Frequency characteristics of lung tissue strip during passive stretch and induced pneumoconstriction

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DURING THE LAST DECADE, studies on lung tissue mechanics after constrictive agonist challenge have shown a behavior compatible with a process of “pneumoconstriction,” i.e., the activation of contractile structures closely related to the connective matrix structure. Increases of tissue elastance (E), tissue resistance (or viscance; R), and tissue hysteresivity (η) have been reported in studies performed both in vivo (1, 13, 15, 19, 23, 25–27, 30) and on tissue samples oscillated in vitro (5, 6, 16, 28, 29).

Lung parenchyma can be simplified as a viscoelastic connective matrix connected to a contractile system that modulates its mechanical properties. It is currently considered that connective matrix is passively driven by contractile cells along its functional kernel characteristics to a new operating level without intrinsic structural changes and, therefore, without significant changes in the transfer of tissue mechanical properties. On the other hand, contractile cell systems show prominent hysteretic properties related to the structure of the intracellular contractile machinery (6, 8). Structural changes are observed in contractile cells during constriction because of the bridge dynamics (8) and the plastic remodeling of the cytoskeleton (10). Therefore, it is to be expected that, to the extent that oscillated parenchymal strips express the mechanical properties of contractile cells, the frequency dependence of lung tissue mechanical properties would change during constrictive challenge.

Mechanical properties of lung tissue during agonist challenge in vitro have been studied by using a single-frequency sinusoidal input. Results obtained thereby reflect changes at that frequency, but they say nothing about the frequency dependence of tissue mechanical properties. On the other hand, the changes in lung mechanical properties during constrictive lung challenge are time variant. To determine transfer characteristics in a system submitted to transient changes, a multisinusoidal input is a better experimental approach than the sequential application of single-frequency sinusoidal inputs.

In this study, a pseudorandom input covering the spectrum between 0.2 and 3.1 Hz was applied instead of the traditional single-frequency approach. Transitional periods were not avoided to give more consistency to the findings, during both multiple-step stretch and graded histamine challenge in an organ bath. The primary purpose of this study was to detect whether frequency dependence of tissue mechanics changes significantly during pneumoconstriction. For this purpose, we measured the dynamic properties of lung parenchymal tissue strips during passive graded stretching and during increasing histamine stimulation. A pseudorandom input signal with oscillations that contain energy at five discrete frequencies between 0.2 and 3.1 Hz was used. The confrontation of
the responses to stretching and agonist challenge will lead to new data on the nature of the parenchymal mechanics.

**METHODS**

**Viscoelastic model.** In a simple linear viscoelastic system the total stress \( \sigma \) is related to the strain \( \varepsilon \) by the classical equation of motion

\[
\sigma_t = E \varepsilon_t + R(\varepsilon_t/dt),
\]

where \( E \) and \( R \) represent tissue elastance and resistance, respectively, and the subscript \( t \) indicates the time domain. In the frequency domain, such a relationship is expressed by multiplying the Fourier transform of the strain by \( j \omega \); thus Eq. 1 becomes

\[
\sigma_v = E \varepsilon_v + j\omega R \varepsilon_v,
\]

where the subscript \( \omega \) indicates the frequency domain.

When lung tissue strips are mechanically cycled over a range of frequencies, the impedance amplitude \( (R_v) \) is an inverse power law function of the cycling frequency (31)

\[
R_v = R_0 \cdot \omega^{-n}
\]

where \( \omega \) is the angular frequency, \( R_0 \) is the value of tissue \( R \) at \( \omega = 1 \) rad/s, and \( \alpha \) is a constant describing the frequency dependence of \( R \). Recent modeling of the viscoelastic behavior of lung tissue postulates the existence of a Newtonian element (31), expressed either as a term proportional to strain rate and solvent viscosity or as a frequency-invariant component of tissue \( R \) in series with the \( R \) component hyperbolically decreasing with frequency. The existence of such a component has been confirmed for chest wall (3, 11), airway walls tissue (32), and lung parenchyma (33). In the presence of a frequency-invariant component of \( R \), frequency dependence of tissue \( R \) can be expressed as follows

\[
R_v = R_0 + R_0 \cdot \omega^{-n}
\]

where \( R_0 \) represents the frequency-invariant component, and \( R_0 = R_0 - R_0 \) is the \( R \) component hyperbolically decreasing with frequency at \( \omega = 1 \) rad/s.

