Decrease of airway smooth muscle contractility induced by simulated breathing maneuvers is not simply proportional to strain

Chris D. Pascoe,1 Chun Y. Seow,1,2 Peter D. Paré,1,3 and Ynuk Bossé1

1University of British Columbia James Hogg Research Center, St. Paul’s Hospital Vancouver, British Columbia, Canada; 2Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada; 3Department of Medicine, Respiratory Division, University of British Columbia, Vancouver, British Columbia, Canada

Submitted 16 July 2012; accepted in final form 25 November 2012


The lung is a dynamic organ and the oscillating stress applied to the airway wall during breathing maneuvers can decrease airway smooth muscle (ASM) contractility. However, it is unclear whether it is the stress or the attendant strain that is responsible for the decline of ASM force associated with breathing maneuvers, and whether tone can prevent the decline of force by attenuating the strain. To investigate these questions, ovine tracheal strips were subjected to oscillating stress that simulates breathing maneuvers, and the resulting strain and decline of force were measured in the absence or presence of different levels of tone elicited by acetylcholine. In relaxed ASM, high stress simulating 20 cm H2O-tranpulmonary pressure excursions strained ASM strips by 20.7% and decreased force by 17.1%. When stress oscillations were initiated during measurement of ACh concentration—response curves, tone almost abrogated strain at an ACh concentration of 10^-6 M (1.1%) but the decline of force was not affected (18.9%). When stress oscillations were initiated after ACh-induced contraction had reached its maximal force, strain was almost abrogated at an ACh concentration of 10^-6 M (0.9%) and the decline of force was attenuated (10.1%). However, even at the highest ACh concentration (10^-4 M), substantial decline of force (6.1%) was still observed despite very small strain (0.7%). As expected, the results indicate that tone attenuated the strain experienced by ASM during breathing maneuver simulations. More surprisingly, the reduction of strain induced by tone was not proportional to its effect on the decline of force induced by simulated breathing maneuvers.

asthma; airway hyperresponsiveness; lung maneuvers; tone; spasmogen; stress; strain

THE MOVEMENT OF AIR in and out of the lung is caused by changes in pressure generated by cyclical expansion of the thoracic cavity. The airways, like any structure inside the thoracic cage, are stressed to change shape by these oscillating pressures. The strain (i.e., changes in shape) of the airway wall implies that all of its constituents, including the airway smooth muscle (ASM), are continuously subjected to length changes during breathing. There is considerable interest in the effect of these length oscillations on ASM contractility and the bulk of the evidence suggests that the effects of oscillating strain on ASM mechanics are not small; that is, they decrease ASM contractility considerably (9–11, 25, 28, 31). Because it is the strain experienced by the ASM rather than the applied stress that seems to be better correlated with the decline of ASM force (24, 27), an important variable related to the effect of breathing on ASM contractility is airway wall stiffness.

One of the most important factors that modifies airway wall stiffness is ASM tone (i.e., ASM activation). In lung disorders such as asthma, the overexpression of many inflammation-derived spasmogens such as leukotrienes, histamine, and others, can contribute to ASM tone (2). Even changes in pH were recently shown to affect ASM tone (30). No matter its origin, increasing tone stiffens the airway wall, which would be expected to decrease the amount of strain and possibly the decline of ASM contractility induced by breathing maneuvers. The effect of increasing tone on the strain and decline of force caused by simulated breathing maneuvers has never been systematically investigated. However, previous studies have shown that ASM preshortened with high concentrations of ACh relengthens when subjected to force oscillations simulating breathing maneuvers (7, 16, 17, 20, 25). This phenomenon was dubbed force fluctuation-induced relengthening (7, 16, 20). These studies confirmed the results of earlier studies showing that length oscillations decrease ASM contractility (9–11, 25, 28, 31). The concordance of these studies using either force or length oscillations is counterintuitive, because with high tone, the strain induced by force oscillations would be expected to be very low. It raises the possibility that the stress itself in the absence of strain (or in the presence of very small strain) may be sufficient to decrease ASM contractility. These later studies did not assess strain induced by force oscillations in a relaxed ASM and compare them with the strain obtained at different levels of activation (only one concentration of ACh was used). Thus, these studies were not designed to determine whether preventing the strain by increasing tone would attenuate the decline of ASM contractility induced by the oscillating stress of breathing.

