Dissociation between hysteresivity and tension in constricted tracheal and parenchymal strips

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Salerno, Francesco G., and Mara S. Ludwig. Dissociation between hysteresivity and tension in constricted tracheal and parenchymal strips. J. Appl. Physiol. 85(1): 91–97, 1998.—The object of this study was to investigate how changes in the contractile state of smooth muscle would modify oscillatory mechanics of tracheal muscle and lung parenchyma during agonist challenge. Guinea pig tracheal and parenchymal lung strips were suspended in an organ bath. Measurements of length (L) and tension (T) were recorded during sinusoidal oscillations under baseline conditions and after challenge with 1 mM ACh. Measurements were also obtained in strips pretreated with the calmodulin inhibitor calmidazolium (Cmz) or staurosporine (Stauro), a protein kinase C inhibitor. Elastance (E) and resistance (R) were calculated by fitting changes in T, L, and ΔL/Δt to the equation of motion. Hysteresivity (η) was obtained from the following equation: η = (R/E)2πf, where f is frequency. Finally, maximal unloaded shortening velocity during electrical field stimulation was measured in Cmz-pretreated and control tracheal strips. In tracheal strips, pretreatment with Cmz caused a significant decrease in the η response to ACh challenge and in maximal unloaded shortening velocity measured during electrical field stimulation; Stauro decreased the T, E, and R response to ACh. In parenchymal strips, Cmz decreased the η response, whereas Stauro had no effect. These results suggest that modifications in the contractile state of the smooth muscle are reflected in changes in the hysteretic behavior and that T and η may be controlled independently. Second, inasmuch as changes in η were similar in parenchymal and tracheal strips, the contractile element is implicated as the structure responsible for constriction-induced changes in the mechanical behavior of the lung periphery.

calmidazolium; protein kinase C; smooth muscle contraction; elastance; resistance

Hyperresponsive Airways are characterized by excessive airway narrowing during in vivo challenge with smooth muscle agonists. However, studies in vitro have been largely unsuccessful at defining differences in the contractile response between asthmatic airway smooth muscle (ASM) and normal control ASM (4, 5). Solway and Fredberg (27) hypothesized that the lack of a difference in the contractile behavior of asthmatic ASM relates to the measures of contractility that have been employed to characterize smooth muscle response. For the most part, isometric force generation, which reflects the tonic component of smooth muscle contraction, has been measured, and little difference has been found between the asthmatic and the normal ASM. However, a few investigators (1, 7, 13, 18) have instead focused on measures of maximal shortening velocity (V₀), which reflects the rate of cycling of actin-myosin cross bridges and myosin phosphorylation (14). With this approach, provocative data have been obtained demonstrating differences between hyperresponsive and/or sensitized and normal control ASM. Whereas V₀ is altered in sensitized muscle, force generation is not. This implies that the difference in hyperresponsive airways may lie in events occurring earlier in the contractile process.

Measurement of V₀ is technically difficult. Fredberg and co-workers (9) recently published data showing that measurement of mechanical friction in ASM (hysteresivity, η) gives information equivalent to that provided by measurement of V₀; they propose that both reflect the rate of cross-bridge cycling. Measurement of η offers certain advantages, in that mechanical friction or cross-bridge cycling rate can be continuously obtained without unloading the in vitro preparation and perhaps, more importantly, measurements can be obtained in vivo.

We reasoned that if mechanical friction and force generation could be dissociated in sensitized ASM, as suggested by the above studies, then it could also be dissociated by biochemical modulation of the proteins that regulate contraction. Specifically, we examined how calmidazolium (Cmz) affected mechanical friction during ACh-induced contraction in guinea pig tracheal rings. Cmz antagonizes calmodulin, which is involved in the cascade leading to myosin light chain phosphorylation and cross-bridge activation (11). We also assessed how Cmz affected V₀ during electrical field stimulation (EFS). In addition, we incubated tissues with staurosporine (Stauro), an inhibitor of protein kinase C (PKC), which is thought to be involved in the sustained, tonic component of smooth muscle contraction (31).