Tissue \( E \) has been considered to be related to the frequency by a power function, at least for frequencies in the physiologic domain (31). This model derives from the power law type of stress relaxation described for rubber balloons (14)

\[
E_v = E_0 \cdot \omega^{-\beta}
\]

where \( E_0 \) is the value of tissue \( E \) at \( \omega = 1 \) rad/s and \( \beta \) is a constant describing the frequency dependence of \( E \) (\( E_v \)).

According to these equations, elastic or Young complex modulus \( (\Psi = \sigma_v/\varepsilon_v) \) as a function of frequency can be derived from Eqs. 2, 3, and 4

\[
\Psi(\omega) = E_0 \cdot \omega^{-\beta} + j(R_0 \cdot \omega + R_0 \cdot \omega^{(1 - n)})
\]

Note that for \( \alpha = 1 - \beta \), Eq. 5 corresponds to the constant-phase viscoelastic model proposed by Hantos et al. (12) to fit tissue impedance in dog lungs.

The hysteretic properties of lung tissues have been characterized by \( \eta \) (9). The \( \eta \) is an intrinsic mechanical property of lung tissue related to the coupling between the elastic and dissipative forces at the level of the stress-bearing element, when submitted to forced oscillations

\[
\eta = \omega \cdot R \cdot E
\]

The \( \eta \) is considered to be frequency independent in lung tissue, mainly because of the fact that the frequency-invariant component of lung tissue is considered negligible compared with the \( R \) component hyperbolically decreasing with frequency (25). However, when \( R_v > 0 \), \( \eta \) will show positive frequency dependence (9).

**Sample preparation.** Male Hartley strain guinea pigs weighing between 450 and 550 g were anesthetized with pentobarbital sodium (30 mg/kg ip), tracheostomized, and mechanically ventilated during 20 min. The thorax was opened, and a positive end-expiratory pressure of 5 hPa was applied. The guinea pig was then exsanguinated, and the lungs were removed en bloc from the thoracic cavity and rinsed in a modified Krebs-Henseleit (K-H) solution containing \( (\text{in mM}) \) 118.4 NaCl, 4.7 KCl, 2.5 CaCl_2, 1.2 MgSO_4, 1.2 K_3PO_4, 25.0 NaHCO_3, and 11.1 glucose (pH 7.40 at 6°C). A strip of subpleural parenchyma was cut from the periphery of the lung. The lung strip was weighed, and its unloaded resting length (\( L_0 \)) was measured with a caliper. Lung strip volume (\( V_s \)) was measured by simple densitometry as \( V_s = \Delta V/L \), where \( \Delta V \) is the total change in volume before and after strip immersion in K-H solution and \( \delta \) is the mass density of the tissue (1.06 g/cm^3). The strips were kept in a recirculating bath of iced K-H solution that was continuously bubbled with a mixture of 95% O_2 and 5% CO_2.

**Apparatus.** Parenchymal strips were suspended vertically in a K-H organ bath (30 ml internal volume) maintained at 37°C and continuously bubbled with 95% O_2-5% CO_2. Each end of the tissue strip was tied by means of a thin 000 silk, and a small plastic ring was glued at each end of the sample with cyanoacrylate. The upper ring was attached to a linear force transducer (FT03, Grass Instruments, Quincy, MA), and the other one was fastened to a lever arm. The position of the transducer was adjusted by means of a stereomicro manipulator to bring the sample to the vertical position and to adjust for basal tension. The lever arm, introduced inside the bath, was actuated by means of a modified woofer driven by the signal generated by a computer and analogically converted (AT-MIO-16-E-10, National Instruments, Austin, TX). The movement of the woofer cone was transmitted to a linear spring connected to a second force transducer (FT10, Grass Instruments) that allowed length changes to be measured with a precision of 0.01 mm. The whole ensemble was fixed on an antivibration table. The frequency response of the system was studied by using purely elastic silver springs with different elastic Young's modulus, which were oscillated by single and multisinusoidal waves. Neither amplitude dependence (<0.1% change in stiffness) nor phase changes with frequency were detected in the range from 0.01 to 12 Hz.

