Effect of the cytoskeletal prestress on the mechanical impedance of cultured airway smooth muscle cells

DIMITRIJE STAMENOVIĆ,1 ZHUANGLI LIANG,1 JIANXIN CHEN,2 AND NING WANG2

1Department of Biomedical Engineering, Boston University, Boston 02215; and 2Physiology Program, Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts 02115

Received 25 July 2001; accepted in final form 3 December 2001

Stamenović, Dimitrij, Zhuangli Liang, Jianxin Chen, and Ning Wang. Effect of the cytoskeletal prestress on the mechanical impedance of cultured airway smooth muscle cells. J Appl Physiol 92: 1443–1450, 2002. First published December 14, 2001; 10.1152/japplphysiol.00782.2001.—We investigated the effect of the cytoskeletal prestress (P) on the elastic and frictional properties of cultured human airway smooth muscle cells during oscillatory loading; P is preexisting tensile stress in the actin cytoskeleton generated by the cell contractile apparatus. We oscillated (0.1 Hz, 6 Pa peak to peak) small ferromagnetic beads bound to integrin receptors and computed the storage (elastic) modulus (G') and the loss (frictional) modulus (G'') from the applied torque and the corresponding bead rotation. All measurements were done at baseline and after cells were treated with graded doses of either histamine (0.1, 1, 10 μM) or isoproterenol (0.01, 0.1, 1, 10 μM). Values for P for these concentrations were taken from a previous study (Wang et al., Am J Physiol Cell Physiol, in press). It was found that G' and G'', as well as P, increased/decreased with increasing doses of histamine/isoproterenol. Both G' and G'' exhibited linear dependences on P: G'(Pa) = 0.20P + 82 and G''(Pa) = 0.05P + 32. The dependence of G' on P is consistent with our previous findings and with the behavior of stress-supported structures. The dependence of G'' on P is a novel finding. It could be attributed to a variety of mechanisms. Some of those mechanisms are discussed in detail. We concluded that, in addition to the central mechanisms by which stress-supported structures develop mechanical stresses, other mechanisms might need to be invoked to fully explain the observed dependence of the cell mechanical properties on the state of cell contractility.

storage modulus; loss modulus; oscillatory cytometry; actin network; cytoskeletal mechanics

It is well established that mechanical behavior of adherent cells can be characterized as viscoelastic. Mechanical measurements on various types of adherent cells, using various techniques, have shown that adherent cells exhibit creep in response to applied mechanical stress (2, 7, 19, 28, 29), stress relaxation in response to applied mechanical strain (27), and hysteresis in response to cyclic loading (4, 12, 18) and that elastic and frictional stresses within the cell in response to applied cyclic loading are dependent on the loading frequency (4, 12, 20). All these manifestations are believed to be related to intrinsic viscoelastic properties of various molecules that comprise the cytoskeleton (CSK), liquid cytoplasm, cell membrane, and nucleus. On the other hand, adherent cells also exhibit features that characterize stress-supported structures (32). The prime characteristic of those structures is that their mechanical properties are strongly influenced by the preexisting tensile stress (termed “prestress”) carried by their structural components. In the past, investigations have been focused on the effect of the prestress on the elastic response of the cell. Little is known how the prestress may affect frictional stress within the cell. There is a good reason to believe that the CSK prestress may affect frictional properties of the cell. It has been shown recently that, during oscillatory loading, at low frequencies (0.05–0.4 Hz) frictional losses reside within the CSK and not within the liquid cytoplasm (12). The purpose of this study was to elucidate the latter problem.

We have recently shown that the elastic stiffness of cultured airway smooth muscle cells increases linearly with increasing prestress (32). The prestress, defined as a preexisting contractile stress carried by the actin network of the CSK before application of any external load, was modulated pharmacologically by graded doses of either a relaxant agonist (isoproterenol) or a contractile agonist (histamine). We attributed this dependence of the stiffness on the prestress to principal mechanisms of stress-supported structures that are described below.

