Bronchodilatory response to deep inspiration in bronchial segments: the effects of stress vs. strain

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1School of Anatomy, Physiology and Human Biology, University of Western Australia, Crawley, Australia; and 2Centre for Neonatal Research and Education, School of Paediatrics and Child Health, University of Western Australia, Crawley, Australia

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Ansell TK, McFawn PK, Mitchell HW, Noble PB. Bronchodilatory response to deep inspiration in bronchial segments: the effects of stress vs. strain. J Appl Physiol 115: 505–513, 2013. First published May 30, 2013; doi:10.1152/japplphysiol.01286.2012.—During deep inspirations (DI), a distending force is applied to airway smooth muscle (ASM; i.e., stress) and the muscle is lengthened (i.e., strain), which produces a transient reversal of bronchoconstriction (i.e., bronchodilation). The aim of the present study was to determine whether an increase in ASM stress or the accompanying increase in strain mediates the bronchodilatory response to DI. We used whole porcine bronchial segments in vitro and a servo-controlled syringe pump that applied fixed-transmural pressure (P_{tm}) or fixed-volume oscillations, simulating tidal breathing and DI. The relationship between ASM stress and strain during oscillation was altered by increasing doses of acetylcholine, which stiffened the airway wall, or by changing the rate of inflation during DI, which utilized the viscous properties of the intact airway. Bronchodilation to DI was positively correlated with ASM strain (range of r values from 0.81 to 0.95) and negatively correlated with stress (range of r values from −0.42 to −0.98). Fast fixed-P_{tm} DI produced greater bronchodilation than slow DI, despite less ASM strain. Fast fixed-volume DI produced greater bronchodilation than slow DI, despite identical ASM strain. We show that ASM strain, rather than stress, is the critical determinant of bronchodilation and, unexpectedly, that the rate of inflation during DI also impacts on bronchodilation, independent of the magnitudes of either stress or strain.

Bronchodilation: airway mechanics; airway smooth muscle; asthma; bronchoconstriction; rate of inflation

DEEP INSPIRATIONS (DI) in healthy individuals produce a transient reversal of bronchoconstriction (i.e., bronchodilation) (7, 17, 24, 33). The underlying mechanism by which DI produces bronchodilation in healthy individuals is not completely understood but likely involves stretch-induced relaxation of airway smooth muscle (ASM) (9–11, 15). Since the bronchodilatory response to DI is attenuated or abolished in asthmatics (i.e., stress) as well as a change in length (i.e., strain). The relative contribution of ASM stress and strain to the bronchodilatory response to DI remains unclear.

To better understand the role of ASM stress, as opposed to strain, in mediating the bronchodilatory response to DI, we manipulated the relationship between ASM stress and strain during oscillation. The relationship was altered by activation of ASM, which stiffens the airway wall, or by changing the rate of inflation during DI, which utilizes the viscous properties of the intact airway. First, since the stiffness of the airway wall is governed by the level of ASM activation (2), the magnitude of ASM strain produced by fixed-transmural pressure (P_{tm}) oscillations will fall with increasing dosage of contractile agonist. Conversely, if the airway lumen is oscillated by a fixed volume, the stress produced by oscillation will increase during contraction (29). The viscous and inertial properties of the intact airway provide a second method to alter the relationship between ASM stress and strain that is independent of the dose of contractile agonist (and, therefore, independent of the level of ASM activation). A slower rate of inflation for a given P_{tm} (i.e., a fixed-P_{tm} DI) will result in greater ASM strain than a faster maneuver by reducing the viscous and inertial load on the airway wall. Conversely, a faster rate of inflation for a given volume (i.e., a fixed-volume DI) will result in greater stress than a slower maneuver.

The aim of the present study was to determine whether an increase in ASM stress or the accompanying increase in strain mediates the bronchodilatory response to DI. We hypothesized that strain, rather than stress, is the critical determinant of bronchodilation. We used whole porcine bronchial segments in vitro that were contracted to acetylcholine (ACh) under static (i.e., no oscillation) conditions or during tidal breathing with intermittent DI maneuvers. A servo-controlled syringe pump was used to simulate breathing maneuvers. We predicted that fixed-P_{tm} DI would produce significantly less strain with increasing ASM stress or the accompanying increase in strain.

In vitro, length oscillation (i.e., stretch) in isolated ASM strips (11, 12, 37, 39) and oscillation of lumen volume in intact airways (i.e., whole airway segments) (3, 4) attenuates active force. Increasing amplitude of oscillation produces greater attenuation of active force in an amplitude-dependant manner (12, 14, 15). Similarly, in healthy individuals in vivo, a deeper depth of DI produces greater bronchodilation (7, 33). The amplitude dependence of bronchodilation has typically been explained by increased ASM stretch (11, 29, 37). However, a DI maneuver results in both a distending force being applied to ASM (i.e., stress) as well as a change in length (i.e., strain).