The force transducers were calibrated by attaching known loads to them. A precalibrated linear spring (1 g/cm) allowed displacement changes to be calibrated. Calibration was performed in the same range of displacement and frequency used during the experiment. Calibration records using the linear frictionless spring were obtained before each experiment to ensure the linearity and the absence of internal friction in the measurement system. The \( \eta \) was <0.004 and did not show any frequency dependence.

**Preconditioning.** Cross-sectional unstressed area (\( A_0 \)) was determined from volume and unstressed length, according to \( A_0 = V_s/L_0 \). Basal force (\( F_B \)) for a stress of 10 g/cm^2 was calculated as \( F_B = A_0 \cdot (25.0 \times 10^{-6}) \). Vertical displacement of the force transducer. The displacement signal was then set to zero. Once basal force and displacement signals were adjusted, the length between bindings (\( L_B \)) was measured by means of a precision caliper. Instantaneous length (\( L_i \)) during oscillation around \( L_B \) was determined by adding the value of \( L_B \) to the measured value of displacement at any time. Instantaneous average cross-
sectional area \(A_i\) was determined as \(A_i = \frac{V_i}{L_i} \) (cm²). Instantaneous stress \((\sigma_i)\) was calculated by dividing force \((g)\) by \(A_i\) (cm²), i.e., \(\sigma_i = \frac{F_i}{A_i}\). Strain was calculated as \(\Delta = \frac{(L - L_0)}{L_0}\).

Each parenchymal strip was preconditioned by sinusoidally oscillating the tissue (frequency = 0.5 Hz; amplitude large enough to reach a maximal stress of 20 g/cm²) until a stable loop was reached. Thereafter, the sample was driven slowly to its basal length by progressively decreasing amplitude. A further period of 15 min during which the sample was kept at basal length was enough to completely stabilize the sample. No changes in basal tension with time were detected afterward. The bath solution was renewed regularly (~20 min) with 37°C warmed K-H solution.

Experiment protocol. A total of 16 subpleural lung strips were studied. The measured dimensions of the strips were \(V_0 = 0.17 \pm 0.02\) ml and \(L_0 = 1.69 \pm 0.092\) cm (means ± SD). After preconditioning, the samples were submitted to multifrequency forced oscillations. The computer-generated small-amplitude pseudorandom signal contained five noninteger discrete frequency components of 0.2, 0.5, 1.1, 1.9, and 3.1 Hz. Individual \(\Delta\) amplitudes were adjusted to give the same fast Fourier transform (FFT) amplitude (±5% variation) at every frequency (0.0244 ± 0.0002 cm, mean ± SD). Individual phases were then adjusted to minimize peak-to-peak amplitude of the strain (\(\Delta\)) of the composed signal (<0.04 cm peak to peak). Then, baseline recordings (20-s duration each) were done three times every 5 min to confirm the stability of the preparation. Afterward, a 300-s continuous recording was performed. After 60 s of basal recording, three consecutive 60-s-long steps of passive stretching were performed. Each step increased the length of the strip by 0.3 cm, which was performed. Afterward, a 300-s continuous recording was performed. After 60 s of basal recording, three consecutive 60-s-long steps of passive stretching were performed. Each step increased the length of the strip by 0.3 cm, which corresponds to \(17.4 \pm 1.06\%\) of \(L_0\) (mean ± SD). Length was driven back to \(L_0\) at the end of the third step, and recording was continued during 60 s. About 12 min later, a new recording was initiated, and after 60 s of baseline sampling a histamine solution in K-H warmed at 37°C was added to the bath to reach successively bath concentrations of \(10^{-7}\), \(10^{-5}\), and \(10^{-3}\) M. Signals were recorded during a total of 400 s. Both force and displacement signals were preamplified, filtered at 30 Hz (Urelab DSII, Biostec, Begues, Spain), analog-to-digital converted (DT-2801-A, Digital Translation, Marlboro, MA) and sampled at a frequency of 150 Hz (Software Anadat- Labdat, Infodat, Montreal, PQ, Canada).