As a secondary objective, we made similar measurements in lung parenchymal strips. Lung parenchymal strips are considered a good proxy of the peripheral lung tissue and are a commonly used model for the study of the mechanical and pharmacological properties of the lung periphery (12, 17). However, parenchymal strips represent a complex system of alveolar walls, small airways, and small vessels. When lung parenchymal strips are challenged with a smooth muscle agonist, hysteretic properties change (8, 24). However, the extent to which changes in the parenchymal strip reflect changes in the hysteretic behavior of the smooth muscle present in this preparation as opposed to modification of the other elements that comprise the lung strip, e.g., the collagen-elastin-proteoglycan matrix, is not known. We hypothesized that if changes in η reflect the behavior of the smooth muscle present in this preparation, then altering the activity of proteins involved in contractile regulation.
should result in changes in the hysteretic behavior of these strips.

**MATERIALS AND METHODS**

**Strip Preparation**

Male guinea pigs weighing ~400 g were obtained from Charles River (St. Constant, PQ, Canada) and housed in a regular animal facility. Each animal was anesthetized with pentobarbital sodium (30 mg/kg ip). To degas the lung, the guinea pig was tracheotomized and a tracheal cannula was inserted; a small animal respirator (model 683, Harvard Apparatus, S. Natick, MA) was used to mechanically ventilate the animal with 100% O₂. The thorax was opened, and after 10 min the trachea was clamped and the O₂ remaining in the lungs was absorbed into the bloodstream. The animal was then exsanguinated, and the heart, lungs, and trachea were carefully dissected en bloc. The lungs were placed in a recirculating bath of iced solution, which was continuously bubbled with 95% O₂-5% CO₂.

**Apparatus**

Metal clips were glued to either end of tracheal and parenchymal strips with cyanoacrylate. In the case of the tracheal strip the clips were glued to the tracheal cartilage adjacent to the ASM. Steel music wires (0.5 mm diameter) were attached to the clips, and the strip was suspended vertically in an organ bath. A mercury bead was placed in the bottom of the organ bath, allowing the wire to pass through the bloodstream. The animal was then exsanguinated, and the heart, lungs, and trachea were carefully dissected en bloc. The lungs were placed in a recirculating bath of iced solution, which was continuously bubbled with 95% O₂-5% CO₂.

**Oscillatory mechanics: tracheal strips.** Tracheal strips were preincubated with 1 µM Stauro (model 300B, Cambridge Technologies) and the tissue strip. The spring was suspended in the bath by music wire in the same manner as the strip. The frequency and amplitude of oscillation were set by movement of a screw thumb. The lever arm was capable of peak-to-peak length excursions of approximately 10 mm, and a sinusoidal length oscillation of 1% of the resting length was added to minimize spontaneous contractions. The tissue was loaded to 10 µM Cmz and Stauro during the 25 min of stress relaxation. Direct effects of Cmz and Stauro were assessed by recording oscillations during the preincubation period. The tracheal strips included cartilage and connective tissue. Under baseline conditions the mechanical behavior of this preparation includes contributions from the contractile and passive elements of the structure. However, after induced constriction, because of the relative stiffness of these elements, changes in the exponential of the system was independent of frequency.

