Mechanism of partial adaptation in airway smooth muscle after a step change in length

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Ali F, Chin L, Paré PD, Seow CY. Mechanism of partial adaptation in airway smooth muscle after a step change in length. J Appl Physiol 103: 569–577, 2007. First published May 10, 2007; doi:10.1152/japplphysiol.00216.2007.—The phenomenon of length adaptation in airway smooth muscle (ASM) is well documented; however, the underlying mechanism is less clear. Evidence to date suggests that the adaptation involves reassembly of contractile filaments, leading to reconfiguration of the actin filament lattice and polymerization or depolymerization of the myosin filaments within the lattice. The time courses for these events are unknown. To gain insights into the adaptation process, we examined ASM mechanical properties and ultrastructural changes during adaptation. Step changes in length were applied to isolated bundles of ASM cells; changes in force, shortening velocity, and myosin filament mass were then quantified. A greater decrease in force was found following an acute decrease in length, compared with that of an acute increase in length. A decrease in myosin filament mass was also found with an acute decrease in length. The shortening velocity measured immediately after the length change was the same as that measured after the muscle had fully adapted to the new length. These observations can be explained by a model in which partial adaptation of the muscle leads to an intermediate state in which reconfiguration of the myofilament lattice occurred rapidly, followed by a relatively slow process of polymerization of myosin filaments within the lattice. The partially adapted intermediate state is perhaps more physiologically relevant than the fully adapted state seen under static conditions, and it simulates a more realistic behavior for ASM in vivo.

The current theories that have been proposed to explain the adaptive behavior of smooth muscle focus on two structural domains of the cell: the cytoskeleton and the contractile apparatus. The cytoskeleton appears to be malleable (28). That is, it can be shaped plastically by external forces (3, 4, 6, 21). The deformation appears to involve rearrangement and reattachment of protein filaments (mostly actin filaments) within the cytoskeletal filament network (7, 8, 26, 37). Plastic deformation of the contractile apparatus appears to involve addition and deletion of contractile units in series and in parallel (1, 12, 18, 20, 27), and the rearrangement is thought to be facilitated by the ability of the myosin thick filaments to dissolve and reform within the myofilament lattice during the adaptation process (10, 11, 18, 32). The signal that triggers the adaptive response is likely the strain applied to the muscle cells. Through the deformed cytoskeleton (myofilament lattice), the strain appears to result in partial dissolution of myosin filaments in airway smooth muscle (19). The initial response to a change in cell length therefore appears to be the disassembly of some contractile units in the muscle cells in preparation for reassembly of the contractile units in different locations of the myofilament lattice, so that the reconfigured contractile apparatus fits the new cell dimension.

The cytoskeleton and contractile apparatus are intimately connected. There is evidence that the actin filament lattice of the cytoskeleton is where the thick filaments are polymerized to form contractile units or, in a reversed process, depolymerized to remove contractile units (29). Although polymerization and depolymerization of the thick filaments are thought to be associated with the adaptation of smooth muscle to long and short lengths, respectively (18), the exact mechanism is not clear. Previous studies have focused on the functional and structural changes after the muscle has fully adapted to a length change (18, 27, 32). The results of the present study suggest that there is an intermediate state after a length change. Combined with our laboratory’s previous findings in the fully adapted state, the changes in force, velocity, and thick filament mass found in this intermediate state offer important clues on how contractile filaments are reorganized in the muscle cells after a step change in length and how these structural changes affect the muscle function. Because airway smooth muscle in vivo is subject to constant mechanical perturbation due to the action of breathing, full adaptation of the muscle to any particular length likely never occurs. Understanding the muscle properties in a partially adapted state is therefore more relevant.
for understanding the in vivo role of the muscle in regulating airway caliber.

