Length oscillation mimicking periodic individual deep inspirations during tidal breathing attenuates force recovery and adaptation in airway smooth muscle

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Raqeeb A, Solomon D, Paré PD, Seow CY. Length oscillation mimicking periodic individual deep inspirations during tidal breathing attenuates force recovery and adaptation in airway smooth muscle. J Appl Physiol 109: 1476–1482, 2010. First published September 9, 2010; doi:10.1152/japplphysiol.00676.2010.—Airway smooth muscle (ASM) is able to generate maximal force under static conditions, and this isometric force can be maintained over a large length range due to length adaptation. The increased force at short muscle length could lead to excessive narrowing of the airways. Prolonged exposure of ASM to submaximal stimuli also increases the muscle’s ability to generate force in a process called force adaptation. To date, the effects of length and force adaptation have only been demonstrated under static conditions. In the mechanically dynamic environment of the lung, ASM is constantly subjected to periodic stretches by the parenchyma due to tidal breathing and deep inspiration. It is not known whether force recovery due to muscle adaptation to a static environment could occur in a dynamic environment. In this study the effect of length oscillation mimicking tidal breathing and deep inspiration was examined. Force recovery after a length change was attenuated in the presence of length oscillation, except at very short lengths. Force adaptation was abolished by length oscillation. We conclude that in a healthy lung (with intact airway-parenchymal tethering) where airways are not allowed to narrow excessively, large stretches (associated with deep inspiration) may prevent the ability of the muscle to generate maximal force that would occur under static conditions irrespective of changes in mean length; mechanical perturbation on ASM due to tidal breathing and deep inspiration, therefore, is the first line of defense against excessive bronchoconstriction that may result from static length and force adaptation.

airway hyperresponsiveness; asthma; muscle mechanics

IN A MECHANICALLY STATIC ENVIRONMENT, airway smooth muscle (ASM) is able to generate substantial amount of force, and when it is allowed to shorten, the amount of shortening could exceed that needed to close the airways completely (11). Fortunately, the dynamic mechanical environment of the lung (due to tidal breathing and periodic deep inspiration) curtails the ability of airway muscle to generate excessive force. Precontracted ASM can be “relaxed” by fluctuating stress and strain in an amplitude-dependent manner (5–6, 8, 14–15, 17); length oscillation applied to activated ASM with an amplitude comparable to that induced by tidal breathing has a bronchodilating effect equivalent to potent pharmacological bronchodilators at high concentration (9). The “relaxing” effect of mechanical perturbation on activated ASM can be partially explained by the disruption of actomyosin cross-bridge binding (8). The elegant model developed by Fredberg et al. (8) could explain the decrease in force and stiffness with increasing amplitude of oscillation; however, on returning to smaller amplitudes of oscillation, the model failed to account for the increased mean length and decreased force. It is speculated that the oscillation may lead to partial disassembly and/or rearrangement of the contractile apparatus and cytoskeleton that typifies the onset of length adaptation (8, 13), a unique smooth muscle phenomenon characterized by the regaining of the diminished ability to generate force that occurs after a change in muscle length (1).

Length adaptation is observed under static conditions. It is a time-dependent process, and it typically takes tens of minutes for the muscle to regain its ability to generate maximal force; the process is facilitated by brief isometric contractions induced in the muscle periodically after the length change (13, 16, 18, 21). Length adaptation allows smooth muscle to function over a large length range. The large working length range has been demonstrated in various smooth muscles (10, 19, 20, 23), including airway smooth muscle (11–12, 16, 18, 22). This ability is required for the smooth muscle that lines the wall of hollow organs that undergo large changes in volume, but for airway smooth muscle, undiminished force at short muscle length could lead to excessive narrowing and closure of the airways.

Another unique smooth muscle property observed under static conditions is the ability of the muscle to generate more force after prolonged exposure to an elevated tone. This is called “force adaptation” (3–4). Similar to length adaptation, force adaptation could lead to excessive narrowing of the airways.

So far, no study has been carried out to specifically determine whether length oscillation mimicking normal pattern of breathing, i.e., periodic deep inspiration during tidal breathing, can reverse the force increase that would otherwise occur due to length and force adaptation in the absence of length oscillation. In this study, we first examined the effect of mechanical perturbation (mimicking tidal breathing and deep inspiration) on the time course of force recovery after a length change, apart from its effect on the kinetics of cross-bridge binding. To achieve this goal, we applied length oscillation to ASM in the relaxed state. We then examined the effect of length oscillation on force adaptation in ASM with experimentally induced tone. The objective of these experiments was to compare force recovery in the presence and absence of length oscillation (mimicking breathing) and to answer the question of whether tidal breathing alone or in combination with deep inspiration...
can attenuate or eliminate the increase of force associated with length and force adaptation seen under static conditions.

