Responsiveness of the isolated airway during simulated deep inspirations: effect of airway smooth muscle stiffness and strain

Peter B. Noble, Peter K. McFawn, and Howard W. Mitchell

Physiology, School of Biomedical, Biomolecular, and Chemical Sciences, University of Western Australia, Perth, Western Australia, Australia

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BREATHING MOVEMENTS PROVIDE powerful regulation of respiratory mechanics in humans, notably a strong reduction in existing bronchoconstrictor tone after deep inspirations (DIs) or sighs (8, 9, 28, 31–33). DIs may protect against the effects of bronchoconstriction when they are performed before bronchoconstrictor challenge, a phenomenon dubbed bronchoprotection (20, 24, 33). As a result of the dilator and potential protective actions of DI, dose-response parameters (e.g., the maximum effect) of bronchoconstrictor agonists used in provocation studies are depressed by DI (9). The beneficial effects of DI in healthy individuals are reduced or absent in asthma (9, 11, 32). Furthermore, healthy individuals develop asthma-like responses to bronchial provocation when DIs are prohibited (36). Therefore, in addition to providing short-term relief of bronchoconstriction in some populations, the presence or absence of normal inhibitory responses to DI may partly account for bronchial hyperresponsiveness typically seen in asthma and other obstructive diseases.

The mechanism(s) responsible for the effects of lung inflation is uncertain. Several hypotheses include the balance of lung and airway forces (load) across the airway wall during a breathing cycle, which may favor bronchodilation in healthy individuals, but not asthmatic patients, and the effects of dynamic strain on airway cells, especially airway smooth muscle (ASM). For example, small oscillatory strain (>1% change in length) reduces active force in isolated ASM cells, which would presumably produce bronchodilation in vivo (12, 35). However, the airway wall is a complex multilayered structure with nonlinear mechanical and tissue-tissue interactions that may contribute to the effects of lung inflation by modifying responses of ASM within its local environment. Little is known about the intrinsic response of the intact airway wall to oscillatory load produced by breathing (i.e., transmural load) or the factors that may determine its response, such as viscoelastic properties of wall components, which determine the degree of ASM strain under the dynamic conditions of lung inflation. Of particular importance is the stiffness of the airway, which, in addition to passive mechanical properties of the airway wall, could also be subject to the amount of active ASM tone. Contractile agonists, such as those used in provocation testing or present endogenously, have been shown to increase the stiffness of ASM cells in culture (1, 18), which could produce positive-feedback effects on ASM responsiveness under dynamic conditions associated with DI. How the above-described scenario plays out at the airway level or lung is unclear, since relationships between airway stiffness and responsiveness in the presence of DI and ASM tone have not been determined.

The present study examines the intrinsic airway response to dynamic load applied as transmural pressure to the intact airway to determine whether major effects of DI observed in vivo are explained by the effects of load at the airway level. To simulate airway loads during breathing, we cycled the luminal volume of liquid-filled bronchial segments at a fixed airway length. Different breathing patterns (e.g., tidal breaths and DIs) were simulated across the full range of airway responsiveness and at different levels of airway stiffness. We hypothesized that under dynamic conditions the bronchodilator responses to load in intact airways are related to ASM strain and are dependent on the airway stiffness produced by ASM contraction.

Address for reprint requests and other correspondence: P. B. Noble, Physiology, School of Biomedical, Biomolecular, and Chemical Sciences, Univ. of Western Australia, 35 Stirling Hwy., Crawley, Perth, Western Australia, 6009, Australia (e-mail: Peter.Noble@uwa.edu.au).

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METHODS

Animal Handling

All animal experiments conformed to the American Physiological Society’s “Guiding Principles in the Care and Use of Animals” and were approved by the institutional ethics and animal care unit. Female pigs (25 kg body wt) were initially sedated with tiletamine-zolazepam (4.4 mg/kg im) and xylazine (2.2 mg/kg im) and then exsanguinated under pentobarbitone sodium anesthesia (30 mg/kg iv). Lungs were removed and preserved on ice.

Bronchial Segment Preparation

A 2-cm segment of bronchus (~2-mm ID) was dissected from the lower lobe of the left lung, and its side branches were tightly ligated (15, 29). The segments were cannulated at each end and placed horizontally in an organ bath containing gassed (95% O2-5% CO2) Krebs solution (mM: 121 NaCl, 5.4 KCl, 1.2 MgSO4, 25 NaHCO3, 5.0 sodium morpholinopropane sulfonic acid, 11.5 glucose, and 2.5 CaCl2) at 37°C. Intraluminal (therefore, transmural) pressure was set by the height of a Krebs solution-filled reservoir, which was connected at one end of the segment. The opposite end of the segment was connected to a 1-ml syringe via stiff polyethylene tubing, which was also filled with Krebs solution. The length of the segment was stretched to a length shown previously to approximate functional residual capacity (FRC) in the pig lung, i.e., ~105% of the fully deflated length at 0 cmH2O (30). Intraluminal pressure was set to 5 cmH2O.