**MATERIALS AND METHODS**

**Muscle preparation.** Sheep tracheas were obtained from a local abattoir. When the sheep were killed, the tracheas were removed from the carcasses and immediately placed in 4°C physiological saline solution (pH 7.4; 118 mM NaCl, 5 mM KCl, 1.2 mM NaH2PO4, 22.5 mM NaHCO3, 2 mM MgSO4, 2 mM CaCl2, and 2 g/l dextrose). From experiment, a tracheal segment was removed from the midportion of the trachea. The in situ length of tracheal smooth muscle bundle connecting the C-shaped cartilage ring was measured. Caution was taken to ensure that the smooth muscle was relaxed when the measurement was made. If the epithelial layer was thrown into folds, indicating that the underlying smooth muscle was contracted, the trachea was not used for the experiment. Relaxed tracheal rings were then cut open on the ventral side. Connective tissue and the epithelial layer covering the smooth muscle were removed. Muscle bundles ~8 mm long, 1 mm wide, and 0.3 mm thick were dissected out and clipped on both ends with aluminum foil clips for attachment to the force-length transducer. The dimensions of the muscle strip were measured under a dissecting microscope, with the strip stretched to the in vivo length (i.e., the length of the muscle before it was cut from the cartilage “C” ring). In terms of its mechanical properties and ultrastructural appearance, ovine trachealis is very similar to porcine trachealis (12, 18, 19), and in terms of its “plastic” length adaptability, tracheal smooth muscle is qualitatively similar to bronchial smooth muscle (23).

**Establishment of a reference length.** A muscle strip was connected in a tissue bath to two hooks: one stationary and one connected to the lever arm of a servo-controlled force-length transducer. Physiological saline solution in the tissue bath had been previously heated to 37°C, and pH stabilized by bubbling with carbogen gas (mixture of 95% O2 and 5% CO2). During the equilibration and experimental procedures, the muscle strip was activated every 5 min with 12-s electrical field stimulations. Muscle “equilibration” was done before beginning of any experiment to allow the muscle to recover from mechanical and metabolic perturbations caused by dissection, lack of perfusion, and low storage temperature. Equilibration was considered complete when stimulations produced a stable maximal isometric force (Fmax) with low resting tension. The process took ~1.5 h.

After equilibration, the shortest and longest muscle lengths that produced near Fmax were identified. Muscle length was recorded during the plateau of an isometric contraction. Fmax was identified by adapting the muscle at a length ~1 mm less than in situ length. The muscle was then shortened by small decrements until the isometric force produced at that shortened length was ~90% of Fmax after adequate time for adaptation. This length corresponded to the top of the ascending limb of the adapted length-tension curve and was taken as a reference length (Lref). By lengthening the muscle in increments, the longest muscle length that produced >90% of Fmax and <1.5 mN (~3% Fmax) of resting tension after adaptation was also identified. This low resting tension was to ensure that passive elastic components did not contribute excessively to the load transfer during isometric contraction and elastic recoil during a quick length change. A muscle strip was deemed suitable for our experiment if the long length was two times Lref or greater.

It is important to note the difference between the adaptation and equilibration processes. In these experiments, equilibration and adaptation were both accomplished by 12-s isometric contractions at 5-min intervals, but adaptation experiments were performed only after the muscle had been equilibrated. The main purpose of equilibration is to allow the muscle to recover from the trauma of dissection, while the purpose of adaptation is to allow the muscle to recover from a length change. Adaptation was considered complete when isometric force production stabilized at the set length, and it usually took 0.5 h.

**Experiment procedure.** The purpose of this study was to examine ultrastructural and functional changes in a strip of airway smooth muscle after a length change and before the muscle had fully adapted to the new length. Three measurements were taken: 1) isometric force after a step change in length (either a stretch or a release), 2) myosin filament density after the length change, and 3) shortening velocity after the length change. For force measurements at different lengths, stretches and releases were performed at three starting lengths (Lref, 1.5 Lref, and 2 Lref). That is, the muscle was adapted at one of these preset lengths and then stretched or released to the other two predetermined lengths. The same muscle was then adapted at a different starting length and was stretched or released to the other two lengths, followed by force measurements. Length change was done manually, which took ~1–2 s to complete. Also, the length was changed 25 s before electrical stimulation to allow the passive viscoelastic tissue response to settle to a level <3% Fmax. The experiment was repeated with 10 strips from 3 animals.

