Transient oscillatory force-length behavior of activated airway smooth muscle

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AIRWAY SMOOTH MUSCLE (ASM) undergoes cyclic changes in length during breathing. In particular, the expansion of the airway wall during inspiration can force the ASM to lengthen, even when it is activated (6, 27, 33). This mitigates the degree of airway narrowing that would otherwise occur when the ASM is stimulated and is thought to be an important mechanism protecting against excessive bronchoconstriction. Consequently, the force-length behavior of activated ASM has become a subject of considerable interest. Nevertheless, the nature of the link between events occurring within ASM at the microscopic scale and its behavior at the macroscopic scale remain poorly understood. Addressing this link is very important for the design of strategies that might limit excessive airway narrowing in asthma, and indeed for our understanding of asthma pathogenesis in general.

One of the approaches taken to modeling the force-length behavior of activated ASM is that of Fredberg and coworkers (16, 31), who ascribed the macroscopic behavior of ASM to the way in which strain affects the behavior of the crossbridges. They combined the four-state crossbridge model of Hai and Murphy (22), which was developed to explain the “latch-state” in smooth muscle, with Huxley’s 1957 two-state crossbridge model of skeletal muscle (24). While impressive in many of its predictive capabilities, this model has not been particularly successful in accounting for stress-strain nonlinearities (15, 31), and it neglects any contribution from the passive rheological properties of the noncontractile tissue that makes up a substantial fraction of a macroscopic strip of ASM (25). By contrast, Bates and Lauzon (5) developed a model of the ASM strip that accounted for the nonlinear elastic properties of connective tissue but employed a simplistic representation of active force generation based on the Hill force-velocity relationship. This model gave accurate simulations of steady-state force-length loops from ASM, but only when the parameters of the Hill relationship were made to vary with experimental conditions so that peak ASM force during each loop was independent of peak length, a characteristic for which there was no clear explanation.

While it is arguable that these two models each embody key mechanisms underlying the macroscopic dynamic force-length behavior of ASM, it seems equally clear that each model is missing something important. The purpose of the present study was therefore to develop a model of the activated ASM strip capable of accounting for both the transient and nonlinear dynamic behavior of the activated ASM strip, borrowing elements from both modeling approaches described above.

EXPERIMENTAL METHODS

Experimental data collection. All procedures were approved by the McGill University Animal Care Committee and complied with the guidelines of the Canadian Council on Animal Care. Lewis rats (ssNHsd, Harlan Sprague Dawley; n = 6; body mass 280–320 g; age 8–12 wk) were euthanized by overdose of pentobarbital sodium. Tracheas were dissected out and placed in Krebs-Henseleit solution (composition in mM: 118.0 NaCl, 4.5 KCl, 2.5 MgSO4, 1.2 KH2PO4, 25.5 NaHCO3, 10.0 glucose, 2.5 CaCl2, pH 7.4; aerated with 95% O2/5% CO2 for at least 30 min) on ice. All experiments were performed on at least three muscle strips.

A strip of ASM was dissected from each trachea. Krebs-Henseleit solution aerated with 95% O2/5% CO2 was circulated through the dissection chamber at a rate of ~3 ml/min throughout the dissection. Loose connective tissue was removed, and the trachea was cut transversely to separate out a single cartilage ring with adjoining muscle tissue. In situ muscle length was measured to the nearest 0.1 mm by holding a scale next to the tracheal ring under a microscope at ×45 magnification. The strip of ASM was cut out of the ring with ~1.5 mm of cartilage at either end, and aluminum foil clips (14) were folded around the cartilage, as close as possible to the muscle. These clips were used to attach the ASM strips to the experimental apparatus.

The ASM strips were mounted in a horizontal muscle bath by placing the foil clips over wire hooks attached to a length controller (model 322C-I; Aurora Scientific, Ontario, Canada) and a force transducer (model 404A, Aurora Scientific). Krebs-Henseleit solution was stored in an adjacent reservoir, where it was aerated with 95%
O2/5% CO2 and circulated through the muscle bath at ~3 ml/min throughout the experiment (except when specified below). A water jacket maintained the bath at ~37°C. The ASM strips were set to an initial length (Lref) of 1.1 × in situ length. The strips were then equilibrated at Lref for 1 h during which time they were stimulated electrically (5 ms square wave, 50 V, 30 Hz) for 10 s every 4 min. All subsequent stimulations were performed using 5 × 10−4 M methacholine (acetyl-β-methylcholine chloride, Sigma Chemical). Preliminary experiments demonstrated that this concentration generated a maximal isometric force response.