The present experiments were undertaken to determine the influence of increasing tone on the strain caused by stress oscillations that simulate breathing maneuvers and to determine the relative effects of strain and stress on ASM force. Our results demonstrate that decreasing strain by increasing tone was slightly but significantly associated with an attenuated decline of ASM contractility induced by simulated breathing maneuvers. This finding is pertinent to individuals who have greater ASM tone, such as asthmatics. The ASM of such individuals may not be strained as much by the stress imposed by breathing maneuvers. Consequently, they may not fully benefit from the decrease in ASM contractility caused by breathing, rendering them more likely to be hyperresponsive due to ASM hypercontractility. However, our results also show that preventing the strain almost entirely by high tone was not...
was chosen because it generates a consistently large response. The decline of force caused by oscillations was calculated by subtracting the force generated by either EFS or ACh under static conditions from the force generated by the same stimulus when the oscillations were momentarily stopped following the start of the oscillations.

Protocol 2. In protocol 2, ACh concentration-response curves from $10^{-10}$ to $10^{-4}$ M were performed in a cumulative fashion with or without stress oscillations that simulated either repetitive pressure excursions of 20 cm H$_2$O or the pressure excursions occurring during normal breathing pattern [i.e., tidal breathing + a deep inspiration (DI) every 5 min]. Hence, three ACh concentration-response curves were performed in each tissue strip. The ASM was allowed to recover completely between every concentration-response curve. ACh was washed away repeatedly with Krebs solution and the muscle strip was allowed to sit for 30 min while stimulated to contract at 5-min intervals with EFS before the next concentration-response curve. The purpose of the repeated EFS was to gauge tissue recovery over time. The order of the oscillating conditions (none, 20 cm H$_2$O, and breathing pattern) was randomized. The oscillations were initiated just before adding the first concentration of ACh. The force values were taken at the maximal force obtained in the first 5 min of every ACh concentration. During breathing pattern simulation that included a DI, the force values were taken 2 to 2.5 min after the simulated DI. In the presence of oscillations, the force values were taken at the trough when the ASM strip was at $L_{in situ}$ [functional residual capacity (FRC) for the simulation of breathing]. The strain at every ACh concentration was the average strain of three oscillations taken at the maximal force obtained during the 5-min stimulation. The decline of force caused by oscillations was calculated by subtracting the force generated by ACh under static conditions from the force generated by the same concentration of ACh under oscillating conditions.

Protocol 3. In protocol 3, the maximal force that can be achieved in response to a given concentration of ACh was attained under static conditions prior to the instigation of stress oscillations. The force value at 100 s after ACh administration was always chosen because it allowed time for the muscle to reach its near maximal force in response to any of the ACh concentrations used ($10^{-7}$, $10^{-6}$, $10^{-5}$, or $10^{-4}$). This force was the reference value for calculating the changes of force over time that occurred either spontaneously or in response to the oscillations. The stress oscillations simulated transpulmonary pressure excursions of 10 cm H$_2$O from FRC, 20 cm H$_2$O from FRC, or those occurring during normal breathing (tidal breathing + intercalated DI). Each tissue was exposed to these three oscillating conditions and also stimulated once for the same amount of time in the absence of oscillations in a randomized fashion. Therefore, each muscle was stimulated to contract four times in response to the same concentration of ACh. Every tissue was exposed to the four conditions, but was exposed to only one concentration of ACh. Different sets of tissues were used for different concentrations. The ASM was allowed to recover completely between ACh stimulations (ACh was washed away repeatedly with Krebs solution and the tissue was allowed to sit for 30 min while stimulated to contract at 5-min intervals with EFS, before the next ACh stimulation). The ACh stimulation in the absence of oscillations was a time-control to measure the time-dependent changes of ACh-induced tone. These changes were taken into account to calculate the effect of the oscillations on ASM force. This was to distinguish changes in ACh-induced tone that sometimes occur over time from the effect of the oscillations. The force values in the oscillating state were taken 5 min after the beginning of the stress oscillations, which allowed sufficient time to reach a new dynamic plateau. During breathing pattern simulation that included a DI, the force value was taken 2.5 min after the simulated DI. These values were taken at the trough of the oscillations when the muscle was back to its length under static conditions (back to simulated FRC).

Simulations of breathing maneuvers. The stress oscillations imposed on ASM strips simulated the tension oscillations experienced by the wall of a fourth-generation airway during transpulmonary

