Mechanical strain increases cell stiffness through cytoskeletal filament reorganization

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Mechanical strain increases cell stiffness through cytoskeletal filament reorganization. Am J Physiol Lung Cell Mol Physiol 285: L456–L463, 2003. First published April 18, 2003; 10.1152/ajplung.00329.2002.—We tested the hypothesis that cytoskeletal reorganization induced by cyclic strain increases cytoskeletal stiffness (G'). G' was measured by optical magnetic twisting cytometry in control cells and cells that had received mechanical strain for 10–12 days. G' was measured before and after both contractile and relaxant agonists, and in the strained cells both parallel (Para) and perpendicular (Perp) to the aligned cytoskeleton. Before activation, G' Para was 24 ± 5% (± SE) greater compared with Perp (P < 0.05), and 35% ± 6 greater compared with control (Cont, P < 0.01). The difference between strained and control cells was enhanced by KCl, increasing G' 171 ± 7% Para compared with 125 ± 6% Perp and 129 ± 8% Cont (P < 10−5 both cases). The decrease in G' from baseline due to relaxant agonists isoproterenol and dibutyryl cAMP was similar in all groups. Long-term oscillatory loading of airway smooth muscle (ASM) cells caused stiffness to increase and become anisotropic. These findings are consistent with the hypothesis that cytoskeletal reorganization can enhance ASM stiffness and contractility. They imply, furthermore, that oscillatory loading of ASM may contribute to airway narrowing and failure of airway dilation in asthma. Smooth muscle contraction; plasticity; optical magnetic twisting cytometry; anisotropy; airway smooth muscle cell

Bronchial hyperresponsiveness leads to excessive narrowing of the airway and is ultimately driven by the contractile activity of the airway smooth muscle. Why the airway narrows excessively in asthma is not clear, however. The evidence for alteration of airway smooth muscle in asthma and other diseases, both structurally and functionally, is equivocal (26, 27), and the cellular mechanisms responsible for changes that have been reported are uncertain (13, 18). One factor that could alter smooth muscle behavior may be the mechanical stress experienced by the smooth muscle cell. For example, in asthma, there are changes in the mechanical microenvironment of the smooth muscle cell due both to connective tissue remodeling, which alters the stresses and strains impinging on the cell from the tidal action of breathing (13, 28), and to the excessive activation and shortening of the airway smooth muscle itself.

We have shown previously using cultured airway smooth muscle cells that long-term periodic mechanical strain induces changes in cytoskeletal and contractile filament organization as well as contractile function. Strain induces dramatic differences in the formation and organization of focal adhesions and stress fibers (19). Strain also induces increased force production and greater shortening capacity (22, 23). Whether such cytoskeletal changes translate into changes in active or passive cell mechanics in vivo is unknown, but such changes might explain mechanisms of abnormal function of smooth muscle.

Stiffness is an important determinant of stress generation in any tissue experiencing mechanical strain and, in muscle, is also a good index of active force development (29). Here we report the changes in smooth muscle cytoskeletal stiffness (G') induced by cyclic deformational strain before and after application of contractile and relaxant agonists. Stiffness was measured by optical magnetic twisting cytometry (OMTC). OMTC is a technique whereby microscopic (4.5 μm) ferromagnetic beads are passively attached to the cytoskeleton of adherent cells via focal adhesion linkages. We measured G' by optically tracking bead motions through an inverted microscope during application of an oscillating magnetic field.

We used identical protocols because we had earlier employed using cyclic deformational strain to induce parallel alignment of the cells and organization of the cytoskeleton (19). We found that, functionally, airway smooth muscle cells subjected to mechanical strain displayed increased G' at baseline and that these differences in stiffness were greatly enhanced after exposure of the cells to contractile agonists. In addition, the G' was found to be anisotropic, being greater when measured parallel to the cell long axis compared with when measured in the transverse direction. By selectively using relaxant agonists and contractile agonists and by probing cell stiffness both parallel and perpen-

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icular to the cell long axes, we show that filament reorganization greatly enhanced the generation of $G'$ with contractile activation.