Response of ASM to sinusoidal length change. The ASM strips were stimulated for periods of 14.5 min by stopping the circulating Krebs-Henseleit solution and injecting methacholine directly into the muscle bath. Each activation period was followed by a 14-min washout period. Two isometric contractions were performed, followed by three contractions during which ASM strip length was held constant for 2 min, then oscillated sinusoidally for 10 min, and then held constant for a further 2 min. Oscillation amplitudes of ± 1, ± 2 and ± 4% Lref (in that order) were used for the three-10 min oscillation periods in each muscle strip. An oscillation frequency of 2 Hz (approximately resting breathing frequency in the rat) was used throughout. Finally, a third isometric contraction was performed. Force during the isometric contractions immediately before and after the oscillations always differed by less than 20%.

Unstimulated ASM was tested in calcium-free Krebs-Henseleit solution to prevent a stretch activation response. The ASM strips were stretched until their passive force was approximately equal to their active isometric force and then held isometric for 1 min. Length was oscillated sinusoidally with an amplitude of ± 4% Lref and frequency of 2 Hz for 10 min and then held constant for a further 2 min.

Response of stimulated muscle to deep inflation. Deep inflations (DI) were simulated by performing single, large-amplitude, half-sinusoidal loading cycles of 1-s duration. Before and after each DI, length was oscillated for 1 min with an amplitude of ± 2% Lref and frequency of 2 Hz. In two experiments, DIs with amplitudes of 15%, 5%, and 25% Lref were performed, in that order (the largest DI was performed last in case it damaged the tissue). After each DI, an isometric contraction was maintained for at least 20 min and until force had recovered to 90% of its initial value or was no longer increasing, to try to ensure that the latter two DIs were unaffected by the previous DIs. Because this required the muscle to remain activated for long periods, stimulation was achieved by adding methacholine to the circulating Krebs-Henseleit solution and performing all the DIs within a single period of activation. No DIs were performed within the first 8 min of activation because preliminary experiments indicated that the response to a DI differed during this time.

We examined the possible protective effects of a DI by performing four or five 100-s isometric contractions separated by 8-min washout periods. Immediately before the final contraction, a DI of 25% Lref was performed on the ASM strip, preceded and followed by 1 min of ± 2% length oscillation at 2 Hz.

EXPERIMENTAL RESULTS AND MODEL DEVELOPMENT

Passive oscillatory force-length behavior. Figure 1A shows the passive viscoelastic behavior of a representative strip of unstimulated ASM, which is typical of the other strips examined. The immediate increase in force produced by sudden stretch from Lref relaxed progressively. When length oscillations of ± 4% Lref were imposed on the strip ~1 min later, peak force was further increased but again relaxed progressively with time. These data demonstrate the passive viscoelastic behavior of ASM tissue. The nonlinear nature of this behavior is evident in the curvilinear shape of the force-length loops also plotted in Fig. 1A.

To account for the key features of the data shown in Fig. 1A, we extended the model of Bates and Lauzon (5). Their model consists of an active force generator in series with a nonlinear spring. However, it has long been pointed out, for example, by Gunst (18), that the passive tissue connected in series with the contractile proteins is viscoelastic. Accordingly, we replaced the series spring with the standard viscoelastic model, a Kelvin body, consisting of two springs and a dashpot as shown in Fig. 2. This parallel Kelvin body ensures that the model retains mechanical integrity when the force generator is not active. In fact, when the muscle is passive, we assume that the force across the force generator is zero so that it follows the length changes of the entire model without resisting. This assumes that there are no crossbridges that remain bound when the muscle is inactive, although there has been some debate about this (18, 34).

Smooth muscle cells have been reported to make up 75% of a tracheal strip (36). Presumably not all of a smooth muscle cell is spanned by contractile proteins, however, so we will assume that the force generator makes up 70% of the resting length of the model strip. Accordingly, we set the resting length of the series Kelvin body to be L0 = 30 in arbitrary length units (Fig. 2). Consequently, the resting length of the entire model, and also the length of the main spring in the parallel Kelvin body, is L0 = 100 length units. The resting length of the series Maxwell body spring is L0 = 30 length units, and the resting length of the parallel Maxwell body spring is w0 = 100 length units.