Intraluminal pressure was measured by a calibrated transducer (model P23ID, Gould) and a PowerLab data-acquisition system (ADInstruments). Closure of a tap inserted between the segment and the Krebs solution-filled reservoir created a closed liquid-filled state. In the closed state, ACh-stimulated ASM contraction produced an increase in intraluminal pressure, referred to as “active pressure,” and is a measure of ASM contractility (16). In the closed state, intraluminal pressure also increased cyclically with volume oscillation (see below).

Volume Oscillation

When required, the luminal volume of the airway was cycled via the Krebs solution-filled syringe. The syringe plunger was driven by a direct-current motor (model JDTH-2250-FX-1C, Litton Clifton Precision) via a BioPWM sequential motor controller (model V1.0) and custom-designed software (Shane De Catania, ©2005), which allowed sinusoidal or ramp changes in volume and, therefore, pressure. The amplitude, frequency, and duration of volume oscillation were set, and absolute changes in bronchial volume were determined from the linear movement of the syringe after correction for system compliance (0.12 μl/cmH2O). Airway luminal volume at 5 cmH2O was determined from fully deflated luminal volume at 0 cmH2O, estimated at the end of the day by the volume of Krebs solution that filled the lumen at atmospheric pressure (26) plus the volume of fluid required to inflate the airway from 0 to 5 cmH2O after stress relaxation.

Experimental Protocol

After dissection, airways were allowed to equilibrate to organ bath conditions for ~1 h under a passive luminal pressure of 5 cmH2O, viability of tissue stimulated with 10−4 M was confirmed, and tissue was allowed 30 min of recovery. Two experimental protocols were used to investigate the effects of dynamic load on airway responsiveness, as assessed from cumulative dose-response curves (DRCs) to ACh (10−7–3 × 10−3 M).

Protocol A. DRCs to ACh were recorded under the following conditions: 1) static conditions, i.e., without volume oscillations, 2) simulated tidal volume oscillations, and 3) simulated tidal oscillations and intermittent DIs (see below). Each bronchial segment was subjected to all three conditions, conducted in randomized order. To simulate tidal breaths, volume oscillations in relaxed airways were adjusted so that they produced a sinusoidal pressure swing from ~5 to 10 cmH2O (i.e., a trough-to-peak pressure of 5 cmH2O, a ~9-μl increase in bronchial volume) at 0.25 Hz (i.e., 15 breaths/min). To simulate DI, pressure was ramped from ~5 to 30 cmH2O (i.e., a trough-to-peak pressure of 25 cmH2O, ~27-μl increase in bronchial volume) with a cycle period of 4 s. Any change in stiffness produced by ACh (see RESULTS) led to an increase in trough-to-peak tidal and DI pressure oscillations. The increased pressure in the presence of ACh was not adjusted back to the pressure in the relaxed airway; i.e., this was an unconstrained pressure protocol.

Protocol B. Luminal pressures produced by tidal and DI volume oscillations in the presence of ACh, which increased airway stiffness, were adjusted so that the trough-to-peak pressures remained constrained to the pressure in the relaxed airway (i.e., 5 cmH2O for tidal and 25 cmH2O for DI). This was achieved by reducing the volume of fluid cycled into the airway lumen at each dose of ACh, which was established separately in each airway preparation before the analysis. A different group of bronchial segments was used in protocol B (cf. protocol A). However, each airway was also used to generate an unconstrained DRC to ACh (same method as protocol A), allowing direct comparisons of bronchodilation, airway stiffness, and ASM strain (see RESULTS) in the same airway when the oscillation pressures were constrained or unconstrained to their relaxed values.

In protocols A and B, airway contraction was assessed from the increase in trough pressure at isovolume points, i.e., active pressure. When DRCs included DIs, DIs were delivered at the plateau in response of each ACh dose. The resulting pressures were tracked for 1 min, then the next ACh dose was administered (see RESULTS). Protocols A and B were preceded by a ~20-min preconditioning period designed to fully adapt airways to each mechanical condition described above: static pressure, tidal oscillation, or tidal oscillation with DIs at the rate of one every 6 min, which is the rate of spontaneous sighs in humans (3). During the preconditioning period, airway viability was continuously monitored by transient quickly reversible contractions to electrical field stimulation. Field stimulation (60 V, 3-ms pulse duration at 30 Hz) was induced using a Grass S44 square-wave stimulator via platinum electrodes encircling the segment.