**METHODS**

**Cell culture and strain apparatus.** Canine tracheal muscle was harvested and digested in collagenase and elastase with soy trypsin inhibitor as previously described (20). Freshly dissociated cells were seeded into flasks at a density of $5 \times 10^4$ cells/cm$^2$ in Ham’s F-12/DMEM with 10% fetal bovine serum, penicillin, streptomycin, and amphotericin. Cells were passaged to collagen type I-coated silastic membranes in a six-well plate (Flexcell, McKeensport, PA) when 80–90% confluent. First- and second-passage cells were used for these studies. To subject cells to mechanical stress, we positioned the plates over a manifold connected to a vacuum source. The vacuum was programmed by computer software (Flexcell) to cause a 10% increase in surface area of the membranes for a quasisinusoidal positive half-wave (tensile only) strain at 0.25 Hz. Cells were subjected to strain as described above for 10–12 days.

The mechanical strain on the membranes is nonuniform, varying with position on the membrane. At the center, the strain is uniform and isotropic, but at the periphery the strain is directed radially. This radial strain is maximal near the rigid boundary of the well and decreases toward the center where it is a minimum (7). Cultured cells migrate and reorient in response to this cyclic strain gradient, resulting in circumferential alignment of the cells around the periphery of the wells, giving the impression of a ring of aligned cells, which is absent in unstrained control cells (19). In all experiments, medium was replaced with serum-free medium for 24–48 h before measurements of $G'$.

**OMTC.** To determine $G'$ using OMTC, we grew cells to ~90% confluence and then changed them to serum-free media supplemented with insulin and transferrin as previously described (21). After a rinse with PBS, membranes were cut from the six-well plates with a scalpel and placed in serum-free media. Sections were cut from the membranes for experimental measurement and maintained at 37°C with 5% CO$_2$. Depending on membrane size and cell density, we placed 100–120 µl of solution containing 1 mg/ml beads on the cells for 15 min to maintain about two beads per cell to allow bead attachment. The beads were Fe$_3$O$_4$ ferrimagnetic (diameter 4.5 µm) and coated with an Arg-Gly-Asp-containing peptide (5). After 15 min, the cells with beads attached were washed once to remove any unbound beads. The 35-mm dish containing the membrane section was placed on the heated (37°C) stage of an inverted microscope (Nikon Diaphot). We magnetized beads in the horizontal plane by using Helmholtz coils to cause a 10% increase in surface area of the membrane to $0.16$ Hz frame rate. For example, at twisting frequencies of 100 Hz, the beads were oscillated for 160 cycles, and the 16 phases for a single were recorded from every 10th twisting cycle, giving an ~10-Hz frame rate.

**Twisting protocols.** To determine the response of cell stiffness to varying twisting frequencies, we used a protocol of increasing and decreasing oscillation frequencies of 0.1, 0.3, 0.76, 4.2, 9.1, 30, 100, and 300 Hz, for five cycles each, excepting 300 Hz, which was recorded for 10 cycles. This ascending pattern of frequencies was repeated in reverse order. The total measurement required <5 min. To determine the time course of drug effects on cell stiffness, we fixed the oscillation frequency at 0.76 Hz, and either control measurements were made or a drug was administered at 60 s, and the response was recorded for 4 min. Before and after every recording (varying frequency and time course), a single cycle of 0.3-Hz oscillation was recorded to avoid large transients during data analysis. For all samples, a varying frequency measurement was recorded first, which we refer to as baseline. After the baseline measurement, a time-course response to one of the stiffness-altering drugs was recorded. After the time course, we made a repeated measurement of the varying frequency experiment, which we refer to as the plateau measurement. Only one chemical was added to any single preparation of cells, and separate samples of each population were tested with each chemical.