The model is driven with an imposed length perturbation L(t), beginning with the dashpots in the two Kelvin bodies each at a length of zero and allowing for negative dashpot lengths. When the force generator is not active, the model dynamics are determined only by the parallel Kelvin body. In this case, the force, F(t), across the model is

$$F(t) = E_p(L(t))L(t) + E_{mp}(w(t))w(t)$$

where L(t) is the extension beyond Lref of the main spring in the parallel Kelvin body, and w(t) is the extension beyond w0 of the spring in the parallel Maxwell body. To give the model the requisite nonlinear stress-strain behavior, the elastic stiffnesses of the two springs, $E_p$ and $E_{mp}$, are functions of their respective lengths. In the original model of Bates and Lauzon (5), stiffness was proportional to extension raised to the third power. We use the same degree of nonlinearity for both springs in the present model. The elastic functions in Eq. 1 are thus

$$E_p(L) = \alpha_p|L|^3$$

and

$$E_{mp}(w) = \alpha_{mp}|w|^3$$

where $\alpha_p$ and $\alpha_{mp}$ are positive constants.

The stress relaxation following a step in strain measured experimentally in strips of ASM (Fig. 1A) followed a time course that is close to a power law, as has been reported for numerous other biological soft tissues (7, 13, 17). It is problematic to get a Kelvin body to reproduce power-law stress relaxation (2), so we took the conventional approach in the
model of having the stress decay exponentially. To achieve this when the spring stress in the Maxwell body depends on strain, it is necessary to make the dashpot resistance exhibit the same strain dependence so that the stress relaxation has a well-defined time constant (i.e., the ratio of resistance to elastance is constant). We therefore made the resistive pressure drop across the dashpot inversely proportional to the stiffness of the spring of the parallel Maxwell body. This gives the balance between elastic and resistive forces across the Maxwell body as

$$\frac{\alpha_M p_{w(t)}}{R_p} \frac{d}{dt} [L(t) - w(t)]$$  \hspace{1cm} (4)

where $R_p$ is a constant.

We further assumed that the main spring in the parallel Kelvin body generates tension only in extension, and is flaccid in compression, since shortening a passive strip of trachealis below $L_{\text{ref}}$ does not immediately generate a significant opposing force. The Maxwell element spring does have to generate compressive tension, however, so that it is able to reverse the direction of the dashpot to which it is connected to simulate viscoelastic stress recovery. However, we assume that this tension only causes internal reorganization within the tissue and does not manifest as a compressive tension across the tissue as a whole, so that when the tissue is shortened below its resting length it remains completely flaccid.

Since in the nonactivated model the length of the force generator follows changes in $L$ without resisting, we solved the model equations in this case simply by determining $w(t)$ from *Eq. 4* and then integrating it at 1,000 Hz using the first-order Euler scheme with $w(0) = 0$. When the viscoelastic parameters were given the values $\alpha_p = 0.0001$, $\alpha_M = 0.0003$, and $R_p = 100$ (arbitrary length and force units, time in seconds), we obtained the simulated force-time and force-length profiles shown in Fig. 1B. These simulations were generated by imposing on the model an $L(t)$ matching that used experimentally to obtain the data shown in Fig. 1A. That is, the model was subjected to sinusoidal length oscillations of $\pm 4\% L_{\text{ref}}$ about a length that yielded the appropriate force levels. The starting length in the model was 110% of $L_{\text{ref}}$, whereas in the actual strips themselves it was $\sim 127\%$. Thus, passive force was apparently not manifest in the actual strip, as predicted by the model, until it had been strained by $\sim 17\%$. However, this is not important for the active model (developed below) because

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Fig. 1. *A*: passive force-time (*top*) and force-length (*bottom*) plots from a passive strip of rat trachealis oscillated at 2 Hz with a peak-peak amplitude of $\pm 4\%$ baseline length. The strip was initially stretched to achieve a force close to the isometric force generated by maximally stimulating the muscle with methacholine. *B*: corresponding model simulations produced with the force generator in the model inactivated. The position of zero on the length axis is set at the length at which the tissue, or model, had to be set to generate the necessary passive tension.
the force generator, when activated, simply stretches the series Kelvin body by whatever length is required to generate the necessary reactive force.

**Active oscillatory force-length behavior.** Figure 3 shows representative force-time and force-length measurements made in a stimulated strip of ASM. These typify the data obtained in all strips studied. Exposure to methacholine at length \( L_{\text{ref}} \) beginning at \( t = 0 \) caused a transient rise in force to a steady-state level of slightly less than 3 mN. Length oscillations beginning after ~1 min initially gave rise to a force that oscillated about the isometric force level. Peak force decreased very rapidly, however, so that after only a few seconds it had become equal to or even slightly less than isometric force (isometric force obtained without length oscillations is also shown in Fig. 3). Peak force continued to decrease very slightly until the oscillations ceased at 700 s. Force immediately following the termination of length oscillation was substantially less than isometric, but it recovered progressively toward the isometric level, with the rate of force recovery being slightly less than the rate of onset of force at the beginning of the experiment. This pattern was qualitatively similar when the experiment was repeated using oscillation amplitudes of \( \pm 1\% \), \( \pm 2\% \), and \( \pm 4\% \) \( L_{\text{ref}} \). The isometric force curves obtained before and after the length oscillation experiments are virtually identical, showing that the oscillations themselves did not affect the subsequent contractile ability of the muscle. Figure 3 also shows the data plotted as force vs. length, again highlighting the observation that peak oscillatory force decreased rapidly to a level almost independent of oscillation amplitude. Also, the shape of the force-length loops changed markedly with amplitude, moving from more or less elliptical to quite “banana shaped” as amplitude increased from \( \pm 1\% \) to \( \pm 4\% \) \( L_{\text{ref}} \).