---

MATERIALS AND METHODS

Muscle preparation. Sheep tracheas used in these experiments were obtained from a local abattoir. The use of the tissue was approved by the Committees on Animal Care and Biosafety of the University of British Columbia. Tracheas were removed soon after the animals were killed and put in Krebs solution (118 mM NaCl, 4 mM KCl, 1.2 mM NaH$_2$PO$_4$, 22.5 mM NaHCO$_3$, 2 mM MgSO$_4$, 2 mM CaCl$_2$, and 2 g/liter dextrose; pH 7.4). Upon arrival at the laboratory, tracheas were cleaned of blood, fat, and loose connective tissue, and stored in Krebs solution at 4°C until further processed. ASM strips for experiments were dissected from 2 cm-long tracheal segments. The in situ length of a relaxed tracheal smooth muscle bundle connecting the C-shaped cartilage ring was measured. The tracheal rings were then cut open. Adventitial connective tissue and the epithelial and subepithelial layers were dissected away from the tracheal smooth muscle layer, and muscle strips (~6 mm long, 1 mm wide, and 0.3 mm thick) were isolated. The muscle strips were attached on both ends with aluminum foil clips. The ASM strips flanked by foil clips were then mounted vertically in a muscle bath. The bottom clip was attached to a stationary hook and the upper clip was attached to a hook connected to the lever arm of a servo-controlled force-length transducer by a surgical thread (size 6). The distance between the clips during the dissection was used to adjust the length of the ASM strips once installed in the muscle bath to the length that it was in situ. This length is hereafter called $L_{in situ}$. The organ bath was filled with Krebs solution that had been preheated to 37°C and aerated with a gas mixture containing 95% O$_2$ and 5% CO$_2$. The temperature of the bath was also maintained by circulating 37°C water through a jacket that surrounded the organ bath.

Conditioning period. ASM strips were subjected to a conditioning period before the start of the experiments. During the conditioning, the ASM strips were activated every 5 min with a 9-s electrical field stimulation (EFS) (60 Hz, 12 volts). Krebs solution was replaced every 5 min following EFS with warmed (37°C), aerated Krebs solution. The conditioning was completed when a plateau in isometric force was reached (i.e., after which there was no further increase in force in response to subsequent EFS). The force produced by EFS at that time was called $F_{max}$. Therefore, $F_{max}$ is the force generated by the ASM in response to EFS at in situ length after a stable plateau was achieved minus the resting tension. The conditioning period took ~1 h.

Protocol 1. In protocol 1, we set out to determine the level of oscillating stress that was required not only to strain the relaxed ASM (i.e., not activated with exogenous ACh) but also to decrease its force-generating capacity sufficiently so that the effect of adding increasing levels of tone could be investigated. A physiological range of stress oscillations that simulated transpulmonary pressure excursions of 3, 5, 10, 15, 20, and 25 cm H$_2$O was tested. The methodology employed for these simulations is explained below. ASM length changes (i.e., strain) induced by the simulated pressure swings were recorded and ASM force-generating capacity was assessed periodically in response to either EFS or ACh. For EFS, the oscillations were present throughout the 5-min cycle except during the measurement of isometric force, when the oscillations were stopped for 40 s (10 s prior, 9 s during, and 21 s after EFS). A representative trace showing simulated normal breathing prior, during, and after EFS-induced force has been previously published (26). The 5-min cycles of oscillations were continued until no further decrease in ASM force was observed (i.e., when the force in response to EFS had reached a new plateau in oscillating conditions). The same procedure was performed for force measurement in response to ACh at $10^{-5}$ M, except that a 2-min period without oscillations was used after every ACh administration, which was the time needed for ASM to reach its near maximal force in response to this concentration of ACh. The concentration of ACh
pressure excursions of different magnitude [e.g., 3 cm H₂O from 5 to 8 cm H₂O to mimic tidal breathing, and 25 cm H₂O from 5 to 30 cm H₂O to mimic a DI from FRC to total lung capacity (TLC)]. We chose airway generation 4 because the thickness of the wall is approximately equal to the thickness of the trachealis (~0.3 mm) (12, 13). Therefore, assuming that the transmission is transmitted homogenously across the entire thickness of the wall, the stress experienced by our tracheal strips would be the same as the stress experienced by the ASM of an airway of the fourth generation in vivo. Representative force (upper) and length (lower) traces are shown in Fig. 1 in the absence (A) and presence (B) of tone elicited by ACh. The stress oscillations at any chosen magnitude were performed at breathing frequency (0.2 Hz) with half sine waves (without a trough) to mimic maneuvers from, and back to, FRC (i.e., one semi-sine wave every 5 sec).

The changes in wall tension caused by such excursions were calculated on the basis of the Laplace relationship (tension = pressure × radius), assuming that the airways resemble a thin-walled cylinder [a reasonable assumption because the ratio of wall thickness/airway luminal diameter is ~1/22 in a nonasthmatic airways of the fourth generation (12, 13)]. Because the airway wall is also strained during these swings in transpulmonary pressure, the calculated change in wall tension also has to take into account the change in radius (at the middle of the ASM layer). Equations developed by Lambert and coworkers (14) and morphological data for a fourth generation airway taken from James et al. (12), Kwano et al. (13), and Wiggs et al. (32) were used to make this adjustment. The magnitude of the tension oscillations was the same for every ASM strip (e.g., 0.74 mN/mm for tidal breathing and 6.24 mN/mm for the DI) but the magnitude of the oscillations was the same for every ASM strip (e.g., 0.74 mN/mm for tidal breathing and 6.24 mN/mm for the DI).