The mechanisms by which stress-supported structures develop mechanical stresses to resist distortion of their shape are changes in spacing, reorientation, and elongation of their structural components (6, 21). The greater the prestress carried by the structural elements, the smaller the distortion that the structure must undergo before attaining an equilibrium configuration. In other words, as the prestress increases, the less deformable (i.e., the more rigid) the structure is. Among these three mechanisms, changes in spacing...
and in reorientation are associated only with redistribution of the prestress within the network. Because the prestress is static stress, it follows that these two mechanisms contribute only to the static, i.e., elastic stress within the network. In contrast, the change in length of structural elements can directly contribute to both elastic and frictional stresses of the network, depending on the rheological properties of structural elements and the type of applied load to the network. If the structural elements were viscoelastic, then dynamic shortening and lengthening of those elements would produce both elastic and frictional stresses.

In adherent cells, the prestress is carried by the actin network and is partly balanced by microtubules that are supported by intermediate filaments (25). These three filamentous networks of the CSK are known to exhibit viscoelastic behavior in vitro (8). Thus one should expect that viscoelastic properties of the CSK network would be affected by changes in the CSK prestress. In this study we investigated this idea.

We used the oscillatory magnetic twisting technique (12) to measure the dynamic properties (mechanical impedance) of cultured airway smooth muscle cells whose contractility was modulated by graded doses of either isoproterenol or histamine. We found that both elastic and frictional components of the impedance increased with increasing cell contraction and decreased with increasing cell relaxation in a dose-dependent fashion. The central mechanisms of the stress-supported structures could fully account only for the observed dependence of the cell elastic properties on cell contractility. However, to explain dependences of both elastic and frictional properties on cell contractility, additional mechanisms may need to be invoked. Some of those mechanisms were discussed in detail.

MATERIALS AND METHODS

The working hypothesis of this study is that the CSK of adherent cells is a stress-supported structure in which the prestress is carried primarily by the actin network. This does not rule out possible contributions of microtubules and intermediate filaments to the cell mechanical response because these two structures contribute to balancing the prestress and stabilizing the CSK (25). To get insight into how the CSK prestress affects elastic and frictional properties of the cell, we measured the oscillatory response of cultured airway smooth muscle cells treated with graded doses of contractile or relaxant agonists. Data from the oscillatory measurements were correlated with data for prestress measured previously in airway smooth muscle cells (32).

We used the oscillatory magnetic cytometry technique to measure the dynamic modulus (mechanical impedance) in cultured airway smooth muscle cells at different states of cell contraction. This technique can separate the contributions of elastic and frictional stresses to the impedance (12).

Cell culture. Human airway smooth muscle (HASM) cells (passage 3–6) were used for all experiments. These cells maintain smooth muscle cell morphology and physiological responsiveness to agonists until at least passage 8 (16). The reason we used HASM cells was that their contractility could be modulated pharmacologically in a dose-dependent fashion (7, 32). After cells reached confluence in plastic dishes, they were serum deprived for 48 h before being trypsinized. The cells were then plated in a serum-free medium on collagen-1-coated (0.2 mg/ml) dishes (96-well plate, Immunon II, Dymetec) (20,000 cells per well, subconfluent).

Oscillatory magnetic twisting cytometry. This technique applies a twisting, sinusoidally varying magnetic field to ferromagnetic beads attached to integrin receptors on the cell apical surface (12). Integrins are linked to the actin network of the CSK through a series of linking proteins. A vertical magnetic field (0.1 Hz, 6 Pa peak to peak) was applied after the beads were magnetized at 45° from the horizontal direction and resulting bead rotation (ranging from 0.052 to 0.157 rad for 10^{-5} M histamine and 10^{-5} M isoproterenol, respectively; baseline strain was ~0.087 rad) was determined by measuring the oscillating remnant magnetic field produced by the beads. The dynamic modulus (GԴ) was defined in the frequency domain as a complex ratio of the twisting specific torque and the corresponding angle of rotation. The in-phase component of GԴ was the storage (elastic) modulus (G’s), and the out-of-phase was the loss (frictional) modulus (G”). This method greatly reduces the effects of heterogeneous bead rotations and abolishes contribution of plastic deformation. Arg-Gly-Asp (RGD)-coated ferromagnetic microbeads (4.5-μm diameter; average 2 beads per cell) were added to the cells plated on collagen-1-coated wells for 20 min. Unbound beads were washed away with serum-free medium, and then the magnetic twisting was performed 6–10 h after plating.

