Plasticity in Skeletal, Cardiac, and Smooth Muscle

Selected Contribution: Effect of chronic passive length change on airway smooth muscle length-tension relationship

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Wang, Lu, Peter D. Pare´, and Chun Y. Seow. Selected Contribution: Effect of chronic passive length change on airway smooth muscle length-tension relationship. J Appl Physiol 90: 734–740, 2001.—The ability of rabbit trachealis to undergo plastic adaptation to chronic shortening or lengthening was assessed by setting the muscle preparations at three lengths for 24 h in relaxed state: a reference length in which applied force was 1–2% of maximal active force ($P_o$) and lengths considerably shorter and longer than the reference. Passive and active length-tension ($L$-$T$) curves for the preparations were then obtained by electrical field stimulation at progressively increasing muscle length. Classically shaped $L$-$T$ curves were obtained with a distinct optimal length ($L_o$) at which $P_o$ developed; however, both the active and passive $L$-$T$ curves were shifted, whereas $P_o$ remained unchanged. $L_o$ was 72% and 148% that of the reference preparations for the passively shortened and lengthened muscles, respectively. The results suggest that chronic narrowing of the airways could induce a shift in the $L$-$T$ relationship of smooth muscle, resulting in a maintained potential for maximal force production.

rabbit trachealis; isometric contraction; tension recovery; plasticity

The dependence of isometric force on muscle length is thought to be governed mainly by the amount of overlap between myosin thick filaments and actin thin filaments. Although this is true for striated muscle, recent experiments have suggested that a different mechanism may underlie the length-tension ($L$-$T$) relationship in smooth muscle (3, 9). The ability of smooth muscle to adapt to length change confers a broad plateau on the $L$-$T$ curve. Harris and Warshaw (4) found that, in single isolated smooth muscle cells, the $L$-$T$ curve for the preparation shifts according to the initial length of the muscle, if the initial length is set when the muscle is at rest. The notion of optimal length ($L_o$) in such an $L$-$T$ curve is meaningless because the length at which optimal tension ($P_o$) occurs will shift depending on the length at which the muscle is adapted. Shifting of the $L$-$T$ curve (or $L_o$) in response to chronic passive shortening has been described in skeletal muscle such as the diaphragm in emphysema- tous hamsters (2, 7). The diaphragm muscle overcomes the disadvantage of the short working length because of emphysema by deleting some of the sarcomeres in series; this enables the thick and thin filaments in the muscle to achieve optimal overlap. The mechanism for such adaptation in skeletal muscle is probably different from that in smooth muscle in which rearrangement of the contractile filaments occurs within a relatively short period of time. Smooth muscle normally functions over a large length range; rapid onset of adaptation (plasticity) is therefore required. Our previous finding suggests that the recovery of isometric tension from length oscillation (which has disrupted filament organization) is complete within 30 min (12). The rate of recovery, however, depends on the frequency of activation; that is, the more frequently the muscle is stimulated, the faster the recovery. A similar observation was described for skeletal muscle stimulated periodically at short lengths (6). Adaptation of skeletal muscle to chronic shortening occurs by deleting sarcomeres in series. The process of adaptation is...
initiated by both a change in muscle length and repeated electrical stimulations. Adaptation occurs slowly (if at all) in unstimulated preparations set at short lengths in skeletal muscle (6). In rat soleus muscle, a period of 12 h of repeated stimulation is required for the adaptation process to complete (6). This is much longer than the 30 min required for airway smooth muscle stimulated at 5-min intervals (12).

An unanswered question is whether length adaptation occurs in the absence of stimulation, and, if it occurs, what is the minimal period of time required for the adaptation process to complete. The present study addresses these questions.

In patients who have chronic airway diseases such as asthma and chronic obstructive pulmonary diseases (COPD), prolonged active and passive shortening of smooth muscle contributes to airway narrowing. If adaptation of the muscle’s L-T relationship to shorter length occurs under these circumstances, the ability of the smooth muscle to narrow the airways could be enhanced. The present study therefore addresses not only mechanisms of normal airway smooth muscle function but also mechanisms that may underlie pathological conditions of asthmatic or COPD airways.