**Cytoskeletal organization and OMTC stiffness measurements.** We sought additional evidence for cytoskeletal contributions to cell stiffness by taking advantage of the fact that ~90% of the cells under cyclic deformational strain are oriented perpendicular to the direction of the strain (19). This orientation is accompanied by increased alignment of actin filaments parallel to the long axis of the cell. In contrast, cells not grown under the influence of cyclic strain lie in random...
directions with less organized stress fibers. We reasoned that if cyclic deformational strain increased cell stiffness by organizing cytoskeletal elements, stiffness measurements should be greatest when the twisting forces were parallel to the stress fibers (along the long axis of strained cells). Therefore, we made baseline measurements of cell stiffness by OMTC in three different manners (Fig. 2): direction of twisting force parallel to long axis of strained cells (Para), direction of twisting force perpendicular to the long axis of the cells (Perp), and OMTC measurements from control (randomly oriented) cells (Cont). To further demonstrate the contribution of the stress fibers to the stiffness measurements, we exposed cells to cytochalasin D (2 μM) to disrupt actin filaments. To determine the level of baseline activation of contractile elements of the cells contributing to stiffness, we made OMTC measurements before and after administration of the relaxant agonists db-cAMP (1 mM) or isoproterenol (10 μM). To determine whether baseline increases in cell stiffness that might be caused by cytoskeletal reorganization might enhance cell stiffening to contractile agonist, we measured stiffness before and after KCl (80 mM). Significant differences between baseline and plateau values were determined by t-test with $P < 0.05$. Statistical tests were conducted at either 0.76 or 0.3 Hz. These frequencies are physiologically relevant, as they are close to breathing frequencies, and 0.76 Hz is the sampling rate chosen in the time-course recordings limited by the camera frame rate. In any case, the choice was largely arbitrary because the stiffness dependencies on frequency were parallel among groups (Fig. 3).

**Reagents.** All tissue culture reagents and chemicals were from Sigma Chemical (St. Louis, MO) with the exception of the trypsin-EDTA solution, which was purchased from Gibco (Grand Island, NY). KCl was diluted in PBS at 240 mM, and 1 ml was added to the 35-mm dish for a final concentration of 80 mM. Db-cAMP was dissolved at $10^{-1}$ M in distilled water, frozen in aliquots, and thawed on the day of use. Isoproterenol was prepared 1 h before use, and cytochalasin D was frozen in aliquots and thawed on the day of use. Solutions of db-cAMP, cytochalasin D, and isoproterenol were added in volumes of 1 ml of medium to 2 ml of medium containing the cells.

**Data analysis.** The recorded data were digitally filtered with a band-pass filter 48 points in length to remove baseline drift and high-frequency noise in the bead positions. For frequency recordings, the data were then separated according to oscillation frequency and separately analyzed by Fourier transform. The Fourier-transformed bead motion signal from a single frequency, $\tilde{d}$, and the specific torque $\tilde{T}$, were used to compute a complex modulus, defined here as

$$G = \tilde{T}/\tilde{d} = G' + jG''$$

where $G$ has dimensions of Pascals per nanometer and is related by a geometric factor to the complex shear modulus of the cell, and $j$ is the unit imaginary number $\sqrt{-1}$. The component of bead displacement that is in phase with $T$ is the real part of $G$ and is denoted $G'$. $G'$ is a measure of the elasticity or stiffness, which we use to quantify the cell stiffness. $G''$ is a measure of the friction, representing the out-of-phase bead motion to the applied torque and is typically $<0.2 G'$ (3). For time-course recordings, each cycle of the 0.76-Hz oscillations was analyzed cycle by cycle, giving 1.3-s time resolution. Because the distribution of $G'$ from OMTC is approximately log-normal, results are reported as the medians, together with either SE or geometric standard deviation (GSD) where indicated. GSD of $G'$ was calculated as the SD of the natural logarithm of the $G'$ from all beads for a given treatment group (Para, Perp, or Control). Measurements

![Fig. 2. Images of cells with beads showing the alignment of the cells relative to the twisting direction (arrows). Images are bright field, with contrast digitally enhanced to reveal the cell edges. A: control cells (Cont) with no apparent alignment; B: strained cells aligned in the parallel twisting direction (Para); C: strained cells aligned in the perpendicular direction (Perp).](image)

![Fig. 3. Cytoskeletal stiffness ($G'$) of the cell groups at baseline conditions as a function of bead oscillation frequency ($f$) on a log-log scale with least-squares regression linear fit for each group (error bars show SE), demonstrating power-law frequency dependence [grouped slopes = 0.172 (SE 0.008)]. Groups are, from bottom to top, control cells (v) and strained cells measured perpendicular (•) and parallel (△) to cell long axes. Insert: difference between cell groups on nonlog scale at 0.76 Hz. Control and strain were measured perpendicular and parallel to the cell long axes. Para (◆) was significantly stiffer than both Perp ($P < 0.05$) and Cont ($P < 0.01$).](image)
were recorded from 4–8 membranes, yielding from a minimum of 600 beads (~300 cells) to as many as 1,800 beads, which was more than sufficient to accurately determine the median G’ for each group (5).