Protocol. All measurements were done at 0.1 Hz and with the applied specific torque of 6 Pa unless indicated otherwise. Measurements were done at baseline and after cells were treated with either graded doses of constricting agonist, histamine (0.1, 1, 10 μM), or graded doses of relaxant, isoproterenol (0.01, 0.1, 1, 10 μM), 1 min after administration of each dose. To investigate the relative contributions of actin filaments, microtubules, and intermediate filaments to the elastic and frictional properties of the CSK, we selectively disrupted those networks by adding cytochalasin D (1 μg/ml), colchicine (1 μM), and acrylamide (4 mM), respectively. Cytochalasin D was added at baseline for 30 min. Colchicine was added after a saturated dose of histamine (10 μM). We have shown previously that in maximally activated HASM cells (10 μM histamine) addition of colchicine does not cause further cell contraction (31). Measurements were done after 1 min of histamine and after 15 min of colchicine. Acrylamide was added at baseline for 45 min. On disruption of each of those networks, the impedance measurements were repeated as described above. To investigate the cumulative contribution of the actin, microtubule, and intermediate filament networks to the elastic and frictional CSK properties, we measured the impedance in HASM cells in which all three filamentous networks were disrupted by a combination of cytochalasin D (1 μg/ml), colchicine (10 μM), and acrylamide (10 μM) for 30 min. To test for linearity of the oscillatory response, measurements were done at baseline for a series of specific torque amplitudes of 1, 2, 4, 6, and 8 Pa. In this study, we used data for the prestress from our laboratory’s recent report (32). A brief description of those measurements and definitions are as follows. The prestress was measured in cultured HASM cells by using the traction microscopy technique (3, 17). This technique is used to measure traction at the cell-substrate interface. The substrate is an elastic polycrylamide gel block that is used as a strain gauge to detect contraction of cells that adhere to the gel. The prestress is defined as the net contractile force transmitted by the actin network across a cross-sectional area of the cell. It was determined from measured traction by considering the mechanical balance of traction forces and prestress forces on a section of the cell. Measurements were done at baseline and
RESULTS

In general, both $G'$ and $G''$ increased on average with increasing doses of histamine (Fig. 1) and decreased with increasing doses of isoproterenol (Fig. 2). Changes in $G'$ were greater than changes in $G''$: from the baseline to 10 $\mu$M histamine $G'$ increased by ~65% whereas $G''$ increased by ~33% (Fig. 1); both $G'$ and $G''$ decreased between the baseline and 10 $\mu$M isoproterenol by ~50%, although at the highest dose they exhibited a slight increase (Fig. 2). Taken together, these results showed that stimulating or relaxing the cell contractile apparatus had a greater effect on the elastic stiffness than on the frictional losses of the cell.

We also calculated the coefficient of hysteretic damping (hysteresivity; $\eta = G'/G''$) and found that it changed little with drug doses, $\eta = 0.3$ (Fig. 3); $\eta$ tended to decrease with increasing doses of histamine (Fig. 3A) and slightly increased with increasing doses of isoproterenol (Fig. 3B).

To find out how $G'$, $G''$, and $\eta$ changed with the prestress $P$, we used data for $P$ as function of the graded dozes of histamine and isoproterenol that we

after cells were stimulated or relaxed by exactly the same doses of histamine and isoproterenol as in the impedance measurements described above. For details, see Ref. 32.

Statistically significant differences between groups of data were assessed by the t-tests. The statistical significance of the dependence of measured $G'$ and $G''$ on the prestress and on the amplitude of forcing was assessed using a two-way ANOVA. In both tests, the differences with $p < 0.05$ were considered as significant.

Fig. 1. Storage modulus ($G'$) and loss modulus ($G''$) increased with increasing doses of histamine. For $G'$, $p < 0.05$ between 0.1, 1.0, and 10 $\mu$M histamine and baseline; $p < 0.05$ between all successive treatments except between 1.0 and 10 $\mu$M histamine. For $G''$, $p < 0.05$ between 1.0 and 10 $\mu$M and baseline; $p = 0.319$ between 0.1 $\mu$M histamine and baseline; $p > 0.05$ between all successive treatments. Data are means \pm SE; $n = 9$ wells. *Significantly different from previous dose; #significantly different from the baseline ($p < 0.05$).