A different group of bronchi was used in separate experiments from protocols A and B to evaluate the time course of airway responses to DI (see Fig. 3) and the effects of different amplitudes of DI on airway contraction (Table 1).

Analysis and Statistics

Active pressure was calculated from total intraluminal pressure at each dose of ACh minus passive pressure. In the case of cycling airways, pressures were measured at isovolume points in the cycle, i.e., trough pressures. Sigmoidal (variable-slope) DRCs were fit to data using Prism data analysis software, which computed the maximal response (Emax) and the negative logarithm of the dose producing half-maximal response, i.e., sensitivity (pD2). Prism software was also used for statistical comparisons. Differences in Emax and pD2 were compared using matched ANOVA or paired t-tests (depending on group size). Linear relationships were determined from Pearson’s correlation analysis. Values are means ± SE, where n represents the number of airways and pigs.

RESULTS

Effect of Tidal Breathing on Airway Responsiveness

As described in METHODS, simulated tidal volume oscillations produced pressure cycles from 5 to 10 cmH2O (i.e., trough-to-peak amplitude of 5 cmH2O) in relaxed airways. However, in
the presence of ACh, i.e., in contracted bronchi, pressure cycles increased in amplitude (tough-to-peak value; Fig. 1A), typically approaching ~20 cmH2O, indicating that the airway was stiffer in the presence of ACh. Airway stiffness was determined from the trough-to-peak pressure change divided by the change in bronchial volume. Airway wall stiffening exhibited a sigmoidal dose-response behavior (Fig. 1B) and increased from 0.57 ± 0.03 cmH2O/μl in relaxed airways to 3.75 ± 0.58 cmH2O/μl after a maximal dose of ACh (n = 5), a nearly six- to sevenfold increase.

ACh-induced airway contractions recorded under tidal cycling conditions were reduced compared with those recorded under static conditions (protocol A). Static and tidal ACh DRCs are shown in Fig. 2. Emax was 74.7 ± 2.9 cmH2O in static airways and 65.7 ± 3.4 cmH2O in tidally cycled airways (P < 0.05, n = 6). pD2 values were 4.23 ± 0.09 and 4.30 ± 0.05 in static and tidally oscillated airways, respectively (not significant).

Effect of DIs on Airway Responsiveness

The time course of responses to DI was assessed in a group of bronchial segments (n = 4), each of which was contracted to a submaximal (~pD2) dose (10−4 M) of ACh. A DI produced an immediate reduction in intraluminal pressure followed by rapid recovery in pressure, which then stabilized after ~1 min and remained almost unchanged for ≥5 min (Fig. 3). In subsequent dose-response studies, airway responses to ACh were therefore recorded up to 1 min after DI. Bronchial responsiveness in the presence of a DI at each dose of ACh (protocol A) was greatly reduced compared with that of tidally oscillated airways. Percent bronchodilation (i.e., percent decrease in intraluminal pressure compared with pre-DI level) was relatively constant across all ACh concentrations and ranged from 40 to 50%. In Fig. 4, tidal DRCs are compared with DI DRCs (n = 6) measured immediately and 1 min after DI. Emax fell immediately after DI from 65.7 ± 3.4 to 38.6 ± 3.6 cmH2O (P < 0.001 vs. tidal) and recovered partially to 51.0 ± 2.7 cmH2O 1 min later (P < 0.001). There was also a small but significant reduction in sensitivity immediately after DI. Values of pD2 were 4.30 ± 0.05 in tidally oscillated airways and 4.10 ± 0.06 immediately after DI (P < 0.05). However, the decrease in sensitivity was no longer present 1 min after DI.