For the measurement of myosin filament mass (i.e., number of myosin filaments per cell cross section, or density), length change was performed between two lengths (Lref and 1.6 Lref). The result was obtained using a 60% length change (instead of the 50 and 100% length changes used in the length-tension measurement protocol described immediately above) was that in this group of experiments force-velocity measurement was also carried out. A 60% stretch from Lref was the maximal length change that could be achieved without increasing the passive tension for >2% Fmax. The low resting tension is important for accurate measurement of velocity. After adaptation at Lref or 1.6 Lref, a length change was applied, force was recorded, and the muscle strip was immediately fixed for electron microscopy. The change in filament density was observed in pairs of muscle strips from the same trachea. For the stretch (from Lref to 1.6 Lref) experiment, the reference value was obtained from a muscle strip adapted at Lref. For the release (from 1.6 Lref to Lref) experiment, the reference value was obtained from a muscle strip adapted at 1.6 Lref. Four pairs of muscle strips from four animals were used for this group of experiments. The muscles were all fixed in the relaxed state.

For the measurement of shortening velocity, a force-velocity (F-V) curve from a muscle strip adapted at either Lref or 1.6 Lref was determined by measuring the maximal shortening velocity during isotonic contractions at loads equal to 75, 50, 30, 20, and 15% of Fmax. Fmax was determined before every isotonic shortening. The F-V points at Lref were fitted by Hill’s hyperbola (13), which is defined by the equation F + a (V + b) = b (Fmax + a) where F is the isotonic load, Fmax is the maximal isometric force, V is the shortening velocity, and a and b are Hill’s constants. To find the change in velocity from Lref to 1.6 Lref, F-V points at 1.6 Lref were fitted by scaling up the velocity values with a constant (scaling factor) from the F-V curve obtained at Lref. For the stretch experiment, a muscle adapted at Lref was stretched to 1.6 Lref and immediately followed by electrical stimulation and shortening of the muscle under a constant load of ~18% Fmax. Measurement of the maximal velocity of shortening yielded one single F-V point, which was then compared with the F-V curve obtained when the muscle was adapted at Lref. For the release experiment, a muscle adapted at 1.6 Lref was released to Lref and immediately followed by electrical stimulation and shortening of the muscle under a constant load. Measurement of the maximal velocity of shortening again yielded one single F-V point, which was then compared with the F-V curve obtained when the muscle was adapted at 1.6 Lref. Four of muscle strips from four animals were used for this group of experiments.

**Apparatus.** The servo-controlled force-length lever system had a force resolution of 10 μN and a length resolution of 1 μm. Electrical field stimulation was provided by a 60-Hz alternating-current stimulator with platinum electrodes and a voltage of 20–30 V that produced stable, maximal response from the muscle. The analog signals were converted to digital signals by a National Instrument analog-to-digital converter and then recorded by a computer that also controlled the...
onset and duration of stimulation. This apparatus measured both isometric and isotonic contractions of the muscle, i.e., muscle contractions at a constant length or muscle shortening at a constant load, respectively. More details about the apparatus can be found in our laboratory’s previous publications (18, 36).