**MATERIALS AND METHODS**

Animal handling. All animal experiments conformed to institutional ethics and animal care unit regulation (Animal Ethics Committee, University of Western Australia, Perth, Australia). Male White Landrace pigs, ~35 kg, were initially sedated with tiletamine-zolaz-
epam (4.4 mg/kg im) and xylazine (2.2 mg/kg im) and then exsanguinated under pentobarbitone sodium anaesthesia (30 mg/kg iv). The lungs were removed and transported on ice to the laboratory.

Airway segment preparation. Airway segments were dissected from the main stem bronchus of the left or right lower lobe. All side branches were ligated with surgical silk, and a ~20-mm-long airway segment was cannulated at both ends, as previously described (3, 4). The mode generation was 18 at the distal and 12 at the proximal end (where trachea = 0), with an internal diameter of ~2 mm at the distal end and ~3 mm at the proximal end. Following cannulation, the airway was mounted horizontally in an organ bath containing gassed (95% O₂ and 5% CO₂) Krebs solution (in mM: 121 NaCl, 5.4 KCl, 1.2 MgSO₄, 25 NaHCO₃, 5 sodium morpholinopropane sulfonic acid, 11.5 glucose, and 2.5 CaCl₂; pH 7.3) at 37°C. The length of the segment was stretched to 105% of its length in the fully deflated lung, shown previously to approximate the length at functional residual capacity (30).

The proximal end of the airway lumen was connected to a reservoir filled with Krebs solution, the height of which set the initial Pₘ (5 cmH₂O) and which was used to flush the lumen with Krebs solution between experiments. The distal end of the airway was connected to a liquid-filled syringe pump. The syringe pump was capable of simulating breathing maneuvers in one of two ways: fixed-Pₘ oscillations or fixed-volume oscillations (see below). All protocols were performed in a closed system, created by closure of a tap between the airway and the Krebs solution reservoir. The system was leak free with negligible compliance (0.0113 μmH₂O/cm with a ~7.0-mL system volume).

Airway narrowing and fixed-Pₘ oscillations. A custom-built servo-controlled syringe pump and pressure transducer was used to measure airway narrowing and to apply fixed-Pₘ oscillations (i.e., tidal breathing and DI maneuvers), as previously described (28). Briefly, airways were connected to a 1-mL glass syringe driven by a feedback-controlled servomotor (model M540; McLennan Servo Supplies, Surrey, U.K.) and motor controller (Shane De Catania, Perth, Australia). Pₘ was measured via a calibrated pressure transducer (model MLT0380/D; ADInstruments, Bella Vista, Australia) with feedback to a servomotor. In these experiments, Pₘ was set to the desired level (i.e., static or oscillatory; see Experimental protocols), and ASM activation resulted in a decrease in lumen volume (i.e., airway narrowing; Fig. 1A). Changes in airway luminal volume (i.e., airway narrowing and fixed-Pₘ oscillations) were measured via a calibrated displacement transducer (model HEDS-5540A06; RS Components, Smithfield, Australia) that measured the rotation of the syringe motor. Both pressure and volume displacement were recorded by a PowerLab data acquisition system (model 4/30; ADInstruments) and displayed on a computer monitor.

Active pressure and fixed-volume oscillations. Measurement of ASM force and fixed-volume oscillations were applied using the same syringe pump oscillator described above but using the displacement transducer and not pressure transducer as the feedback control to the servomotor. In these experiments, lumen volume does not decrease in response to ASM activation but instead results in an increase in Pₘ (i.e., active pressure) that represents ASM force production (Fig. 1B). For comparisons with protocols that used fixed-Pₘ oscillations, the volume of oscillation (i.e., tidal breathing and DI maneuvers) was that which produced the same Pₘ in the relaxed state (i.e., before the administration of the contractile agonist), unless otherwise stated, and was fixed thereafter (see Experimental protocols).

Experimental protocols. After dissection and mounting, airways were initially equilibrated to organ bath conditions for ~60 min under a static Pₘ of 5 cmH₂O, approximating the mechanical environment present at functional residual capacity in vivo. The Krebs solution in the organ bath and lumen was replaced every 10 min to remove the effects of metabolites and bronchoactive mediators released from the epithelium. Viability of the tissue was confirmed through stimulation with ACh (10⁻⁴ M) added to the organ bath. Three different protocols were followed (see below). In protocol 1, the amplitude dependence of the bronchodilatory response to DI was established, whereas protocols 2 and 3 were designed to determine the relative contribution of ASM stress and strain to the bronchodilatory response to DI.

