Intraluminal pressure oscillation enhances subsequent airway contraction in isolated bronchial segments

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Noble, P. B., P. K. McFawn, and H. W. Mitchell. Intraluminal pressure oscillation enhances subsequent airway contraction in isolated bronchial segments. J Appl Physiol 96: 1161–1165, 2004. First published November 21, 2003; 10.1152/japplphysiol.01082.2003.—A period of deep inspiration in humans has been shown to attenuate subsequent bronchoconstriction, a phenomenon termed bronchoprotection. The bronchoprotective effect of deep inspiration may be caused through a depression in the force production of airway smooth muscle (ASM). We determined the response of whole airway segments and isolated ASM to a period of cyclic stretches. Isovolumetric contraction to electrical field stimulation (EFS) was assessed in porcine bronchial segments before and after intraluminal pressure oscillation from 5 to 25 cmH₂O for 10 min at 0.5 Hz. Morphometry showed that this pressure oscillation stretched ASM length by 21%. After pressure oscillation, the response to EFS was not reduced but instead was modestly enhanced (P < 0.01). Airway responses to EFS returned to preoscillation levels 10 min after the end of oscillation. The increase in EFS response after pressure oscillation was not altered by the addition of indomethacin. In a separate experiment, we assessed isometric force in isolated ASM strips before and after length oscillation. The amplitude, frequency, and duration of length oscillation were similar to those induced in bronchial segments. In contrast to bronchial segments, length oscillation of ASM produced a significant depression in isometric force induced by EFS (P < 0.01). These results suggest that the response of ASM to length oscillation is modified by the airway wall. They also suggest that the phenomenon of bronchoprotection reported in some in vivo studies may not be an intrinsic property of the airway.

airway smooth muscle; bronchoconstriction; bronchoprotection; bronchus; deep inspiration

METHODS

Bronchial segments. Animal experiments were conducted in conformance with APS’s Guiding Principles in the Care and Use of Animals. This procedure was approved by the institutional ethics and animal care unit. Pigs, 20 kg, were sedated with tiletamine-zolazepam (4.4 mg/kg im) and xylazine (2.2 mg/kg im) and anesthetized with sodium pentobarbitone (25 mg/kg iv). After exsanguination, left and right lungs were removed. Lower lobe bronchial segments were dissected free of parenchyma and were cannulated at each end, and the side branches were ligated as previously described (26) (1–2 bronchial segments were used from each animal). Segments were otherwise stored in Krebs-Henseleit buffer at 4°C. Before the experiment, segments were equilibrated at 37°C in the presence of 5% CO₂ and 95% O₂. After equilibration, segments were exposed to different conditions for periods of 3–5 min.

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intact from luminal to adventitial surfaces. The bronchial segments were 2 cm long and 1.8–2.5 mm in internal diameter at the distal and proximal end, respectively. Bronchial segments were mounted horizontally in an organ bath containing gassed Krebs solution (95% O<sub>2</sub>–5% CO<sub>2</sub>) at 37°C. Segments were stretched to 110% of their relaxed length to produce optimum responses (26). Once segments were mounted, airway length was fixed and could not change during recordings or pressure oscillations. The segments were connected in series to a 30-cm-high jacketed Krebs reservoir. When required, airways were subject to periods of intraluminal pressure oscillation achieved by cycling the height of Krebs solution in the reservoir by using an elliptical piston pump. Closure of taps positioned at each end of the segment produced isovolumetric conditions that were used when stimulating the airway (see below). A calibrated pressure transducer (MPX2010DP, Motorola Semiconductors) was inserted between the taps for measurement of airway luminal pressure. The transducer was connected to a Powerlab data-acquisition system (ADI Instruments), and the signals were displayed on a computer monitor.

Isovolumetric contraction was induced by electric field stimulation (EFS) via platinum electrodes encircling the segment by using a Grass S44 square-wave stimulator. Stimulation parameters (300 mA, 3-ms pulse duration at 30 Hz) were chosen to produce maximal ASM activation. Each train of EFS was maintained until a plateau in active pressure response was observed (~20 s). Active pressure response was calculated by subtracting baseline intraluminal pressure from the total pressure developed during EFS.