Bead acceptance criteria. Beads with erratic motions below the noise level of the OMTC system were eliminated according to the following criteria. Because all frequencies were recorded twice, beads were eliminated if G’ changed by more than a factor of two at any frequency. Beads were also eliminated if the waveform response of the bead motion approached a square wave rather than that of a sinusoidal wave. This was done by eliminating beads with a 2nd harmonic of >0.18 of the fundamental magnitude. The choice of 0.18 of the fundamental was because this is the halfway point between the 2nd harmonic magnitude of a sinusoid (zero) and a square wave (0.36 of the fundamental). Beads were eliminated if they did not maintain the elastic component of the bead motion within 30 degrees of the twisting direction (at 0.76 Hz). The top 5% and lower 5% of all beads (motions measured at 0.76 Hz) for any measurement were also rejected (this had no effect on the median for any recording). The method of rejection was for bead angle outside the ±30-degree range followed by the upper and lower 5%. The 2nd harmonic test eliminated the least number of beads. Together, these criteria eliminated ~1/5 of the beads.

RESULTS

Under baseline conditions, G’ measured parallel to the cell’s long axes was significantly greater than G’ measured either perpendicular in the strained cells or G’ measured from the control unstrained cells (P > 0.05 Para vs. Perp, and P > 0.01 Para vs. Cont determined at 0.76 Hz, see Fig. 3 insert). All G’ data exhibited a weak power law fβ with exponent β = 0.172 ± 0.008 (±SE), with no significant difference of the exponents between groups as indicated by the parallel regressions on the log-log plot (Fig. 3). The rank ordering of baseline stiffness among groups (Cont, Perp, Para, respectively) was the same regardless of oscillatory frequency (Fig. 3).

The response to contractile agonists KCl is shown in Fig. 4. There was a rapid rise in stiffness after administration of KCl at 60 s (arrow), then increased to a plateau below baseline, and the stiffness (G’, bottom panel) increased then decreased to a plateau above baseline. The dotted traces show the median divided by geometric SD.

db-cAMP and isoproterenol, there were no significant differences in the decrease as a percentage of baseline for any group (Cont, Para, or Perp); 3) cytochalasin D decreased stiffness from baseline significantly for each group; and 4) cytochalasin D also decreased G’ significantly more in strained cells Para and Perp compared with Cont (P < 0.05, Fig. 6).

DISCUSSION

The primary results of this study are that cultured airway smooth muscle cells exposed to 10–12 days of mechanical strain have increased G’, and this stiffness is greatly enhanced by contractile agonists compared with unstrained, time-matched controls. Furthermore, we found that the stiffness after stimulation was greatest when measured in the parallel direction to the cell long axis, whereas contractile activation only modestly increased stiffness measured in the perpendicular direction. In the following sections, we discuss the methodology and similarities of our results with others. We then discuss the relevance and implications of increased airway smooth muscle stiffness in asthma.

These results are consistent with and extend previous results. We have previously shown that mechanical strain causes several changes in smooth muscle cell structure and function that suggest enhancement of contractile function. In addition to increases in smooth muscle-specific protein content (21) and activity (20), mechanical strain causes cytoskeletal filaments to be more aligned and the cells more elongated (Fig. 2 and Ref. 19). Functionally, strain increases the shortening

Fig. 4. Time course of response to KCl obtained parallel to long axes of the cells. Median displacement (D, solid top trace) decreased after administration of KCl at 60 s (arrow), then increased to a plateau below baseline, and the stiffness (G’, bottom panel) increased then decreased to a plateau above baseline. The dotted traces show the median divided by geometric SD.
capacity and velocity and increases force generation (22, 23). In those studies, force production increases could not be entirely accounted for by increases in myosin or myosin light chain kinase activity, and our speculation was that enhanced contractile filament organization may have led to the enhanced force production. The results we now report support this.