**METHODS**

**Tissue preparation.** A total of 12 rabbits (New Zealand White, 2–4 mo) were used for this study, 3 for each of the subprojects: 1) comparison between passively shortened and control, 2) comparison between passively lengthened and control, 3) tension recovery of control preparations after length oscillation, and 4) tension recovery of passively shortened preparations. Tracheas were removed from rabbits immediately after the animals were euthanized with CO2 asphyxiation. The trachea was immersed in physiological saline at 4°C (composition in mM: 118 NaCl, 5 KCl, 1.2 NaH2PO4, 22.5 NaHCO3, 2 MgSO4, 2 CaCl2, and 11.1 glucose). The solution was aerated with 95% O2-5% CO2 to maintain a pH of 7.4. The trachea was then dissected free of connective tissue. Four specimens (each containing 2 cartilage rings, ~2.5–3 mm wide) were obtained from the central region of the trachea. The entire length (in situ) of the tracheal smooth muscle layer between the tips of cartilage in each double ring was measured while the cartilage rings were still intact. The cartilage ring was then cut open, leaving the smooth muscle preparation attached to cartilage at both ends. The epithelial layer and most of the connective tissue were removed from the smooth muscle layer. Aluminum foil clips were placed on the cartilage ends, precisely along the insertion points of the smooth muscle so that the distance between the edges of the clips was the entire length of the smooth muscle preparation. The dissected specimens were placed on wax at the bottom of a plastic container and covered in physiological saline solution. Pins were inserted through the aluminum clips to set the length of the smooth muscle. From each trachea, two specimens were used as control samples, i.e., the length of the smooth muscle was set at a reference length in which applied force was just enough to keep the muscle straight; another two specimens were either passively shortened or passively lengthened. To passively shorten a specimen, one end of the specimen was fixed by a pin and the other end of the specimen was allowed to be free of constraint. To passively lengthen a specimen, pins were placed such that the smooth muscle length was stretched to the in situ length. The prepared specimens were kept at 4°C in the physiological saline for 24 h before being mounted vertically in a tissue bath with one end fixed to a stationary hook and the other to a servo-controlled lever of a length-force transducer. The tissue bath contained physiological saline solution at 37°C that was bubbled with a 95% O2-5% CO2 mixture. The initial lengths of the muscle preparations were chosen to be a length at which there was no buckle or visible slacking of the preparations and at which the passive force was zero. The muscle preparation was equilibrated for 1–1.5 h at the initial length. During this time, the muscle was activated by electrical field stimulation (EFS) at 5-min intervals using a 60-Hz AC stimulator with platinum electrodes.

**L-T measurements.** After reaching equilibration at their initial length, the muscle preparations were stretched by 0.25-mm increments at 5-min intervals. An isometric contraction was elicited after each stretch. There was a 1-min delay between the stretch and stimulation to allow the passive tension to stabilize before activation. Both passive and plateau active forces were measured during each contraction. The stretches continued until the active force passed a maximum and declined to approximately the value at the initial length.

**Tension recovery.** To examine the rate and extent of tension recovery of passively shortened preparations, two groups of muscle preparations were set passively for 24 h at two lengths: the reference and the shortened length. First, an L-T curve for the preparation set at the reference length (first group) was obtained by stretching the muscle incrementally with 0.25-mm-length steps. A partial L-T curve for the shortened preparation (second group) was then obtained by stretching the muscle incrementally until it reached the L0 of the reference L-T curve (obtained in the first group). At this length, the preshorted muscle was on the descending limb of its L-T curve. While the muscle was maintained at this length, a series of isometric contractions was elicited at 5-min intervals to allow the muscle to adapt, and the active and passive tensions were recorded. The rate of tension recovery (or reversal of chronic adaptation) of the preshorted muscle was compared with the rate of tension recovery of a muscle subjected to a brief period of length oscillation at reference length (acute adaptation). The oscillation protocol was adopted from our previous study (12) to induce a temporary decrease in the potential for generating active tension, so the time course of tension recovery could be examined.

**Correction for system compliance.** The compliance of the lever system itself is negligible, but the surgical silk that connected the muscle to the lever system had a small but significant compliance. The total system compliance (lever plus silk) was obtained by applying calibrated forces to the system and the silk thread without a muscle preparation and recording the length changes. The system compliance was then subtracted from the measured muscle compliance to obtain the correct length of the muscle preparations and the changes in length applied to the muscle.