To estimate the width of the ASM strips, we assumed that the ASM generates a stress (force/cross-sectional area) of 100 kPa (4) in response to EFS following the conditioning period (so Fₘₐₓ = 100 kPa or 100 mN/mm²) and that the thickness of the ASM strips was 0.3 mm. The value of Fₘₐₓ (in mN) was thus used to calculate the width of the ASM strips. Knowing the length of the airway wall that would be covered in situ by this estimated width of the ASM strip and the changes in wall tension occurring during breathing maneuvers, we were then able to calculate the force oscillations (in mN) that had to be imposed on tracheal strips to simulate the amount of stress to which the ASM is subjected in vivo. This approach is an efficient way to calculate the force oscillation required to obtain a given stress oscillation without the need for measuring the cross-sectional area of ASM strips by histology, which would only be possible a posteriori (i.e., after the mechanical measurements).

Statistical analyses. Resting tension, EFS-induced force, and ACh-induced tone obtained for each muscle strip were normalized to Fₘₐₓ before averaging. Data shown are means(SD). Linear regression analysis of individual tissue and the mean of their slope were used to measure the relationship between stress vs. strain, stress vs. decline of force, and strain vs. decline of force. Two-way ANOVAs were used to compare the effect of different oscillating conditions and different ACh concentrations on strain and the decline of force, as well as their interactions. Repeated measures for one or two factors were performed whenever appropriate. Two-way repeated measures ANOVA for both factors were conducted in R (version 2.15.1) using the ANOVA function from the car package. All the other statistical analyses were performed using Prism 5 (GraphPad Software, San Diego, CA). A value of P < 0.05 was considered significant.

RESULTS

Stress oscillations on relaxed ASM (protocol 1). In protocol 1, the oscillations were always performed when the ASM was relaxed (i.e., not activated with ACh). ASM force generation was assessed by measuring the response to both EFS and ACh (Fig. 1, left and right, respectively) while the oscillations were interrupted. In Fig. 2A, the strain is plotted against the magnitude of stress. Linear regression analyses show that the strain was related to the magnitude of the stress oscillations. Tidal breathing and simulated repetitive DI strained the relaxed ASM strips by 5.7%(SD1.2) and 21.2%(SD2.0), respectively, in the set of experiments using EFS to measure force and by 4.5%(SD1.3) and 23.2%(SD4.2), respectively, in the set of experiments using ACh to measure force. In Fig. 2B, the decline of force is plotted against the magnitude of stress. The decline of force was calculated as the force generated by the muscle in response to either EFS or ACh prior to oscillations minus the force generated by the same tissue after a new plateau of force was reached following the start of the oscillations (see Protocol 1 in Materials and Methods). The linear fit of these relationships indicates that the decline of EFS-induced force was related to the magnitude of the stress oscillations. However, this was not always the case for ACh-induced force. Two linear regressions were not significant (from the triangle symbols pointing up, Y = 0.50X + 3.38, r² = 0.58, P = 0.07; and from the hexagon symbols, Y = 0.12X + 5.06, r² = 0.11, P = 0.53). Tidal breathing and repetitive simulated DI in relaxed ASM strips decreased force by 1.2%(SD0.5) and 18.2%(SD1.2), respectively, in the set of experiments using EFS to measure force and by 3.4%(SD1.7) and 17.6%(SD5.2), respectively, in the set of experiments using ACh to measure force. In Fig. 2C, the strain and the decline of force induced by the oscillating stress are plotted together. The fit of these linear regressions indicates that they were related when EFS was used as the contractile stimulus. Again, this was not always the case when ACh was used as the contractile stimulus. Two linear regressions were not significant (from the square symbols, Y = 13.08X + 5.20, r² = 0.06,
that simulations of 20 cm H₂O-transpulmonary pressure excursions were required to cause sufficient strain [21.4%(SD1.5) and 20.1%(SD3.5)] and decline of force [19.3%(SD2.8) and 19.8%(SD4.0)] so that the effect of tone on both strain and decline of force induced by the oscillating stress can be measured. Simulations of 20 cm H₂O-transpulmonary pressure excursions were therefore performed in the following experiments. A normal breathing pattern (i.e., tidal breathing + a DI every 5 min) was also investigated due to its in vivo relevance.

Figure 3 shows representative force (left) and length (right) traces illustrating the sequence of interventions used in protocol 2. The simulated breathing maneuvers were started just before the stepwise increases of ACh concentration. ACh concentration was changed every 5 min. In this example, the magnitude of the stress oscillations simulated transpulmonary pressure excursions of 20 cm H₂O from functional residual capacity. The effect at 10⁻⁸ M is not shown because even at 10⁻⁹ M (first concentration shown) no measurable force was generated. Notice that as the tone increased, the strain induced by the stress oscillations decreased.