**Stiffness anisotropy.** We were able to probe G’ and enhancement of stiffness during cell activation relative to cell orientation. Using strain regimens identical to those applied here, we had previously demonstrated that stress fibers of airway smooth muscle cells become aligned in thick bundles with a unified vector along the long axis of the spindle-shaped cells (Ref. 19 and shown in Fig. 2). Because the beads were magnetized along a particular vector, the twisting direction could be chosen relative to cell orientation, and we took advantage of this and tested stiffness both parallel and perpendicular to cytoskeletal organization in strained cells and made comparisons before and after stimulation in both strained and control cells. We found a large difference between stiffness measured in the parallel direction compared with the perpendicular direction, relative to the cell long axis, both at baseline and after stimulation with KCl. On the other hand, KCl only modestly increased stiffness measured in the perpendicular direction (Figs. 5 and 6). In control cells that had much less alignment and less apparent cytoskeletal organization compared with the strained cells (19), only modest increases in stiffness were observed. Together, these findings are important primarily because they imply that the orientation of the contractile lattice is aligned to produce efficient contraction and stiffness generation. This interpretation of G’ from OMTC is in agreement with earlier interpretations that stiffness reflected the number of attached acto-myosin cross bridges (5) and also reflects the stiffness of the actin scaffolding (2). Furthermore, we found that there were no significant differences in the magnitude of decrease in baseline stiffness measured after either db-cAMP or isoproterenol when comparing the decreases between strained and unstrained cells (Fig. 6). This would seem to indicate that baseline stiffness is more dependent on the cytoskeletal organization than contractile protein activation.

However, organization is not likely to have been the only contributor to enhancing baseline stiffness, because control cells were not as stiff as the cells mea-
sured perpendicular to filament direction. If organization of cytoskeletal elements was the only difference between unstrained cells and the strained cells, then stiffness of control cells would be between the stiffness perpendicular and the stiffness parallel to the cytoskeletal alignment, because in randomly oriented cells some lie parallel and some lie perpendicular to the bead twisting. Other changes that we have previously documented in the contractile protein content may account for this finding (21). Additionally, strain may activate other signaling events that enhance contractile function. For example, we have recently described strain-induced activation of RhoA, a small GTPase that is known to regulate smooth muscle contractility through several effects (24). Strain has an immediate effect enhancing contractile response in smooth muscle through stretch-activated calcium channels (1, 16, 25). We previously found that strain causes an increase in calcium sensitivity of force production in permeabilized cells that had been subjected to strain. However, even when calcium concentration was controlled for, increases in force production demonstrated that other effects of strain were responsible (23). Thus strain apparently leads to increased contractile function through different mechanisms, which may or may not be independent: increased cellular organization, increased contractile-associated proteins, and changes in regulation of contractile function.

**OMTC.** We used OMTC (3, 5) to measure the stiffness of the cytoskeleton of canine airway smooth muscle cells. This technique probes the mechanical properties of the cytoskeleton through sinusoidal mechanical twisting of a ferromagnetic bead that is linked to the cytoskeleton through ligand-receptor binding (3, 5). We found that stiffness was highly heterogeneous and was distributed approximately log-normally, as has previously been reported with this technique (3, 5) and its progenitor, magnetic twisting cytometry (4, 15). Heterogeneity from OMTC has been largely attributed to differences in bead attachment characteristics, such as the number of binding sites linking the bead to the focal adhesion and the focal adhesion linkage to the cytoskeleton, but a significant portion of the heterogeneity is likely from variations in properties among cells (5). The heterogeneity inherent in the method and in the cells themselves requires that a sufficient number of beads (and cells) be probed (5). We were able to show significant differences in our data from groups including from 600 to >1,800 beads, as we describe in METHODS.

With contractile agonist, both the time course of stiffening and degree of stiffening we report in control canine smooth muscle cells (Figs. 4 and 5) were similar to previous results in human airway smooth muscle cells (5, 15). Similarly, the time courses (not shown) and decrease in stiffness with relaxant agonists (Fig. 6) are in agreement with earlier findings (4, 10, 15). We attribute the stiffness and its changes measured here and obtained previously to the elastic properties of the airway smooth muscle cytoskeleton and contractile machinery (3, 15). However, it may have been that other components, such as the lipid membrane, submembrane organelles, etc., contributed to the stiffness. Possibly these structures would impede bead motion along the cell axis compared with that perpendicular to it, leading to measured stiffness anisotropy. For a number of reasons we believe this to be unlikely. The stiffness of these structures is thought to be much less than that of the actin cytoskeleton (3). Also with disruption of the cytoskeleton, we found that G′ decreased to <20% of its original value, in agreement with earlier studies (3). Furthermore, the percent decreases with cytochalasin D were comparable whether measured parallel or perpendicular to the cell long axis (Fig. 6), implying that an intact actin cytoskeleton contributed the dominant part of the measured stiffness. Moreover, in studies where the beads are bound only to the lipid membrane with nonspecific binding or via acetylated LDL receptors, the G′ recorded are much lower than with integrin binding (3) and are similar to the levels we find after actin disruption. Finally, the disproportionate increases in cell stiffness we measured parallel to the cell long axis compared with the perpendicular direction or with the control cells also implicates underlying cytoskeletal and contractile filaments as the origin of the measured stiffness rather than membrane structures.