Protocol 1: the amplitude-dependence of the bronchodilatory response to DI. Airways were narrowed to a single, moderate dose of ACh (10⁻⁷ M) and subjected to five fixed-Pₘ DI from submaximal Pₘ (20-, 30-, 40-, 50-, and 60-cmH₂O Pₘ). For these experiments, DI maneuvers comprised of a 2-s inflation, a 2-s hold at the peak of inflation, and a 2-s deflation (a 6-s maneuver). DI were applied amidst a background of tidal breathing (Δ5 cmH₂O at 0.25 Hz) and the bronchodilatory response was allowed to fully recover before application of the next DI maneuver (typically ~1 min).

Protocols 2 and 3: the contributions of ASM stress and strain to the bronchodilatory response to DI. The relative contributions of ASM stress and strain to the bronchodilatory response to DI were assessed by manipulating the strain produced by a fixed-Pₘ DI or the stress produced by a fixed-volume DI. In protocol 2, the ASM stress and strain relationship was altered by activation of ASM with ACh, which increases airway wall stiffness (2). With increasing airway wall stiffness, the magnitude of strain (i.e., the ΔDI volume) to a fixed-Pₘ change decreases, whereas the magnitude of stress (i.e., the ΔDI Pₘ) to a fixed-volume change increases in a dose-dependent manner. Full-dose response-curves (DRC) were constructed to ACh (10⁻⁷ to 3 × 10⁻³ M) under both static (5-cmH₂O Pₘ) and oscillatory conditions in a randomized order. The oscillatory protocol comprised tidal breathing (Δ5 cmH₂O at 0.25 Hz) and intermittent DI maneuvers (Δ25 cmH₂O, a 6-s manoeuvre, described above) applied once contraction at each dose of ACh had plateaued. In the fixed-volume experiments, the volume changes used were adjusted for each airway so that tidal breathing was Δ5 cmH₂O and DI maneuvers were Δ25 cmH₂O in the
relaxed state. Experiments conducted using the fixed-$P_{tm}$ or fixed-volume approach were performed in separate groups of airways. Although protocol 2 allowed us to assess the relative contributions of ASM stress and strain on the bronchodilatory response to DI, increasing ACh dose and, therefore, ASM activation may have independent effects (related to the biology of the cell, i.e., contractile filament remodeling) beyond that of a simple change in airway wall stiffness. To address this possibility, in protocol 3, the relationship between ASM stress and strain was not altered by increasing dose of contractile agonist but by varying the rate of inflation during DI. Due to the viscoelastic properties of the intact airway, slower DI produce greater strain (i.e., greater $\Delta DI$ volume) during a fixed-$P_{tm}$ oscillation than faster DI. Conversely, for a fixed-volume oscillation, slower DI produce less stress (i.e., less $\Delta DI$ $P_{tm}$) than faster DI of the same volume. Airways were contracted to a single, moderate dose of ACh ($10^{-5}$ M) and subjected to DI ($\Delta 25$ cmH$_2$O) of three different rates in a randomized order: a slow DI comprised of a 5-s inflation and a 5-s deflation (a 10-s maneuver, i.e., no hold at the peak of inflation), a moderate DI comprised of a 2-s inflation and a 2-s deflation (a 4-s maneuver), and a fast DI comprised of a 1-s inflation and a 1-s deflation (a 2-s maneuver). DI were applied amidst a background of tidal oscillations ($5$ cmH$_2$O at $0.25$ Hz), and the bronchodilatory response was allowed to fully recover before application of the next DI maneuver. For fixed-volume oscillations, the tidal and DI volumes used were adjusted for each airway to match the volume measured during fixed-$P_{tm}$ tidal breathing and in the moderate rate DI maneuver under contracted conditions, respectively.

**Morphometry.** Morphometric analyses were carried out to estimate the magnitude of strain on the ASM during the various DI maneuvers. Following experimentation, airways were removed from the organ bath and fixed in 4% formaldehyde solution under atmospheric pressure (i.e., $0$ cmH$_2$O $P_{tm}$). Distal and proximal regions of the airway segment were processed into paraffin blocks. Transverse airway sections were cut at thickness of $5$ $\mu$m and stained with hematoxylin and eosin. Inner wall area ($WA_i$) was calculated from the area enclosed by the outer ASM perimeter ($A_{mo}$) minus the area enclosed by the lumen internal area ($A_l$) using ImageJ (version 1.45j, National Institutes of Health). Measurements at distal and proximal locations were averaged and corrected for horizontal stretch (105% of its length in the fully deflated lung), which reduces the cross-sectional area of the wall, assuming tissue volume is constant. The calculated inner wall area was also corrected for tissue shrinkage that occurs during histological processing. In our recent study using human bronchi, shrinkage was estimated to be $\sim 15\%$ following processing (27).