MATERIALS AND METHODS

Muscle Preparation

Ovine tracheas obtained from a local abattoir were used in these experiments. The use of tissue was approved by the Committee on Animal Care of the University of British Columbia. Soon after the sheep were euthanized, tracheas were removed and put in Krebs solution (pH 7.4; 118 mM NaCl, 4 mM KCl, 1.2 mM NaH2PO4, 22.5 mM NaHCO3, 2 mM MgSO4, 2 mM CaCl2, and 2 g/l dextrose). After transportation to the laboratory, tracheas were cleaned and kept in Krebs solution at 4°C for not more than 4 days before use. Muscle strips for experiments were prepared from a tracheal segment of ~2 cm in length taken from the middle portion of the trachea. Before dissection, the tracheal rings were examined under the dissecting scope for signs of muscle contracture. A wrinkled epithelial layer above the muscle layer was an indication that the ASM was in a contracted state. These tracheas were discarded because the in situ length of trachealis could not be measured accurately and also because the muscle might be adapted to a length different than its true in situ length due to prolonged contracture. Only the tracheas with relaxed ASM were used for the experiments. The in situ length of relaxed tracheal smooth muscle bundle connecting the C-shaped cartilage ring was measured. The tracheal rings were then cut open, and adventitial connective tissue and the epithelial layer were removed from the muscle layer. Muscle strips of ~7 mm long and 1.5–2 mm wide (with thickness of ~0.2–0.3 mm) were dissected, and aluminum foil clips were attached on both ends for attachment to the force-length transducer.

Equilibration of ASM Strips at Reference Length

The muscle strip was mounted vertically on a force-length transducer between two hooks. The bottom hook was fixed, and the upper hook was connected to the lever arm of a force-length transducer through surgical thread (size 6). The muscle strip was then immersed in a muscle bath containing Krebs solution aerated with a gas mixture of 95% O2 and 5% CO2 at 37°C. The 37°C bath temperature was maintained by circulating warm water through the jacket around muscle bath. The muscle strip was stretched to its premeasured in situ length. This in situ length was used as a reference length (Lref) for normalization of all length measurements. Muscle strips were equilibrated before the experiments were started to allow the muscle to recover from mechanical and metabolic perturbations caused by dissection, lack of perfusion, and low storage temperature. During the equilibration period, the muscle strip was activated every 5 min with a 9-s electric field stimulation (EFS). Krebs solution in the muscle bath was refreshed every 5 min with prewarmed Krebs at 37°C. Equilibration was considered complete when stimulations produced a stable maximal EFS-induced force (Fmax) with low resting tension. (The EFS-induced force referred to active force only, that is, total force minus passive force). The process took ~1.5 h.

Experimental Protocols

To study the effect of length oscillation mimicking tidal breathing and deep inspiration on length adaptation, the muscle strip was first preconditioned at Lref. The maximal isometric force (Fmax) produced at Lref was used as a reference for normalization of force measurements. The seven different protocols (a–g) of length oscillation used in the present study are schematically illustrated in Fig. 1. Fmax was established before the commencement of each of the protocols.

Protocols a and b. In protocol a, sinusoidal length oscillation with a peak-to-peak amplitude of 8% Lref at a frequency of 0.25 Hz (mimicking tidal breathing in sheep) were applied to relaxed ASM preparations for 250 s. The oscillation therefore applied repeated 4% Lref stretches from the muscle’s static length. After oscillation and for the next 25 min, EFS-induced force was elicited and measured at 5-min intervals (Fig. 1a). Protocol b was the same as protocol a, except a single stretch of 25% Lref amplitude (mimicking deep inspiration) was applied after 125 s of 4% Lref, 0.25 Hz stretches (Fig. 1b). Protocol c. In protocol c, EFS-induced force was measured for a 25-min period at 5-min intervals without intervening length oscillations (Fig. 1c). This was carried out to determine the force decline (or the lack thereof) over the time period examined. There was a small (2%) force decline over the 25-min period. EFS-induced force was then measured for the next 25-min period with intervening length oscillations as shown in Fig. 1c; the same force decline (2% over 25 min) was assumed to exist in the latter series of force measurements, and the results were corrected accordingly. This was done to exclude the effect of muscle deterioration over time. The pattern of length oscillation was the same as that described for protocol b. The muscle was length oscillated in the relaxed state, and EFS-induced force was measured after every 5-min period of oscillation for 25 min.

