produced at that length. It is important to note that because passive force is dependent on the strain and activation history of the tissue, passive and total force at a particular length should be remeasured following a length change. Furthermore, when passive force values are reported, the recent strain and activation history should also be reported. Additional studies are necessary to further characterize passive force in DSM and to establish a more detailed procedure for measuring and reporting passive and active force values. There may be a need to develop similar guidelines for other smooth muscles.

**An APS model.** Two generally accepted analog models of smooth muscle are the Kelvin (Maxwell) (Fig. 5A) and the Voigt (Fig. 5B) viscoelastic models (15, 16, 37), which each contain three elements: a series elastic component (SEC), a parallel elastic component (PEC), and a contractile component/damper (CC/D) that generates active force when the cross bridges are cycling and acts as a dashpot when the cross bridges are passive. Exponential functions for the PEC and SEC elements have been defined for the Kelvin model and parameters estimated for rat portal vein (18), and the Voigt model has been used to model plasticity in airway smooth muscle (30). The Kelvin model also has been used to model the
behavior of rubber using hyperelastic stiffness elements and a VPEC element to account for strain softening (7, 33).

We recently adapted the Kelvin model for smooth muscle to create an APS model by including a VPEC element (defined in Ref. 56) to account for APS (Fig. 5C) (56). The VPEC can also be added to the Voigt model (Fig. 5D). Figure 5E illustrates seven locations in the Kelvin model to be considered for the placement of the VPEC to account for the adjustable component of passive stiffness, and Fig. 5F illustrates similar locations within the Voigt model. The VPEC was not placed in series with the PEC in either the Kelvin or Voigt models (Fig. 5, E and F, box 1) because this would be equivalent to replacing the PEC with a VPEC, and there is empirical evidence for modeling the static and variable components of passive stiffness separately. Placing the VPEC in series (box 2) or parallel (box 3) with the SEC is not appropriate because this configuration would enable a contraction to cause strain softening, which is inconsistent with our data. In Fig. 5, E and F, the D and CC components are drawn separately (34) to allow the VPEC to potentially be placed in series with either element. It is important to note the CC is strictly a force generating model element and that any elastic or damping behavior within the contractile component is modeled by the PEC, SEC, and D elements and possibly our new VPEC element. Placing the VPEC in series with the D element (box 4) would allow the force in this element to approach zero at steady state, which is not appropriate because we previously showed that APS does bear force at steady state (56). Placement of the VPEC in series with the CC (box 5) is also not appropriate because this configuration would enable a contraction to cause strain softening. Our present data indicate that the structures responsible for APS act in parallel with the contractile apparatus, indicating that the VPEC could be placed in either box 6 or box 7 in either the Kelvin (Maxwell) or the Voigt model. This finding supports our previously proposed APS model (Fig. 5C) (56), in which we placed the VPEC in parallel with the PEC (i.e., in box 7 of Fig. 5E). If APS is due to cross bridges, then placing the VPEC in box 6 would be appropriate, whether or not these cross bridges are slowly cycling or cross-linked, and whether or not they are a fraction of the cross bridges responsible for active force generation or a distinct population of cross bridges specifically responsible for APS. Further studies are necessary to identify the specific structures responsible for APS in DSM and the physiological mechanisms by which they are adjusted.

A number of other models have been developed to account for the dynamic L-T relationship in smooth muscle. Gunst et al. (22) accounted for adaptation by shifting the sites where actin filaments connect to dense bodies. Seow et al. (1, 27, 29, 30, 45) have developed a model in which contractile units are added in series as a smooth muscle adapts to longer lengths, and Solway et al. (53) proposed that changes in actin filament length and the parallel-to-series arrangement of the contractile units contribute to plasticity. Fredberg and colleagues (3, 10, 11, 21, 31) have described smooth muscle as a soft glassy material in which the cytoskeleton can deform, flow and reorganize. Furthermore, Fredberg et al. (50, 51) have developed a network model consisting of fibrous and contractile links. The cross-links identified by us as being responsible for APS may represent a subset of the links and attachments described in these other models. APS may be distinct from adaptation because it specifically describes instantaneous cross-link breakage affecting the passive L-T relationship and because stiffness reformation is dependent on strong muscle activation at short muscle lengths (54). However, additional studies are clearly warranted not only to identify all of the mechanisms regulating the complex mechanical behavior of smooth muscles but also to understand similarities and differences in the mechanical behaviors of smooth muscles serving different physiological roles.

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REFERENCES


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