Fig. 2. $G'$ and $G''$ decreased with increasing doses of isoproterenol. For $G'$, $p < 0.05$ between 0.01, 0.1, 1.0, and 10 $\mu$M isoproterenol and baseline; $p < 0.05$ between all successive treatments except between 1.0 and 10 $\mu$M isoproterenol. For $G''$, $p < 0.05$ between 0.01, 0.1, 1.0, and 10 $\mu$M isoproterenol and baseline; $p < 0.05$ between all successive treatments. Data are means \pm SE; $n = 8$ wells. *Significantly different from previous dose; #significantly different from the baseline ($p < 0.05$).

Fig. 3. Hysteresivity ($\eta$), calculated as the ratio $\eta = G'/G''$, did not change significantly in response to histamine (A) and isoproterenol (B). In A, $p < 0.05$ between 0.1, 1.0, and 10 $\mu$M histamine and baseline; $p < 0.01$ between baseline and 0.1 $\mu$M, $p = 0.238$ between 0.1 and 1.0 $\mu$M and $p = 0.863$ between 1.0 and 10 $\mu$M histamine. In B, $p < 0.05$ only between 10 $\mu$M and baseline; $p = 0.902$ between baseline and 0.01 $\mu$M, $p < 0.001$ between 0.01 and 0.1 $\mu$M, $p = 0.769$ between 0.1 and 1.0 $\mu$M, and $p < 0.05$ between 1.0 and 10 $\mu$M isoproterenol. Data are means \pm SE; $n = 9$ wells for histamine and $n = 8$ wells for isoproterenol. *Significantly different from previous dose; #significantly different from the baseline ($p < 0.05$).
obtained in our previous study (32). According to those data, \( P \) increases with increasing doses of histamine and decreases with increasing doses of isoproterenol (Fig. 4). We found that both \( G' \) and \( G'' \) increased with increasing \( P \) but that \( G' \) exhibited a much greater dependence than \( G'' \); \( G' (Pa) = 0.20P + 82 (r^2 = 0.97, p = 5.5 \times 10^{-6}, \text{ANOVA}) \) and \( G'' (Pa) = 0.05P + 32 (r^2 = 0.94, p = 3.95 \times 10^{-5}, \text{ANOVA}) \) (Fig. 5A). On the other hand, \( \eta \) exhibited a slight but a significant hyperbolic decrease with increasing \( P; \eta = 2464/(P + 7502) \), with \( P \) in Pa (\( r^2 = 0.62; \) for coefficients of the hyperbola \( p < 0.04) \) (Fig. 5B).

Changes of the specific torque amplitude from 1 to 8 Pa caused both \( G' \) and \( G'' \) to increase systematically (Fig. 6) (\( p < 0.012, \text{ANOVA} \)). However, the increase of \( G' \) and \( G'' \) at each amplitude was not significant (\( p > 0.09 \)). Disruption of microtubules and intermediate filaments caused minor (\( \leq 15\% \)) but not statistically significant changes in both \( G' \) and \( G'' \) (\( p > 0.14 \)) (Fig. 7). Disruption of the actin network caused a \( \sim 30\% \) decrease of both \( G' \) and \( G'' \) (\( p < 0.05 \)) (Fig. 7). Disruption of all three major CSK networks (actin filaments, microtubules, and intermediate filaments) caused a substantial decrease in both \( G' \) and \( G'' \) by \( \sim 63 \) and \( \sim 50\% \), respectively (\( p < 0.05 \)) (Fig. 7).

**DISCUSSION**

The most significant result of this study is that both elastic and frictional components of the HASM cell impedance depend on of the status of cell contractility, i.e., both \( G' \) and \( G'' \) increase linearly with increasing contractile prestress \( P \). The linear dependence of \( G' \) on \( P \) has been observed previously (32) and could be explained by the mechanisms that are embodied in the stress-supported structure. The dependence of \( G'' \) on \( P \) is a novel finding. There are a number of mechanisms that may explain this behavior, including the mechanisms of stress-supported structures, CSK remodeling nonlinear rheological properties of the CSK filament networks, friction that arises at the interface of sliding CSK filaments or at filament junctions, myosin crossbridge kinetics, and myosin light chain phosphorylation. Before addressing those mechanisms in more detail, we first critically evaluate major assumptions and potential experimental artifacts.