After dissection, airways were allowed to equilibrate at a resting pressure of 5 cmH<sub>2</sub>O for 1 h, and the viability of the bronchi was confirmed by using 10<sup>−4</sup> M ACh followed by a 30-min recovery period. Bronchial segments were then electrically stimulated every 10 min (at a 5 cmH<sub>2</sub>O resting pressure) until consistent isovolumetric responses were observed (typically after 4 responses). Passive intraluminal pressures of 5 cmH<sub>2</sub>O produced maximal active pressures during contraction (see RESULTS). Once baseline response to EFS was established, airway luminal pressure was oscillated from 5 cmH<sub>2</sub>O to either 10, 15, or 25 cmH<sub>2</sub>O for 0.5 Hz for 10 min. The trough-to-peak amplitudes of pressure oscillation were, therefore, 5, 10, and 20 cmH<sub>2</sub>O, respectively. EFS-induced responses were resumed immediately (~5 s) after the period of pressure oscillation. In some bronchi (after the oscillation protocol was completed), we also determined the relationship between passive intraluminal pressure and active pressure response to EFS. Active pressure was recorded at passive pressures between 0 and 25 cmH<sub>2</sub>O.

To assess the change in smooth muscle length occurring with airway inflation, airways removed from left and right lungs were fixed for ~12 h in 9% formaldehyde at either 5 or 25 cmH<sub>2</sub>O. After fixation, a 0.5-cm length of airway was cut from the midportion of each bronchial segment and embedded in wax blocks. Sections, 10 µm, were subsequently stained with hematoxylin and eosin. The outer perimeter of ASM (P<sub>mo</sub>) was measured in four sections per airway by use of image analysis software (Optimus) and averaged.

**Isolated ASM.** ASM strips were isolated from three pig tracheas. Using forceps under a dissecting microscope, we teased away the mucosal and epithelial layers from the ASM so that none remained. ASM strips (~0.5 cm long and ~0.3 cm wide) were cut away from the cartilage and mounted in an organ bath containing gassed Krebs solution at 37°C. One end of the strip was connected to an isometric force transducer (Grass model FT03), and the other was connected to an attachment post. The attachment post was glued to the cone of a loudspeaker (18 cm, 6 Ω) driven by a signal generator (Interstate sweep generator F44) to produce cyclic change in ASM length. A Powerlab data-acquisition system was used to record force.

Isometric contractions were induced by EFS via platinum plate electrodes. Each train of pulses was maintained until a plateau in force was observed, and stimulation parameters were chosen to produce maximal ASM activation (300 mA, 0.5-ms pulse duration at 50 Hz). Active force was calculated by subtracting baseline force from the total force produced with EFS.

A passive-active tension curve was first constructed to determine the muscle optimum length (L<sub>o</sub>). Once at L<sub>o</sub>, contractions to EFS were recorded every 10 min until a stable baseline response was observed. The length of the muscle strips was then oscillated at an amplitude matched to the measured length change of ASM in the bronchial wall (determined by histology). The average trough-to-peak amplitude of length oscillation was 22.7 ± 2.6% L<sub>o</sub>, cycled at 0.5 Hz for 10 min. ASM strips were stimulated immediately and 10 min after oscillation had ceased.

**Statistical analysis.** Responses to EFS before and after oscillation, and at different pressures, were compared by using repeated-measures ANOVA and a Newman-Keuls posttest. Unpaired Student’s t-test was used to compare the percent increase in EFS response with and without indomethacin. The effect of fixation pressure on P<sub>mo</sub> in airways removed from left or right lungs was analyzed by using unpaired ANOVA. Data are given as means ± SE where n represents the number of airway tissues.

**RESULTS**

**Bronchial segments.** We established a relationship between intraluminal passive pressure and active responses to maximum EFS in isolated bronchial segments. Passive intraluminal pressure was plotted against active pressure responses, and the resultant curve is shown in Fig. 1. Maximum active pressure response to EFS occurred at an intraluminal pressure of 5 cmH<sub>2</sub>O (P < 0.05, n = 4). Increasing passive intraluminal pressure above 5 cmH<sub>2</sub>O significantly reduced active pressure response to EFS (P < 0.05).