**Measurement of V₀.** Tracheal strips (n = 9) were mounted in the organ bath as described above. Indomethacin (1 µM) was added to minimize spontaneous contractions. The tissue was loaded to 2 g, and a baseline recording was obtained for 5 min. Ach was then added to the bath, and data were collected for an additional 10 min. Strips were challenged with 1 mM Ach (n = 16), 1 mM Ach after 20 min of preincubation with 10 µM Cmz (n = 8), or 1 mM Ach after 20 min of preincubation with 1 µM Stauro (n = 8). Tracheal strips were preincubated with Cmz and Stauro during the 25 min of stress relaxation. Direct effects of Cmz and Stauro were assessed by recording oscillations during the preincubation period. The tracheal strips included cartilage and connective tissue. Under baseline conditions the mechanical behavior of this preparation includes contributions from the contractile and passive elements of the structure. However, after induced constriction, because of the relative stiffness of these elements, changes in the exponential of the system was independent of frequency.
at the same time point. In some tracheal preparations, T and E had not peaked 10 min after challenge. In these cases, we took the final value as the maximum response.

**Measurement of Strip Mechanics**

E and R were estimated by applying the recursive least-squares algorithm to the equation of motion (16)

\[ T = E \Delta l + R \Delta l / \Delta t + K \]  

where \( \Delta l \) is length, \( \Delta l / \Delta t \) is the length change per unit time, and \( K \) is a constant term reflecting the baseline T. Baseline results were standardized for strip size by multiplying the values of E and R by \( L / A_0 \), where \( A_0 \) is the unstressed cross-sectional area of the lung parenchymal strip obtained from the following formula

\[ A_0 (cm^2) = W_0 / [\rho L r] \]  

where \( \rho \) is the density of the tissue taken as 1.06 g/cm³ and \( W_0 \) is the strip weight. Values of E and R were multiplied by \( L / A_0 \). Values of \( E \) and \( R \) varied between 0.018 and 0.038 cm². The \( \eta \), defined by Fredberg and Stamenovic (10) as a dimensionless variable coupling the dissipative and elastic behavior, was calculated by using the following equation

\[ \eta = (R/E) * 2\pi f \]  

where \( f \) is frequency.

\( V_0 \) was calculated as the change in length over time between 100 and 200 ms after the unloading of the tracheal strip.

**Data Analysis**

Unpaired t-tests were used to determine whether a response different from baseline was obtained and whether preincubation with Cmz or Stauro affected the contractile response. Paired one-tailed t-test was used to compare \( V_0 \) before and after Cmz preincubation. The Dunnett multiple comparison procedure, after Bonferroni correction for the number of time points studied, was used to compare the groups preincubated with Cmz or Stauro with the ACh control group. Results were considered statistically significant at a probability level of 5%. Values are means ± SE.

**RESULTS**

Tracheal Strip Responses

There were no significant differences in baseline mechanics between groups. Representative responses in a typical tracheal strip after ACh challenge are shown in Fig. 1. The response in \( \eta \) peaks before that of T, E, and R. The peak values of the different mechanical parameters after ACh alone, ACh after preincubation with Cmz, and ACh after preincubation with Stauro are presented in Table 1. In all groups, for all parameters, ACh induced increases that were statistically different from baseline. Cmz preincubation significantly decreased the peak \( \eta \) response, whereas T, E, and R were not significantly affected. Stauro significantly decreased the peak response of T, E, and R to ACh.

The mean responses 30, 60, 180, 300, and 600 s after ACh challenge in tracheal strips challenged with ACh alone and those preincubated with Cmz and Stauro are shown in Fig. 2. At 180, 480, and 600 s there was a significant difference in \( \eta \) response between Cmz-pretreated strips and strips treated with ACh alone. The other parameters were not statistically different between the two groups. In the Stauro-preincubated tracheal strips, responses in T, E, and R were significantly smaller 60, 180, 300, and 600 s after ACh challenge than after ACh alone.

A typical tracing of changes in T and length with unloading of the tracheal strip during EFS is shown in Fig. 3. The \( V_0 \) was significantly lower in the group preincubated with Cmz than in control strips (Fig. 4; \( P < 0.05 \)). Maximal shortening, on the other hand, was comparable: 0.85 ± 0.08 and 0.88 ± 0.10 mm for control and Cmz-preincubated strips, respectively.