**Electron microscopy.** Strips were fixed while they were still attached to the length-force transducer for 15 min with a 37°C fixative solution of 2% gluteraldehyde, 2% formaldehyde and 2% tannic acid, in 0.1 M Na-cacodylate buffer, pH 7.3. Care was taken not to disturb the strips during this initial fixation. Methods for tissue fixation, dehydration, and embedding were exactly the same as previously described by our laboratory (10–12, 18, 19). Blocks were sectioned on a Leica EM UC6 ultramicrotome with a diamond knife at 50- to 70-nm thickness, and sections were placed on 400-mesh copper grids. Sections on grids were stained with 1% uranyl acetate for 4 min and Reynolds lead-citrate for 3 min before being viewed under a FEI Technai 12 transmission electron microscope. For each experimental group, a minimum of two blocks were sectioned and multiple grids were imaged per block.

In each group, images of 15 cells that were cut in cross section were collected; because images were acquired using a digital camera (model 792, Gatan BioScan) at a magnification of ×37,000, capturing the whole cross section of a single cell often required taking multiple images and reconstructing the whole cell cross section in Photoshop.

**Morphometric analysis.** Image-Pro Plus software (version 4.0) was used to determine the thick filament density in cell cross sections. Thick filaments were counted in a cell cross section using the “manual tag” function. The cytoplasmic area of a cell cross section was found by subtracting the area taken up by the nucleus and other organelles from the area of the entire cell. Thick filament density was calculated by dividing the number of thick filaments in a cross section by the cytoplasmic area to give a value in no./µm², where no. represents the number of thick filaments. Thick filament density was compared within pairs of airway smooth muscle strips.

Samples were “masked” on day 2 of the EM preparation protocol and remained masked until after morphometric data had been analyzed. Thus the group identity of the samples was hidden from the experimenter until morphometric analysis was completed. This reduced potential bias in the counting of thick filaments.

**Statistical analysis.** In all experiments, data from each animal was averaged before averaging the means from different animals. Unless otherwise noted, data shown as means ± SE. Significant difference was determined using ANOVA.

**RESULTS**

**Changes in force after a stretch or release.** The average F_{max} for the three animals (with 2 muscle strips from each trachea) in this group was 129.6 ± 5.3 kPa. Figure 1 shows changes in force in relative terms such that the force produced immediately after a stretch or release is expressed as percentage of F_{max} produced at the final length. For large changes in length, the direction-dependent asymmetry of force decrease is obvious. Stretching the muscle from L_{ref} to twice that length caused a ~20% decrease in force, whereas in release, the decrease was nearly 50%; multiple comparisons using ANOVA indicated a statistically significant difference between the force after a stretch and that after a release (P < 0.05). The asymmetry is also suggested in the smaller changes in length. Releasing the muscle from 1.5 L_{ref} to L_{ref} caused a significant decrease in force, whereas the decrease in force caused by stretching the muscle from 1.5 L_{ref} to 2.0 L_{ref} was not significant. The decrease in force was not a linear function of the length change, even if the length change was in the same direction. For example, the force decrease was smaller when the muscle was released from 2.0 L_{ref} to 1.5 L_{ref}, compared with the decrease in force from 1.5 L_{ref} to L_{ref} as indicated by ANOVA.

**Changes in myosin filament density after a stretch or release.** An example of electron micrograph used for measuring myosin filament density is shown in Fig. 2. The myosin thick filaments have an irregular cross-sectional profile, with an average “diameter” of 15–20 nm. The actin thin filaments have a well-defined round profile with a rather uniform diameter of 6–7 nm. The experiments were carried out in pairs of trachealis strips from the same trachea. Comparison was made only within the pair, because there was a large variation in force and filament density in different tracheas. Also, in each pair, the initial resting lengths of the muscle strips were the same. For each experiment, four strips (in 2 pairs) of trachealis were fixed under four conditions. In the first pair, one strip was fixed after being adapted at L_{ref}, and the other one was adapted at L_{ref} and then quickly stretched to 1.6 L_{ref} before it was fixed. In the second pair, one was fixed after being adapted at 1.6 L_{ref}, and the other was adapted at 1.6 L_{ref} and then quickly released to L_{ref} before it was fixed. Figure 3 shows representative micrographs of cross sections of cells fixed under the four conditions. The changes in filament density along with the changes in force (measured from the same muscle strips before they were fixed for electron microscopy) are shown in Fig. 4. The average thick filament density of muscle adapted at L_{ref} is 62.3 ± 2.3 (no. of filaments/µm²). Changes in force and filament density after release from 1.6 L_{ref} to L_{ref} are statistically significant (P < 0.05) (Fig. 4).