To assess whether DI conditioned the airway to subsequent contraction to ACh (i.e., caused bronchoprotection), airway

<table>
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<tr>
<th>DI Amplitude, cmH2O</th>
<th>ASM Strain, %</th>
<th>Bronchodilation, %</th>
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<tr>
<td></td>
<td>Immediate</td>
<td>After 1 min</td>
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<tr>
<td>49.9 ± 3.5†</td>
<td>10.6 ± 2.4*</td>
<td>60.7 ± 4.5†</td>
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<td>39.5 ± 2.3‡</td>
<td>7.3 ± 1.7‡</td>
<td>41.4 ± 5.6‡</td>
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<tr>
<td>21.7 ± 1.7</td>
<td>3.5 ± 0.5*</td>
<td>9.6 ± 5.8</td>
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Values are means ± SE (n = 4). DI amplitude, trough-to-peak pressure change produced by deep inspiration (DI); ASM, airway smooth muscle; strain, change in ASM circumference with inflation from an intraluminal pressure of 5 cmH2O; bronchodilation, decrease in intraluminal pressure compared with pre-DI levels. Airways were precontracted to 10−6 M ACh 3 times; on each occasion, effect of 1 of 3 different-sized DIs was determined. Magnitude of bronchodilation produced by DI was reduced with decreasing DI amplitude and ASM strain. Significantly different from subsequent row: *P < 0.05, †P < 0.01; ‡P < 0.001 (matched 1-way ANOVA and Newman-Keuls post test).
contraction was also measured at the plateau of each ACh dose immediately before DI produced bronchodilation and was compared with the contraction in the tidal breathing simulation (protocol A). The “pre-DI” DRC (n = 6) was similar to the DRC in tidally oscillating airways (without DI; Fig. 5) with no significant difference in Emax or pD2, suggesting that DI did not affect contraction induced subsequent to DI.

As with tidal oscillations, the amplitude of DIs (25 cmH2O from trough to peak in relaxed airways) increased with ACh-induced stiffening and approached ~75 cmH2O after maximal contraction. When DI pressure cycles were constrained to values in the relaxed airway (protocol B), the bronchodilation immediately after DI was substantially reduced (Fig. 6A). When expressed as percent decrease in intraluminal pressure compared with pre-DI levels, the amount of bronchodilation fell with increasing ACh concentrations (Fig. 6B) and was greatly reduced compared with that in experiments where the pressure was unconstrained. Furthermore, when pressure cycles were constrained, airway contraction returned to pre-DI levels by 1 min after DI, in contrast to unconstrained pressure conditions, where relaxation persisted for >1 min (Fig. 4). The above-described differences in bronchodilation produced by constrained and unconstrained DIs were observed above the contractile threshold dose (>3 × 10⁻⁶ M) of ACh. At lower ACh doses, i.e., without ASM contraction, DI-induced relaxation was similar in both groups of airways.

![Fig. 3. A: effect of deep inspiration (DI) on intraluminal pressure in an isolated bronchial segment. Airway was contracted to a submaximal dose (10⁻⁴ M) of ACh; after plateau in response, DI was induced, and change in pressure was tracked from 0 min (immediately after DI) to 5 min after DI. B: mean DI-induced changes in intraluminal pressure. Responses to DI are compared with responses to tidal oscillations recorded over the same time period in the same airway. DI induced >5 min of bronchodilation followed by partial recovery, which stabilized after ~1 min. Values are means ± SE (n = 4). *P < 0.05; **P < 0.01; ***P < 0.001 vs. tidal oscillation (matched 2-way ANOVA and Bonferroni post test).](http://jap.physiology.org/)
Relation of Airway Stiffness, ASM Strain, and Bronchodilation

We investigated whether, under pressure-constrained conditions (protocol B), the bronchodilatory response to DI is limited by airway stiffening produced by contractile activation. Percent bronchodilation immediately after DI was plotted against airway stiffness for each dose of ACh (same data as Fig. 1B). Only bronchodilation and stiffness data at or above the ACh contraction threshold, i.e., $\geq 3 \times 10^{-6}$ M, were used. Airway stiffness was negatively correlated with DI-induced bronchodilation (Fig. 7).

We further assessed whether the reduction in bronchodilation with increasing ACh doses in airways in which the oscillation pressure was constrained (protocol B) was a result of reduced ASM strain. ASM strain is defined as the change in ASM circumference (i.e., length of ASM within the airway wall) from intraluminal pressure of 5 cmH2O. To estimate ASM strain with airway inflation, assuming a cylindrical airway wall, we first calculated airway luminal cross-sectional area from luminal volume (5 cmH2O) and the length of the segment. ASM perimeter (i.e., circumference or $P_{mo}$) was then determined from the total cross-sectional area enclosed by ASM (i.e., $A_{mo}$), including inner wall area, which was measured morphologically in a group of similar-sized bronchi (inner wall area = $0.9 \pm 0.1$ mm$^2$, $n = 9$). As for correlations with airway stiffness, only bronchodilation and ASM strain data at or above the ACh contraction threshold, i.e., $\geq 3 \times \ldots$