Analysis and parameter estimation. For a given data set (either stretch or histamine), a 10-s-long time rectangular window centered at \(t = 5\) s was first applied. Then the single-sided transfer function (frequency response) was computed from the time-domain strain (stimulus) and the time-domain stress (response) as

\[
\Psi_\omega = \frac{\text{FFT}(\sigma)}{\text{FFT}(\epsilon)}
\]

and this result was transformed into single-sided magnitude (\(\Psi\)) and phase (\(\phi\)). \(E, R,\) and \(\eta\) were calculated according to

\[
E_\omega = \Psi \cdot \cos (\phi) \\
\eta_\omega = \tan (\phi) \\
R_\omega = E_\omega \cdot \eta_\omega / \omega
\]

for the values of magnitude and phase corresponding to the relevant frequencies. The window was shifted ahead (\(\Delta = 7\) s), and the same process was repeated. Rectangular windowing and a 10-s interval gave the best spectral resolution. For each segment, parameters \(\beta\) and \(E_0\) were obtained by linear log-log correlation between \(\omega\) and \(E_\omega\) according to Eq. 4. \(R_0, R_\eta,\) and \(\alpha\) were obtained by using the Levenberg-Marquardt method to determine the least squares set of coefficients that best fit the set of input data points as expressed by Eq. 3. Analysis and parameter estimation were performed by means of a specific software elaborated with Labview 5.1 (National Instruments, Austin, TX).

One-way ANOVA with replications was applied to test the significance of variations of measured and calculated parameters, either during step stretch or under histamine challenge. Values of \(F\) statistic and probability of error \((P)\) are given. In all instances we have assumed a level of two-tailed significance of 0.01, except when specifically stated in the text.

RESULTS

Figure 1 shows the mean squared error of the fitting of the Eq. 5 to the experimental values, as a function of time during stretch and histamine challenge. As expected, the fitting was poorer during the transition phases. During histamine challenge, the duration of the transition periods increases with increasing histamine concentration. Mean squared error values return, however, to basal values during the steady phases.

The left sides of Figs. 2, 3, and 4 show the change of \(E, R,\) and \(\eta\) at the frequencies 0.2 and 3.1 Hz as a function of time during step stretch changes and after return to basal length. Left bottom plots show the average value for each frequency plotted together against time for trend comparison. Both \(E\) and \(R\) have a similar time course for the different frequencies, as average curves are roughly parallel. However, the time course of \(\eta\) depends on the frequency component of the composite signal. At low frequencies (0.2 and 0.5 Hz), \(\eta\) does not change either with stretch or after release. However, at the medium or highest frequency components, \(\eta\) tends to decrease proportionally to the degree of stretching, recovering basal values after releasing (paired \(t\)-test between basal and release was not significant, \(P > 0.01\) for all frequencies). Therefore, the frequency dependence of \(\eta\) decreases proportionally to the degree of stretching.

Fig. 1. Trends of mean squared error (MSE) obtained by fitting the model (Eq. 5) to the experimental results during passive stretch (A) and histamine challenge (B).
The right sides of Figs. 2, 3, and 4 show the changes of E, R, and \( \eta \) at the frequencies 0.2 and 3.1 Hz as a function of time during histamine challenge. Right bottom plots show the average value for each frequency plotted together against time for trend comparison. As in the case of passive stretch changes, both E and R have a similar time course for the different frequencies. Conversely to what happens during passive stretch changes, however, \( \eta \) remains practically invariant during histamine challenge at 1.9 and 3.1 Hz but tends to increase at the lowest frequencies (0.2 and 0.5 Hz), the behavior of \( \eta \) at 1.1 Hz being intermediate. As a result, the frequency dependence of \( \eta \) decreases in a concentration-related way.