To simulate the data in Fig. 3, we extended Eq. 1 to include an active force term, \( F_A(t) \), due to the force generator (Fig. 2).

\[
\text{The total force across the muscle is now increased by the contribution from the force generator, which produces an equivalent force across the series viscoelastic element. That is,}
\]

\[
F_A(t) = E_A(y) y(t) + E_{M_s}(z) z(t)
\]

where \( E_A \) and \( E_{M_s} \) are the elastic constants of the two springs in the series Kelvin body and \( z \) is the extension of the spring in the series Maxwell body (Fig. 2).

The force balance across the series element itself is expressed as

\[
\alpha_{M_s} |z(t)|^3 z(t) = \alpha_{M_s} |z(t)|^3 R_s [\dot{y}(t) - \dot{z}(t)]
\]

where \( R_s \) is the dashpot resistance in the series Maxwell body and we have assigned the same strain-cubed dependence of stiffness to the springs of the series viscoelastic element as used above for the parallel element. The force across the entire model is now

\[
F(t) = E_A(L) L(t) + E_{M_s}(w) w(t) + F_A(t)
\]

and the length of the force generator changes with time according to

\[
L(t) = \dot{x}(t) + \dot{y}(t)
\]

As in the model of Bates and Lauzon (5), the relationship between \( F_A(t) \) and the rate of change of force generator length \( \dot{x} \) is determined by the Hill equation,

\[
\dot{x}(t) = \frac{b[F_A - F_A(t)]}{a + F_A(t)} \quad \text{when} \quad F_A(t) < F_0
\]

\[
= \frac{bF_0}{a + F_0} - \frac{bF_A(t)}{a + F_0} \quad \text{when} \quad F_A(t) \geq F_0
\]

where muscle velocity is negative when the muscle is shortening. Equations 4, 6, 8, and 9 constitute the velocity equations for the active model and were again solved numerically using Euler integration at 1,000 Hz.

To simulate the active force-length data shown in Fig. 3, the parameters of the Hill equation were set proportional to those used by Bates and Lauzon (5), with the values \( F_0 = 3 \), \( a = F_0/4 \), and \( b = 1.5 \) (again in arbitrary units of force and length, with time in seconds). The parameters of the parallel Kelvin body remained the same as for the passive case above. We assume that the values of the series Kelvin body parameters bear the same relationship to each other as the corresponding parameters in the parallel Kelvin body. The actual parameter values themselves in the series Kelvin body were chosen, through trial and error, to make the model behavior similar to that of real ASM strips. Interestingly, the best results were obtained when the values of the series Kelvin body parameters were the same as those in the parallel body.

Figure 4 shows force-time and force-length curves predicted by the model for oscillation amplitudes of \( \pm 1\% \), \( \pm 2\% \), and \( \pm 4\% \) \( L_{\text{ref}} \). These simulated curves differ from the experimental data shown in Fig. 3 in several important ways. First, while the onset of length oscillation causes a small transient decrease in force amplitude in the model, as a result of viscoelastic settling of the series Kelvin body, the decrease in force is nowhere near as large or as rapid as in the experimental data. Second, the steady-state peak in force during the length oscillations in the model increases progressively with oscillation amplitude, in
contradistinction to the experimental data in which the steady-state peak force is virtually independent of amplitude. Third, upon cessation of length oscillations, the experimental data show a marked recovery in force that manifests over 50 to 100 s, whereas the model exhibits no such force recovery. These discrepancies are mirrored in the force-length plots shown in Fig. 4 compared with those in Fig. 3.