Fig. 6. A: relative effects of DI when DI-evoked pressure oscillations were constrained to a trough-to-peak amplitude of $-25$ cmH2O (heavy trace) or left unconstrained (light trace). Traces, which are superimposed for clarity, are from the same airway contracted to $10^{-4}$ M ACh on 2 separate occasions. Trough pressures in both traces, before and after DI, are at isovolume points. B: bronchodilation (% decrease in intraluminal pressure vs. pre-DI levels) measured immediately after DI for pressure-constrained airways ($n = 5$, protocol B). At $>3 \times 10^{-6}$ M ACh, where contraction occurred, percent bronchodilation fell in proportion to ACh dose. $**P < 0.01$; $***P < 0.001$ vs. relaxed airway “R” (matched 1-way ANOVA and Newman-Keuls post test). Compared with pressure-constrained airways, percent bronchodilation in unconstrained airways was relatively constant at 40–50% across all ACh concentrations (see Fig. 4).

Fig. 7. Relationship between airway stiffness and immediate DI-induced bronchodilation for 5 airways. Trough-to-peak pressure amplitude of DI was constrained to $-25$ cmH2O (protocol B). Data points for each airway are stiffness and bronchodilation at 7 ACh doses ($3 \times 10^{-6}$–$3 \times 10^{-3}$ M, which are at or above contraction threshold). Airway stiffness was negatively correlated with DI-induced bronchodilation [Pearson’s correlation analysis ($R^2$)].

Relation of Airway Stiffness, ASM Strain, and Bronchodilation

We investigated whether, under pressure-constrained conditions (protocol B), the bronchodilatory response to DI is limited by airway stiffening produced by contractile activation.
$10^{-6}$ M, were used. ASM strain was positively correlated with percent bronchodilation (Fig. 8). The magnitude of ASM strain depended on the state of contraction and ranged from 11.2 ± 1.9% in a relatively relaxed airway (threshold ACh dose) to 3.0 ± 0.3% after maximal contraction. The magnitude of ASM strain at which zero bronchodilation was observed (i.e., x-intercept in Fig. 8) was 0.75 ± 1.05% ($n = 5$), suggesting that ASM strains of $>0.75\%$ are required to produce bronchodilation. In comparison, when pressure was unconstrained, the magnitude of ASM strain produced by DI remained relatively constant across all ACh doses and was, on average, 11.4 ± 0.6%.

In a group of four bronchi, we determined the relationship between the amplitude of DI and the bronchodilation produced by DI. Each bronchial segment was contracted to a submaximal ACh dose ($10^{-4}$ M) three times and subjected to one of three different amplitudes of DI. Table 1 lists DI amplitude (trough-to-peak pressure), ASM strain, and the amount of bronchodilation produced immediately and 1 min after DI. The amount of DI-induced bronchodilation decreased proportionally with reduced DI amplitude and ASM strain.

**DISCUSSION**

It is well documented that DIs in humans provide powerful regulation of respiratory system responsiveness (8, 9, 20, 24, 28, 31–33); however, the mechanism(s) is poorly understood. The effect of oscillating the length of isolated ASM on muscle force has been previously documented (12, 35) and could explain some of the beneficial effects of DI in vivo. In the present study, we have examined airway contraction in intact midsized bronchial segments and compared airway responsiveness (sensitivity and reactivity) under static conditions or in the presence of oscillatory load (applied as transmural pressure) to simulate tidal breathing and, importantly, tidal breathing with periodic DI. The approach is physiological, since ASM in the airway wall is subject to the viscoelastic properties of all airway structures and, thus, is exposed to stresses encountered in vivo, which is more difficult, if it is even possible, using isolated ASM or culture techniques. To our knowledge, this is the first study of responsiveness at the airway level that has replicated complex physiological breathing patterns (see below). The full range of airway responses with respect to effects of different oscillatory loads has not previously been reported. We have demonstrated substantial reductions in airway responsiveness in the presence of DI. However, critical levels of ASM load are required to produce the bronchodilator effects of inflation, which may, under some conditions, exceed those normally present in vivo. Our findings show that the capacity of DI to reduce bronchoconstriction is markedly restricted by stiffening of the airway wall in response to contractile stimulation, which reduces ASM strain. These findings have important implications to our understanding of possible mechanisms through which DIs exert their effects on respiratory system responsiveness in the healthy and asthmatic states.