Results of the parametric analysis of the model are presented in Tables 1 and 2. Differences between basal mean values before step and histamine were tested by paired Student’s t-test, and no significant difference was observed for any of the parameters of the model at the two-tailed \( P \) threshold of 0.05. ANOVA showed significant changes for all parameters during histamine stimulation (Table 1). Step stretch changes significantly modified tissue E, the frequency-dependent component of tissue R, and \( \alpha \) but failed to modify the frequency-independent component of tissue R, as well as \( \beta \) (Table 2). \( \alpha + \beta \) did not change significantly during passive stretch. The reliability of the constant-phase hypothesis was tested by paired comparison of \( \alpha \) and \( \beta - 1 \) values (null hypothesis \( \alpha = \beta - 1 \)). No significant difference at a probability threshold of 0.05 was observed during passive stretching or after \( 10^{-7} \) M histamine challenge. However, significance was achieved at \( 10^{-5} \) and \( 10^{-3} \) M histamine (\( t = 4.73 \) and 9.63, respectively; \( P < 0.001 \) in both cases), which allowed us to reject the null hypothesis and, consequently, the constant-phase hypothesis.

**DISCUSSION**

In agreement with previous studies, contractile stimulation of lung tissue modified its mechanical properties in a dose-dependent way, as also did passive lengthening of a lung tissue strip. Previous authors agree that the connective tissue network dominates parenchymal mechanics, which can also be influenced by the tone or contraction of interstitial cells, due to
actin-myosin cross-bridge cycling (6, 9, 18). Recent findings suggest that mechanical properties of lung tissue in basal conditions are actually dominated by the connective tissue fiber network, whereas interstitial cells play a less significant role (33). Less is known about the effect of tissue contraction on the rheological properties of lung tissue.

The majority of previous studies use sinusoidal inputs to the tissue strip because the signal is easier to generate and simpler to analyze. However, to obtain data at different frequencies, it is necessary to repeat the measurements and to assume that the system is stationary between and during the measurement times. In basal conditions, the long-lasting memory of lung tissue may vary the mechanical response of the system from one measurement to another (31, 33). Furthermore, during tissue contraction the transitory nature of mechanical changes makes unreliable the comparison between discrete measurements separated in time. These are the reasons that led us to chose the multifrequencial approach to characterize the rheological properties of living lung tissue in both resting and constricted states.

The baseline values of $E_0$ were close to those obtained by previous authors in rat or guinea pig lung strips using sinusoidal oscillations with the same or similar basal stress (16, 21, 22, 34). Higher values have been reported, but these data are not directly comparable to ours because either the operative stress was much higher or the preconditioning protocol was different (5, 6, 18, 28). In general, for a similar lung size and species, tissue stiffness is roughly proportional to the operative stress after conditioning. Values of $h$ are in the same range (0.08–0.15) as in previous studies at similar operative stress (6, 16, 21, 22, 33). The $h$ showed only slight but consistent frequency dependence, which contrasts with some previous data in the literature that show a small concave upward variation with a minimum at $0.5 \text{ Hz}$ (18, 24) or a slight consistent decrease with frequency (21). These data have been obtained by using a sequential discrete frequencies approach, which is different from the one used in our study. In a previous study using the multifrequency approach, a small but not constant positive frequency dependence of $h$ ranging between 0.10 and 0.14 can be observed (33).

Fig. 3. Time course of tissue resistance ($R$, $g \cdot s^{-1} \cdot cm^{-2}$) during step stretch changes (left) and histamine stimulation (right). Top and middle plots show mean ± SD values at frequencies of 0.2 and 3.1 Hz, respectively. Bottom plot depicts average values at all 5 frequencies plotted together for comparison. Note that a logarithmic scale was used on the y-axis. Histamine was added to the organ bath at times indicated by arrows.
The model in Eq. 5 predicts that \( \eta \) will show some frequency-dependent behavior in the following conditions: 1) if the model does not meet the constant-phase requirement \( \alpha + \beta = 1 \) and 2) if there is a nonnegligible Newtonian or frequency-invariant resistive element. Methodological factors that can influence the trade-off of the power law phenomena, such as nonlinearity or internal frictions in the system, have been discarded by analyzing the frequency response of the measurement system before each study by means of a linear spring. Measured \( R \) and \( E \) were never significantly different from zero or from the elastic modulus of the spring, respectively, at any of the experimental frequencies. Furthermore, our results did not allow rejecting the constant-phase hypothesis either during baseline state or during passive stretching of the sample, but only at the higher degrees of constrictor challenge. Operative length of the sample was not modified by constriction, and average tensions during constriction were in the range of tensions during passive stretching. All these arguments allow discarding any extrinsic mechanism responsible for the observed changes during constriction.