**Data analysis.** Both active and passive tensions were analyzed in relation to the length of the muscle preparations. A Weibull four-parameter function (SigmaPlot 5.0) was found to best fit the active force data, and the peak value was obtained from the fitted curve and taken as the maximal active force (Po). L0 was determined as the length at which Po occurred. Because the control and passively shortened preparations were from the same animals, paired t-test was used to compare the calculated L0. Paired t-test was also used to compare L0 between control and passively lengthened preparations. One-way ANOVA was used to compare the L0 of the
two control groups as well as the two passively altered groups. The time course of active force recovery was fitted to a two-parameter exponential growth equation, \( y = a(1 - e^{-bx}) \), where \( a \) and \( b \) are constants. The passive L-T curves were fitted with a two-component exponential decay function, \( y = a(e^{-bx}) + c(e^{-dx}) \), where \( a, b, c, \) and \( d \) are constants. For all statistical tests employed, \( P > 0.05 \) was not considered statistically significant. In each case, \( n \) is the number of muscle preparations used.

RESULTS

Length-tension measurements and \( L_o \). The Weibull curve fit for all active force data yielded \( r^2 \) values between 0.96 and 0.99. The \( P_o \) values measured from the control and test preparations from the same trachea were similar. The mean and SD of \( P_o \) for the control preparations was 19.8 ± 2.5 mN. The estimated mean cross-sectional area of each preparation was ~0.3–0.5 mm². It was found that the \( L_o \) of passively shortened preparations was shifted to lengths shorter than the reference length from the same animal, and the \( L_o \) of passively lengthened preparations shifted to longer lengths. An example is shown in Fig. 1. Figure 1A shows a pair of muscle preparations adjacent to each other from the same trachea. One preparation was used as the control (set at the reference length), and the other was passively shortened for 24 h. Figure 1B shows a pair of muscle preparations obtained adjacent to each other from another trachea. One preparation was the control, and the other was passively lengthened for 24 h. Figure 1, top, shows that \( P_o \) values of these preparations were similar, and the lengths at which the \( P_o \) occurred were clearly different (passively shortened: 1.00 mm, control: 1.58 mm; passively lengthened: 2.43 mm, control: 1.64 mm). The same shift was repeated in the passive force measurements, which are shown in Fig. 1, bottom. The passive force remained at a level close to zero and increased at lengths longer than \( L_o \). The passive force curve of the shortened preparation shifted to the left of its control, and the lengthened preparation shifted to the right. The amount of shift in passive force was consistent with that found in the active force. The means and SD of the \( L_o \) measurements are shown in Fig. 2. On average, the \( L_o \) values for passively shortened preparations were 28% shorter than the control. The preparations that were stretched to the in situ length for 24 h had an average \( L_o \) that was ~48% shorter than the control.

![Fig. 1. Length-tension curves of rabbit trachealis. A: comparison between a passively shortened muscle preparation (○) and a reference preparation (●) from 1 trachea. B: comparison between a passively lengthened preparation (○) and reference preparation (●) from another trachea. Top: active force; circles are measured force at various lengths, and lines are the Weibull 4-parameter function fitted to data. Bottom: passive force at various lengths. See text for definition of reference length.](image)

![Fig. 2. Means and SE (n = 6 muscle preparations) of the measured optimal length (\( L_o \)). Passively shortened and control (PS) preparations were from the same trachea. Passively lengthened and control (PL) preparations were from the same trachea.](image)
Tension recovery. Passively shortened preparations were stretched to $L_o$ of their paired controls. Active force increased with repeated stimulation at 5-min intervals. An example is shown in Fig. 3. The active $L$-$T$ curve of the passively shortened preparation shifted to the left of the control (Fig. 3A). When the preparation was allowed to adapt at $L_o$ of its paired control, the active force increased with time (Fig. 3). The measured isometric force from each contraction was converted to a fraction of total recovery. The means ($n = 6$ for the first 7 points and $n = 5$ for the last 3 points) and SE of the calculated fraction of recovery as well as the exponential fit are shown in Fig. 4. The level of active force at the start of recovery was 16.1 ± 6.5 mN (mean and SD). It took ~45–50 min for the active force to reach a plateau. The fully recovered level was 22.7 ± 10.8 mN (mean and SD). A two-parameter exponential growth function, $y = a(1 - e^{-bx})$, was found to best fit the time course of the active force, where $y$ was the percentage of total recovery and $x$ was time. The $r^2$ values of the fit ranged between 0.997 and 0.999. The $b$ constant indicates the rate of recovery and was found to be 0.11 ± 0.04 (s$^{-1}$). At the plateau, the recovered active force (22.7 ± 10.8 mN) exceeded the $P_o$ of the paired controls (19.6 ± 9.9 mN). Paired $t$-tests showed that this difference was significant ($P = 0.029$).