**Analysis and statistics.** Lumen volume was measured by the volume that could be withdrawn until closure in the relaxed airway at 5-cmH$_2$O $P_{tm}$ (14). Airway narrowing to ACh (in the fixed-$P_{tm}$ experiments) was expressed as % lumen volume (where $100\%$ airway narrowing indicates airway closure). As described above, morphometry allowed the outer ASM perimeter ($P_{mo}$) to be calculated using the following equation:

$$P_{mo} = \sqrt{4 \times \pi \times \left( \frac{WA_i + \frac{\text{lumen volume}}{\text{Airway length}}}{} \right)}$$

where, lumen volume is volume of the lumen at the trough of the pressure cycle at the time of measurement and airway length is the length of the airway segment mounted in the organ bath. Active pressure to ACh (for the fixed-volume approach) was expressed as $\Delta P_{tm}$. Comparisons between static and oscillatory conditions were made at troughs of the oscillation cycle (volume or pressure, depending on the approach used). Dose-response curves had variable slope sigmoidal curves fitted to individual airways. The maximum response ($E_{max}$) and sensitivity ($PD_2 = -\log EC_{50}$) was calculated for individual airways under static and oscillatory conditions. During fixed-$P_{tm}$ oscillations, ASM strain was calculated using the following equation:

$$\frac{\Delta DI}{\text{Pre-DI}} \times 100$$

where, $\Delta DI$ is the trough-to-peak change in $P_{tm}$ during DI and Pre-DI is the $P_{tm}$ immediately before DI. During fixed-volume oscillations, stress was defined as $\Delta DI$ $P_{tm}$. The bronchodilatory response to DI was defined as % reversal of contraction to ACh using the equation (29):

$$\frac{\text{Pre-DI} - \text{Post-DI}}{\text{Pre-DI}} \times 100$$

where, for the fixed-$P_{tm}$ approach, Pre-DI and Post-DI are the airway narrowing immediately before and immediately after DI, respectively. For the fixed-volume approach, Pre-DI and Post-DI are both the active pressure immediately before and after DI, respectively. Therefore, $100\%$ reversal indicates that the post-DI airway narrowing or active pressure returned to precontraction values (i.e., full reversal of the response to ACh). Comparisons of the bronchodilatory response were not possible at low doses of ACh ($\leq 3 \times 10^{-6}$ M), which produced little to no contraction. Scatter plots of bronchodilation to DI against either ASM strain or stress had linear lines of best fit fitted to individual airways. The intercept, slope, and Pearson’s correlation coefficient ($r$) were calculated for individual airways. Following DI, the bronchodilatory response was measured for a further 1 min, and one-phase exponential decay curves were fitted to individual airways. The decay constant ($k$) following DI was calculated for individual airways and was used to assess the kinetics of airway re-narrowing following DI.

Specific airway compliance was calculated from the $\Delta$volume in relation to the $\Delta$volume during the inflamatory limb of the tidal oscillation cycle using the equation:

$$\frac{\Delta \text{Tidal volume}}{\Delta \text{Tidal } P_{tm} \times \text{Lumen volume}}$$

where $\Delta$tidal volume and $\Delta$tidal $P_{tm}$ are the trough-to-peak changes in volume and pressure during tidal oscillation, and lumen volume is the volume of the lumen at the trough of the pressure cycle at the time of measurement.

Differences between groups were analyzed using one-way, repeated-measures ANOVA and Newman-Keuls post hoc test, unless otherwise stated below. Airway narrowing and active pressure DRC were analyzed using two-way, repeated-measures ANOVA and Newman-Keuls post hoc test with dose of ACh and the condition (i.e., static or oscillatory) as the repeat measures variables. Sensitivity to ACh under static and oscillatory conditions was analyzed using paired $t$-tests. DRC of bronchodilation to DI were analyzed using two-way ANOVA and Newman-Keuls post hoc test. Data analysis and statistical tests were performed using Statistica (version 8.0; StatSoft, Tulsa, OK) and GraphPad Prism (version 5.0d; GraphPad Software, La Jolla, CA). Data are presented as means ± SE, where $n$ = number of animals.

