Airway Hyperresponsiveness: From Molecules to Bedside
Selected Contribution: Adaptation to chronic length change in explanted airway smooth muscle

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1Departments of Medicine and 2Department of Pathology and Laboratory Medicine, University of British Columbia, V6T 1Z3; and 3The McDonald Research Laboratories, The iCAPTURE Center, St. Paul’s Hospital, Providence Health Care, Vancouver, British Columbia, Canada V6Z 1Y6
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Naghshin, Jahanbakhsh, Lu Wang, Peter D. Paré, and Chun Y. Seow: Selected Contribution: Adaptation to chronic length change in explanted airway smooth muscle. J Appl Physiol 95: 448–453, 2003. First published March 7, 2003; 10.1152/japplphysiol.01180.2002.—It has been shown that airway smooth muscle in vitro is able to maintain active force over a large length range by adaptation in the absence of periodic stimulations at 4°C (Wang L, Paré PD, and Seow CY. J Appl Physiol 90: 734–740, 2001). In this study, we show that such adaptation also takes place at body temperature and that long-term adaptation results in irreversible functional change in the muscle that could lead to airway hyperresponsiveness. Rabbit tracheal muscle explants were passively maintained at shortened and in situ length for 3 and 7–8 days in culture media; the length-tension relationship was then examined. The length associated with maximal force generation decreased by 10.5 ± 3.8% (SE) after 3 days and 37.7 ± 8.5% after 7 or 8 days of passive shortening. At day 3, the left shift in the length-tension curve due to adaptation at short lengths was reversible by readapting the muscle at a longer length. The shift was, however, not completely reversible after 7 days. The results suggest that long-term adaptation of airway smooth muscle could lead to increased muscle stiffness and force-generating ability at short lengths. Under in vivo condition, this could translate into resistance to stretch-induced relaxation and excessive airway narrowing.

AIRWAY SMOOTH MUSCLE (ASM) plays an important role in controlling the caliber (and hence flow resistance) of the airways. The ability of ASM to narrow an airway depends, among other factors, on how much force the muscle is able to generate at a given length. It has been shown that both active and passive length-tension (L-T) relationships in ASM in vitro are not static; they can be shifted along the length axis by adapting the muscle to different lengths (18). Adaptation of ASM to short lengths, if it occurs in vivo, could contribute to airway hyperresponsiveness by 1) producing greater shortening due to the increased ability to generate active force (left shift in the active L-T relationship) and 2) resisting stretch-induced relaxation due to the higher resting tension and greater stiffness (left shift in the passive L-T relationship). There are a variety of conditions under which ASM in vivo could be adapted to pathologically short lengths, especially when inflammation of the airways is involved. In chronic asthma and chronic obstructive pulmonary disease, ASM shortening could happen due to airway wall remodeling and increase in the thickness of all airway wall layers (1–3, 5, 6, 9, 11, 12). In addition, the thicker adventitial layer could uncouple the smooth muscle from the stretching effect of lung tissue caused by deep inspiration and tidal breathing that have been shown to cause bronchodilation (4, 16). ASM shortening could also be a consequence of episodes of acute airway inflammation, as it occurs in acute asthmatic exacerbations and in infectious settings. In these situations, either edematous adventitial tissue or the release of a variety of local mediators could cause a sustained shortening of ASM.

Knowing that ASM in vitro is able to adapt to length changes by restructuring its contractile apparatus (13) and regain its ability to generate maximal force (17, 18), the consequence of prolonged shortening of ASM in vivo, although it has not yet been demonstrated, could be excessive airway narrowing. The importance of the dynamic L-T relationship in ASM is further investigated in the present study under conditions closer to that in vivo, with an emphasis on long-term length adaptation at body temperature and reversibility of the functional change that accompanies length adaptation.