After length oscillation, the active force of the control preparations decreased by ~10–15% and then recovered fully in 25–30 min (Fig. 4). The rate of recovery (6) is 0.27 ± 0.05. This rate of recovery is significantly higher ($P = 0.0004$) than that of the passively shortened preparations.

Passive force, as seen in Figs. 5 and 6, also adapted to length change. An example is shown in Fig. 5. The passive $L$-$T$ curve of the preshortened preparation shifted to the left (Fig. 5A). Whereas the active force increased during adaptation at $L_o$, the passive force decreased toward zero (Fig. 5, A and B). The means ($n = 6$ for the first 7 points and $n = 5$ for the last 3 points) and SE of the calculated fraction of recovery as well as the exponential decay fit are shown in Fig. 6. The passive force at the start of recovery was 4.5 ± 2.1 (SD) mN. Passive force decreased with each stimulation. When the active force was fully recovered, the passive force was 1.7 ± 0.4 (SD) mN; this value is significantly higher than the passive force of the control at $L_o$, which is virtually zero. A two-component exponential decay function, $y = a(e^{-bx}) + c(e^{-dx})$, was found to best fit the time course of the passive force decline. The $r^2$ values ranged between 0.998 and 0.999. The $b$ value, a measure of the rate of decay of the first component, was found to be 0.43 ± 0.36 (mean ± SD). The $d$ value, the rate of decay of the second component, was found to be 0.07 ± 0.05 (mean ± SD).

**DISCUSSION**

Adaptation to length change in unstimulated muscles. The present finding of a bidirectional shift in the $L$-$T$ curve of unstimulated airway smooth muscle implies that the subcellular components governing the
The shift in the passive $L$-$T$ curve shown in Fig. 1 is as drastic as the active $L$-$T$ curve, and the physiological implication is just as profound. Passive tension is thought to be determined by the amount of stretch the muscle preparation is subjected to. With stress relaxation (due to the viscous property of the muscle preparation), it is expected that the passive $L$-$T$ curve will change with time. The change in passive tension observed (Fig. 1) in the passively adapted muscle preparations is enormous, and this raises a question of whether the change is all because of passive stress relaxation. It is likely that restructuring of the cytoskeleton and/or the extracellular scaffolding filaments are involved in the process of adaptation. The possibility of cytoskeletal rearrangement in airway smooth muscle contraction has been suggested by Gunst et al. (3). The diameter of an airway is largely determined by the airway muscle tone, whether the tone is passive or active. Results from this study indicate that the “tone” is critically influenced by the length at which the muscle is adapted. Take Fig. 1 as an example: a muscle preparation adapted at a long length has virtually no muscle tone unless it is stretched beyond 2.5 mm (Fig. 1B, bottom). A similar preparation adapted at a short length shows a great amount of tone when it is stretched just beyond 1 mm! It is not difficult to imagine how difficult it would be to distend a narrowed airway with an adapted muscle layer just to overcome the passive tension.

**Adaptation and tension recovery.** Whereas the shift in the $L$-$T$ relationship occurs slowly in relaxed muscle at 4°C, repeated activation at 37°C greatly speeds up the process, as shown in Figs. 3 and 4; a new $P_o$ (with the associated $L_o$) is achieved in $<30$ min after an acute length perturbation. Our previous study (12) indicates that the frequency of muscle activation is positively correlated to the rate of tension recovery after a length perturbation.
The active tension recovery that occurs when the muscle is allowed to adapt at a constant length (Fig. 3A) follows a monoexponential process (Fig. 3B). We have previously observed a similar process of tension recovery in swine trachealis after length perturbation (12). The rate of tension recovery from acute length perturbation \((b = 0.27)\) is faster than the rate of recovery from chronic adaptation \((b = 0.11)\). This is likely due to the possibility that chronic adaptation may have resulted in a more permanent structural alteration that renders reorganization of the subcellular components a slower process.

Recovery of active tension shown in Fig. 3 always surpasses the \(P_o\) values of the \(L-T\) curves. This is expected because the \(P_o\) values associated with the \(L-T\) curves were not obtained in the adapted state. If we allow the muscle to adapt, no distinct peak tension \((P_o)\) could be identified. To obtain \(L-T\) curves with narrow peaks, such as the ones shown in Figs. 1 and 3, it is necessary to carry out the measurements with minimal recovery (or adaptation) occurring in the muscle.