The above simulations demonstrate that passive viscoelasticity in the noncontractile components of ASM cannot account for either the rapid fall in peak oscillatory force seen experimentally or the near independence of steady-state peak force on oscillation amplitude. Something crucial is obviously missing from the model. The most likely possibility is that we failed to include some important aspects of crossbridge dynamics that were originally postulated by Huxley (24) for skeletal muscle, and which have been invoked more recently for smooth muscle by Fredberg and coworkers (16, 27, 31). Here, the notion is that myosin crossbridges do not attach instantaneously to their respective actin binding sites as soon as the muscle is stimulated, but rather there is a certain time scale associated with their attachment. Furthermore, relative movement of actin and myosin filaments increases the crossbridge detachment rate relative to an isometric contraction. Huxley (24) proposed a specific dependence of crossbridge binding and unbinding probabilities on displacement, which Mijailovich et al. (31) extended to smooth muscle by including latchbridges, as well as conventional crossbridges, according to the scheme of Hai and Murphy (22). These models, however, are based on the notion that the muscle filaments are all lined up in the direction of bulk strain. While this makes perfect sense for the crossbridges of skeletal muscle with its highly ordered linear arrays of sarcomeres, smooth muscle has a much less geometrically constrained structure. This raises some doubt as to whether the level of detail inherent in the crossbridge binding probabilities of the Huxley model is justifiable for ASM.

We therefore decided to implement a simpler and purely empirical mechanism for modeling crossbridge attachment and detachment dynamics based on the following considerations. There are presumably many actin-myosin fiber pairs arranged in both series and parallel throughout a strip of ASM. If crossbridges were to become detached randomly throughout the strip, there would be, on average, fewer crossbridges acting in parallel across any cross-section through the strip. This would reduce isometric force correspondingly. However, provided all of the fiber pairs retained at least one working crossbridge, there would still be the same number of fiber pairs acting in series. Assuming that the shortening velocity of a completely unloaded fiber pair is determined solely by the crossbridge cycling rate, regardless of how many crossbridges are involved, the unloaded shortening velocity of the entire strip would be unaffected by random crossbridge detachment. These assumptions lead to the conclusion that the number of
attached crossbridges should influence the value of \( F_0 \) in Eq. 9, and hence the value of \( a \), which remains equal to \( F_0/4 \), but should not affect the value of \( b \). In this way, the maximum (unloaded) shortening velocity, given by \( bF_0/a \), remains unchanged.

The simplest way to incorporate attachment dynamics is to assume that those crossbridges that are not yet attached will become so according to first-order kinetics. Thus, if \( F_{0,\text{max}} \) is the maximum value of force achieved in an isometric steady-state contraction, then the rate at which isometric force increases from some submaximal value, \( F_0 \), is given by \( k_{\text{on}}(F_{0,\text{max}} - F_0) \), where \( k_{\text{on}} \) is a rate constant with units of \( s^{-1} \). We assume a similarly simple relationship between the rate of crossbridge detachment and the relative motion of actin and myosin filaments; the detachment rate is proportional to both the rate of change of muscle length and the current value of \( F_0 \), and is governed by a rate constant \( k_{\text{off}} \) having units of inverse length. Combining these two concepts gives

\[
\dot{F}_0(t) = k_{\text{on}}[F_{0,\text{max}} - F_0(t)] - k_{\text{off}}|\dot{x}(t)|F_0(t) \quad (10)
\]

This scheme for crossbridge attachment and detachment was incorporated into the model by using Eq. 10 to determine the value of \( F_0 \) used in Eq. 9 at each time step, beginning with \( F_0 = 0 \).

Figure 5 shows the simulated force-time and force-length curves obtained using \( k_{\text{on}} = 0.1 \) \( s^{-1} \) and \( k_{\text{off}} = 0.15 \) (arbitrary length units)\(^{-1} \), all other model parameters remaining the same as above. Now the model is able to reproduce the key features in the real data shown in Fig. 3 that were not accounted for in the previous simulations shown in Fig. 4. In particular, immediately after the start of oscillations, the model demonstrates a large and rapid decrease in peak oscillatory force. Furthermore, the steady-state force peak is virtually identical to isometric force and is independent of length amplitude, again similar to experimental observations (although the experimental data exhibit a slight negative dependence of peak force on amplitude). Finally, the model also reproduces the transient force recovery seen experimentally upon cessation of length oscillations.

**Effects of DI.** Figure 6A shows a representative example of the effects of a DI on the force-time behavior of ASM during \( \pm 2\% \) \( L_{\text{ref}} \) oscillations. A DI of \( 5\% \) \( L_{\text{ref}} \) caused a transient decrease in isometric force, most of which recovered with a time course similar to that of the initial onset of force at the beginning of stimulation (see Fig. 3), although full recovery took much longer (it took 14 min for force to recover to 99% of its pre-DI level, averaged from 3 strips). The DIs to 15% and 25% \( L_{\text{ref}} \), however, cause progressively greater decrements in isometric force, as shown in Fig. 6A. Force recovery was also delayed, with force reaching 91% of its pre-DI level by 34 min following the 15% DI and 76% of its pre-DI level at 65 min following the 25% DI.