We explored the frequency-dependent stiffness of the stiffness between 0.1 and 300 Hz and found a power-law dependence of G′ on frequency. This observation has been previously reported and is generally consistent with the notion that the cytoskeleton behaves as a glassy system as reported by Fabry et al. (3). In these systems, changes in the structural state are mechanistically linked to changes in a single parameter, the “noise temperature,” which is defined by the exponent of the power-law stiffness minus one (3). We observed a power-law frequency dependence for G′ (Figs. 4 and 6) with exponent 0.17, which is in agreement with the report by Fabry et al. and found, as they did (3), that the exponent was increased after administration of muscle relaxants and cytoskeletal disruption. The notion that cytoskeletal remodeling follows the framework of glassy systems may have important implications. We had speculated that the changes in cytoskeletal organization induced by mechanical strain would alter the mechanical state of the cells and that we would see differences in exponent between groups. However, we did not find any significant differences in the slope of the G′ frequency dependence between groups (Cont, Perp, or Para) at baseline (Fig. 4) or after contractile or relaxant agonists; the reasons for this are unclear.

**Mechanical plasticity of the airway smooth muscle cell and chronic strain.** It is now well established that the contractile function of the airway smooth muscle cell is highly dependent on the length-tension history and is highly adaptable on the time scale of minutes (18). For example, the muscle is able to generate near maximal force and stiffness over more than a twofold change in length, and oscillatory length perturbations are able to decrease average force and stiffness to 20%.
These and similar length-tension adaptation behaviors have been described as mechanical plasticity, and a number of molecular mechanisms have been postulated (9, 17, 18). For example, Pratusevich et al. (17) postulated that with length changes, changes occur in the organization of contractile units in series to optimize force production, implying a change in cytoskeletal organization. Gunst and colleagues (8, 9) postulated that changes in length may stimulate remodeling of the actin lattice by either changing the length of the filaments or changing the sites of attachment to the cell membrane or both in a manner that optimizes force production. Additionally, airway smooth muscle exhibits its changes in myosin filament density when contracted at different lengths, indicating that myosin filaments may reform between activations, permitting changes in force generation and stiffness (14). Each of these mechanisms occurs within minutes. In comparison, the differences we observe in cell structure and function from mechanical strain occurred after several days of continuous cyclic strain of a magnitude approximating physiological movement of the airways. In this regard, the strain cells more closely mimic physiological conditions than the control cells grown in static conditions. Thus the changes in mechanical stiffness, shortening velocity and capacity, and force generation due to long duration mechanical strain we report here and previously are likely due to different mechanisms than the changes seen after an acute length change in other studies. However, it is likely that mechanical strain has altered the mechanical plasticity of these cells, although this remains to be tested. The present data indicate that alignment of cytoskeletal elements contributes to cell stiffness and the efficiency of contraction.

Airway smooth muscle stiffness and asthma. Therefore, the data we present here demonstrate an important means whereby increased smooth muscle G’, in response to chronic strain, might contribute to the observed failure of deep inspirations to induce bronchodilation in asthmatics. It is known that a normal individual can dilate constricted airways with a deep breath, whereas an asthmatic fails to do so (11, 12). The mechanism for airway dilation may reside within the smooth muscle. The normal response of activated airway smooth muscle to acutely delivered oscillatory strain is a rapid decrease in stiffness and force generation (6). This decrease requires that activated smooth muscle receive sufficient oscillatory loading to adequately stretch the acto-myosin cross bridges. Independent of changes in passive parenchymal lung tissue components, increases in smooth muscle stiffness would decrease the oscillatory stretch imparted to the smooth muscle. Thus, if chronic activation and/or mechanical stress increases cytoskeletal organization, this could translate into increased stiffness, with reduced stretch precipitating a vicious cycle of stiffness preventing relaxation and enhancing further contractility.

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DISCLOSURES

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