**Table 1. Peak increases in different mechanical parameters in tracheal strips**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tension (g)</th>
<th>Elastance (g/mm)</th>
<th>Resistance (g·mm⁻¹·s⁻¹)</th>
<th>Hysteresivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td>73.6 ± 7.9</td>
<td>94.4 ± 12.2</td>
<td>123.3 ± 13.7</td>
<td>48.5 ± 5.9</td>
</tr>
<tr>
<td>Cmz + ACh</td>
<td>61.1 ± 12.3</td>
<td>85.1 ± 15.6</td>
<td>92.6 ± 17.2</td>
<td>29.9 ± 6.1*</td>
</tr>
<tr>
<td>Stauro + ACh</td>
<td>38.1 ± 5.8*</td>
<td>49 ± 10.5*</td>
<td>75.7 ± 11.0*</td>
<td>37.3 ± 8.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as percentage of baseline. Cmz, calmidazolium; Stauro, staurosporine. *Statistically different from ACh alone.
Parenchymal Strip Responses

There were no significant differences in the baseline mechanics between groups. A representative tracing of the parenchymal strip response to ACh is shown in Fig. 5. The response in $\eta$ again peaks before that of $T$, $E$, and $R$. The peak values of the different mechanical parameters after ACh alone, ACh after preincubation with Cmz, and ACh after preincubation with Stauro are presented in Table 2. In all groups, for all parameters, ACh induced increases that were statistically different from baseline. Cmz preincubation significantly decreased the peak response in $\eta$ to ACh alone (P < 0.05); increase in $T$, $E$, and $R$ was statistically lower in Stauro-preincubated strips than in strips challenged with ACh alone (P < 0.05).

Figure 6 shows the mean responses at 30, 60, 180, 300, and 600 s after ACh challenge in parenchymal strips challenged with ACh alone, preincubated with Cmz, and preincubated with Stauro. At 180 and 480 s the response in $\eta$ in the Cmz-pretreated group was significantly different from that in the group treated with ACh alone. The other parameters, $T$, $E$, and $R$, were not statistically different between the two groups. There were no differences between Stauro-preincubated strips and those treated with ACh alone.

Challenge with Cmz and Stauro alone did not significantly modify the different mechanical parameters in

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**Fig. 2.** Response in ACh-challenged tracheal strips (n = 16) and strips preincubated with calmidazolium (Cmz, n = 8) and staurosponine (Stauro, n = 8) at 30, 60, 180, 300, and 600 s after ACh challenge. *Increase in $\eta$ was statistically lower in Cmz-preincubated strips than in strips challenged with ACh alone (P < 0.05); # increase in $T$, $E$, and $R$ was statistically lower in Stauro-preincubated strips than in strips challenged with ACh alone (P < 0.05).

**Fig. 3.** Representative trace of tension and length during unloaded shortening.

**Fig. 4.** Maximal shortening velocity ($V_0$) in control (C) and Cmz-preincubated tracheal strips. *P < 0.05.
the lung parenchymal strip. Cmz pretreatment caused a mild increase in the measured mechanical parameters in the tracheal preparation (3.3 ± 0.9, 4.4 ± 1.2, 7.5 ± 2.1, and 2.9 ± 1.7% for T, E, R, and η, respectively).

DISCUSSION

In this study we have shown that modulation of the activity of the proteins that regulate ASM contraction differentially affects hysteresis and force generation in isolated tracheal rings and lung parenchymal strips. Numerous investigators have attempted to define differences in the smooth muscle contractility between asthmatic and normal control airways (4, 5). Generally, studies have focused on isometric force generation, and little difference has been found in the responsiveness of smooth muscle between these two groups. Stephens and colleagues (1, 7, 13), on the other hand, looked at the velocity of tracheal smooth muscle shortening ($V_0$). $V_0$ has been shown to be an index of cross-bridge cycling rate and myosin phosphorylation (14). Stephens and colleagues (1, 13) showed that $V_0$ is altered in the tracheal smooth muscle of sensitized dogs, while isometric force generation is unchanged. Similarly, Mitchell et al. (20) showed that $V_0$ measured during EFS was increased in human bronchial smooth muscle passively sensitized with atopic serum. Most recently, Fan et al. (7) showed in sensitized mice that $V_0$ and maximal shortening capacity of tracheal smooth muscle are increased compared with other mouse strains, whereas isometric force generation was similar in all strains studied.