Figure 6B shows the results of applying the same DI maneuvers in the model used to generate the simulations shown in Fig. 5. Here, the model was activated for 1 min and then subjected to \( \pm 2\% \) length oscillations in the middle of which a
DI was applied with the same timing and magnitude as used experimentally. The simulations reproduce many of the key features of the experimental data, including a transient decrease in oscillatory force following DI. Indeed, in the case of the 5% DI, the experimental and simulated data are very similar in all important respects. However, the larger DIs produced sustained, amplitude-dependent decreases in experimentally measured force, accompanied by decreases in tissue stiffness reflected in reduced amplitudes of oscillatory force. This effect is not evident in the simulated data. Instead, the simulated force curves return to pre-DI levels with the same relatively rapid dynamics regardless of DI magnitude. This indicates that when ASM is vigorously stretched in the activated state, some mechanism not present in the model causes a temporary reduction in the ability of the ASM to generate active force.

Possibly related to the effects of stretching activated ASM is the phenomenon known as bronchoprotection, where the application of a DI to the lungs in vivo just before bronchial challenge reduces the subsequent degree of bronchoconstriction (35). The mechanisms responsible for bronchoprotection remain poorly understood. However, we reasoned that if bronchoprotection is somehow the result of stretching ASM in situ, then prestretching the ASM strips in our experiments just before activation might produce a similar effect. Figure 7B shows a DI caused a significant and sustained reduction in passive oscillatory force in the ASM strip before it is stimulated. The levels of forced produced by the ± 2% \( L_{ref} \) oscillations in the passive strip were very much less than in activated strips (compared with Fig. 3). However, the relative magnitude and timing of the force reduction evident in Fig. 7B is highly reminiscent of that seen experimentally with the larger DIs in Fig. 6.

**DISCUSSION**

In this study, we have attempted to identify the essential elements required to account for the force-length behavior of an activated strip of ASM. Our criterion for deciding whether an element is essential in this regard is whether a computational model is unable to predict experimental data without it. There is, of course, substantial existing literature on the measurement and modeling of the mechanical properties of ASM. These properties have been examined over a wide range of scale, exemplified at one extreme by measurements of the force and displacement produced by a single crossbridge (28–30), and at the other by studies of airway responsiveness in living animals (3, 10, 38). We note, however, that because of the
possible effects of averaging, a computational model of ASM mechanics at one particular level of scale does not automatically have to include all phenomena demonstrable at all others levels of scale. Our approach in the present study has therefore been to take a hierarchical approach to the problem of modeling the mechanical behavior of an isolated piece of ASM. That is, we begin with the simplest configuration we can think of, and then proceed to add features as the experimental data demand.

The initial step in this process has actually already been taken in a previous study from our laboratories (5) in which we showed that a Hill-type force generator in series with a highly nonlinear spring gives accurate reproductions of steady-state force-length loops from activated ASM. We know, however, that this model has two serious shortcomings. First, it only accounts for force data obtained at different amplitudes of length oscillation if isometric force is made to decrease with oscillation amplitude in a purely ad hoc fashion. Second, this model is only able to account for steady-state force-length behavior. It has no means of mimicking the transient decreases in peak force that occur following onset of length oscillations. We (5) have previously suggested that these force transients might be due to viscoelastic stress relaxation in the connective tissue elements of the ASM strip. The need for a viscoelastic mechanism in the model is demonstrated by our observations of stress relaxation following stretch and oscillation of a passive ASM strip, where crossbridge dynamics are presumably absent (Fig. 1A). This stress relaxation follows a course that is close to a power law in time, as has been reported for numerous other biological soft tissues and which has been suggested to reflect the complex structural nature of the tissue (2, 7, 13). For ease of analysis, however, we chose to model viscoelasticity using a nonlinear Kelvin body (Fig. 2). This model exhibits stress relaxation that, while not identical to that of the ASM strip, nevertheless shows a similar magnitude decrease in peak force over a corresponding period of time (Fig. 1B).