Protocols d, e, and f. After muscle equilibration at Lref, length was changed to one of three preselected lengths (1.2, 0.8, and 0.6 Lref, as shown in Fig. 1, d, e, and f, respectively). EFS-induced force was measured soon (15–20 s) after the length change to determine the decrease in force due to length change. EFS-induced force was then measured at 5-min intervals for a 25-min recovery period without further change in length. During this period, the muscle adapted to the new length and the force produced by the muscle reached a plateau. To examine the effect of length oscillation on length adaptation, the same muscle was then brought back to Lref and readapted for 25 min. During this period, EFS-induced force was measured at 5-min intervals. After the muscle was readapted to Lref, its length was changed to the same predetermined length as in the previous measurements. EFS-induced force was again measured soon (15–20 s) after length change to determine the drop in force due to length change. This time, intervening length oscillation (the same as mentioned in protocol b)
and is shown as a dotted line in all graphs presented in this section. Control observations were done by following protocols a and b (as described in MATERIALS AND METHODS). Figure 2 shows active force measured during a 25-min period after 250-s 4% $L_{ref}$ periodic stretches (protocol a, circles) and 250-s, 4% $L_{ref}$ periodic stretches with a single 25% $L_{ref}$ stretch (protocol b, squares) applied to the ASM strip in the relaxed state. Note that the length oscillations in protocols a and b were applied between 0 and 5 min. There was no change in active force during protocol a, whereas during protocol b (which included a “deep inspiration”), there was a ~10% decrease in force immediately following the period of oscillation; nearly complete force recovery was observed in the next 20 min, during which the muscle was stimulated with EFS once every 5 min. For protocol b, two-way ANOVA indicated that the effect of length oscillation on force development was significant ($P < 0.001$). Oscillation protocol b was used in subsequent experiments.

In the next set of experiments (using protocol c), the effect of length oscillations (between every EFS) on force development was determined (Fig. 3). Two-way ANOVA showed a significant effect for this pattern of length oscillation ($P < 0.001, n = 9$). A decrease in active force was observed immediately after the first length oscillation (this was also shown in Fig. 2). Unlike that seen in protocol b (Fig. 2), the force recovery was absent in the presence of length oscillations between each EFS.

The effect of length oscillation on the time course of force recovery after a length change was then investigated by changing the muscle length from $L_{ref}$ to three preselected lengths: 1.2, 0.8, and 0.6 $L_{ref}$ as shown in Fig. 1, d, e, and f, respectively. A decrease in active force was always observed immediately after the muscle length was changed.

With protocol d, a small decrease (~7%) in EFS-induced active force was seen under both control (circles, no length oscillation) and intervention (squares, length oscillation) conditions when the muscle length was changed from $L_{ref}$ to 1.2 $L_{ref}$ (Fig. 4A). After a 25-min recovery period, the active force under the control condition (no length oscillation) recovered completely with respect to $F_{max}$ whereas under the intervention condition (with length oscillation), force recovery was significantly less than the recovery in the absence of length oscillation (2-way ANOVA, $P < 0.01$).
With protocol e, a moderate decrease in force was observed when the muscle length was changed from \( L_{\text{ref}} \) to 0.8 \( L_{\text{ref}} \) in both control (10.11\%\%) and intervention measurements (18\%) (Fig. 4B). After the 25-min recovery period, active force was 0.93 of \( F_{\text{max}} \) and 0.87 of \( F_{\text{max}} \) in control and intervention measurements, respectively (\( n = 8 \)). Two-way ANOVA shows that the force recoveries in the presence and absence of length oscillation were significant (\( P < 0.001 \)).

With protocol f, a large decrease (40\%) was observed when the muscle length was changed from \( L_{\text{ref}} \) to 0.6 \( L_{\text{ref}} \) in both control and intervention measurements (Fig. 4C). During the 25-min recovery period, the active force under the control condition at 0.6 \( L_{\text{ref}} \) reached a plateau force of 0.86 \( F_{\text{max}} \) (\( n = 12 \)). Under the intervention condition, the initial decrease in force was the same as that under the control condition. During the recovery period, active force reached a plateau force of 0.77 \( F_{\text{max}} \). Two-way ANOVA shows that there is no difference in the time course of force recovery in the presence and absence of length oscillation (\( P = 0.5276 \)).