The number of myosin filaments per cell cross section (minus the area occupied by organelles) was used to indicate the mass (or amount) of myosin filaments inside the cell. This density measurement is independent of cell length; that is, as long as the mass of the filaments inside a cell does not change, the filament density will be constant, regardless of the cell length. This argument was elaborated in our laboratory’s previous studies (18).
Changes in velocity of shortening after a stretch or release.

In this group of experiments, we examined the acute effects of a length change on the shortening velocity of trachealis muscle. The F-V points obtained shortly after a length change and the adapted F-V curves are plotted in Fig. 5. These experiments were carried out using four tracheas, with an average Fmax of 162.2 ± 14.3 kPa. The velocity after a release from 1.6 Lref to Lref is not different from the velocity expected from a muscle adapted at Lref (Fig. 5, vertical arrow). The velocity after a stretch from Lref to 1.6 Lref is also not different from the velocity expected from a muscle adapted at 1.6 Lref (Fig. 5, horizontal arrow). These conclusions were based on Chauvenet’s criterion for outlier rejection (16).

DISCUSSION

The function of a muscle cell, unlike other cell types, is very much influenced by the cell geometry. This is because a change in cell geometry, due to either external or internal forces, alters the arrangement of the contractile filaments, most importantly, the overlap between myosin and actin filaments. This in turn alters the ability of the muscle cell to generate force. In airway smooth muscle, this geometric influence can be quickly reversed (at least partially) due to the cell’s ability to compensate for any adverse effect on filament overlap brought on by the change in cell geometry. By reorganizing subcellular structures in an adaptive process, optimal overlap between contractile filaments can be restored, thus maintaining efficient force generation. This adaptive response is crucial for the regulation of airway smooth muscle tone and airway diameter, which are modulated by various physiological and pathological conditions.
filaments can be restored after large changes in cell length, as evidenced by the recovery of force (7, 27) and reestablishment of velocity appropriate to the adapted length (12, 18, 27). The adaptation does not occur instantaneously. In vitro experiments suggest that the process of force adaptation takes ~30 min (12, 18, 27). The present results suggest that the process of velocity adaptation (or recovery) does not proceed in tandem with the force adaptation but that it occurs in a much faster pace. Understanding the changes in muscle function and structure during the adaptation process is crucial for understanding the adaptation phenomenon and the in vivo behavior of the muscle.