While the addition of parallel viscoelasticity to the model appeared to be sufficient to account for the main force-length characteristics of passive ASM, the inclusion of series viscoelasticity was less successful for describing our measurements in activated ASM. It is clear, in fact, that the rapid transient reductions in peak force during each length oscillation that occurred immediately after onset of the oscillations (Fig. 3) cannot be attributed to viscoelasticity alone (Fig. 4). We are thus forced to conclude that although the effects of nonlinear viscoelasticity certainly need to be included in a model of ASM strip mechanics, we are still missing a major feature necessary to account for activated force-length behavior. The obvious candidate for the missing feature in the model is strain-induced detachment of crossbridges. This mechanism has been modeled in detail previously in a series of studies by Fredberg and coworkers (16, 31). These investigators attributed the dynamic force-length behavior of activated ASM, with considerable success, entirely to the kinetics of crossbridge attachment and detachment. In fact, the notion that crossbridge kinetic effects
should be apparent at the macroscopic level dates back to the classic skeletal muscle work of Huxley (24). Fredberg and colleagues elegantly extended this work to include the additional complexities of crossbridge biochemistry that are peculiar to smooth muscle (22). Our concern about going this far, however, is that because smooth muscle is much more amorphous than skeletal muscle, the assumption made in skeletal muscle, that bulk muscle strain is mirrored by the relative movement between actin and myosin filaments, may not be as justified in ASM. Also, despite its complexity, a model based purely on the kinetics of crossbridge cycling is still not able to account for the highly nonlinear banana-shaped force-length loops exemplified in Fig. 3 (16, 31).

For these reasons, and in the general interests of parsimony, we therefore employed a much simpler empirical scheme (Eq. 10) for encapsulating the notion that a population of myosin heads takes a finite amount of time to become fully engaged with its associated population of actin binding sites, and that relative movement of actin and myosin fibers increases the probability that bound crossbridges will detach. With this simple scheme, we obtained a dramatic improvement in the ability of the model to mimic the experimental data (Fig. 5). This is not to say that the theory of Hai and Murphy (22) is not relevant here; it just does not seem to be crucial in all its details for the present data. In any case, we conclude that accounting for the dynamic force-length behavior of activated ASM cannot be explained purely in terms of tissue viscoelasticity. In particular, even though one might quibble about the details, Fredberg and colleagues (15, 16, 31) are likely correct in their assertion that crossbridge attachment and detachment kinetics are needed to account for the force-length behavior of activated ASM.

At this point, it seems that both connective tissue rheology and crossbridge kinetics are on relatively solid ground in terms of their roles in explaining the mechanical behavior of ASM. However, our understanding of the situation is challenged again when we compare predicted and measured responses of activated ASM force to a large transient stretch (Fig. 6, B and A, respectively). Although the model predicts a post-DI force transient due to crossbridge detachment that matches experimental measurement when the DI amplitude is small (Fig. 6), with larger DI amplitudes (Fig. 6) the force remains depressed for much longer than predicted by the model. Force in the strip of trachealis muscle eventually did recover much of its pre-DI value if we waited long enough (over an hour in some cases), but it is clear that the mechanism behind this recovery is not related to the way we modeled crossbridge attachment kinetics in Eq. 10. Importantly, this is not a phenomenon limited only to in vitro preparations of ASM, as DI-induced reductions in contractility of ASM have been observed in vivo (4, 9). The same phenomenon may also be behind the slight decreases in steady-state oscillatory force that we observed with increasing oscillation amplitude (Fig. 3), and which have been reported by others (15, 18, 27).

This raises the intriguing question as to what could be behind the sustained post-DI force decrement seen in Fig. 6. One possibility is that the answer lies with the latch theory of ASM, according to which activated crossbridges progress from the conventional phosphorylated form to a slowly cycling dephosphorylated form termed the latchbridge (11). The time scale over which our post-DI force measurements climbed back toward baseline is similar to that over which force was reported to be maintained in the original study that gave rise to the latch hypothesis (11). Thus, if a DI causes latchbridges to detach, perhaps the post-DI force transient reflects a slow transition from detached latchbridges back to faster cycling crossbridges. However, this would mean that detached latchbridges take a significant amount of time to regain the capacity to act as conventional crossbridges, and we are not aware of evidence to support this.

Another possible explanation for the post-DI force decrement is physical disruption of contractile filaments. There is evidence that the actin and myosin filaments within a smooth muscle cell are quite labile, and that activation of the cell can induce rapid polymerization of filament subunits (26). Indeed, this has been one of the mechanisms postulated to be behind the length plasticity of ASM (20, 21), and might even have played a role in our observation that the muscle strips had to be stimulated for some time before the DI response became stable. Perhaps a vigorous DI causes interacting contractile filaments to break up in a way that is only amenable to reassembly over an extended period of time. In support of this explanation are some recent observations by Kuo et al. (25, 26), who showed that, after a maneuver similar to our bronchoprotection test (Fig. 7), force followed a trajectory similar to the recovery of thick filament density. Furthermore, following passive length oscillations, Kuo et al. (26) found that force and thick filament density recovered with a time course not too dissimilar to that...
of the force recovery we found following large DIs (Fig. 6). It is thus possible that the post-DI force reductions we observed in the present study have a similar mechanistic basis to the force-length plasticity that has been well documented by other investigators (19, 23, 40).