Adaptation occurs in passive tension as well. The passive \(L-T\) curves shown in Fig. 5A are from the same muscle preparation that produced the active \(L-T\) curve shown in Fig. 3A. As active tension increases with each activation, passive tension decreases. The time course of passive tension decline is best fitted by a two-term exponential function (Fig. 5B), unlike the monoexponential fit for active tension recovery. This implies that the underlying mechanisms for the two processes are likely different. Part of the passive tension “recovery” likely involves stress relaxation from a pure passive structure and part of it from an active component that may involve restructuring of the cytoskeleton of the muscle cells. These may contribute to the mixed exponential decay of passive tension during the adaptation process. The recovery of passive tension in the chronically shortened preparation is not complete (Figs. 5 and 6); that is, passive tension does not reach the control level, at least within the time frame of the experiment. The averaged passive tension after 45 min of adaptation reaches a value of 1.72 mN; the reference value at the same length is 0.69 mN. Chronic shortening therefore seems to have left a long-lasting “tone” in the muscle.

\(L-T\) curve shift and implications in asthma and COPD. Chronic inflammation and the associated mediator and cytokines release seen in asthmatic and COPD patients result in a chronically narrowed airway due to smooth muscle activation and shortening. In addition, passive shortening of smooth muscle may occur in these conditions because of loss of lung elastic recoil and airway wall edema as well as airway wall edema that effectively uncouples the muscle from lung recoil. Our in vitro results suggest that smooth muscle cells in chronically constricted airways may adapt to an abnormally short length. A direct mechanical consequence of this adaptation is the shifting of the \(L-T\) relationship, which in turn results in a maintained maximal force production and passive tension. According to Laplace law, the level of wall tension required to maintain a constant transmural pressure is proportional to the radius of the airway. In a constricted airway, less wall tension is therefore needed to maintain the same pressure. With muscle adaptation, the same maximal force produced by muscle at normal length can be generated by shortened muscle. This could create a catastrophic situation in which chronically narrowed airways can further constrict due to the mismatch of maximal force and the caliber of the airways. The shifting of the passive \(L-T\) curve further exacerbates the situation. Even without smooth muscle contraction, the leftward shift of the passive \(L-T\) curve in the shortened muscle makes distension of the airways more difficult.

Relation to early studies. When skeletal muscle is chronically lengthened or shortened it undergoes remodeling so that the length at which \(P_o\) is generated shifts to longer or shorter lengths, respectively (2, 7, 13). Skeletal muscle has been shown to achieve this adaptation by adjusting the number of sarcomeres in series in response to chronic static length changes (1, 5, 6, 8, 10, 11, 13). Marked changes occur in the size and shape of hollow organs that contain smooth muscle in certain physiological conditions (the uterus during and after pregnancy) and in pathological states (the bladder during chronic outflow obstruction). Smooth muscles have likely evolved a mechanism similar to that for skeletal muscle to accommodate large changes in length. It seems likely that smooth muscle has a series arrangement of identical contractile units (9). We have found in the present study that the maximal force generated by smooth muscle is independent of muscle length, similar to that found for canine trachealis (9). This suggests that, although there is variation in the number of contractile units in series due to chronic shortening or lengthening, manifested as shifts in \(L_o\), the individual filament pairs are kept at their optimal overlap. This is similar to the situation in skeletal muscle in which the number of sarcomeres is adjusted to preserve the \(L-T\) relationship of individual sarcomere but shifting the whole muscle \(L-T\) relationship.

There are similarities between the present study and the above-mentioned studies in both smooth and skeletal muscles. The major difference is that the present study has used a protocol in which the muscle is adapted passively, that is, without periodic stimulation.

In conclusion, passive adaptation of airway smooth muscle to length change has resulted in a shift in the muscle’s \(L-T\) curve similar to that observed in active adaptation in which the muscle is subjected to periodic stimulation. The time required for completion of the adaptation is, however, greatly increased in the passive process. The shift in the \(L-T\) curve produced in a 24-h passive adaptation of the shortened muscles does not appear to be permanent; it can be reversed in ~50 min by allowing the muscle to readapt at a longer length with periodic stimulation.

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