**RESULTS**

**Protocol 1: the amplitude dependence of the bronchodilatory response to DI.** Airways produced $35.4 \pm 7.4\%$ narrowing in response to a single, moderate dose of ACh ($10^{-5}$ M). We observed a strong amplitude dependence to the bronchodilatory response to DI (Fig. 2A). Bronchodilation increased from $18.5 \pm 3.7$ to $50.6 \pm 7.1\%$ reversal of narrowing when DI amplitude increased from $20$ to $60$ cmH$_2$O $P_{tm}$ (i.e., $\Delta 15$ to $\Delta 55$ cmH$_2$O). Airways rapidly re-narrowed following DI, and the decay constant (average $k$ of $0.280 \pm 0.0246$ s$^{-1}$) was independent of amplitude (Fig. 2B). As expected, increasing amplitudes of DI also produced greater ASM strain (Fig. 3). Deep inspiration to 20-cmH$_2$O $P_{tm}$ produced $0.08 \pm 0.01$ ASM strain (i.e., an $8\%$ increase in ASM perimeter), which increased...
to 0.26 ± 0.02 (i.e., a 26% increase in ASM perimeter) with DI to 60 cmH2O Ptm.

Protocols 2 and 3: the contributions of ASM stress and strain to the bronchodilatory response to DI. In protocol 2, the relationship between ASM stress and strain was altered by increasing airway wall stiffness using cumulative doses of ACh (10−7 to 3 × 10−3 M). Specific compliance of the airway wall fell from 0.0086 ± 0.0009 cmH2O−1 in the relaxed state to 0.0029 ± 0.0001 cmH2O−1 at the maximal dose of ACh (Fig. 4).

Scatter plots of bronchodilation against either ASM strain produced by fixed-Ptm DI (Fig. 5A) or stress produced by fixed-volume DI (Fig. 5B) had linear lines of best fit fitted to individual airways. Bronchodilation was positively correlated with ASM strain. The average slope and intercept of the lines fitted between bronchodilation and ASM strain was 814.1 ± 206.3 (i.e., ~8% bronchodilation per 1% ASM strain) and 0.026 ± 0.005 (i.e., only ASM strains greater than ~3% produced bronchodilation), respectively. Unexpectedly, bronchodilation and stress were negatively correlated with an average slope and intercept of −0.34 ± 0.08 cmH2O−1 and 505.3 ± 202.3, respectively. Pearson’s correlation coefficients for individual airways are shown in Table 1.

The effect of ACh dose on the bronchodilatory response to DI differed between experiments where Ptm change was held fixed compared with fixed-volume change. Bronchodilation to fixed-Ptm DI fell substantially with increasing ACh dose from 54.5 ± 12.0% reversal of narrowing at 10−5 M ACh (the lowest dose that produced contraction) to 0.80 ± 0.33% reversal at 3 × 10−3 M (P < 0.001, Newman Keuls post hoc test against 10−5 M). Although bronchodilation to fixed-volume DI also fell with increasing dose, this was far less pronounced. Fixed-volume DI produced 88.9 ± 7.6% reversal of active pressure at 10−5 M ACh, which fell to 67.8 ± 5.1% reversal at 3 × 10−3 M (P < 0.01, Newman Keuls post hoc test against 10−5 M).

The greater dependence (i.e., steeper slope) of the bronchodilatory response on strain, rather than stress, of oscillation and the pronounced stiffening to ACh meant that attenuation of...
contraction was much weaker in fixed-Ptm experiments compared with fixed-volume experiments. Airways produced a maximum of 81.9 ± 4.8 cmH₂O active pressure under static conditions, which fell to 16.4 ± 4.3 cmH₂O under oscillatory conditions (Fig. 6B). Interestingly, the PD₂ (sensitivity) under oscillatory conditions for both airway narrowing and active pressure was less than that for specific compliance, indicating that changes in specific compliance were more sensitive to stimulation with ACh than the observed bronchoconstrictor response (Table 2).

Finally, in protocol 3, the relationship between ASM stress and strain was altered by changing the rate of inflation during DI at single, moderate dose of ACh (10⁻⁵ M). As predicted, the ASM strain produced by fixed-Ptm DI varied in a rate-dependent manner. Taking a slow DI produced 0.13 ± 0.01 ASM strain, which fell to 0.11 ± 0.01 with a fast DI (Fig. 7A). Despite producing less ASM strain than a slow DI, a fast or moderate DI produced a greater bronchodilatory response than a slow DI (Fig. 7B). Interestingly, despite less ASM strain, fixed-volume DI produced greater stress than the fixed-Ptm experiments (i.e., >30-cmH₂O Ptm). This is likely explained by the observation that activation of ASM resulted in greater airway wall stiffening in the fixed-volume (0.019 ± 0.0001 cmH₂O⁻¹) than the fixed-Ptm experiments (0.029 ± 0.0006 cmH₂O⁻¹; P < 0.05).