Fig. 3. Electron micrographs of cross sections of ovine trachealis cells fixed under different conditions: fixed at $L_{ref}$ (A), fixed shortly after a stretch from $L_{ref}$ to 1.6 $L_{ref}$ (B), fixed after full adaptation at 1.6 $L_{ref}$ (C), and fixed shortly after a release from 1.6 $L_{ref}$ to $L_{ref}$ (D). The filament densities (i.e., the number of filaments in a cell cross section divided by the cytoplasmic area of the cell, in the unit of no./$\mu m^2$) for the first pair (cells A and B) are 64.0 and 63.7, respectively, and for the second pair (cells C and D) are 60.2 and 49.8, respectively. Comparison of results only made within the pairs (See text for more details). Image size, $1 \times 1 \mu m$. Arrows point to some selected myosin filaments.
Changes in force and myosin filament density with length steps. In the absence of adaptation, there are at least two factors associated with acute length change that could alter muscle force: a change in the contractile filament overlap and partial dissolution of thick filaments. Evidence for the latter was first shown by Kuo et al. (19), who have found that mechanical agitation (length oscillation) applied to relaxed airway smooth muscle caused a decrease in both the cell content of thick filaments and the ability of the muscle to generate force. When the muscle was allowed to recover in the absence of mechanical perturbation, force and myosin filament density returned to the preoscillation level over a similar time course (19). A decrease in the muscle’s ability to generate force after an acute step change in length in the relaxed state is well known (27, 36), and a similar phenomenon is observed in the present study (Fig. 1). In addition to the force decrease, we notice an asymmetrical decrease depending on the direction of length change; that is, a greater decrease in force is associated with length release. An obvious question is why does a step decrease in length lead to a larger decrease in force than a step increase in length? A possible answer is that the thick filament length (and thus force) is limited by the dimension of the actin filament lattice, which is altered by the length change. As shown in Fig. 6, a step release in length, compared with a step stretch, will cause a greater decrease in force (which is assumed to be proportional to the amount of overlap between myosin and actin filaments) if the actin filaments are longer than the myosin filaments. This explanation is based purely on geometric considerations. Using a stochastic model, Silveira et al. (31) have simulated the muscle behavior based on geometric considerations similar to that outlined in Fig. 6. To account for the symmetric length-force relationship in rabbit tracheal smooth muscle (36), they assumed that the lengths of myosin and actin filaments are equal (31). It is unclear why the length-force relationships obtained by Wang et al. (36) and that from the present study are different (i.e., one is symmetric and one is not). It could be due to species difference or the fact that in the studies of Wang et al. the rabbit trachealis had been chronically adapted to a length in vitro and that somehow affected the dynamically regulated actin filament length.

As shown in Fig. 4, a significant decrease in myosin filament mass is associated with a length decrease from 1.6 $L_{ref}$ to $L_{ref}$, and no change in the filament mass is associated with a length change in the reversed direction. Similar changes in force were also observed (Fig. 4). Unlike the decrease in force, the decrease in the myosin filament mass cannot be explained by geometric factors alone; additional assumptions have to be made. The argument that myosin thick filaments are stable only when bounded by adjacent actin filaments (see Fig. 6) is supported by in vitro results that showed unstable thick filaments at physiological levels of ATP, ionic strength, and pH (33), and in unphosphorylated state (15, 34). The argument is also supported by the observation that actin filaments (2, 22) and their associated proteins (14, 17, 25) facilitate thick filament formation. Therefore, there may be a physiological mechanism that eliminates the ends of the thick filaments once they slide pass the dense bodies, because the “out of bound” myosin heads may interact with actin filaments possessing the “wrong” polarity and adversely affect the contractile process. The schematics in Fig. 6 may be able to explain the force data (Fig. 1), but when velocity data are included, a more elaborate scheme is needed, as explained below.

Changes in velocity with length steps. As shown in Fig. 5, a step change in length led to an immediate change in velocity that matched the velocity obtained after the muscle had been fully adapted at the new length. This is different from the pattern of force change after a step change in length (Fig. 1). That is, recovery of force takes time (~30 min) whereas recovery of velocity appears to be “immediate.” (Note that there was a time gap between length change and measurement of velocity due to the fact that velocity could only be measured when the muscle was activated. See MATERIALS AND METHODS and RESULTS for more details). This suggests that the processes governing the changes in force and velocity after a length step are different. We propose the following explanation, again, based on geometric considerations, as illustrated in Fig. 7. In
this explanation, we assume that there is a state immediately after the length change where no structural adaptation has occurred and that force change is as depicted by Fig. 6. Because no change in the number of contractile unit is associated with the instantaneous length step, shortening velocity before and after the step will be the same, assuming the velocity is determined by the number of contractile units in series (30). This theoretical state, however, is not attainable in our experiments, because there is always a delay between the length change and the time when shortening velocity is measured. Partial adaptation of airway smooth muscle has been shown to occur within seconds during an isometric contraction (30). The acute effects of length change on muscle properties that we have observed in this study therefore may be manifestations of an intermediate state where partial adaptation of the muscle to the length change has occurred. As shown in Fig. 7, in this state, the actin filament lattice has been reconfigured to fit the new length; the myosin filaments are, however, still in the process of filling up the lattice space. Such a time course could explain the observed quick recovery of velocity and the relatively slow recovery of force.