METHODS

Tissue preparation. New Zealand White rabbits (2–4 mo old) were killed by intravenous injection of overdosed pentobarbital sodium. Tracheas were removed, and muscle strips were dissected from each trachea and randomly assigned as control (CTL) day 0 (CTL0), CTL day 3 (CTL3), CTL day 7 (CTL7), CTL day 8 (CTL8), passively shortened (PS) day 3 (PS3), PS day 7 (PS7), and PS day 8 (PS8). The day number...
refers to the number of days the preparations were to be incubated in culture medium before assessment of their mechanical properties. Some data from days 7 and 8 were grouped together; the reason for having day 7 and day 8 preparations was that the experiments were too long to be finished in a single day. Each strip was -2.2-2.5 mm wide, 2.0-3.5 mm long, 0.1-0.2 mm thick, with two cartilage bits attached at each end. Length of the muscle between the insertion points with the cartilage was measured in the intact rings and was designated as the in situ length (L_in_situ). For day 3, day 7, and day 8 preparations, each pair was dissected from adjacent tracheal rings with comparable L_in_situ. Dissection was performed with the tissues submerged in physiological saline solution (PSS; composition in mM: 118 NaCl, 5 KCl, 1.2 NaH2PO4, 22.5 NaHCO3, 2 MgSO4, 2 CaCl2, and 11.1 glucose).

The L-T curve of the CTL0 preparations was determined on day 0 immediately after finishing the dissection. The rest of the preparations were placed on sterilized wax. Under a dissecting microscope, CTL preparations (CTL3, CTL7, and CTL8) were set at their L_in_situ. PS preparations (PS3, PS7, and 11.1 glucose) were set at their reference length of the muscle preparation (L_ref) and were stretched to reach either the passive force (L_Pass) or the maximal active force (L_max). A high-K+ PSS (80 mM K+) was used to induce smooth muscle contraction in all experimental protocols, except in setting the initial PS lengths for PS preparations, where electrical stimulation was used. The average time to reach the plateau or F_max was between 450 and 550 s. Moreover, 2% of the maximal decay function (F_t) was used to determine the cross-sectional areas of the muscle preparations. All the preparations were embedded in paraffin wax and sectioned with H&E staining. Digital images were obtained, and Image Pro-Plus 3.0 software was used to measure the total cross-sectional area as well as smooth muscle area of the preparations. Smooth muscle area values were then used individually to normalize the measured F_max.

Data analysis. The active and passive forces as functions of muscle length were plotted to obtain the L-T curves for both the CTL and PS groups. A Weibull 4-parameter equation of peak function (Sigma Plot 5.0) was used to fit the active force data and to measure the F_max and L_max. Paired t-tests were used to compare the values between CTL and PS preparations. Smooth muscle area was measured and used to normalize the F_max values and calculate the maximal stress (in kPa). The values of maximal stress of CTL and PS groups were also compared with the paired t-test. To show how stretchable the preparations were after 3 and 7 (or 8) days of passive shortening, the L/CTL L_max (%) index was used, where L is the final length of the PS muscle being stretched. This index indicates the length of PS preparations as a percentage of CTL L_max at which the rate and extent of tension recovery were measured. The poststretch maximal active force and the corresponding passive force were compared with the maximal prestretch active force (i.e., F_max) and the corresponding passive force (i.e., passive force at L_max) of PS preparations, respectively. A single three-parameter exponential rise-to-maximum function (F = y0 + a (1 - e^(-bt)) where b represents the rate of recovery, and y0 and a are constants) was used because it provided the best fit for the active force recovery. A single three-parameter exponential decay function (F = y0 + ae^(-bt)) was used to fit the passive force recovery (relaxation). With the use of a paired t-test, the active and passive force recovery rates of days 3 and 7 (or 8) PS preparations were compared with those of freshly dissected muscle preparations. To examine the effect of periodic stimulation on passive force recovery, a comparison was made between the passive force recovery rates obtained in the presence and absence of periodic stimulation. For all statistical tests, P > 0.05 was not considered statistically significant.
RESULTS

L-T measurements. The \( L_{\text{in situ}} \) values of day 0, day 3, and day 7/8 groups were not significantly different \((P > 0.20; \text{Table 1})\). However, the mean \( L_{\max} \) \((2.77 \pm 0.14 \text{ mm})\) for CTL3 was significantly greater than the \( L_{\max} \) for PS3 preparations \((2.48 \pm 0.17 \text{ mm})\) \((P = 0.025)\). The \( L_{\max} \) of CTL7/8 and PS7/8 preparations were \( 2.47 \pm 0.14 \) and \( 1.54 \pm 0.20 \) mm, respectively, and they were also significantly different \((P = 0.0039; \text{Table 1})\). In brief, after 3 and 7/8 days of PS, \( L_{\max} \) decreased by 10.5 \pm 3.8 and 37.7 \pm 8.5%, respectively, compared with the corresponding CTL preparations.

The isometric stress of PS7/8 preparations was significantly lower than that of the CTL7/8 preparations.