The effect of stepwise increases of ACh concentration on ASM strain induced by different stress oscillations is shown in Fig. 5. As observed under relaxed conditions (Fig. 2), the strain was related to the magnitude of the stress oscillations. Increasing ACh concentrations decreased ASM strain to an extent at which the strain was almost abolished irrespective of the magnitude of the stress oscillations [at 10⁻⁷ M, the strain induced by simulated 3, 20, and 25 cm H₂O-transpulmonary pressure excursions were 0.09%(SD0.02), 0.64%(SD0.05), and 4.90%(SD0.11)]. From this study, it was concluded that simulations of 20 cm H₂O-transpulmonary pressure excursions were required to cause sufficient strain [21.4%(SD1.5) and 20.1%(SD3.5)] and decline of force [19.3%(SD2.8) and 14.8%(SD4.0)] so that the effect of tone on both strain and decline of force induced by the oscillating stress can be measured. Simulations of 20 cm H₂O-transpulmonary pressure excursions were therefore performed in the following experiments. A normal breathing pattern (i.e., tidal breathing + a DI every 5 min) was also investigated due to its in vivo relevance.

Stress oscillations during force development (protocol 2). Figure 3 shows representative force (left) and length (right) traces illustrating the sequence of interventions used in protocol 2. The force oscillations were consistent throughout and the force generated by ACh was concentration-dependent. The length oscillations (strain) caused by the stress oscillations decreased as the concentration of ACh increased (Fig. 3, right).

Figure 4 shows the concentration-response curves under oscillating conditions that simulate either breathing pattern (tidal breathing with periodic DI; A) or 20 cm H₂O-transpulmonary pressure excursions from FRC (B). They are compared with the concentration-response curves obtained under static conditions (same tissues in A and B). Interestingly, the simulated breathing pattern (tidal breathing + periodic DI) had a weak but significant effect on ASM force (A). The significant effect due to condition (oscillating vs. static), together with the lack of significant interaction, suggests that the oscillations reduced the ASM capacity to generate force by the same amount at all concentrations of ACh. The effect of simulated 20 cm H₂O-transpulmonary pressure excursions was more pronounced (B). The oscillations reduced the ASM response to exogenous ACh, and this effect was significantly increased with increasing ACh concentrations (the interaction was significant). It should also be noted that the two curves did not converge at the higher doses of ACh, where the strain was lowest.
The effect of increasing stress on the strain of ASM stimulated to contract with different concentrations of ACh is shown in Fig. 7. The stress-strain relationship found in relaxed muscle is also shown as a reference. These later results are from Fig. 2A (right). As observed in Figs. 2 and 5, the strain was proportional to the magnitude of the stress oscillations. Also observed in Fig. 7 is the attenuating effect of increasing concentrations of ACh on strain. The effect was especially evident beyond $10^{-6}$ M, when even high stress became ineffective for straining ASM. The bottom of the y-axis has been magnified to better visualize the concentration-dependent decrease of ASM strain. The significant interaction ($P \leq 0.0001$) indicates that the stress-dependent ASM strain was greatly affected by ACh concentration.

The data showing the decline of force induced by simulating breathing maneuvers in both protocol 2 and protocol 3 are presented in Fig. 8. The word “history” in the figure legend refers to the results of protocol 2, when oscillating history was present before taking force measurements at different ACh concentrations. The words “no history” refer to the results of protocol 3, when the oscillations were instigated after ACh had reached its maximal contractile effect. Only the results obtained with the simulated 20 cm H$_2$O-transpulmonary pressure excursions are shown, because neither breathing pattern nor 10 cm H$_2$O-transpulmonary pressure excursions affected force significantly. The strain obtained at every ACh concentration in the two protocols is also shown. The strain obtained in both protocols is almost overlapping. The strain was reduced by more than half with $10^{-7}$ M of ACh compared with the strain experienced by ASM strips exposed to the same oscillating stress in a relaxed condition [compare the upper horizontal dashed line, taken from results shown in Fig. 2 (right) when ASM was exposed to simulated 20 cm H$_2$O pressure excursions without ACh, with the strain obtained in the presence of $10^{-7}$ M of ACh in Fig. 8]. A further increase of ACh to $10^{-6}$ M almost completely abolished ASM strain. In contrast to strain, the decline of force induced by the oscillating stress was very different between the two protocols. With a prior history of oscillations (protocol 2), the decline of force was similar to the decline produced by oscillations in relaxed ASM (protocol 1); and this was true no matter the concentration of ACh. To visualize this effect, compare the lower horizontal dashed bar in Fig. 8, which was taken from results shown in Fig. 2 (right)
(obtained by using protocol 1 in which the muscle was oscillated in a relaxed condition and stimulated to contract with $10^{-5}$ M ACh when the oscillations were momentarily stopped), with the decline of force obtained by the same stress at every ACh concentration (black bars). The larger SD at $10^{-7}$ M is due to the lack of response of some ASM strips to respond to that concentration of ACh under oscillating conditions (and if there was no force, there was no decline of force). Without oscillating history (protocol 3), $10^{-7}$ M of ACh attenuated the decline of force by 35.3% [compare the lower horizontal dashed bar, taken from results shown in Fig. 2 (right), with the decline of force obtained at $10^{-7}$ M in Fig. 8]. However, further stepwise increases of ACh only slightly attenuated, in a concentration-dependent manner, the decline of ASM force. This was different from the results obtained with protocol 2 (with oscillating history), when no reduction in the decline of force was observed with increasing concentrations of ACh. The results obtained with both protocols demonstrate a striking difference between the effect of tone on strain vs. its effect on the decline of force.