The stress produced by a fixed-volume DI also varied in a rate-dependent manner. Taking a slow DI produced 37.2 ± 1.5 cmH₂O stress, which increased to 49.0 ± 2.2 cmH₂O with a fast DI (Fig. 7C). Despite identical ASM strains, fast DI and moderate DI also produced a greater bronchodilatory response than a slow DI (Fig. 7D).

**DISCUSSION**

The present study determined the relative contributions of ASM stress and strain to the bronchodilatory response to DI using an intact airway preparation. We manipulated the relationship between ASM stress and strain by either increasing dose of ACh or varying the rate of inflation during DI. We show that ASM strain, rather than stress, is the critical deter-

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**Table 1. Pearson’s correlation coefficients for scatter plots of bronchodilation against either ASM strain (airway 1–6) or stress (airway 7–12)**

<table>
<thead>
<tr>
<th>Airway</th>
<th>ASM Strain</th>
<th>P Value</th>
<th>Stress</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.83</td>
<td>0.0415</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.91</td>
<td>0.0122</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.95</td>
<td>0.0036</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.93</td>
<td>0.0078</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.81</td>
<td>0.0493</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.82</td>
<td>0.0451</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>–0.42</td>
<td>0.4087</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>–0.96</td>
<td>0.0025</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>–0.96</td>
<td>0.0019</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>–0.92</td>
<td>0.0084</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>–0.98</td>
<td>0.0008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>–0.98</td>
<td>0.0007</td>
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Bronchodilation is the % reversal in airway narrowing or active pressure following deep inspiration (DI), airway smooth muscle (ASM) strain is the change in ASM perimeter produced by fixed-transmural pressure (Ptm) DI, and stress is the change in Ptm produced by fixed-volume DI.
compared the effect of increasing doses of ACh on the magnitudes of bronchodilation to fixed-Ptm or fixed-volume DI. A known effect of contractile agonists, such as ACh, is an increase in airway wall stiffness (2), which decreases the ASM strain to a fixed-Ptm DI and increases the stress to a fixed-volume DI. This allowed us to plot relationships separately between strain and bronchodilation and stress and bronchodilation. We show that the bronchodilatory response to DI and the magnitude of strain were positively correlated, whereas there was a negative correlation between bronchodilation and stress (discussed below). These data suggest that mechanical length-change (i.e., strain), rather than the applied force (i.e., stress) underlies ASM relaxation following DI. The intercept of the linear line of best fit between strain and bronchodilation suggests that only ASM strain greater than \( \sim 3\% \) produce bronchodilation. This finding is comparable to our previous study (29), where only ASM strain exceeding \( \sim 1\% \) produced bronchodilation, although in this study bronchodilation referred to reversal of active pressure, as opposed to airway narrowing as in the present study. The cellular mechanism(s) underlying bronchodilation to DI may involve perturbed crossbridge binding (10, 11) and/or remodeling (i.e., de-polymerization and re-polymerization) of the contractile apparatus (13). Physical breaking of ASM cross bridges could, theoretically, result from either stress or strain on the ASM fibers, although we suggest it is the latter. Alternatively, remodeling of the contractile apparatus is thought to involve mechanosensors (i.e., integrins) that respond to the physical deformation (i.e., strain) of cells and initiate the downstream remodeling process. Our finding that ASM strain rather than stress underlies bronchodilation is consistent with cellular mechanotransduction (16).

Two recent studies have examined the effect of ASM stress and strain on contraction. Lavoie and colleagues (21) assessed the behavior of human lung slices under oscillatory conditions and concluded that reversal of bronchoconstriction was dependent on strain but did not measure stress. In contrast, Pascoe and colleagues (32) demonstrated attenuation of ASM force development despite negligible strain using ovine tracheal strips, implicating a role of stress. In their study, when constant oscillations of \( \Delta 20 \) cmH\( \text{2} \)O were introduced before ASM activation, oscillations attenuated ASM force at high doses of contractile agonist despite \( <1\% \) strain. We suggest that this finding reflects the ability of strain to inhibit ASM contraction (i.e., bronchoprotection) rather than reversing existing contraction (i.e., bronchodilation), which is the subject of the present study. The apparent disparity between the present findings and those by Pascoe and colleagues may, therefore, relate to the different underlying mechanisms governing bronchodilation.