Tension recovery measurement. The mean and standard error of \( L/(\text{CTL } L_{\max}) \) (%) for day 3 \((n = 6)\) and day 7/8 \((n = 14)\) of PS preparations were 99.9 \pm 1.2 and 87.2 \pm 3.7%, respectively \((\text{Table 2})\). A t-test \((P < 0.05)\) showed a significant difference between the two groups. It was observed that on day 3, after the PS preparations were stretched to the predetermined length \( L_{\max} \), the active force of the first contraction was very close to the maximal adapted \( L_{\max} \), and the recovery rates were measured by fitting the data with exponential equations described in the text. Numbers beside the symbols in A and B indicate the time sequence of contractions.

Table 1. \( L_{\text{in situ}}, L_{\max}, \) and \( \sigma_{\max} \) of the muscle preparations

<table>
<thead>
<tr>
<th></th>
<th>CTL Day 0 ((n = 14))</th>
<th>CTL Day 3 ((n = 6))</th>
<th>PS Day 3 ((n = 6))</th>
<th>CTL Day 7/8 ((n = 14))</th>
<th>PS Day 7/8 ((n = 14))</th>
</tr>
</thead>
<tbody>
<tr>
<td>( L_{\text{in situ}}, \text{ mm} )</td>
<td>2.60 \pm 0.19</td>
<td>2.87 \pm 0.09</td>
<td>2.87 \pm 0.09</td>
<td>2.73 \pm 0.18</td>
<td>2.73 \pm 0.18</td>
</tr>
<tr>
<td>( L_{\max}, \text{ mm} )</td>
<td>2.28 \pm 0.29</td>
<td>2.77 \pm 0.14</td>
<td>2.48 \pm 0.17*</td>
<td>2.47 \pm 0.14</td>
<td>1.54 \pm 0.20*</td>
</tr>
<tr>
<td>( \sigma_{\max}, \text{ kPa} )</td>
<td>57.7 \pm 7.5</td>
<td>66.1 \pm 8.9</td>
<td>62.0 \pm 5.7</td>
<td>42.0 \pm 9.6</td>
<td>25.3 \pm 4.7*</td>
</tr>
</tbody>
</table>

Values are means \pm \text{ SE}. \( L_{\text{in situ}} \) in situ length; \( L_{\max} \), length associated with maximal force generation; \( \sigma_{\max} \), maximal stress; CTL, control; PS, passively shortened. *Statistically significant difference between the PS group and their respective CTL \((P < 0.05)\).
ness of $L_{\text{max}}$ values of CTL and PS preparations (and hence a small stretch required). As shown in Table 2, on day 3, the poststretch maximal adapted active force reached the same level as the prestretch active force. As in active force, recovery in passive force after 3 days of passive shortening was also complete. Unlike the behavior observed on day 3, it was found that in most cases the PS preparations on day 7/8 were either too stiff to be stretched to their CTL $L_{\text{max}}$ and that at the stretched length the active force could not recover to the prestretch value (Table 2). The rates of passive force recovery between day 3 and day 7/8 PS preparations (after stretch) were compared. The rates were not different ($P = 0.381$).

**Force recovery in the presence and absence of stimulation.** The initial passive forces at the beginning of the recovery measurement (Fig. 2) were not different between the group subjected to periodic stimulation and the group that was not stimulated ($P = 0.25, n = 8$). The rate of passive force recovery ($b$ value) was $0.063 \pm 0.007 \text{ min}^{-1}$ for the group with stimulation and $0.074 \pm 0.014 \text{ min}^{-1}$ for the group without stimulation. These rates were not significantly different. In Fig. 3A, the active force was measured periodically after a stretch of the muscle (with a protocol similar to that described for Fig. 1C). Results from eight experiments with muscle preparations from eight animals were combined. The equation used for curve fitting was the same as that described for Fig. 1C. In Fig. 3B, the muscle was not stimulated for ~2 h after the stretch, but at the end of the “resting” period, the muscle was stimulated periodically as that in Fig. 3A, and the recovery of active force was plotted. The starting level of active force for the group shown in Fig. 3B was $93.4 \pm 1.4\%$ of the maximum value. In contrast, this average starting value was achieved in the group shown in Fig. 3A by the fourth contraction.

