Pharmacological modulation of the mechanical response of airway smooth muscle to length oscillation

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Pharmacological modulation of the mechanical response of airway smooth muscle to length oscillation. J. Appl. Physiol. 83(3): 739–745, 1997.—Stretch and retraction of the airways caused by changes in lung volume may play an important role in regulating airway reactivity. We studied the effects of different pharmacological stimuli on airway smooth muscle to determine whether the muscle behavior during length oscillation can be modulated pharmacologically and to evaluate the role of different activation mechanisms in determining its behavior during the oscillation. Active force decreased below the static isometric force during the shortening phase of length oscillation, resulting in an overall depression of force during the length oscillation cycle. This pattern of response was unaffected by the contractile stimulus or level of activation, suggesting that it was caused by a mechanism that is independent of the level of activation of cross bridges. The normalized area of the length-force hysteresis loop (hysteresivity) differed depending on the stimulus used for contraction. Effects of different stimuli on hysteresivity were not correlated with their effects on isotonic shortening velocity or isometric force, suggesting that the pharmacological modulation of the behavior of airway smooth muscle during length oscillation at these amplitudes cannot be accounted for by the effects on the cross-bridge cycling rate.

METHODS

Tissue preparation. Mongrel dogs weighing 20–25 kg were anesthetized with pentobarbital sodium and killed by exsanguination. A 10- to 15-cm segment of trachea was immediately removed and immersed in physiological saline solution at 22°C [composition (in mM): 110 NaCl, 3.4 KCl, 2.4 CaCl₂, 0.8 MgSO₄, 25.8 NaHCO₃, 1.2 KH₂PO₄, and 5.6 glucose]. The solution was aerated with 95% O₂-5% CO₂ to maintain a pH of 7.4. Rectangular strips of trachealis muscle were dissected from the trachea after removal of the epithelium and connective tissue layer. Muscle strips (2-3 mm wide × 5–8 mm long) were mounted horizontally in a 25-ml rectangular Plexiglas tissue bath containing 37°C physiological saline solution that was bubbled with 95% O₂-5% CO₂. One end of each strip was fixed tightly to a stationary platinum hook while the other end was attached to a servo-regulated electromagnetic lever (model 300B, Cambridge Technology). The static compliance of the entire system excluding the muscle was negligible with respect to muscle compliance.

After placement in the tissue bath, muscles were equilibrated for 60–90 min. During this time, they were stimulated by electrical field stimulation at 2- to 5-min intervals using 20-V, 15 pulses/s, 0.5-ms duration square waves by means of rectangular platinum electrodes (55 × 10 × 0.3 mm) connected to Grass stimulator and a current amplifier. To determine the muscle length at maximal active force (L₀), muscle length was increased progressively after each stimulation until the force of active contraction reached a maximum. After L₀ was determined, muscles were subjected to different experimental protocols. Pharmacological agonists used for subsequent contractions were injected into the tissue bath by micropipette. Contractions induced by KCl were induced by

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replacement of the bathing medium with 60 mM KCl in the presence of \(10^{-6}\) M atropine.

Length oscillation of muscle strips. Length oscillation of muscle strips was performed by using a waveform generator (model 75A, Wavetek, San Diego, CA). Triangle waves were used so that the rate and amplitude of the length oscillation cycle could be varied independently. Muscles were oscillated at an amplitude of 10% \(L_o\) (from 0.80 to 0.70 \(L_o\)) or 6% \(L_o\) (from 0.80 to 0.74 \(L_o\)) and at rates of length change (ramp rates) ranging from 0.005 to 0.02 \(L/o/s\). All data were captured on a Nicolet digital oscilloscope and transferred to a computer for further analysis. The area of the force-length loop for each cycle was computed by integration using the digitally recorded data.