Our results show that the mechanical response to passive length changes obeys the constant-phase be-

### Table 1. Model parameters during step stretch of lung parenchymal strips

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>( F )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R_0 ), g·s·cm(^{-2})</td>
<td>8.98 ± 2.04</td>
<td>10.55 ± 1.89</td>
<td>12.44 ± 2.22</td>
<td>14.39 ± 2.28</td>
<td>160.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>( \alpha )</td>
<td>-0.909 ± 0.039</td>
<td>-0.926 ± 0.043</td>
<td>-0.950 ± 0.049</td>
<td>-0.985 ± 0.066</td>
<td>4.95</td>
<td>0.009</td>
</tr>
<tr>
<td>( E_0 ), g/cm(^2)</td>
<td>97.3 ± 17.15</td>
<td>113.8 ± 18.99</td>
<td>130.2 ± 21.05</td>
<td>145.9 ± 23.46</td>
<td>395.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>( \beta )</td>
<td>0.066 ± 0.0058</td>
<td>0.064 ± 0.0048</td>
<td>0.063 ± 0.0051</td>
<td>0.063 ± 0.0057</td>
<td>3.65</td>
<td>0.029</td>
</tr>
<tr>
<td>( R_\gamma ), g·s·cm(^{-2})</td>
<td>0.23 ± 0.204</td>
<td>0.24 ± 0.204</td>
<td>0.23 ± 0.234</td>
<td>0.24 ± 0.334</td>
<td>0.004</td>
<td>0.99</td>
</tr>
<tr>
<td>( \alpha + \beta )</td>
<td>0.975 ± 0.040</td>
<td>0.990 ± 0.044</td>
<td>1.014 ± 0.049</td>
<td>1.048 ± 0.064</td>
<td>4.66</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Values are means ± SD of 16 lung parenchyma strips. \( R_0 \), difference between the value of tissue resistance at an angular frequency of 1 rad/s (\( R_0 \)); \( \alpha \), constant describing the frequency dependence of resistance; \( E_0 \), tissue elastance at an angular frequency of 1 rad/s; \( \beta \), constant describing the frequency dependence of elastance.

Fig. 4. Time course of tissue hysteresivity (\( \eta \)) during step stretch changes (left) and histamine stimulation (right). Top and middle plots show mean ± SD values at frequencies of 0.2 and 3.1 Hz, respectively. Bottom plot depicts average values at all 5 frequencies plotted together for comparison. Histamine was added to the organ bath at times indicated by arrows.
behavior in the frequency domain, whereas at higher degrees of constrictor challenge the constant-phase hypothesis is not satisfied. Recently, it has been postulated that a mechanistic basis for the constant-phase tissue viscoelasticity based on the power-law stress relaxation behavior is a consequence of the structural disposition of fibers and their instantaneous configuration during motion (31). The fibers rearrange through a series of highly constrained “wormlike” undulations under the influence of external stresses, a mechanism called “reptation.” Slow reptation of fibers contributes to the viscoelastic properties of the tissue, whereby tissue stiffness and dissipation moduli would depend on the amount of fibers and the average distance among them. Interaction between fibers is another microstructural mechanism based on the exchange of energy between fibers in close proximity (21). Both mechanisms contribute to maintain the viscoelastic properties of lung tissue, so long as the architectural integrity of its microstructure is not modified. This is expected to happen when lung is submitted to passive stretch or the amount of constriction is low. Larger constrictions are expected to rearrange the connective matrix, mainly if interstitial cells in close contact with the fiber system are stimulated. Modification of the architectural characteristics of the microstructure of the lung is expected to have substantial implications in its viscoelastic behavior, so challenging the power law-like stress relaxation and, therefore, the constant-phase behavior.