DISCUSSION

In the present study, we sought to determine whether it is the stress or the attendant strain that is responsible for the decline of ASM force associated with breathing maneuvers and whether tone can prevent the decline of force by attenuating the strain. Other investigators have examined the effects of simulated breathing oscillations on ASM force generation but have done so using a different protocol involving ASM shortening and subsequent relengthening in response to oscillations (7, 16, 17, 20, 25). Our study examined the strain and the decline of force triggered by different oscillating conditions at different levels of ASM activation elicited by different ACh concentrations. Our results demonstrated that the decline of force caused by oscillating stress is related to the strain, especially when the oscillations were performed when the ASM was relaxed. However, when the stress oscillations were applied during force development, decreasing the strain by increasing tone had much less, or no effect, on the decline of force. This suggested that the oscillations may be sufficient to decrease ASM force, or that the decline of force is disproportional to the magnitude of strain under some circumstances. This lack of proportionality is further supported by the finding that a significant decline of force was still observed at high ACh concentrations when the strain was very small (protocol 3), indicating that there is sometimes a clear mismatch between the pronounced effect of tone on strain and its more modest effect on the decline of force.

Strain induced by simulations of breathing maneuvers. It is important to determine whether and to what extent the ASM is strained by the oscillating stress acting on the airway wall during breathing maneuvers. In this study, a physiological range of stress was applied to ASM to measure its effect on strain. In relaxed ASM, our results showed that stress excursions of 2.5 and 20.8 kPa, which simulate tidal breathing and DI, respectively, strained the ASM strips by 4.5%(SD1.3) and 23.2%(SD4.2), respectively. These values are also similar to the strain obtained in a previous study using the same experimental setting [6.5%(SD2.6) for tidal breathing and 24.2%(SD2.6) for DI] (26). These values are also very close to the ones predicted on the basis of changes in lung volume during these breathing maneuvers (4% and 25% for tidal breathing and DI, respectively, which was based on the assumption that the airway perimeter changes in proportion to the cube root of the changes in lung volume) (9).

Effect of tone on strain. Recent ex vivo and in vivo studies have demonstrated that the decline of contractility or the relief of bronchoconstriction is better correlated with strain than with stress (24, 27). These findings have urged the search for factors affecting airway wall stiffness. One of the more obvious factors affecting airway wall stiffness in vivo is ASM tone (i.e., muscle activation). In the presence of tone, the amount of stress imposed by breathing maneuvers presumably remains the same, but the level of strain is likely to be negatively affected. In the present study, we measured the impact of tone on the stress-strain relationship. The results suggested that concentrations of ACh that are sufficient to trigger a measurable amount of force are very effective in preventing ASM strain. When the stress generated by ASM was approximately equal to the oscillating stress (e.g., the stress generated by ASM in response to ACh at $10^{-7}$ M was 16.4KPa(SD2.8) and the stress imposed by simulation of 20 cm H2O-transpulmonary pressure excursions was 16.7 kPa), the strain was reduced by more than half (20.7%(SD3.5) vs. 7.92%(SD1.7)). A critical point was also observed at $10^{-6}$ M, when the strain almost disappeared even at the highest stress tested. This was not surprising because $10^{-6}$ M of ACh generated 72.6KPa(SD5.3), which represents almost four times the stress caused by a simulated DI (20.8 kPa). Together, these results suggest that very little tone (i.e., small percentage of the maximal force-generating capacity of the ASM) can almost abrogate the strain caused by any physiological level of stress.