The intraluminal pressure of isolated bronchial segments was oscillated at 0.5 Hz for 10 min. EFS responses were recorded before and after oscillation. The effect of pressure oscillation on response to EFS was dependent on the amplitude of the pressure oscillation. Intraluminal pressure oscillation at a trough-to-peak amplitude of 5 cmH<sub>2</sub>O or 10 cmH<sub>2</sub>O did not alter the response to EFS (n = 4). Pressure oscillation at 20 cmH<sub>2</sub>O also produced no depression in the response to EFS but instead modestly increased active pressure, on average by 11.3 ± 4.0% (n = 11, P < 0.01) (Fig. 2B). Active pressure response to EFS returned to preoscillation levels 10 min after oscillation concluded. The response of the airway to pressure oscillation was not significantly different between airways.

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Fig. 1. Relationship between passive intraluminal pressure (cmH<sub>2</sub>O) and active pressure response to electrical field stimulation (EFS; cmH<sub>2</sub>O) in isolated bronchial segments (n = 4). Values are means ± SE. Airways were subject to EFS at a range of passive pressures. Maximum responses were obtained at a passive pressure of 5 cmH<sub>2</sub>O (*P < 0.05; #P < 0.001 compared with preceding lower pressure).
RESULTS

A major aim of the present study was to assess the contribution of the airway wall to the bronchoprotective effects of DI observed in vivo. We isolated whole airway segments from the lung and measured maximum airway responses to EFS before and after a period of cyclic stretch to the airway wall. In this manner, the airway wall was partitioned from other potential targets of DI such as neural pathways, while preserving the ASM in situ. It has previously been shown that, in precontracted canine bronchial segments, cyclic stretch of the airway wall reduces airway contraction (10). Our methodology dif-

Fig. 2. A: example trace of active pressure responses (cmH2O) to EFS observed before and after intraluminal pressure oscillation from 5 to 25 cmH2O for 10 min at 0.5 Hz. Active pressure responses to EFS were recorded every 10 min (first EFS is labeled). B: mean ± SE (n = 11) active pressure response (cmH2O) to EFS in airways before and after intraluminal pressure oscillation as specified in A. Immediately after pressure oscillation [time (T = 0)], airway response to EFS was increased (**P < 0.01). Responses to EFS returned to preoscillation levels 10 min later (T = 10).

Fig. 4. Airway smooth muscle (ASM) perimeter (Pmo; mm) morphometrically determined in bronchial segments fixed at an intraluminal pressure of 5 or 25 cmH2O. Values are means ± SE. Airway Pmo was significantly greater at 25 cmH2O than at 5 cmH2O (**P < 0.01).

removed from left or right lungs. A sample trace of airway response to EFS before and after intraluminal pressure oscillation at a trough-to-peak amplitude of 20 cmH2O is shown in Fig. 2A. This airway had the greatest response to pressure oscillation and produced a 21% increase in active pressure response to EFS. In a separate group of bronchi (n = 11), the effect of 10−5 M indomethacin (cyclooxygenase inhibitor) on the response to pressure oscillation was examined. The response of the airway to pressure oscillation was not affected by the addition of indomethacin (Fig. 3).

The Pmo was measured in airways from left and right lungs at 5 and 25 cmH2O (Fig. 4). Increasing intraluminal pressure from 5 to 25 cmH2O produced a 21% increase in airway Pmo from 7.9 ± 0.4 to 9.5 ± 0.2 mm (n = 8–9, P < 0.01). There was no difference in Pmo between airways removed from left and right lungs (Table 1).

Isolated ASM. Force response to EFS was recorded in ASM strips before and after length oscillation (n = 7). Length oscillation of ASM produced a significant decrease in active force response to EFS immediately after oscillation (P < 0.01) (Fig. 5). The percent decrease in EFS response after pressure oscillation was 15.2 ± 2.3%. Force responses to EFS returned to preoscillation levels 10 min after oscillation had concluded.

DISCUSSION

The principal finding of the present study is that intraluminal pressure oscillation in isolated bronchial segments does not inhibit subsequent airway contraction but instead modestly enhances it. In contrast, oscillating the length of isolated ASM depresses force development, confirming previous reports (28). These results suggest that the response of ASM to a period of length oscillation is not expressed at the level of the whole airway. To our knowledge, this is the first study that has investigated the response of the intact airway wall to oscillation carried out in the relaxed state before the induction of bronchoconstriction.