An alternate explanation arises from the natural complexity of lung tissue and a random interaction between its molecular constituents. These phenomena compose the stochastic model of interactions in complex systems, in which a cascade of molecular fractal interactions has an occurrence of 1/\( \omega \) noise (4). In this model, substantial mechanical nonlinearities at microstructural level and a neighboring sequence of fractal interactions (connectivity) induce a very long power law-like stress relaxation at the macrostructural level. Actually, the link between 1/\( \omega \) noise and power law-like relaxation processes is achieved by a phenomenon called self-organized criticality (SOC) (2). SOC occurs in a system of interconnected components that are each able to absorb energy up to a certain point. Energy initially stored in the stress-bearing element flows into the neighboring elements when the threshold is reached. These elements act in the same way, and the result is a cascade of energy spillover with a spatial and temporal extension, covering several orders of magnitude. This energy-shearing organization requires a widespread fractal organization of the complexity. In other words, the probability of the constituents of the cascade to be activated (connectivity sequence) must follow a log-normal distribution function. The activation of the contractile apparatus in the interstitium might reorganize the spatial disposition of molecules in such an extent that it would generate nonrandom paths. Consequently SOC may be disturbed, therefore challenging the \( t^{-\alpha} \) form of soft tissue stress adaptation and consequently the constant-phase behavior of lung tissue.

The time course of tissue stiffness and dissipation after passive stretching and histamine challenge is consistent with the findings in the literature. Both situations increased \( E \) and \( R \) at all studied frequencies. However, according to previous studies in vivo (25) and in vitro (6, 33), \( \eta \) behaved differently after histamine response and passive stretching. This suggests that contraction and passive stretching correspond to different hysteretic states of the tissue, as previously suggested (6). Our results thus need to address the question: which are the mechanisms underlying the frequency dependence of the temporal time course of hysteresis during tissue constriction and passive stretching?

Lung tissue \( \eta \) is expected to decrease slightly but systematically with passive stretch (6, 21, 24, 25), coming back to basal values when released after stretching (6). This behavior was observed in our samples only for frequencies \( >0.5 \) Hz, whereas in frequencies \( \leq 0.5 \) Hz \( \eta \) did not change with passive stretch. According to Fredberg et al. (6), the locus of stretch induced changes in \( \eta \) lie predominantly in the rheological behavior of the connective matrix. From our results it can be inferred that both the small frequency dependence of \( \eta \) and the frequency dependence of the stretch-related changes of \( \eta \) are dynamic phenomena that seem to depend on \( R_0 \), the frequency-independent resistive element found in the tissue. In fact, after recalculation of \( \eta' = (R - R_0) \cdot \omega^2 / E \), the so called “true” \( \eta \) was fully frequency invariant, as predicted by the constant-phase model, and the behavior of \( \eta \) during passive stretching showed a very small decrease, similar in all frequencies (Fig. 5A). However, during histamine-induced contraction of lung parenchyma (Fig. 5B), correction of \( \eta \) for the \( R_0 \) effect shows a progressive development of negative frequency dependence of “true” \( \eta \) (\( \eta' \)) with increasing challenge. According to the structural damping hypothesis proposed by Fredberg and

| Table 2. Model parameters during histamine stimulation of lung parenchymal strips |
|--------------------------------------|-----------------|-----------------|-----------------|-------|-------|
|                                     | Basal           | His (10^{-7} M) | His (10^{-5} M) | F     | P     |
| \( R_0 \), g·s·cm^{-2}              | 9.14 ± 2.08     | 9.61 ± 2.27     | 11.39 ± 2.84    | 14.19 ± 3.16 | 51.3  | <0.0001 |
| \( \alpha \)                        | -0.949 ± 0.024  | -0.964 ± 0.053  | -1.002 ± 0.039  | -1.056 ± 0.036 | 15.5  | <0.0001 |
| \( E_0 \), g/cm²                    | 97.8 ± 17.79    | 99.3 ± 17.08    | 107.2 ± 19.24   | 126.0 ± 23.2  | 72.1  | <0.0001 |
| \( \beta \)                         | 0.066 ± 0.0056  | 0.066 ± 0.0050  | 0.067 ± 0.0063  | 0.068 ± 0.0049 | 7.6   | 0.0013  |
| \( R_\alpha \), g·s·cm^{-2}         | 0.31 ± 0.159    | 0.32 ± 0.159    | 0.35 ± 0.193    | 0.46 ± 0.207   | 12.3  | <0.0001 |
| \( \alpha + \beta \)                | 1.015 ± 0.021   | 1.029 ± 0.053   | 1.070 ± 0.042   | 1.124 ± 0.036  | 15.8  | <0.0001 |