A key assumption is that the dynamic behavior of HASM cells is primarily determined by the actin network of the CSK. Several findings are consistent with this assumption. First, disruption of microtubules and intermediate filaments caused minor and nonsignificant changes in \( G' \) and \( G'' \) (Fig. 7). This, in turn, suggests that microtubules and intermediate filaments of the CSK are not important determinants of \( G' \) and

---

**Fig. 4.** Prestress increased gradually with decreasing doses of isoproterenol and with increased doses of histamine [replotted from the data of Wang et al. (32)]. B, baseline. \( p < 0.04 \) between all successive treatments except between 0.1 and 1.0 \( \mu \text{M} \). Data are means \( \pm \text{SE} \); baseline \( n = 17 \) cells, histamine-treated (His) cells \( n = 13 \) and isoproterenol-treated (Iso) cells \( n = 4 \). *Significantly different from previous dose (\( p < 0.05 \)); \( \rho \) values were calculated starting from baseline.
G’’, at least not at the low frequency (0.1 Hz) used in this study. This is consistent with our previous finding that microtubules, supported by intermediate filaments, balance a relatively small fraction (~14%) of P (25). Disruption of actin filaments, however, caused a substantial decrease in both G’ and G’’ relative to the basal values. This, in turn, suggests that the actin network is a key determinant of both G’ and G’’ (Fig. 7). Furthermore, disruption of all three filamentous networks of the CSK caused an even greater decrease of both G’ and G’’ (Fig. 7). This is consistent with previous measurements of stiffness in endothelial cells (30). The fact that the changes caused by selective disruption of each of those networks did not add up to the changes caused by disruption of all three networks suggests that there is a mechanical synergy between them. On the basis of the above, we concluded that the assumption that the actin network of the CSK plays the principal role in determining the cell dynamic response was reasonable.

In calculating values of G’ and G’’ from the twisting measurements, we assumed that on average ~30% of the volume of twisting beads was internalized in the cell. If, however, we assumed that only 10% of the bead volume was internalized, the values of G’ and G’’ would be higher by nearly a factor of three relative to the values obtained with the assumed internalization of 30%. We have no data to show the degree of bead internalization for individual beads in individual HASM cells. Thus the values of G’ and G’’ should be taken with caution. However, after the beads are added for 0.5–2 h (the duration of the experiments for each well) in these HASM cells, it is estimated that the average internalization is ~30–40% (12). Regardless of the degree of bead internalization, the qualitative dependences of G’ and G’’ on P (Fig. 5A) would not change, only the slopes may increase or decrease depending on the degree of internalization.

The contribution of cytoplasmic viscosity to G’’ was not taken into account. However, it may not be significant at 0.1 Hz. Fabry et al. (4) showed that in HASM cells the viscous contribution of the cytoplasm to G’’ becomes evident only above 10 Hz. Maksym et al. (12) showed that in HASM cells oscillated from 0.02 to 0.4 Hz, frictional and elastic stresses are primarily developed within the CSK, not the cytoplasm. The cytoplasmic viscosity alone cannot explain the observed dependence of G’’ on P. The reason is that, in general, viscosity does not depend on the pressure in the liquid. Thus we believe that the frictional stress, as well as the elastic stress, arises from within the CSK.

Potential mechanisms. To analyze the contribution of the mechanisms of the stress-supported structures, we consider the following microstructural model of the CSK. The CSK was depicted as a network of initially tensed, pin-joined, and randomly oriented cables. The cables played the role of CSK filaments. The initial tension in the cables corresponded to the force generated by the cell contractile apparatus. The cables were assumed to be viscoelastic and joints frictionless.

As mentioned above, tensed filaments of the network develop mechanical stress through their change in spacing, reorientation, and lengthening. To obtain a quantitative description of these mechanisms, we utilized an approach that we used previously in studies of cell and pulmonary mechanics (22, 23) (APPENDIX). The model predicted (see APPENDIX, Eq. A5) the following relationship between the storage modulus G’ and the loss modulus G’’ of the network and the prestress (P),

\[
g'
\]

\[
g''
\]

Fig. 6. G’ and G’’ as a function of amplitude of applied specific torque. Data are means ±SE (n = 3 wells for the amplitude of 1 Pa, n = 4 wells for the amplitude of 2 Pa, n = 5 wells for all other amplitudes). Dependences of G’ and G’’ on the amplitude are significant (p < 0.05, ANOVA).