Cross-bridge cycling rate and $V_0$ may be important factors in determining the magnitude of smooth muscle shortening and airway hyperresponsiveness. As pointed

### Table 2. Peak increases in different mechanical parameters in lung parenchymal strips

<table>
<thead>
<tr>
<th></th>
<th>Tension</th>
<th>Elastance</th>
<th>Resistance</th>
<th>Hysteresivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td>14.6 ± 2.3</td>
<td>18.6 ± 2.9</td>
<td>31.2 ± 4.6</td>
<td>14.1 ± 2.0</td>
</tr>
<tr>
<td>Cmz + ACh</td>
<td>12.0 ± 2.4</td>
<td>15.0 ± 2.5</td>
<td>21.3 ± 3.8</td>
<td>9.2 ± 1.9*</td>
</tr>
<tr>
<td>Stauro + ACh</td>
<td>14.7 ± 2.5</td>
<td>17.6 ± 3.4</td>
<td>30.0 ± 5.2</td>
<td>15.0 ± 2.4</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as percentage of baseline.

*Statistically different from ACh alone.
out by Solway and Fredberg (27), when bronchoconstriction occurs in vivo, the muscle is constantly oscillated; i.e., tidal breathing is ongoing. During ASM length oscillations, velocity of contraction would be especially critical; the amount of airway narrowing achieved between stretches could be a function of the velocity of shortening.

Fredberg and co-workers (8) suggested that the dissipative processes responsible for the changes in hysteretic behavior of the smooth muscle during constriction are largely accounted for by the mechanical friction between myosin heads and actin filaments as cross bridges rupture during cycling. More recent evidence in canine tracheal smooth muscle from this same group showed striking correlations between $\gamma$ and two established measures of cross-bridge cycling rate: actomyosin ATP utilization and shortening velocity (9). It has been observed that the viscoelastic behavior of ASM is modified during contraction (9, 25). Sasaki and Hoppin (25) and Fredberg et al. (9) showed that ASM demonstrates hysteretic behavior and that $\gamma$ is increased after agonist challenge. In addition, a particular timing in the contractile process has been demonstrated, with the hysteretic behavior being affected primarily in the early phase of activation and the elastic behavior affected later (8, 9, 24), a pattern observed in the current experiment. These data are consistent with the changes in cross-bridge cycling rate described for smooth muscle activation (21, 29).

We postulated that manipulating the biochemical pathways involved in cross-bridge cycling rate should result in alterations in the hysteretic properties. In smooth muscle the contractile response to different agonists shows an initial phase, where myosin phosphorylation and isometric tension increase, and a steady-state phase, where the isometric tension is maintained with a decrease in the phosphorylation rate (21). The latter condition characterized by low levels of phosphorylation and a reduced rate of cross-bridge cycling is sometimes referred to as the latch state (21). Calmodulin is involved in the regulation of smooth muscle contraction through a number of potential mechanisms (22, 30). The principal mechanism involves activation of myosin light chain kinase, phosphorylation of the myosin light chain, and stimulation of the cyclic interaction of the myosin and actin filaments. Increased myosin light chain phosphorylation is believed to increase the actin-myosin cross-bridge cycling rate (14, 22). In addition, calmodulin is thought to modulate the thin filament-associated proteins caldesmon and calponin (30). Finally, calmodulin may affect movement of Ca"2+ across the sarcoplasmic reticulum membranes (30).