Measurement of isotonic shortening velocity. The isotonic shortening velocity was measured as previously described (6, 11). Briefly, each muscle was isometrically contracted at \(L_o\) for 5 min. A step decrease in muscle length from \(L_o\) to 0.8 \(L_o\) was then imposed on the muscle. At the same time, the muscle was subjected to an afterload equal to 10% of the isometric force at \(L_o\). The muscle was then contracted isometrically at 0.8 \(L_o\), until the force equaled the afterload, after which it began to shorten isotonically. During shortening, both muscle length (L) and the rate of change of muscle length (dL/dt) were recorded.

Data analysis. Muscle force during length oscillation at the shortest length of the oscillation cycle was computed as a proportion of the static isometric force at 0.80 \(L_o\) (the peak length of the oscillation cycle). Hysteresis area for each rate and amplitude of length oscillation was computed cycle by cycle from three to six successive force-length loops. The first loop obtained after a change in the rate of length oscillation was not included. The hysteresivity constant (\(\eta\)), which normalizes hysteresis area for differences in length and force excursions, was computed from the hysteresis area of length-force loop (A), the peak-to-peak length excursion (\(\Delta L\)), and the peak-to-peak force excursion (\(\Delta F\)), as described by Fredberg et al. (3)

\[
\eta = \tan \phi = \sin^{-1}(4A/\Delta F \Delta L)
\]

where \(\phi\) is the phase lag between length and force.

Statistical analysis. All data are presented as means ± SE. Comparisons between two groups were made using a two-way analysis of variance with repeated measures or paired t-tests. For all statistical tests employed, \(P < 0.05\) was considered statistically significant. In each case, \(n\) is the number of muscle strips used for each experiment.

RESULTS

Time course of changes in force and hysteresivity during isometric contractions elicited by acetylcholine (ACH), KCl, and McN-A-343. The behavior of the muscle was analyzed during length oscillation to determine whether it can be pharmacologically modulated. Hysteresivity and dynamic force during length oscillation were evaluated during contractions elicited by three contractile stimuli that differ in the mechanisms by which they activate tracheal smooth muscle. The time course of changes in hysteresivity was evaluated during isometric contractions of up to 10-min duration in separate sets of experiments in which contractions were induced by \(10^{-5}\) M ACh, \(10^{-4}\) M McN-A-343, or 60 mM KCl. A length oscillation of 6% \(L_o\) at a rate of 0.02 \(L/o/s\) (0.166 Hz) was imposed on the resting muscle before administration of the contractile stimulus. The oscillation was then continued throughout the period of isometric contraction.

Each of these stimuli elicited an increase in isometric force to a plateau that was sustained for a full 10 min (Fig. 1). Muscles were oscillated continuously before stimulation and throughout contraction by using a 6% \(L_o\) oscillation amplitude and a rate of length change of 0.02 \(L/o/s\). With each of these stimuli, hysteresivity exhibited a phasic increase during the force development phase of contraction but was relatively constant after 5 min, when force had reached a steady state. The peak increase in hysteresivity was greatest during contraction with ACh, smaller for KCl, and lowest during contraction with McN-A-343. However, the magnitude of the peak increase in hysteresivity for these stimuli was not correlated with the values of hysteresivity during the plateau phase of the contraction. Hysteresivity during the plateau phase of contraction was greater for KCl than for ACh and McN-A-343.

Dynamic force and hysteresivity during the plateau phase of contractions induced by ACh, KCl, and McN-A-343. The effect of the level of contractile activation on the dynamic response of the muscle was assessed in six muscle strips by using different concentrations of ACh. Each muscle strip was contracted successively with \(10^{-4}\) M and \(10^{-6}\) M ACh. After 5 min of isometric contraction, the muscle was subjected to length oscillations of 10% \(L_o\) at rates of 0.005, 0.01, and 0.02 \(L/o/s\). Both hysteresivity and the dynamic muscle force at 0.7 \(L_o\), the short length of the oscillation cycle, were calculated. Mean results are shown in Fig. 2A. The concentration of ACh did not significantly affect the decrease in force during imposed shortening or the hysteresivity at any oscillation rate. Isometric force in response to \(10^{-6}\) M ACh was 48.5 ± 1.8% of the isometric force in response to \(10^{-4}\) M ACh.