Finally, with protocol g, the effect of length oscillation was examined at \( L_{\text{ref}} \) in the presence and absence of ACh-induced tone (Fig. 5). After equilibration of the muscle at \( L_{\text{ref}} \), a tone of \(-20\% F_{\text{max}} \) (ranging from 19.6 \( \pm \) 2.1 to 21.9 \( \pm \) 2.5\% \( F_{\text{max}} \))

Two-way ANOVA indicated a significant difference (\( P < 0.05 \)) between the 2 groups of data (without and with oscillations) in A and B, but not in C.
maintained over a period of 25 min) was induced by administra-
tion of ACh (1–3 × 10⁻⁷ M) after the first EFS (Fig. 5). Activeorce (on top of the muscle tone) was then induced by
EFS 5 min later and thereafter once every 5 min for the rest of
the 25-min period. In the absence of length oscillation, the total
force (EFS-induced force plus tone) increased monotonically
toward a plateau (Fig. 5B), whereas the ACh tone was rela-
tively constant over the 5- to 25-min period of the protocol
(Fig. 5C). The EFS-induced force (which equals total force
minus tone) increased monotonically after an initial decrease
(Fig. 5A). In the presence of intervening length oscillations, the
same concentration of ACh induced a tone that was about
60–65% of that induced in the static state (without length
oscillation) (Fig. 5C). The EFS-induced force decreased by
about 18% immediately after tone induction and did not re-
ter the remaining 20 min of the protocol (Fig. 5A).
Similarly, no significant recovery was observed in total force
(Fig. 5B) in the presence of intervening length oscillation.

**DISCUSSION**

Findings from this study suggest that length oscillation
experienced by airway smooth muscle (in either the relaxed or
contracted state) in vivo due to periodic individual deep inspira-
tions during tidal breathing will have a significant inhibitory
effect on the muscle’s ability to generate force. The mechan-
ically dynamic lung environment is therefore likely the first
line of defense against the tendency of ASM to adapt to new
lengths and new levels of tone and generate increasing force
(observed under static conditions), with the consequence of
excessive airway narrowing.

Most of the in vitro studies on the effects of length oscilla-
tion on ASM were carried out using fully activated muscle
(5–6, 8–9, 14–15, 17). Except during an asthma attack, ASM
in vivo is likely in the relaxed or partially contracted state. We
therefore have chosen in this study to apply length oscillation
to relaxed and partially activated ASM. The effects of length
oscillation on force recovery after a change in length or tone
have never been studied. In the present study we have chosen
a length oscillation scheme that mimics what ASM experiences
in vivo (7), with the limitation that the use of periodic EFS in
the protocol is not physiological.

As shown in Fig. 2, length oscillation applied to a relaxed
ASM mimicking tidal breathing (4% L_ref in amplitude) did not
affect the muscle’s ability to generate force. With this ampli-
tude of length oscillation applied to fully activated ASM, a
significant decrease in force was observed (7–9, 17). The effect
of length oscillation on ASM is therefore dependent on the
state of activation of the muscle. When a single stretch (25%
L_ref in amplitude) mimicking a deep inspiration was applied to
a relaxed muscle being subjected to tidal oscillation during a
250-s period, a significant decrease in the muscle’s ability to
generate force was observed (Fig. 2). When the tidal oscillation
was suspended, the muscle force recovered toward the maxi-
mal level of force generated by the muscle before oscillation.
This force recovery is a typical behavior of ASM (13, 21) and
is likely the consequence of length adaptation (1).

In contrast to what is shown in Fig. 2, force recovery was
absent when length oscillation (mimicking periodic individual
deep inspirations during tidal breathing) was applied to the
relaxed ASM throughout the recovery period (Fig. 3). Without
length oscillation, F_max remained unchanged throughout the
25-min period; force obtained in the presence of oscillation
was therefore expressed as a fraction of the paired F_max
(measured at the same time point). Although there is a highly
significant difference (P < 0.001) between the forces measured
in the presence of oscillation and their respective F_max, one-
way ANOVA indicates no difference among the forces mea-
sured between the 5th and 25th minutes of protocol with
oscillation (Fig. 3); that is, the applied length oscillation caused
an initial decrease in force, and thereafter no force recovery
was observed as long as the oscillation was present. The results
indicate that with the specific length oscillation protocol mim-
icking normal breathing, the maximal force produced by the
muscle (dynamic F_max) is reduced by about 7% compared with
that produced under static condition. Since periodic deep
inspirations and sighs are a normal in vivo occurrence (2), this
suggests that ASM in vivo in a healthy lung never achieves its
full capacity in terms of force generation and that in the
absence of muscle tone, periodic individual deep inspirations
during tidal breathing reduce the maximal capacity of the
muscle to generate force by about 7%. If we assume that the
shortening ability and muscle force are linearly related (11), a
7% increase in airway circumference translates into 31% re-
duction in airway resistance, because airway resistance is
inversely related to the 4th power of airway diameter.