Lack of decline of force with tidal breathing. Ultimately, our experiments were designed to determine whether a physiological range of stress before or during ASM stimulation could affect ASM force and whether the changes of force are correlated with the level of strain. At a level of stress that simulates tidal breathing, the declines of force were very small, irrespective of whether the oscillations were finished before stimulations (protocol 1) or were initiated during (protocol 2) or after (protocol 3) force development. It was previously demonstrated using ex vivo airway preparations that simulated tidal breathing has very little effect on contractility (15, 22). However, the real strain experienced by the ASM in those ex vivo airway preparations is difficult to determine. It was argued that the smaller effect (or the lack of effect) of tidal breathing simulations on isolated airways compared with ASM subjected to length oscillations may be due to a failure to transmit the changes in airway wall tension to the ASM cells (so that the changes in tension may be dissipated by other structures in the airway wall). Our isolated ASM strip was ideal to answer this unresolved question. It allowed complete control over the stress acting on the ASM strips and its concomitant effect on strain and on force. Our results suggest that the lack of bronchodilating effect of tidal breathing may not be due to the failure to transmit the swings in tension to the ASM, because tidal breathing simulations had no effect in our ASM preparations. The controversial issues concerning the bronchodilating effect of tidal breathing have been discussed previously (21).

Decline of force induced by strain at higher oscillating stress. At higher levels of oscillating stress, the decline of force...
became significant. The decline of force in conditions simulating 20 cm H2O-transpulmonary pressure excursions was about 20%. This was true when the oscillations were performed in a relaxed muscle prior to stimulation (protocol 1), as well as when the oscillations were initiated prior to stimulation, irrespective of the concentration of ACh (protocol 2). This later observation suggests that strain is not the only contributing factor affecting ASM force. It raises the possibility that strain history may be sufficient to attenuate ASM force. However, the decline of force in protocol 3 was slightly but significantly attenuated as the concentration of ACh increased (Fig. 8). So in the absence of strain history, the magnitude of strain is related (although not proportionally) to the decline of force, suggesting that preventing strain by increasing tone does attenuate the decline of force induced by oscillating stress. This is consistent with a very recent study discussed next (18).

As we were writing this manuscript, Lavoie and coworkers reported a similar relationship between the diminution of strain induced by tone vs. the alteration of ASM contractility in precision-cut lung slices (PCLS) from human lungs that were subjected to oscillating conditions simulating breathing (18). The sequence of the intervention they used resembled protocol 3 of the present study, in which muscle stimulation was initiated and the maximal level of contraction achieved before instigating stress oscillations. Not only did they show that tidal breathing was inefficient to bronchodilate stimulated airways no matter the initial amount of bronchoconstriction, but they found that increasing the severity of bronchoconstriction attenuated the strain and the level of bronchodilation induced by higher magnitudes of stress. Lavoie and coworkers measured airway narrowing (ASM shortening), which is more relevant to in vivo physiology than our approach of measuring ASM force. On the other hand, our approach offers a better control over the load. It is difficult to estimate the amount of stress that is actually transmitted to the muscle during breathing maneuver simulations in PCLS. The authors used the stress that was necessary to strain the airways by a physiological amount in the relaxed airway smooth muscle and exposed the activated airways to the same stress. However, they cannot quantify this stress. It is also difficult to set the preload in PCLS. Our experimental setup allowed precise control of the stress acting on the muscle and its effect on changes in ASM length (strain) at any given level of tone. Nevertheless, despite using two different approaches, tissues from different locations within the lung (trachea vs. intraparenchymal airways) and different species (sheep vs. humans), the conclusions drawn are similar. Collectively, these studies suggested that limiting strain by increasing tone attenuates the decline of force caused by oscillating stress. This suggests that the results of Lavoie and coworkers (18) may be explained at least partially by the effect of strain on ASM force.