In our study, simulated tidal maneuvers were volume oscillations that produced trough-to-peak pressure swings in relaxed airways from 5 to 10 cmH$_2$O, approximating inflation from FRC to end-inspiratory volume. The airway segments were stretched to a length corresponding to FRC as determined in anesthetized pigs in our laboratory (30). Tidal oscillation frequency was 0.25 Hz, which corresponds to the human breathing frequency and also the duration of spontaneous sighs, which is reported to be $\sim$4–6 s (3). The amplitude of DIs was chosen to simulate in vivo conditions, with a trough-to-peak pressure change from 5 to 30 cmH$_2$O approximating the pressures at FRC and total lung capacity, respectively. Airways were exposed to a preconditioning period ($\sim$20 min) to allow for adaptation to the dynamic conditions and to eliminate a possible artifact from a static-to-dynamic transition. The time interval between DI delivery is known to influence the effects of DI before (20, 23, 24) and after (23) bronchial challenges. In the present study, the DI preconditioning period consisted of tidal oscillations and intermittent DIs delivered at a rate of one every 6 min, which is the rate at which humans spontaneously sigh (3). The rate of DI delivery during the DRC was somewhat higher ($\sim$3–5 min), since it was determined by the plateau in bronchoconstrictor response for each dose of ACh. Finally, the length of bronchial segments...
was fixed, such that pressure oscillation produced radial expansion of the airway. Although the effects of lung inflations are thought to arise predominantly through an increase in airway diameter, airways also elongate with lung inflation, and this may contribute to effects on airway caliber. In a previous study from our laboratory (22), we evaluated the response of the airway to cyclical elongation and observed transient bronchodilation at large oscillation amplitudes but a paradoxical, small constriction at tidal oscillation amplitudes. With the techniques used in that study (in which the luminal volume was fixed during cycling), airway elongation likely caused cyclical shortening of ASM length, which may have been responsible for the physiological effects that were observed. The integrated geometrical and physiological effects produced by airway inflation in vivo are therefore more complex than those in vitro models.

Using the above-described tidal and DI volume oscillation parameters (i.e., “unconstrained” pressure oscillations), we showed that otherwise-normal pig airways were essentially converted to a hyperresponsive state when contraction was assessed in the absence of DIs, as shown by shifts in DRC parameters. When responsiveness was measured in the presence of DIs, airway reactivity (E_max) was ~40–50% less than in the absence of DIs, and there was a small decrease in airway sensitivity (pD_2). The amount of bronchodilation was probably underestimated because of system compliance. In other words, as the airway became stiffer with ACh, the constant-volume oscillation would deliver a slightly smaller volume to the airway compared with the system tubing. The resulting small reduction in airway strain in the presence of ACh could reduce the amount of bronchodilation. The suppression of airway responsiveness by DI or, in other words, the increased responsiveness after DI was denied, was a consequence of the transient DI-induced ASM stress or strain. Several groups have proposed that the abnormal response to DI in asthmatic patients may give rise to airway hyperresponsiveness, which is a primary characteristic of human asthma. Skloot et al. (36) showed that healthy human subjects began to exhibit asthma-type symptoms if DIs were prohibited from their normal breathing rhythm. Previously healthy individuals began to mimic asthma patients purely as a result of exclusion of DIs; i.e., a hyperresponsive state was reached. Similar findings were reported by Brusasco et al. (9), although in their study it was clear that DI alone could not account for all the differences observed between healthy subjects and asthmatic patients. Our findings are conceptually similar to the aforementioned studies (9, 36) but represent a significant advance in our understanding of underlying factors, since we show not only that the airway contributes to the effects of DI but, also, that switching between normoresponsive and hyperresponsive states (as shown from DRCs) occurs at an airway level, which had not previously been established.

In ACh-contracted airways, DIs produced substantial bronchodilation immediately after DI and, additionally, a sustained bronchodilation that persisted for >5 min. Although the immediate effect was present across the entire range of airway response, the sustained effect was only seen once airways attained high levels of contraction. These effects of DI on toned airways were present when the volume of oscillation was held constant throughout the ACh DRC (i.e., unconstrained pressure oscillations). With increasing levels of ASM contraction by ACh, airway stiffness increased greatly, which is consistent with measurements of stiffness at the single ASM cell level (1, 18). Agonist-induced cell stiffening is likely to be related to cross-bridge recruitment and/or contractile filament remodeling and, similar to our findings, is dose dependent (1). The additional stiffness of the airway wall produced by ACh then greatly increased the amplitude of the pressure oscillations produced by tidal and DI maneuvers. For example, although a DI in the relaxed state increased pressure by 25 cmH_2O (from trough to peak), at the highest dose of ACh the pressure swing was ~75 cmH_2O, which is likely to be significantly greater than the transmural pressure in vivo. Gunst et al. (17) were the first to investigate the effect of oscillatory load on airway contraction in dogs. Similar to our findings, they showed a reduction in airway contraction with volumetric expansion, suggesting that responsiveness is regulated at the airway level. Also similar to our results, the oscillations in volume that depressed contraction were accompanied by large trough-to-peak pressure fluctuations of ~30–50 cmH_2O.