The effects of different pharmacological stimuli on the dynamic response of the muscle to length oscillation were evaluated by using a protocol similar to that described above. In five muscles, the effects of \(10^{-5}\) M ACh and the muscarinic agonist McN-A-343 (\(10^{-4}\) M) were compared in successive contractions. In another group of six muscles, the effects of ACh (\(10^{-6}\) M) and 60 mM KCl were compared. In each set of experiments, stimulus concentrations that elicit similar levels of isometric force were chosen. Each muscle was contracted isometrically for 5 min and then subjected to length oscillations at rates of 0.005, 0.01, and 0.02 \(L/o/s\) at an amplitude of 10% \(L_o\). Dynamic force at 0.7 \(L_o\), the short length of the oscillation cycle, was not significantly different during contractions induced by ACh (\(10^{-5}\) M) and McN-A-343 (\(10^{-4}\) M) or during contractions elicited by ACh (\(10^{-6}\) M) and KCl (60 mM) at any oscillation rate (Fig. 2B and C). In contrast, hysteresivity was significantly lower at all three oscillation rates during contractions induced by McN-A-343 than during contractions induced by \(10^{-5}\) M ACh (Fig. 2B). Contra-
tion with KCl elicited significantly higher levels of hysteresivity at oscillation rates of 0.01 and 0.02 L_o/s than did contraction with ACh. The static isometric force obtained with each of these pairs of stimuli did not differ significantly.

**Relationship between hysteresivity and isotonic shortening velocity.** Shortening velocity and hysteresivity were measured in paired contractions in the same muscle strip activated under different pharmacological conditions. After 5 min of isometric contraction, the muscle was subjected either to length oscillation or to the measurement of isotonic shortening velocity. Hysteresivity was assessed during oscillations at a rate of 0.01 L_o/s and an amplitude of 10% L_o. Shortening velocity was measured by using a 10% afterload.

The effects of 10^{-6} and 10^{-5} M ACh on hysteresivity and shortening velocity were compared in seven muscle strips (Fig. 3). The isotonic shortening velocity elicited by 10^{-6} M ACh (0.023 ± 0.004 L_o/s) was 62.5 ± 3.6% of that elicited by 10^{-5} M ACh (0.036 ± 0.005 L_o/s), and the isometric force in response to 10^{-6} M ACh was 57.3 ± 2.8% of that elicited by 10^{-5} M ACh. In contrast, hysteresivity was significantly higher during contraction with 10^{-6} M ACh than during contraction with 10^{-5} M ACh at this oscillation rate.

The effects of ACh (10^{-5} M) vs. McN-A-343 (10^{-4} M) and of ACh (3 × 10^{-5} M) vs. KCl (60 mM) on hysteresivity and shortening velocity were determined in five muscle strips for each comparison. The shortening velocity elicited by 10^{-4} M McN-A-343 (0.017 ± 0.005 L_o/s) was 58.7 ± 6% of that elicited by 10^{-5} M ACh (0.027 ± 0.007 L_o/s), whereas hysteresivity was only 6.3 ± 2.2% lower during contraction with McN-A-343 than during contraction with ACh (Fig. 4). Similar protocols were performed on another set of five muscles to compare the effects of 60 mM KCl and 3 × 10^{-5} M ACh on hysteresivity and shortening velocity. Whereas shortening velocity during contractions induced by KCl (0.016 ± 0.001 L_o/s) was 48.2 ± 3.7% of that measured during contractions induced by 3 × 10^{-5} M ACh (0.034 ± 0.003 L_o/s), hysteresivity was 19.3 ± 4.3% higher during stimulation with KCl than during stimulation with ACh. Differences in isometric force induced by these concentrations of ACh and KCl and ACh and McN-A-343 were not statistically significant.