**Freshly dissected vs. chronically adapted ASM.** As shown in Table 3, the rate of active force recovery was $0.081 \pm 0.008 \text{ min}^{-1}$ for the PS group on day 7/8 during readaptation to longer lengths and $0.176 \pm 0.014 \text{ min}^{-1}$ for the group subjected to periodic stimulation during readaptation to longer lengths.

### Table 2. Pre- and poststretch values of active and passive forces obtained from the PS day 3 and day 7/8 ASM preparations

<table>
<thead>
<tr>
<th></th>
<th>Day 3</th>
<th></th>
<th>Day 7/8</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Prestretch</td>
<td>Poststretch</td>
<td>Prestretch</td>
<td>Poststretch</td>
</tr>
<tr>
<td>Active force, mN</td>
<td>$10.38 \pm 1.73$</td>
<td>$11.25 \pm 1.58$</td>
<td>$6.51 \pm 0.84$</td>
<td>$5.58 \pm 0.67^*$</td>
</tr>
<tr>
<td>Passive force, mN</td>
<td>$1.34 \pm 0.24^*$</td>
<td>$0.84 \pm 0.26^*$</td>
<td>$1.55 \pm 0.19$</td>
<td>$4.19 \pm 0.81^*$</td>
</tr>
<tr>
<td>$L/(\text{CTL } L_{\text{max}})$, %</td>
<td>$99.9 \pm 3.0 (n = 6)$</td>
<td>$87.2 \pm 3.8 (n = 14)^{\dagger}$</td>
<td></td>
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</table>

Values are means $\pm$ SE. ASM, airway smooth muscle; $L/(\text{CTL } L_{\text{max}})$, index where $L$ is the final length of the PS muscle being stretched. *Statistically significant difference from respective prestretch values. †Statistically significant difference between day 3 and day 7 values.

![Fig. 2. Passive force decline as a function of time in ASM preparations after 7–8 days of length adaptation. Solid line and ● describe the time course of decline in passive force when the muscle was activated periodically (5 stimulations over a period of 120 min). Dashed line and ○ describe the time course of decline in passive force when the muscle was left unstimulated.](image)

![Fig. 3. Time course of active force recovery after a length change. The force values are expressed as fractions of maximal (plateau) active force (pooled data, $n = 8$). A: active force of PS preparations of day 7 and day 8 as a function of time measured immediately after a step increase in length. B: active force of PS preparations of day 7 and day 8 as a function of time measured with a 2-h delay after a step increase in length. The muscle was not stimulated during the delay period.](image)
Table 3. Rates of active and passive force recovery after stretching preshortedened muscles

<table>
<thead>
<tr>
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<th>Day 0 (Acutely Shortened)</th>
<th>Day 7/8 (Chronically Shortened)</th>
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<tbody>
<tr>
<td>Active force rate, min⁻¹</td>
<td>0.176 ± 0.029* (n = 5)</td>
<td>0.081 ± 0.008 (n = 14)</td>
</tr>
<tr>
<td>Passive force rate, min⁻¹</td>
<td>0.046 ± 0.006</td>
<td>0.068 ± 0.008</td>
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</table>

Values are means ± SE. Note: the rate of force recovery is taken as the b value (see text) of the exponential fit. *Statistically significant difference between day 0 and day 7/8 groups.

0.029 min⁻¹ for the freshly dissected smooth muscle preparations during readaptation to longer lengths. The difference in the rates was statistically significant (P < 0.05). The passive force recovery rates were 0.068 ± 0.008 and 0.046 ± 0.006 min⁻¹ for PS7/8 and the freshly prepared smooth muscle preparations, respectively (Table 3). The difference in the rates was not significant (P > 0.05). The passive force at the beginning of the force measurements was 13.42 ± 1.58 mN for day 7/8 and 13.82 ± 1.26 mN for freshly prepared ASM. There was no significant difference between them. In the first poststretch contraction, the active force was only 11.6 ± 6.1% of the prestretch value for the group of day 7/8. However, with the use of the same stretching protocol, the active force was significantly greater (39.2 ± 5.3% of the prestretch value) for the freshly dissected ASM preparations. The ability to generate force in fresh preparations, therefore, was less affected by the length change compared with the chronically shortened preparations.