<table>
<thead>
<tr>
<th>Table 2. PD(_2) to ACh (10(^{-7}) to 3 \times 10(^{-3}) M) for airway narrowing, active pressure, and specific compliance</th>
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<tbody>
<tr>
<td><strong>Fixed-P(_{tm}) Experiments</strong></td>
</tr>
<tr>
<td>Airway narrowing</td>
</tr>
<tr>
<td>Active pressure</td>
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<tr>
<td>Specific compliance</td>
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</table>

Values are means ± SE (\( n = 6 \)). Changes in specific compliance with ACh were more sensitive than airway narrowing in the fixed-P\(_{tm}\) experiments (\( P < 0.001 \)) and active pressure in the fixed-volume experiments (\( P < 0.001 \)).
and bronchoprotection and their relative sensitivity to ASM stress and strain.

Although bronchodilation was positively correlated with strain, paradoxically, we observed a negative correlation between bronchodilation and ASM stress. Since high stress occurred when ASM stiffness was also high (i.e., at high doses of ACh), it is possible that the stiffness of the airway wall approached that of the system, and consequently a lesser proportion of the volume during DI was taken up by airway. The resulting decrease in strain would favor a reduction in bronchodilation. However, the compliance of the organ bath setup is low (relaxed airway/organ bath system absolute compliance ratio of \( \frac{5}{219} \)) and, even under conditions of maximal airway wall stiffness (ratio of \( \frac{34}{34} \)), the volume taken up by the system was negligible. We believe a more likely explanation for reduced bronchodilation with greater stress is that there is an independent effect of dose (i.e., the level of pharmacologically induced ASM activation) on the bronchodilatory response to DI. Since increased stress occurred at high doses of ACh, the negative correlation between bronchodilation to DI and stress could indicate that high doses of ACh inhibit the bronchodilatory response to DI directly. Previous studies with increasing levels of pharmacologically induced ASM activation have produced conflicting results on the relaxant response to strain. A study by Shen and colleagues (38) showed no effect of ACh dose on the relaxant response to length oscillation using isolated canine ASM strips. However, in vivo, Scichilone and colleagues (34) showed greater bronchodilation to DI with increasing dose of methacholine in healthy individuals. Importantly, if as suggested in the present study the degree of contractile activation negatively impacts the bronchodilatory response, then we would expect that this mechanism would also affect the observed relationship between strain and bronchodilation. The implication is that low strain/high doses of contractile agonist may together explain the fall off in bronchodilation. However, we predict that the strain will be by far the dominant effect, since at high doses of contractile agonist bronchodilation was pronounced to fixed-volume DI (and therefore high strain) despite maximal contractile activation.

Our findings that the magnitude of strain, rather than stress, determines the bronchodilatory response to DI have implications for the role of DI in the regulation of airway responsiveness and, therefore, airway hyperresponsiveness. Under high levels of ASM activation, the airway stiffens greatly, so that the strain on the ASM becomes negligible, and, therefore, the bronchodilatory response to DI is attenuated. Furthermore, the present study also showed that sensitivity to ACh was significantly greater for stiffness than for narrowing (i.e., airways stiffen before narrowing as the dose of contractile agonist increases). The apparent disconnect between airway wall stiffening and narrowing (or active pressure) raises the possibility that the capacity for an airway to stiffen in addition to its ability to narrow may regulate bronchodilation to DI in health and disease. We suggest that asthmatic airways will not only narrow more at low doses of ACh compared with healthy individuals but will also be stiffer. In vivo, studies of DI-induced bronchodilation have typically matched for changes in airway narrowing (i.e., resistance) with contractile agonist (40) between healthy individuals and asthmatics (6). The disconnect between airway narrowing and stiffening raises the possibility that, even if matched for the same level of narrowing by using
low doses of contractile agonist in asthmatics, airways may reach a greater level of stiffness in asthmatic subjects and the ASM be strained less during DI. Increased airway stiffening separate from airway narrowing may contribute to the reduced bronchodilatory response to DI in asthmatics.

To further assess the relative effects of ASM stress and strain on the bronchodilatory response to DI, we adopted an additional protocol (protocol 3). In this protocol, the relationship between ASM stress and strain during DI was not altered by dose of contractile agonist but by varying the rate of inflation during DI. By changing the rate of inflation, the viscous and inertial loads of the airway will determine the resulting strain produced by a fixed-P_{tm} DI and the stress produced by a fixed-volume DI. We show that, when the pressure change for a DI is held fixed, faster DI produce less strain than a slower DI, whereas when the volume change of DI is held fixed, faster DI produce greater stress than slower DI.