Fig. 7. Disruption of microtubules by colchicine (Col) (n = 5 wells) and intermediate filaments by acrylamide (Acrl) (n = 4 wells) caused nonsignificant changes of G’ and G’’. Disruption of actin filaments by cytochalasin D (CytoD) (n = 4 wells) as well as disruption of actin filaments, microtubules, and intermediate filaments by a CytoD + Col + Acrl (n = 5 wells) caused substantial decreases in both G’ and G’’. Data are mean fractional changes ±SE. Before disruption of microtubules, cells were first maximally activated by histamine (His), and then Col was added. Changes were calculated relative to histamine (n = 5 wells). Changes in cells treated with Acrl, with CytoD, and with CytoD + Col + Acrl were calculated relative to the baseline (n = 5 wells). *Significantly different from zero (p < 0.05).

J Appl Physiol • VOL 92 • APRIL 2002 • www.jap.org
the storage \((E')\) and loss \((E'')\) moduli of individual network filaments and their volumetric fraction \((\phi)\)

\[
G'(f) = 0.80P + 0.07\phi E'(f) \tag{1a}
\]

\[
G''(f) = 0.07\phi E''(f) \tag{1b}
\]

where \(f\) indicates frequency dependence. The first term on the right-hand side of Eq. 1a represents the contribution of both change in spacing and reorientation of the CSK filaments. The second term on the right-hand side of Eq. 1a as well as the term on the right-hand side of Eq. 1b depend on elastic and frictional properties of the filaments, respectively, and represent the contribution of changing of the filament length. According to Eq. 1a, at a given frequency, \(G'\) increases linearly with \(P\), which is consistent with our experimental observations (Fig. 5A). Moreover, \(G'\) also depends on the volumetric fraction of the filaments \(\phi\) and on their elastic properties \(E'\). According to Eq. 1b, \(G''\) does not explicitly depend on \(P\). Therefore, in order for \(G''\) to increase with \(P\) as observed (Fig. 5A), either \(\phi\) has to increase with \(P\), which would suggest agonist-induced CSK remodeling, and/or \(E'\) has to increase with \(P\), which would suggest a nonlinear viscoelastic behavior of the CSK filaments. In this study, we did not consider the effect of forcing frequency on \(G'\) and \(G''\).

According to Eqs. 1a and 1b, the hysteresivity \(\eta = G''/G'\) is

\[
\eta = \frac{0.07\phi E''}{0.80P + 0.07\phi E'} \tag{2}
\]

e.i., it decreases with increasing \(P\), which is consistent with the data in Fig. 5B. Note that in our laboratory’s previous communication (31), we predicted that \(\eta = E'/E'\), i.e., it is independent of \(P\), which differs from the prediction of Eq. 2. The reasons for this discrepancy are twofold. First, we previously assumed ad hoc that the dynamic modulus \(G''\) is directly proportional to \(P\). This implies that only changes in spacing and reorientation and not stretching of the filaments contribute to the CSK resistance to dynamic loading. Our second assumption was that \(P\) itself is dynamic, i.e., it varied with frequency. However, data in the present study suggest that all three mechanisms are likely to contribute to the mechanical properties of the CSK. Moreover, the experimental procedure in which the cell was first stimulated or relaxed and then subjected to oscillatory loading is better described by the model in which the structure is first statically prestressed and then oscillated around the state of static prestress. Thus we believe that the present model provides a more realistic picture of the cell behavior during oscillatory measurements than the one in our previous study.

It is known that the actin CSK undergoes agonist-induced remodeling (13, 26). However, it has been shown that by blocking actomyosin force generation HASM cell stiffness was ablated in response to contractile stimulation, whereas the actin polymerization was not altered (1). This, in turn, suggests that the actomyosin motor and not actin polymerization is the dominant mechanism that determines cell mechanical properties.