Table 1. Mean airway smooth muscle perimeters

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<tr>
<th>Fixation Pressure</th>
<th>Left</th>
<th>Right</th>
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<tr>
<td>5 cmH2O</td>
<td>7.4 ± 0.4</td>
<td>8.4 ± 0.6</td>
</tr>
<tr>
<td>25 cmH2O</td>
<td>9.7 ± 0.2</td>
<td>9.3 ± 0.4</td>
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Values are means ± SE airway smooth muscle perimeters (Pmo; mm) determined morphometrically in airways removed from left (n = 9) and right (n = 8) lungs fixed at either 5 or 25 cmH2O. Pmo values in left and right lobe airways were not significantly different. Combined, Pmo increased 21% (P < 0.01, see text) in airways fixed at 25 cmH2O vs. those fixed at 5 cmH2O.
 Changes in ASM length induced by pressure oscillation may be significantly less than that seen under static conditions. Second, owing to the pitch of the spiraled ASM in the bronchial wall (5), it is unclear whether pressure oscillation also stretched ASM obliquely, in addition to longitudinal stretch, and whether such changes could contribute to the physiological response of the whole airway. Last, the disparity between ASM and bronchial segments could be explained if the response of ASM to length oscillation differed between bronchial and tracheal smooth muscle. Bronchi and tracheal smooth muscle have been shown to exhibit differences both in mechanical properties, such as the length-tension relationship (12), and in their response to contractile agents (27). However, studies on bronchial smooth muscle are carried out in heterogeneous tissue preparations. There is little or no evidence on the response of pure or near-pure bronchial smooth muscle to mechanical stimulation or stretch.

The above considerations may account for why the airway wall limits the effects of DI on ASM. However, even if the airway wall does restrict ASM stretch to the extent that the “protective” mechanism existing in the ASM is reduced or inactivated, an increase in bronchoconstriction would not be expected. The mechanism behind the enhanced airway response to EFS after pressure cycling has not been determined, although several possibilities exist. Cyclic inflation and deflation of the airway lumen exposes the airway to a different volume history to that present under static conditions (i.e., for baseline EFS responses). Therefore, greater airway response to EFS could arise after pressure cycling if ASM operating length changed as a result of hysteresis. We have previously observed in pig isolated airways that airway luminal cross-sectional area is greater on deflation than inflation (18). The larger luminal cross-sectional area may be accompanied by increased ASM length beyond that existing at the same intraluminal pressure before cycling. However, the relationship between passive intraluminal pressure and active pressure response to EFS (see Fig. 1) predicts that lengthening ASM beyond that present before oscillation (at 5 cmH₂O) will reduce, not increase, bronchoconstriction. Therefore, airway hysteresis is unlikely to account for an increase in the response to EFS after intraluminal pressure oscillation. An alternative explanation for the increased airway contraction in the whole airway involves the release of bronchoactive mediators from the airway wall. Airway tissue is a rich source of chemical mediators, including an array of metabolites of arachidonic acid (11, 22). Prostaglandins, endoperoxides, and thromboxane can be released from airway tissue by a variety of specific and nonspecific stimuli, and some are capable of inducing airway hyperresponsiveness (19). However, we saw no evidence that cyclooxygenase metabolites were involved in the responses reported in our study in whole airways, because indomethacin did not alter the response of the airway to pressure oscillation.

In summary, a decrease in active force in isolated ASM in response to length oscillation, reported previously and confirmed here, is not observed in isolated whole airway preparations. Instead, there is a modest increase in bronchoconstriction. These findings reflect modification of ASM responses by the intact airway wall. They also suggest that the phenomenon of bronchoprotection reported in some in vivo studies may not be an intrinsic property of the airway wall.

Fig. 5. Active force (g) responses to EFS in isolated tracheal smooth muscle before and after length oscillation. Values are means ± SE. ASM strips (n = 7) were oscillated for 10 min at a trough-to-peak amplitude of –0.2 optimum length at 0.5 Hz. Active force response to EFS was reduced immediately after length oscillation (T = 0) (**P < 0.01) but returned to baseline 10 min later (T = 10).
REFERENCES


27. Van de Voorde J and Joos G. Regionally different influence of contrac-