**DISCUSSION**

Effects of stimulation conditions on the response to length oscillation. We evaluated the force-length behavior of airway smooth muscle during length oscillation after activation under different stimulus conditions to determine whether the dynamic behavior of the muscle is affected by the intensity of contractile stimulation or by receptor-coupled second-messenger pathways. Muscles were studied after contraction with a variety of...
contractile stimuli that utilize different signaling mechanisms to activate the muscle. Regardless of the stimulus used to contract the muscle, its general pattern of behavior during length oscillation was the same.

at each of the oscillation rates employed: the dynamic force dropped below the static force as the muscle was shortened below the peak length of the oscillation cycle and returned to the level of the static force as the muscle was stretched back to the peak length of the oscillation cycle (5, 15). To compare the effects of different stimuli on the oscillatory behavior of the muscle, the response to length oscillation was characterized in terms of the depression of dynamic force relative to the static force at the shortest length of the cycle and the normalized hysteresis area, or hysteresivity (15). In addition, the active shortening velocity and the response to length oscillation were compared under different stimulus conditions to determine whether the same cellular processes are likely to predominate in regulating both these mechanical responses.

Two different concentrations of ACh, the muscarinic agonist McN-A-343, and KCl were used for these studies. These stimuli were chosen because their effects on the activation of canine tracheal smooth muscle are different and have been well characterized in previous studies (1, 9). Although ACh and McN-A-343 both activate M3 muscarinic receptors in canine tracheal smooth muscle (9), ACh is a much more potent muscarinic stimulus than McN-A-343. It elicits a much larger Ca2+ transient, significantly higher levels of inositol phosphate metabolism, and faster rates of force develop-
Comparisons of effects on each parameter were normalized to values obtained with ACh. *Statistically significant differences between effects of KCl (60 mM) and ACh (3 \times 10^{-5} \text{ M}) or of McN-A-343 (10^{-4} \text{ M}) and ACh (10^{-5} \text{ M}) (P < 0.05). Comparisons of effects on each parameter are from paired contractions obtained with ACh and either McN-A-343 or KCl made in same muscle strip.

The depression of force after shortening as well as the hysteresivity were very similar at concentrations of ACh that elicit maximal and half-maximal levels of isometric force (7, 11, 12, 15). However, we did observe differences in the hysteresivity of loops obtained during contractions elicited by ACh and KCl, and we also observed differences in hysteresivity during contractions elicited by ACh and McN-A-343. This suggests that some of the mechanisms that regulate the response of airway smooth muscle to length oscillation are affected by second-messenger pathways that are differentially activated by these stimuli.

Correlation between hysteresivity and shortening velocity. For all contractile stimuli, hysteresivity increased during the force development phase of contraction, when the rate of cross-bridge cycling and shortening velocity are highest, and then decreased to a constant level during the plateau phase of contraction, when the cross-bridge cycling rate and shortening velocity decline. The magnitude of the transient increase in hysteresivity for each stimulus correlated with the rate of force development of the muscle elicited by that stimulus. The peak increase in hysteresivity also occurred at a time point similar to the peak increase in isometric shortening velocity measured in previous studies (6, 7). These data are consistent with the observations of Fredberg et al. (4), who reported transient increases in hysteresivity in canine tracheal smooth muscle during the force development phase of contraction that correlated with changes in the shortening velocity. As the shortening velocity of the muscle is determined primarily by the cross-bridge cycling rate, these observations suggest that during the force development phase of contraction, the rate of cross-bridge cycling plays a predominant role in determining the hysteresis of the force-length loop during length oscillation.

In contrast, during the plateau phase of isometric contraction, we found that the effects of different stimulus conditions on hysteresivity were not correlated with their effects on shortening velocity. Both shortening velocity and isometric force were significantly lower when the muscle was activated with 10^{-6} \text{ M} ACh than with 10^{-4} \text{ M} ACh; however, hysteresivity was slightly higher during contraction with the lower concentration of ACh. Both hysteresivity and shortening velocity were lower when the muscle was activated with McN-A-343 than when it was activated with ACh; however, during contraction with McN-A-343, the shortening velocity was one-half that obtained during contraction with ACh while hysteresivity was reduced only slightly. Hysteresivity was significantly higher during contractions induced by KCl compared with those induced by ACh, but the shortening velocity was lower during contraction with KCl than during contraction with ACh.