Decline of force not induced by strain at higher oscillating stress. In addition to the Lavoie et al. study, our results also demonstrated that tone results in a change in the slope of the relationship between strain and the decline of force compared with the relaxed ASM (protocol 1). In both protocol 2 and protocol 3 a decline of force was still present at high levels of tone despite the quasi absence of strain. In protocol 3, for example, ACh at 10^{-4} M reduced the strain to 0.7% during simulated 20 cm H2O-transpulmonary pressure excursions while the decline of force was still 6.1%. It is also important to notice that the declines of force are expressed in percentages, not in absolute values. In absolute values, a 6.1% decline of force at 10^{-4} M when the ASM generated 141.2KPa(SD4.0) is greater than the decline of 11.1% that occurred at 10^{-7} M when the ASM generated 16.4KPa(SD2.8) (8.6 vs. 1.8 kPa). This implies that very little strain is sufficient to substantially affect ASM force. However, this was only true when the oscillating stress was high. At the same level of strain induced at lower oscillating stress (such as the one occurring during tidal breathing simulations), no decline of force was observed. If only very little strain is sufficient, one would predict that even simulated tidal breathing would substantially decrease ASM force at low tone. Together, the results suggested that several factors may govern the decline of force induced by breathing maneuvers. One factor may be the magnitude of strain, as previously suggested (18, 24). This is easy to conceive. Greater levels of strain would lead to greater levels of disturbance in myosin cross-bridge cycling (8), as well as in the interconnectivity between the contractile apparatus and the surrounding tissue (33). However, in our hands, strain did not explain everything (in terms of force decline). Another factor that seemed to contribute to the decline of force induced by breathing maneuvers is the magnitude of stress itself. The ASM may be endowed with the ability to sense changes in stress even when the strain induced by the stress is minimal due to the presence of tone. Small strain could cause signaling through such a hypothesized stress sensor. Although we are not aware of any such sensing mechanism, it could explain the results of the present study. It would also reconcile the consistent finding that force oscillations cause relengthening of highly activated ASM even when the strain is expected to be very limited (7, 16, 17, 20, 25).

Relevance of these findings in vivo. Translating our findings to in vivo observations such as bronchodilation and bronchoprotection (i.e., attenuated airway responsiveness due to a DI, or a series of DIs, taken prior the induced constriction) offered by DIs is not an easy task. In vivo studies have suggested that the magnitude of the airway wall strain is an important determinant of the respiratory relief obtained by the oscillatory stress of breathing maneuvers. For example, airway distensibility was demonstrated to correlate positively with the bronchodilator effect of DIs (27). In that study though, the distensibility was not measured during the bronchoconstrictive challenge. More convincingly, the size of lung volume expansion attained during repeated DI maneuvers was positively related to the bronchodilator effect (29). Increasing the depth of breathing, and presumably the strain on the airway wall, during exercise was also associated with a bronchodilator effect, similar in magnitude to high doses of a β2-agonist (19). Even in severe cases of bronchoconstriction such as during a late asthmatic reaction or a spontaneous asthmatic episode, increasing the depth of breathing by exercise has been shown to have a potent bronchodilator effect (6). This may be relevant to the findings of the present study. In inflamed airways, a larger amount of spasmogens are present (1). These spasmogens increase the level of ASM activation and likely limit the strain experienced by the airway wall during breathing; similar to the effect of increasing concentrations of ACh on the strain induced by simulated breathing maneuvers reported in the present study. Despite presumably smaller airway wall strain in inflamed conditions, increasing the depth of breathing still has...
of powerful dilating effect (6). This supports our conclusion stating that high stress with only little strains may be sufficient to decrease ASM contractility and perhaps bronchodilate the airways in vivo.

We do not want to pretend that airway physiology can simply be explained by ASM mechanics. Even comparisons between ex vivo measurements of ASM strips with whole airway segments can be tricky. Whereas oscillations of ASM strips were shown to decrease subsequent isometric force development, equivalent pressure oscillations of whole airway segments prior to ASM stimulation were shown to increase isovolumetric contraction (23). This was also confirmed in vivo. DIs taken prior to bronchoconstriction were shown to increase airway responsiveness when the measure of airway narrowing (therein conductance) did not require a DI maneuver (5). In this later study, the broncho-protective effect of DIs was confirmed when the measure of narrowing did require a DI (therein, forced expiratory volume in 1 s – FEV1). The authors concluded that DIs prior to bronchoconstriction increase airway responsiveness but facilitate bronchodi-lation during a subsequent DI (the later only in healthy subjects) (5). This finding is consistent with the fact that bronchopteprotection (measured using FEV1) is impaired only in asthmatics (reviewed in Ref. 3). To test this observation in our experimental settings, we will need to measure the tension required to relengthen a contracted strip that has been subjected, or not, to previous force oscillations. The ultimate answers to those complicated issues will certainly come from the concerted input of findings obtained by experiments performed at different scales (molecule, cell, tissue, organ, and whole organism).

Conclusion. In conclusion, the results of this study suggest that tone can greatly attenuate the strain and, to a lesser extent, the decline of force experienced by ASM during simulated breathing maneuvers. An additional benefit from therapeutic strategies aimed at decreasing tone may thus be an increase in the efficacy of breathing maneuvers to stretch the ASM and, by doing so, decrease its force-generating capacity. However, the results also suggest that the decline of force induced by oscillating stress cannot be precisely predicted by measuring the magnitude of strain, because the decline of force and the strain were related but their proportionality varies with oscillatory history. Altogether, and to the extent to which the force generated by ASM is involved, the results suggest that a significant part of the bronchodilator effect of DIs should persist with large or small strain that arises at low or high tone, respectively.

REFERENCES