Values are means ± SD of 16 lung parenchyma strips. Hist, histamine.
Stamenovic (9), the frequency-independent behavior of \( \eta \) relates to the coupling of dissipative and elastic processes at the level of the stress-bearing element. Although the structural damping hypothesis does not specify the mechanisms through which such a coupling might come about, it is accepted that a structural relationship between tissue constituents and their respective mechanical properties are the underlying mechanisms (17). In agreement to this hypothesis, the development of a frequency-dependent behavior of \( \eta' \) might be related to structural changes induced by histamine challenge.

When lung tissue is stimulated, tissue stiffness increases as cross bridges are recruited. Fredberg et al. (7) provided experimental evidence that changes in \( \eta \) are directly associated with the cross-bridge cycling rate and hence with the metabolic state of the cells. In the absence of specific stimulation (baseline), the value of \( \eta \) is attributable to the passive mechanical properties of connective tissues. Our results indicate that, at low levels of constriction (challenge with histamine \( 10^{-7} \) M), there are not significant changes in the frequency behavior of the tissue, in the sense it behaves similarly to passive stretching. These results coincide with those of Yuan et al. (34), who show that the frequency characteristics of the tissue are similar during passive stretch and during a light constriction inducing a 30-mg increase in the mean tension (which means an increase in the operating stress of \( -1.5 \) g/cm²). According to our results (Figs. 4 and 5), significant changes of frequency behavior appear at the higher concentrations of histamine, which induce a mean increase of the operating stress of at least 12 g/cm². It is possible that the mechanical coupling of contractile cells and fibers depends on the amount of constriction or on the precise characteristics of contractile machinery involved. In a previous study (16), we found that, after elastase challenge, constriction of tissue strips reveal a transition phase characterized by a quasi-isotonic increase of E compatible with a serial disposition of the contractile system with respect to the extracellular matrix. The mechanical integrity of the matrix seems, therefore, essential for the transmission of the mechanical loads generated by the contractile machinery. In a recent study, Maksym et al. (20) found the \( \eta \) transient at the cellular level to have a shorter time constant than that in isolated muscle strips or the entire tissue. This could be explained by a serial arrangement of fibers and contractile cells in the interstitium and their mechanical interaction. This interaction provides a mechanistic basis to explain the frequency dependence of rheological changes during constriction. Thus, on one hand, contraction-related changes in \( \eta \) reflecting the rate of bridge turnover in the activated contractile cells would be dumped at high frequencies by the connective tissue. On the other hand, the loaded shortening of contractile cells would stretch the surrounding connective matrix during contraction, thereby modifying its mechanical properties. The concentration-related increase of \( R_v \) during histamine challenge could reflect some rearrangement of connective structures and a subsequent increase in internal frictions at the level of the matrix.

To conclude, we can summarize our findings as follows: 1) the mechanical response to passive length changes obeys the constant-phase behavior in the frequency domain, whereas at higher degrees of constrictor challenge the constant-phase hypothesis is not satisfied; 2) during histamine challenge, but not during passive stretch, the Newtonian component of \( R \) increases; 3) correction for \( R_v \) suppresses the frequency dependence of \( \eta \) in a basal situation and during strip lengthening, but during histamine challenge a negative frequency dependence of \( \eta' \) develops; and 4) temporal changes of \( \eta \) (and \( \eta' \)) with tissue constriction depend on the frequency, and the transitional increase observed at low frequencies vanishes as frequency increases. According to these results, we conclude that pneumoconstriction significantly modifies the intrinsic mechanical properties of the connective matrix by means of a mechanism differing from that of passive stretching. Therefore, it can be accepted that the contractile cells are able to modulate the mechanical properties of the connective matrix, including the behavior of lung tissue dissipative processes as a function of frequency. A serial disposition of contractile system and connective matrix can be hypothesized.
REFERENCES