The effect of length oscillation on force recovery after a step
change in length is more complicated, as shown in Fig. 4. With
a ±20% change in length (from L_ref to 1.2 and 0.8 L_ref), the
ability of the muscle to recover force was impaired, as evi-
denced by the significant difference in the time course of force
recovery in the presence and absence of intervening length
oscillation (Fig. 4, A and B). With a larger (40%) reduction
in length, the effect of length oscillation on force recovery was
not significant (Fig. 4C), as evidenced by the same (statistically
speaking) degree of force recovery after the length change. It
has to be pointed out that the lack of statistical difference could
be due to insufficient sample number and large variation.
However, it is likely that the difference in force recovery
shown in Fig. 4C would be smaller compared with those in Fig.
4, A and B, if the sample size were increased. We have no clear
explanation for the length-dependent effect of length oscilla-
tion. However, one can speculate that at very short lengths,
passive stiffness of the muscle is much reduced; the same amplitudes of stretch (length oscillation) applied to the short
muscle would provoke less force response and therefore less
structural disruption in the muscle tissue, and thus more force
recovery. In terms of physiological significance, the results
suggest that tidal breathing and deep inspiration are less effec-
tive in inhibiting force recovery at short muscle length; in other
words, an excessively narrowed airway will become less re-
sponsive to the bronchodilatory effect of length oscillation.

When muscle tone was included in the experiment protocol,
a dramatic effect of length oscillation was revealed (Fig. 5).
Without length oscillation, ASM adapted to the ACh-induced
constant tone and augmented its ability to generate force over
a 25-min period. This is the familiar “force adaptation” that we
have observed in ASM previously (3, 4). When intervening
length oscillation was applied to the ASM with tone, force
adaptation disappeared. The effect of length oscillation on
ASM with tone can be dissected into two components. First, in
the presence of length oscillation, the same ACh concentration
produced about one-half as much tone as in the case without length oscillation (Fig. 5C). This is likely due to disruption of actomyosin cross-bridge interaction described by Fredberg et al. (8). Second, length oscillation abolished the force recovery due to force adaptation that was observed under static conditions (Fig. 5, A and B). As a result, length oscillation applied to ASM with tone led to a large decrease (>20%) in the total force produced by the muscle compared with that produced under static conditions.

A common observation from Figs. 4 and 5 is that the recovery of force is relatively small or absent as long as a large stretch (mimicking deep inspiration) is applied to the muscle periodically, irrespective of changes in mean muscle length. In addition, in the case where muscle tone is present, the true benefit of length oscillation lies in diminishing the tone rather than preventing the small increase of force due to force adaptation.

The effect of tidal breathing and deep inspiration in vivo on ASM is perhaps better mimicked by force oscillation rather than length oscillation, because the parenchymal tethering force and the transmural pressure are the driving force that causes ASM to change length; that is, length change is secondary to force change. The length oscillation protocol used in this study is therefore not a realistic simulation of the effect of tidal breathing and deep inspiration in vivo. However, the advantage of using length oscillation is that we can relate the degree of mechanical perturbation to the amplitude of length oscillation and that the amplitude is not affected by the intrinsic tone in the muscle.

In this study, the phenomena of length and force adaptation were revealed in protocols that involved periodic EFS in the absence of length oscillation; this is clearly not what the ASM normally experiences in vivo. We have demonstrated in a previous study (22) that length adaptation is not an EFS-dependent phenomenon. Allowing the muscle to stay at a chronically shortened length or prolonged exposure of the muscle to an elevated tone will lead to length and force adaptation (3, 4, 22).

In conclusion, whereas length oscillation associated with tidal breathing alone had no effect (statistically) on relaxed ASM in terms of its ability to generate force, length oscillation mimicking periodic individual deep inspirations during tidal breathing applied to relaxed ASM attenuated the length adaptation that is normally seen under static conditions. In partially activated ASM, length oscillation mimicking periodic individual deep inspirations during tidal breathing abolished the force adaptation that is also normally seen under static conditions. Thus increase of force in ASM due to length and force adaptation under static conditions can be curtailed (except in excessively shortened ASM) in the mechanically dynamic lung environment. This implies that the consequence of length and force adaptation is only relevant under pathological conditions where mechanical stretches imposed on ASM due to breathing are diminished or absent. Maintaining a healthy airway-parenchymal interdependence in the lung is therefore crucial in preventing excessive ASM force augmentation due to length and force adaptation seen under static conditions.

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REFERENCES


GRANTS

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