As the calculation of hysteresivity normalizes for the amplitude of both force and length perturbations during oscillation, differences in hysteresivity values reflect only shape differences in the force-length loop. Fredberg et al. (4) have suggested that the hysteresivity associated with small-amplitude length oscillations (0.5% L_0) is an index of the energy required for cross-bridge cycling and that hysteresivity may provide an
index of the cross-bridge cycling rate as reflected by the shortening velocity. Although our data suggest that the cross-bridge cycling rate is an important factor in determining the shape and area of the hysteresis loop of airway smooth muscle during length oscillation, particularly during the force development phase of contraction, the lack of correlation between shortening velocity and hysteresivity under different stimulus conditions suggests that, at these larger oscillation amplitudes, factors other than the rate of active shortening also contribute significantly to the behavior of the muscle during length oscillation. These factors may include cross-bridge properties that are not well characterized by the shortening velocity as well as non-cross-bridge mechanisms that also affect the mechanical properties of the tissue. The relative contributions of these other contributing factors may differ depending on the stimulus conditions as well as the rate and amplitude of length oscillation.

There are a number of possible mechanisms that might affect the hysteresis of the muscle without causing corresponding changes in shortening velocity. During the lengthening phase of the oscillation cycle, cross bridges are forced to cycle under an imposed strain and an increasing load; the isotonic shortening velocity may not provide a good index of the rate of cycling of cross bridges during imposed lengthening under these conditions. As noted above, the muscle contractility may also be affected by changes in contractile filament organization that occur in response to changes in muscle length (8, 11, 12, 15). Cytoskeletal proteins that are regulated independently of the cross-bridge cycle may also contribute to the mechanical properties of the muscle during length oscillation. Actin-binding proteins such as caldesmon and calponin as well as other cytoskeletal proteins (e.g., paxillin and talin) have been implicated in the regulation of smooth muscle contraction; however, the role of these proteins in contraction remains undetermined (2, 13–15, 18). Receptor-coupled pathways have been shown to modulate the phosphorylation and dephosphorylation of myosin light chains by Ca^{2+}-independent pathways in smooth muscle tissues (16); there is evidence that these pathways also differentially activate some of these cytoskeletal proteins (12, 19).

Conclusions. We studied the effects of pharmacological stimuli on the response of airway smooth muscle to low-frequency, large-amplitude (6–10% L_{0}) oscillations to evaluate the cellular mechanisms that play a role in determining the effects of changes in lung volume on airway smooth muscle contractility during breathing. Our results indicate that the shortening phase of length oscillation causes a depression of airway smooth muscle contractility that results from a mechanism that is independent of the stimulus used to activate the muscle. The normalized length-force hysteresis area or hysteresivity, differed under different conditions of stimulation, indicating that the behavior of the muscle during length oscillation is pharmacologically modulated. Differences in hysteresivity elicited under different pharmacological conditions were not correlated with differences in the isotonic shortening velocity, indicating that the effects of different stimuli on hysteresivity could not be accounted for by their effects on the cross-bridge cycling rate. Our results are consistent with the hypothesis that the behavior of airway smooth muscle during length oscillation is determined by a combination of cross-bridge and non-cross-bridge mechanisms.

We thank Dr. Richard Meiss for reviewing the manuscript. This work was supported by National Heart, Lung, and Blood Institute HL-29289 and HL-48522. Address for reprint requests: S. J. Gunst, Dept. of Physiology and Biophysics, Indiana University School of Medicine, 635 Barnhill Dr., Indianapolis, IN 46202-5120 (Email: sgunst@indyvax.iupui.edu).

Received 11 February 1997; accepted in final form 6 May 1997.

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