**Alternative mechanisms.** Other possible interpretations of the changes in force and velocity upon length change could be related to the changes in calcium levels or myosin light chain kinase (MLCK) activity. An increase in calcium levels or MLCK activity will lead to an increase in myosin light chain phosphorylation and therefore more rapid cross-bridge cycling (5), as well as formation of myosin filaments (15, 34). The increase in cross-bridge cycling rate will cause an increase in velocity of muscle shortening and could therefore account for the changes observed after a stretch. Similarly, a decrease in MLCK activity or calcium levels can account for the decreased velocity after a release. More experiments will be needed to determine whether these interpretations are valid. The current theories regarding calcium and MLCK regulation, however, cannot explain the structural changes observed in the present

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**Fig. 6.** Schematic illustration of changes in filament overlap after a release and stretch without adaptation. Note that the release and stretch is seen from the dense bodies’ perspective, because they are part of the cytoskeleton where externally applied strains are transmitted. After a release, the amount of filament overlap responsible for force production (distance between the 2 dense bodies that define the boundary of a contractile unit) is reduced. This explains the reduction in force. To explain the decrease in myosin filament mass, the portions of the filament outside of the contractile unit (shown in gray) are assumed to dissolve. After a stretch, the overlap of actin and myosin is better preserved (assuming that actin filaments are longer than myosin filaments); however, there could be some reduction of force and myosin filament mass if the stretch is sufficiently large (shown in gray). The asymmetric decrease in both force (Fig. 1) and myosin filament mass (Fig. 4) associated with the direction of length change suggests that the actin filaments may be longer than the myosin filaments in ovine airway smooth muscle.

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**Fig. 7.** Schematic illustration of changes in the contractile unit reassembly within a muscle cell resulting from step changes in cell length. In this model, shortening velocity is proportional to the number of contractile units in series and force is proportional to the length of myosin filaments. Immediately following the length change, there is no change in the number of units in series, but by the time that measurements can be made (intermediate states) the number of units has been altered (due to adaptation) but the myosin repolymerization is incomplete.
study. The changes in myosin filament density suggest that there is restructuring of contractile apparatus at the contractile filament level after a length change.

**Physiological relevance.** The focus of this study was to examine the structural and functional state of airway smooth muscle after a length change before full adaptation to the new length. This intermediate state is more physiologically relevant than the fully adapted state, which can only be observed under static conditions. Under in vivo conditions, the airways are continuously perturbed by the action of breathing because of the tethering of lung parenchyma to the airway wall. The perpetual oscillatory strain on the airway smooth muscle prevents full adaptation at any particular length. The present findings have provided us a clearer picture of the dynamic state of mechanical performance of airway smooth muscle during adaptation. If maintaining airway patency is the goal, then reduction of active muscle force is a positive outcome of length oscillation. The asymmetry of force decrease observed in this study also points out another positive aspect of airway smooth muscle behavior; that is, greater force loss is associated with shortening compared with that associated with lengthening. Occlusion of the airways is more likely to occur if force is not diminished at short lengths. Yet another positive aspect of the muscle behavior is the initial reduction in shortening velocity on reduction in muscle length, even before the muscle force has a chance to recover. Airway narrowing is associated with a reduction in muscle length. A greater shortening velocity in an already narrowed airway would increase the likelihood of airway closure, whereas in a distended airway the velocity of shortening may not be as crucial. Static airway smooth muscle has a chance to recover. Airway narrowing is associated with reduction in muscle length, even before the muscle force diminishes at short lengths. 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