In light of the high pressures or stresses associated with bronchodilation, we adopted a second protocol in which the trough-to-peak pressure during oscillation was adjusted, i.e., “constrained,” to 5 cmH_2O for tidal oscillation and to 25 cmH_2O for DI. This was achieved by reducing the volume of oscillation and, thus, the airway strain at each dose of ACh. In these pressure-constrained airways, the immediate bronchodilation after DI was reduced and the reduction in airway contraction normally present 1 min after DI was absent. Most notably, the percent bronchodilation produced by the constant-pressure DIs reduced markedly with increasing doses of ACh and was almost abolished at maximal doses. Increasing doses of ACh were associated with a marked increase in airway stiffness, which in turn reduced ASM strain in response to the oscillatory load applied to the airway wall. If it is assumed that the bronchodilator effects of oscillatory load are based on reduced ASM force, as documented in other studies (12, 35), the reduced bronchodilation discussed above can be attributed to the increase in airway stiffness caused by ASM contraction. Furthermore, the fall in bronchodilation for a constant-pressure DI (i.e., constrained) implies that ASM strain, rather than stress, is essential for ASM force inhibition, as suggested in a previous study (31).

The effect of ACh on stiffness in our study allowed a relationship between airway stiffness, ASM strain, and bronchodilation to be established. The relationship between stiffness and ACh-induced contraction could apply generally to other-sized airways and other factors remaining equal to the reduction in bronchodilation in the presence of different levels of ASM tone. Some in vivo studies have also considered the effectiveness of DI on stiff contracted airways. Scichilone et al. (33) found that, with increasing levels of bronchoconstriction, DIs became progressively less beneficial in healthy individuals, as well as asthmatic patients. Brown and Mitzner (5) used high-resolution computed tomography to measure luminal diameter in dog airways across a range of different lung volumes and showed a reduced effect of lung inflation in the presence of ASM tone. Asthmatic patients often exhibit an increase in airway stiffness (4, 19), which may be the result of thickening of wall compartments (10) or the presence of high levels of endogenous ASM tone, which as shown in the present study reduces the capacity of DI to produce bronchodilation. Given

J Appl Physiol • VOL 103 • SEPTEMBER 2007 • www.jap.org
the importance of airway stiffness in determining DI responses, it is important to comment on the airways used here. Interspecies comparisons of airway stiffness from the literature are difficult because of methodological differences, but overall the passive stiffness of pig airways is similar to that of human airways (26, 27, 37). Clearly, there may also be structural differences among species with regard to wall components (e.g., cartilage content and architecture). More relevant, however, we have found no comparable information from other species on airway stiffness in the presence of different doses of ACh, as reported in our study, where, under dynamic conditions, stiffness increases nearly six- to sevenfold when ASM is in its most contracted state. Our study indicates stiffness associated with ASM contraction, rather than just the passive properties of the airway wall, determines the responsiveness of the airway under these simulated dynamic conditions.

How do our findings in single isolated airways relate to integrated respiratory function? 1) Airway responses to DI are likely to be modified in vivo as a result of airway-parenchymal interactions. The airway-parenchymal hysteresis theorem predicts that the hysteretic behavior of lung parenchyma after DI will reduce elastic recoil pressure, favoring bronchoconstriction (13). Consequently, the magnitude of bronchodilation observed in vitro will be less in vivo because of a fall in lung recoil pressure after DI. Furthermore, in light of the importance of airway stiffness established here, if parenchymal responses with DI are considered, one could imagine that a particularly stiff airway may not dilate at all and could potentially constrict after DI. Such a scenario may have relevance to asthma, where, on occasion, DIs produce transient bronchoconstriction (8, 14, 25). 2) Airway responses to DI may reflect contributions from different-sized airways with various mechanical properties and, perhaps, different responses to DI. For instance, peripheral airways could be more compliant and sensitive to DI than larger airways, as suggested from studies by Brown et al. (8). 3) DIs may regulate respiratory mechanics through other mechanisms not necessarily related to the airway wall, such as liberation of bronchorelaxant mediators (7) or activation of neural pathways (21).