Cmz has been shown to be a potent and relatively specific calmodulin inhibitor that is thought to affect the activity of calmodulin-dependent enzymes (11). In isometric studies on tracheal preparations, calmodulin inhibitors have been shown to affect early force development (2). We reasoned, therefore, that preincubation with Cmz would affect the initial phase of ACh-induced smooth muscle contraction and thereby the rate of cross-bridge cycling. In the current experiment, when tracheal strips were preincubated with Cmz, the response to ACh was altered; the peak $\gamma$ response was decreased. However, maximal force generation was unaffected. In addition, $V_0$ was lower in Cmz-pre-treated tracheal strips than in controls. Hence, there was a dissociation between changes in cycling rate and maximal force generation.

Stauro is a potent inhibitor of PKC (26, 28). It has been suggested that PKC plays a role in the sustained, tonic component of smooth muscle contraction (31). This hypothesis is based on the observation that phorbol esters induce contractions that are not necessarily associated with changes in intracellular Ca"2+ or myosin phosphorylation (31). The mechanism of PKC action is thought to be via myosin-activated protein kinase-induced phosphorylation of thin filament-associated proteins such as caldesmon and calponin. Phosphorylation of these proteins alleviates inhibition of actin-activated myosin Mg-ATPase. Stauro is known to act at the catalytic site of PKC (26) and has been shown to inhibit phorbol 12-myristate 13-acetate-induced changes in guinea pig tracheal smooth muscle tension (28). However, there is evidence to suggest that Stauro may not be completely specific for PKC. Stauro may also affect tyrosine-specific kinases as well as other serine- and threonine-specific kinases (26). Moreover, as stated above, calmodulin may also be involved in the regulation of thin filament-associated proteins. This makes interpretation of data somewhat more difficult. Nonetheless, preincubation with Stauro resulted in a pattern of alteration in the contractile response of the tracheal strip different from that after preincubation with Cmz; the increases in $T$, $E$, and $R$ were modified, whereas the $\gamma$ response was unaffected. Again, there was a dissociation between changes in $\gamma$ and maximal force generation.

In the parenchymal strip, results obtained after pretreatment with Cmz were similar to those in the tracheal strip. Cmz caused a decrease in the peak $\gamma$ response, whereas peak $T$ was unaffected. Conversely, Stauro had no effect on the response to ACh in the parenchymal strips. The difference in the effect of Stauro between tracheal and parenchymal strips may reflect the smaller amount of smooth muscle in the parenchymal preparation. The similarity of the $\gamma$ response to Cmz incubation in the tracheal and parenchymal strip suggests that changes in $\gamma$ of the smooth muscle were responsible for changes in $\gamma$ of lung parenchyma during contraction. We and others have shown in previous experiments that viscoelastic or hysteretic properties of parenchymal strips change after challenge with smooth muscle agonists (8, 24). Modification of the hysteretic behavior of the lung parenchyma during agonist challenge could be the result of changes in the viscoelastic properties of the smooth muscle or modification of the structures in series with the contractile elements, i.e., alterations in parenchymal geometry (23) or modifications in the mechanical behavior of surfactant or in the viscoelastic characteristics of the extracellular matrix (18). Small
airways, vessels, alveolar ducts, and alveolar walls have contractile properties (3, 6, 15), and all could contribute to changes in viscoelastic behavior during agonist challenge (3, 24). Our data showing a similar pattern in tracheal muscle and parenchymal strips implicates peripheral smooth muscle as the element responsible for changes in $\eta$.

In conclusion, our findings on the different effects of Cmz and Stauro on the mechanical properties of tracheal strips during contraction demonstrate that, under certain circumstances, $\eta$, $V_0$, and maximal force generation may be subject to separate controls. These findings support the contention of Stephens and others (1, 7, 13, 18) that measuring only maximal force generation may be subject to separate controls. These findings represent a potentially important mechanism to explain differences in airway responsiveness between asthmatic patients and control subjects.

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REFERENCES