The amplitude dependences of \(G'\) and \(G''\) of HASM cells (Fig. 6) are indicative of their nonlinear behavior. This behavior may result from intrinsic rheological nonlinearities of CSK filamentous biopolymers. If so, then these nonlinearities may also account for the observed dependence of \(G'\) and \(G''\) on \(P\). The only major stress-bearing CSK filaments that are known to exhibit nonlinear stress-strain behavior are intermediate filaments. Rheological measurements in vitro on vimentin gels (8) and on keratin gels (11) show stiffening in response to applied load. This is consistent with the observed positive amplitude dependence of \(G'\) and \(G''\) of HASM cells (Fig. 6). On the other hand, our data show that disruption of the intermediate filament network has minor effects on both \(G'\) and \(G''\) of the cell (Fig. 7). These data also indicate that the actin network plays the dominant role in determining cell’s \(G'\) and \(G''\) (Fig. 7). However, data from the literature show that isolated actin filaments exhibit constant stiffness over a wide range of applied tensile load, indicating a linear behavior (10). On the basis of the above, we concluded that rheological nonlinearities of CSK filaments are not likely to contribute significantly to the observed dependences \(G'\) and \(G''\) on \(P\) (Fig. 5A). However, we cannot rule out the possibility that other proteins, such as titin, which provides passive elasticity to muscle cells, or cross-linking proteins such as spectrin, may contribute significantly to the HASM cell mechanical behavior.

Direct mechanical interaction between CSK filaments may cause frictional loss. Friction may arise from the filament contact and at the filament junctions. Mijailovich et al. (14) showed that an increase in contact stress between two sliding fibers in apposition would lead to an increase in both storage and loss moduli of the fiber network. It is likely that an increase in cell contractile stress may cause an increase in the contact stress between various CSK filaments, which, in turn, may explain the observed dependences of \(G'\) and \(G''\) on \(P\) (Fig. 5A). Alternatively, an increase in cell prestress may cause a shift in the frequency response of \(G''\). This, in turn, might lead to the dependence of \(G''\) on \(P\).

The observed increases in the prestress, elastic, and frictional properties with increased HASM cell contractility may also be explained by myosin cross-bridge kinetics. Fredberg et al. (5) showed that in uniaxially oscillated tracheal smooth muscle strips contractile force, stiffness, and frictional properties of the muscle increase with increasing number of attached cross bridges. As the number of attached cross bridges increases in response to increasing doses of contractile agonists, the observed dependences in Fig. 5A could be nothing more than the reflection of the myosin cross-bridge kinetics. However, the frequency response of uniaxially stretched muscle shows that the stiffness increases with increasing frequency and reaches a plateau as the frequency reaches and exceeds bridge cycling rates (9). In contrast, the frequency response of
the smooth muscle cells is characterized by a weak power low of stiffness on frequency over a very wide range of frequencies (4). Thus we do not believe that the myosin cross-bridge kinetics is entirely responsible for the observed dependence of \( G' \) and \( G'' \) on \( P \) (Fig. 5A).

On the basis of theoretical analysis of myosin cross-bridge perturbed equilibrium, Mijailovich et al. (15) predicted that, at a given frequency and given forcing amplitude, mean tensile force, stiffness, and frictional losses of the airway smooth muscle increase with increasing myosin light-chain phosphorylation. To the extent that maximal stimulation with histamine (10 \( \mu \)M) has been shown to cause an increase phosphorylation in HASM cells (31), the observed increases in \( G' \) and \( G'' \) with increasing \( P \) (Fig. 5A) could be nothing more than the result of increased phosphorylation. However, Mijailovich’s model predicts that muscle \( \eta \) increases with phosphorylation (15), whereas we observed that in HASM cells \( \eta \) decreased with increasing doses of histamine (Fig. 3A).

It is noteworthy that the slopes of \( G' \) and \( G'' \) vs. \( P \) relationships (Fig. 5A) are much less than those of the \( G' \) and \( G'' \) vs. applied stress relationships (Fig. 6). It is important to distinguish the prestress from the applied local stress. The former is the internal global cell contractile stress, and the latter is the externally applied local stress. The \( G' \) and \( G'' \) vs. \( P \) relationships reflect the dependence of elastic and frictional properties on the mechanical status of the cell, i.e., the shape stability of the cell. In contrast, the \( G' \) and \( G'' \) vs. applied stress relationships reflect the dependence of elastic and frictional properties on external mechanical perturbation, an entirely different phenomenon (24).

In summary, results of this study showed that the mechanical response of HASM cells at 0.1 Hz is dominated by the elastic stresses but that viscous stresses are substantial. Both elastic and frictional stresses are influenced by cell contractility, but it appears that the mechanisms that determine these stresses are different. The elastic properties of the cell appear to be determined primarily by the central mechanisms by which the stress-supported CSK network resists shape distortion. However, the frictional stress within the CSK and its dependence on the cell contractility might not be fully explained by these mechanisms. Additional mechanisms might need to be invoked to fully explain the dependence of the dynamic response of HASM cells on pharmacological modulations of cell contractility.