DISCUSSION

We have found in this study that rabbit ASM is able to adapt to chronic shortening at body temperature in the absence of periodic stimulation by changing its L-T relationship (leftward shift); the magnitude of the shift in the L-T curve due to length adaptation increased with time even though the resting (shortened) length remained the same during the period of adaptation. An important implication of these findings is that prolonged shortening of ASM in vivo could change its mechanical properties and enhance its ability to shorten, leading to excessive narrowing of the airways and the associated airway hyperresponsiveness. It should be pointed out, however, that the long-term structural and functional changes found in explanted tissue might not be the same as those that would occur under identical conditions in living tissue in situ.

Possible mechanism producing shifts in the L-T relationship. The plastic behavior of ASM has been attributed to a restructuring of the muscle’s contractile apparatus (13) and cytoskeleton (7, 8), and, it is likely, the underlying mechanism for the observed shifts in L-T relationship. The initiator of the plastic restructuring is often a large change in muscle cell length that cannot be accommodated by sliding of the contractile filaments. The length-perturbation-induced loss of contractile force has been shown to be associated with a decrease in the myosin thick-filament density (10). Previous studies (14, 17) suggest that periodic stimulation accelerates the process of force recovery and also that phosphorylation of the myosin regulatory light chain facilitates formation of myosin thick filaments.

Consequence of a left shift in L-T relationship. When an airway narrows due to contraction of the encircling smooth muscle cells, tension generated in the muscle normally decreases, following the L-T curve, as the muscle cell length decreases. Shortening would stop when the force generated by the muscle is counterbalanced by forces that oppose the shortening, and it therefore prevents excessive narrowing of the airway. If the muscle begins to shorten, leading to excessive narrowing of the airway. If the muscle shortens at a different rate than the airway, then a stretch test will result in the shortening of ASM in vivo could change its mechanical properties and enhance its ability to shorten, leading to excessive narrowing of the airways and the associated airway hyperresponsiveness. It should be pointed out, however, that the long-term structural and functional changes found in explanted tissue might not be the same as those that would occur under identical conditions in living tissue in situ.

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PS7 preparations. It appears that the ability of the muscle to rapidly adapt to length change is partially impaired in the day 7 preparations. The rate of passive force recovery is, however, not different between cultured (PS7/8) and freshly isolated (CTL0) ASM preparations (Table 3). The difference between the time course and characteristics of active and passive force recovery suggest that these two processes may be governed by different mechanisms.

Time dependent change in L-T relationship. When incubated under the same condition, the PS ASM preparations produced different degrees of shifting in the L-T relationship depending on the incubation time; the longer the preparations were incubated the larger the shifts, even though the preparations were set at the same length during the incubation period (Table 1). For the day 3 preparation, the shift in \( L_{\text{max}} \) was \(-10\%\), even though the muscle length was kept at 50–60% of its \( L_{\text{in situ}} \) the whole time during incubation. This suggests that either the adaptation to a 40–50% length shortening was not completed after 3 days or the adaptation was not permanent and that some of the adapted changes were partially reversed during the measurement. The shift in \( L_{\text{max}} \) after 7 or 8 days was \(-38\%\), close to the 40–50% length change imposed on the muscle during the incubation period. This suggests that, after 7/8 days, adaptation was nearly complete.

Readaptation of shortened muscles to longer lengths also revealed time-dependent change in the L-T relationship in terms of its permanence. After 3 days of adaptation at short lengths, the muscle could be restretched to its CTL \( L_{\text{max}} \) where full recovery of isometric force could be achieved (Table 2). On the other hand, after 7 or 8 days of adaptation, most of the preparations became so stiff that it was not possible to restretch them to their CTL \( L_{\text{max}} \). Something more permanent appears to have occurred in the process of restructuring during the period of adaptation. It should be pointed out that the irreversibility of the PS7/8 preparations evidenced in short-term (hours) readaptation to longer lengths may not indicate a permanent condition; it is possible that long-term (days) readaptation could reverse the apparent permanent structural change.

These results suggest that if permanent adaptation to short lengths is to be avoided in ASM, prolonged shortening of the muscle either passively or actively due to stimulation by inflammatory mediators should be avoided. Brief episodes of muscle contraction or passive shortening up to 3 days appear to have no permanent effect on the normal structure and function of ASM.

In conclusion, the acute and subacute changes in the L-T curve (up to 3 days of adaptation) are relatively impermanent in that the L-T behavior appears to be plastic over this time period. The finding of a more striking and less reversible change after a prolonged period of length adaptation may be relevant to disease states in which exaggerated airway narrowing is partially or completely irreversible.

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REFERENCES