Our results show that the bronchodilator effects of DIs are increased in proportion to the strains applied to ASM, which is in general agreement with studies of DI of different amplitudes in human subjects in vivo (31) and in dogs (6). However, our study goes further in defining the relation between airway response and ASM strain in the airway wall under dynamic conditions (Fig. 8). The measurement of ASM strain is indirect and based on several assumptions and should be considered an approximation. Our results suggest that the critical level of strain in ASM in situ necessary to produce bronchodilation is ~1%, which is remarkably consistent with studies in isolated ASM, which show force inhibition once length perturbations exceed 1% (12, 35). On the basis of the relationship between bronchodilation and ASM strain in our study, the reason for the greater degree of bronchodilation produced by unconstrained pressure oscillations than by constrained pressure oscillation becomes apparent. The ASM strain produced by an unconstrained DI was ~11%, which accounts for the pronounced dilation. The magnitude of ASM strain produced by DI in a contracted airway under more physiological conditions (i.e., pressure constrained conditions) is considerably less than that during unconstrained pressure conditions and fell typically from ~11% to ~3% when ASM was in its most stiffened state. On the basis of an ideal geometrical relationship between lung volume and airway circumference (relaxed and static), it has been suggested that a DI could strain ASM by up to 25% (12). Our results show that, under physiological dynamic conditions (i.e., 25 cmH2O trough-to-peak pressure), ASM distension with DI is much less, particularly with the airway in the contracted state. To our knowledge, data on ASM strain in vivo under conditions comparable to those in the present study are sparse or nonexistent.

A secondary aim of the present study was to determine whether mechanical stresses produced by tidal breathing regulate airway responsiveness, as suggested in some studies (12, 34). The present study showed a reduction in maximal airway response to ACh during tidal oscillation of ~12% compared with static responses. When the effects of tidal oscillations were examined without constrained pressure cycles (Fig. 2), we estimated ASM strain to be ~4%, which, on the basis of the relationship shown in Fig. 8, would be expected to produce ~16% bronchodilation, which is close to the observed result. However, under constrained conditions, ASM will be stretched less during tidal oscillation, and, consequently, the extent of bronchodilation will be smaller, although this was not directly tested here. Our findings suggest that, in contrast to DIs, tidal stresses play a relatively minor role in regulating airway contraction in airways of this size.

As discussed in the introduction, DIs may also reduce bronchoconstriction induced subsequent to DIs, a phenomenon dubbed bronchoprotection (20, 24, 33). Recently, possible mechanisms for DI-induced bronchoprotection have attracted considerable interest because of the apparent absence of bronchoprotection in asthmatic patients (20). However, the recent findings of Crimi et al. (11) suggest that DIs induce bronchoprotection only if the methods used to assess bronchoconstriction involve a maximal respiratory maneuver (i.e., forced expiratory volume in 1 s), and, hence, a DI will induce bronchodi-lation. When submaximal respiratory maneuvers (avoiding DI) were used to assess lung function, bronchoprotection was not observed, and DI before ASM activation, instead, enhanced subsequent contraction (11). Interestingly, our laboratory previously showed that repeated dilation and lengthening of airways can enhance subsequent contraction to field stimulation (22, 29). In the present study, the effects of DI on subsequent contraction cannot be easily assessed because of periodic bronchodilation by DI. We attempted to gauge the effects of prior DI by comparing the level of contraction recorded at each ACh dose just before the delivery of each DI, with that without DI, i.e., tidal oscillation only. The magnitude of airway contraction measured before DI is influenced by DIs applied during the preconditioning period and at each preceding ACh dose, but not by immediate bronchodi-latory effects of DI. Results showed that “pre-DI” DRCs were similar to control tidal DRCs, suggesting that DIs before an airway contraction did not attenuate subsequent contractions. Furthermore, compared with tidal curves, each sequential pre-DI dose constricted from a lower level of contraction (active pressure) because of residual dilator effects of DI at the previous dose, suggesting that DI before ACh may have actually enhanced contraction (see above) (11, 22, 29).

The present study defines relationships between dynamic load associated with normal breathing patterns and ASM strain...
and responsiveness at the airway level. Airway responsiveness and ASM strain are critically dependent on airway stiffness, which in turn is dominated by the stiffness of actively contracted ASM. These findings may be important to respiratory disease, particularly asthma, where airway stiffening, e.g., by ASM tone, may be linked to DI dysfunction, which could precede airway hyperresponsiveness because of a loss in the advantageous effects of lung inflation.

GRANTS

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REFERENCES