A better understanding of the HASM cellular response to mechanically applied stimuli is essential for the treatment of conditions such as acute respiratory distress syndrome or ventilator-induced lung injury. Results of this study advance this understanding by explaining the effect of priming the cell, prestressing the cell, and mechanically stimulating the cell.

**APPENDIX**

The network is transected by an arbitrary plane. The total stress \( T \) is defined as the sum of all tensile force transmitted by the filaments across a trans-sectional area \( A \) per unit area (23). A filament carries tensile force \( F \) and lies at angle \( \theta \) from the normal to \( A \). The number of elements intersecting the surface is denoted \( n \). Thus

\[
T = \frac{n(F\cos\theta)}{A} \quad (A1)
\]

where angle brackets denote the average over all orientations. It is assumed that initially, before any external loading is applied, all filament orientations are equally probable. Thus \( \langle \cos\theta \rangle = 3/(8\pi) \) and \( nA = \pi NLV/8V \) where \( L \) is the filament length, \( N \) is the total number of the filaments in the network, and \( V \) is the volume of the network (i.e., cell volume). In this case, the \( T \) equals the prestress \( P \). Thus Eq. A1 becomes

\[
P = \frac{NFL}{3V} \quad (A2)
\]

The change in stress \( \delta T \) due to small distortion is obtained by taking small variations of Eq. A1

\[
\delta T = -\frac{n(F\cos\theta)}{A} - \frac{n(F\sin\theta)}{A} + \frac{n((dF/dL)\cos\theta L)}{A} \quad (A3)
\]

The three terms in Eq. A3 represent the three mechanisms (change in spacing, reorientation, and lengthening of structural elements) by which the network develops stress to resist distortion. The quantity \( dF/dL \) in the last term of Eq. A3 represents the stiffness of the actin filament. To quantitatively evaluate each term in Eq. A3, we assumed that the filaments follow the strain field caused by shear distortion. Suppose that the small distortion is a pure shear deformation (i.e., no volume change) and that \( \delta T \) is the principal stress and \( \delta \epsilon \) is the corresponding principal strain, both perpendicular to \( A \). Then it follows that \( \delta A/A = -\delta \epsilon \), \( \langle F\sin\theta \rangle = -2F\delta \epsilon/\cos\theta \), and \( \langle dF/dL\rangle \cos\theta L/\delta \epsilon = 4(dF/dL)\delta \epsilon/15 \) (22). By substituting these values into Eq. A3 and taking into account Eq. A2, we obtained that

\[
\delta T = 2\frac{4P}{5} + \frac{2N(dF/dL)L^2}{15V} \delta \epsilon \quad (A4)
\]

The term in the parenthesis in Eq. A4 represents the shear modulus. If, however, small variations of stress \( \delta T \) and strain \( \delta \epsilon \) are harmonic (sinusoidal), then the term in parenthesis represents dynamic shear modulus (mechanical impedance \( G' \)) and \( dF/dL \) is the dynamic stiffness of the actin filament. Inertial effects are considered negligible. For convenience, we define the volumetric fraction \( \phi \) of the actin filaments in the network as \( \phi = NLS/V \) and the dynamic modulus of the individual actin fiber as \( E^* = (dF/dL)L/S \) where \( S \) is the cross-sectional area of the filament. Then it follows from Eq. A4 that the dynamic modulus of the network is

\[
G^*(f) = \frac{4}{5}P + \frac{1}{15} \phi E^*(f) \quad (A5)
\]

where \( f \) indicates frequency dependence. \( E^* \) is decomposed into the elastic \( (E') \) and frictional \( (E') \) components: \( E^* = E' + iE'' \), where \( i \) is the imaginary unit indicating the out-of-phase behavior, and the corresponding elastic \( (G') \) and frictional \( (G') \) moduli of the network were obtained (Eq. 1).

We thank Dr. Srboljub Mijailovich for fruitful discussions.

This work was supported by National Heart, Lung, and Blood Institute Grants HL-65371 and HL-33009.
REFERENCES