A further notion about the mechanical effects of a DI is afforded by the observation that stretching a passive strip of ASM gives rise to transient force behavior (Fig. 7B) that is intriguingly reminiscent of that seen in active strips, apart from a large difference in scale. The post-DI force decrement in the passive strip in Fig. 7B is a manifestation of its viscoelastic properties, and not surprisingly recovers with a time course quite close to a power law in time. The active force-recovery curves in Fig. 6 exhibit a similar functional form, suggesting that viscoelasticity may be somehow involved here as well. Exactly how it is involved, however, is not clear. Certainly, it cannot simply be a question of a force generator in series with a viscoelastic element, as our current model embodies (Fig. 2), because any temporary slackness that is produced by stretch of the viscoelastic element will be quickly gathered up by the contractile element. This will return force to its isometric level over a time scale reflective of crossbridge cycling dynamics. Also, a viscoelastic effect would be expected to exert its influence if a DI is given either during bronchoconstriction or before ASM activation. However, our test of bronchoprotection showed only a very minor effect on force generation (Fig. 7A), and it has been suggested that the mechanisms behind bronchodilation and bronchoprotection may be different (39).

Somehow, then, the contractile elements in ASM produce less net force than normal for some period of time after a large DI. Broadly speaking, there are two ways this can happen. One way is that the contractile element generates a force that varies with its length. In fact, although ASM has a much flatter force-length relationship than skeletal muscle, it nevertheless does have a maximum force that falls off significantly if one ventures sufficiently far from optimum length, especially if the muscle does not have time to adapt to the new length (37). Thus, if the viscoelastic element representing series connective tissue was to become sufficiently stretched by a DI, it might allow the contractile filaments to shorten past the plateaus of their force-length relationships. Force would then recover as the viscoelastic element creeps back to its original unstretched configuration and realigns the contractile fibers. Our model assumes that isometric force is independent of length, and so does not account for any mechanisms that might reduce force if length becomes too much greater or smaller than \( L_{ref} \).

The second way that net contractile force could be reduced is if something works to oppose it. This again could be related to viscoelasticity if a large stretch results in compression of internal elastic elements when the strip length is returned to normal. This would generate a passive compressive force opposing the active force from the crossbridges, which might explain the similarities in force recovery dynamics seen experimentally in passive (Fig. 7) and active (Fig. 6A) tissues following a large DI. Also, Bosse et al. (8) recently reported that if bovine trachealis muscle is continually stimulated submaximally, the tension generated appears to be gradually transferred to passive elements within the smooth muscle cell. Presumably, viscoelastic creep within the cell cytoskeleton might be how this transference of tension takes place.

We thus have a variety of possible explanations, most of them problematic in some way, for the sustained force decrement that occurs in isolated ASM following a DI. At this point, we are not in a position to make an informed choice as to which is best. Future studies aimed at elucidating the situation are likely to be important, however, because of ongoing interest in the effects of a deep inflation of the lungs on bronchomotor tone. Changes in ASM length are, of course, part of the normal condition; ASM is continually cyclically stretched to a modest degree by the act of breathing, with much larger stretches being imposed periodically by sighs. Because the degree of airway narrowing elicited by bronchial challenge is modulated to an enormous extent by variations in lung volume (1, 4, 12), breathing and sighing exert major influences on the ability of the ASM to narrow the airways. Furthermore, there is evidence that the effects of a deep inflation of the lungs are different in asthmatic individuals compared with normal controls (1, 9, 32). It is therefore important to understand whether any of these effects are due to a change in the behavior of ASM itself. The results of the present study suggest that this may indeed be the case. Furthermore, in a recent study we (4) found that applying a large rapid DI to mice during the development of bronchoconstriction leads to a subsequent decrement in the ability of the airways to narrow. This decrement was transient and most likely linked to a direct effect of stretch on the ASM, and therefore may be an in vivo correlate of the in vitro post-DI phenomenon we observed in the present study (Fig. 6).

In summary, we have undertaken a systematic study of ASM force-length behavior with a view to establishing those mechanisms that unequivocally have to be included in a model to achieve realistic predictions of experimental data. We have striven to be as minimalist as possible, as we felt that this gave us the best chance of reaching clear conclusions. We have confirmed that tissue viscoelasticity, crossbridge attachment rate, and an effect of strain on crossbridge detachment kinetics all play important roles. We have also shown that these mechanisms alone cannot fully account for the transient effect of large stretches on the subsequent ability of ASM strips to generate active force. A number of explanations can be proposed to explain this phenomenon, each of which has its own attractions and problems.

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