Unexpectedly, irrespective of whether the DI was administered using the fixed-P_{tm} or fixed-volume approach, the faster DI produced greater bronchodilation. This finding suggests a rate of change-dependant component of the bronchodilatory response to DI that is independent of the magnitudes of either stress or strain.

Previous studies, in vivo, have examined the importance of the rate of inflation during DI. Hida and colleagues (17) demonstrated a greater bronchodilatory response with a faster DI in healthy individuals. In contrast, Duggan and colleagues (7) showed no effect of rate of inflation in healthy individuals, although the data suggest a trend. It is possible that, in vivo, the pressure swing (i.e., stress) and, therefore, strain on the airway wall is greater during a faster DI. We suggest that, separate from the magnitudes of stress or strain, the rate of change of ASM stretch during DI also determines the bronchodilatory response. Importantly, the rate of change dependence of DI is preserved in asthmatics (17), suggesting that an abnormality in this mechanism does not contribute to the reduced bronchodilatory response to DI in asthmatics. The rate dependence of a transient inflationary maneuver such as DI is qualitatively similar to the frequency dependence of isometric force production during length oscillation of isolated ASM (1, 12, 37, 39) and, in vivo, to the response to mechanical ventilation (36).

The cellular mechanism(s) may involve the relative rates of cross-bridge cycling and length oscillation (i.e., the frequency of oscillation). If the frequency of oscillation is faster than the rate of detachment and reattachment of cross bridges, then fewer detached cross bridges can reattach during the oscillation cycle (16).

The present study also confirms previous observations in the literature that the modulating effects of breathing are substantially less when modeled using fixed-P_{tm} oscillations than fixed-volume oscillations in whole airway segments (14, 19, 20, 26, 28, 29). Fixed-P_{tm} DI are likely to be the more physiological approach (assuming breathing movements are ultimately pressure limited), where the amplitude of DI and the stiffness of the airway wall determine the magnitude of strain on the ASM. In contrast, fixed-volume DI (a less physiological scenario, analogous to length oscillation in ASM strip studies), which are not dependent on airway wall stiffness, are potent at attenuating force production, even at maximal levels of ASM activation. Importantly, low volume respiratory movements such as tidal breathing may not necessarily be pressure limited during bronchoconstrictor challenge, since pressures may rise to overcome the greater impedance of the respiratory system. The true in vivo scenario may then exist somewhere between the fixed-P_{tm} and fixed-volume scenarios (25). Nonetheless, the introduction of fixed-P_{tm} oscillations in vitro (29) do bring into the question the importance of the dynamic mechanical environment of the lung in the regulation of airway responsiveness.

Several methodological aspects of the study also require discussion. First, we chose to use porcine airways to study the effects of ASM stress and strain in response to DI and the resultant bronchodilation. Our laboratory has used porcine airways previously to assess the role of DI amplitude and to demonstrate the importance of airway wall stiffness to this response, particularly during ASM contraction (29). Many of these findings have been replicated recently in studies utilizing human tissue (21, 27). These findings suggest that the porcine airway behaves similarly to the human airway, although we acknowledge some species differences exist (26). Certainly, the porcine airway is highly cartilaginous, which likely increases wall stiffness (31). We are now able to compare the dynamic specific compliance of the porcine airway to similarly sized human airways using the same recording system (28). In the relaxed state, the porcine airway is approximately three times stiffer. Furthermore, the increase in stiffness during ASM activation is substantial in pigs (which correlates with greater narrowing in vitro compared with humans), which we suggest is likely to further inhibit the bronchodilatory effect of DI. Finally, the system we use to measure airway narrowing is not a direct measure of caliber. Previous studies in our laboratory that directly measured luminal caliber by video endoscopy reported only an ~49% narrowing to a maximal dose of ACh (23), which is considerably less than that reported here (~93% narrowing). One possible explanation is that our measurement of the relaxed lumen volume at the point of collapse underestimates the total volume contained within the bronchial segment. This is possible since only one region along the length of the airway would need to collapse for the airway to be deemed closed. However, although this may affect the level of airway narrowing reported, it does not affect the response to DI observed between protocols and thus the conclusions drawn from the study.

In conclusion, the present study demonstrates that the bronchodilatory response to DI is indeed amplitude-dependant and that ASM strain, rather than stress, is the critical determinant of bronchodilation. We also identified a rate of change-dependant component to the response to DI that is independent of the magnitudes of either stress or strain.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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