Relationship of airway narrowing, compliance, and cartilage in isolated bronchial segments

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Noble, P. B., D. J. Turner, and H. W. Mitchell. Relationship of airway narrowing, compliance, and cartilage in isolated bronchial segments. J Appl Physiol 92: 1119–1124, 2002; 10.1152/japplphysiol.00662.2001.—Structural components of the airway wall may act to load airway smooth muscle and restrict airway narrowing. In this study, the effect of load on airway narrowing was investigated in pig isolated bronchial segments. In some bronchi, pieces of cartilage were removed by careful dissection. Airway narrowing was produced by maximum electrical field stimulation. An endoscope was used to record lumen narrowing. The compliance of the bronchial segments was determined from the cross-sectional area of the lumen and the transmural pressure. Airway narrowing and the velocity of airway narrowing were increased in cartilage-removed airways compared with intact control bronchi. Morphometric assessment of smooth muscle length showed greater muscle shortening to acetylcholine in cartilage-removed airways than in controls. Airway narrowing was positively correlated with airway compliance. Compliance and area of cartilage were negatively correlated. These results show that airway narrowing is increased in compliant airways and that cartilage significantly loads airway smooth muscle in whole bronchi.

airway smooth muscle; asthma; cartilage

DRUGS SUCH AS METHACHOLINE or histamine produce an increase in lung resistance in humans and laboratory animals in vivo (20, 21) and in vitro produce narrowing of isolated airway rings (13) and bronchial segments (17). The degree of airway narrowing to drugs may be determined by several factors, including elastic loads that act as a brake on airway smooth muscle shortening (12). A reduction in load on airway smooth muscle has been proposed to account for the increase in maximum bronchoconstriction seen in patients with asthma (1, 12, 27).

Loads could arise within the airway wall itself, as well as from parenchymal attachments via forces of interdependence (6). The effect of load on shortening of isolated airway smooth muscle cells is clearly established (7, 25). In airway segments in vitro, maximum airway smooth muscle shortening is only about half of the shortening produced by isolated and unloaded smooth muscle cells (7, 17, 22, 25), suggesting that load within the airway wall attenuates airway narrowing. The intact airway wall has a complex three-dimensional structure including airway smooth muscle, mucosa, and cartilage, and each may contribute to load (9–11). A relationship between load and airway narrowing in airways isolated from lung attachments and the contribution to load from different structural components remains unclear.

The first aim of this study was to establish a relationship between airway wall load and airway narrowing. Studies were carried out in segments of bronchus that were separated from lung parenchyma to eliminate parenchymal forces, leaving only those forces associated with the airway wall itself. Airway compliance was used as an index of load. To alter airway compliance, pieces of cartilage were removed from the bronchial wall by dissection. Cartilage pieces are connected to airway smooth muscle via a connective tissue webbing or network in bronchi (16–18, 23, 24). An additional aim of the study was therefore to examine the effect of cartilage on airway wall load. Porcine bronchi were studied because these have abundant cartilage (15) and airways from this species have been used in several studies to model factors affecting the extent of airway narrowing (4, 17, 19, 21, 26). We hypothesized that removing cartilage from the airway wall would increase airway compliance and increase airway narrowing.

METHODS

Eight-week-old female pigs were sedated with tiletamine/zolazepam (4.4 mg/kg im) and xylazine (2.2 mg/kg) and then exsanguinated under pentobarbitone (25 mg/kg iv) anesthesia. Methods for preparing bronchial segments and recording luminal narrowing with an endoscope are fully described elsewhere (19). Briefly, lungs were removed, the right lower lobe stem bronchus was dissected free of parenchyma, and all side branches were ligated. Bronchial segments (~4 cm long and 0.6 cm OD) were cannulated at each end and placed horizontally in an organ bath containing gassed Krebs solution so that segment length was held constant during the experiment. One cannula was connected to a reservoir containing Krebs solution that filled the airway lumen, so that the transmural pressure (Ptm) of the bronchus could be...
varied by changing the height of the reservoir. The cannula at the other end of the bronchus was sealed with a latex membrane to prevent any flow of Krebs solution through the bronchus. The airway lumen at the center of the segment was visualized via a rigid fiber-optic endoscope (Olympus SES1711D) inserted into the lumen through the latex membrane. Airway narrowing was produced by electrical field stimulation (EFS) via platinum ring electrodes placed over the region of interest. Stimulation parameters (299 mA, 30 Hz, 3 ms) were chosen to produce maximum bronchoconstrictor responses. Krebs solution contained 10⁻⁶ M propranolol to prevent the action of inhibitory neurotransmitter released by EFS (3). Color video images of the airway lumen were recorded on videotape and displayed on a monitor. Each train of EFS was maintained until maximum narrowing was observed, typically 20 s.

A modification of this apparatus was used to assess possible changes to airway smooth muscle force produced by dissecting cartilage (see below). In this apparatus, the endoscope was removed and the two ends of the bronchial segment were sealed by three-way taps. A pressure transducer was connected to one of these taps for recording luminal pressure. Dissecting cartilage (see below). In this apparatus, the endo-

The changes in cross-sectional area at each step on inflation were determined by tracing the perimeter of the lumen. Intraobserver variability was 0.67 ± 0.09% (n = 5). Velocity of airway narrowing at 5 cmH₂O Ptm was measured by dividing the change in lumen perimeter to EFS, from the onset of narrowing, by the time taken.

For airway compliance measurements, airway lumen CSA was measured between -15 and 20 cmH₂O Ptm in 5 cmH₂O steps on inflation and deflation. The changes in CSA at each Ptm was divided by the CSA at 0 cmH₂O (i.e., strain = ΔCSA/CSA₀). The compliance was calculated from strain/ΔPtm (i.e., cmH₂O⁻¹). Slope was determined between -5 and 5 cmH₂O of the deflation curve by regression analysis by using commercial software, giving an R² > 0.95.

Airway narrowing and compliance were measured in 14 control bronchial segments and in 9 airways with varying amounts of cartilage removed from the outer airway wall. Cartilage plates were removed from an ~1-cm band of the airway segment near its center by careful dissection under a dissecting microscope. In no experiment was all the cartilage removed to avoid damaging the underlying airway smooth muscle, as determined histologically (see Fig. 4).

Some bronchi (8 controls and 9 cartilage-removed) were subsequently fixed for morphometric evaluation of cartilage and smooth muscle shortening (8). Airways were maximally stimulated with 10⁻¹ M acetylcholine at 5 cmH₂O Ptm and then fixed in 4% formaldehyde. A 0.5-cm length of the airway at the area of interest was processed into wax blocks, sectioned, and stained with hematoxylin and eosin. Airway smooth muscle shortening and the CSA of the cartilage were measured in four sections per airway and averaged. Airway smooth muscle shortening was calculated from the inner wall area, the outer perimeter of the airway smooth muscle, and the perimeter of the epithelium, as detailed previously (8). Cartilage was normalized for airway size by dividing by lumen perimeter (2).

Statistical comparisons between means were made by ANOVA or Student’s t-test, as detailed in the text. Correlations between airway narrowing, deflationary compliance, and airway cartilage were performed by the method of least squares. Correlations used the maximum airway narrowing, obtained at any of the pressures on deflation, in each airway segment. Lines of best fit were generated by using the built-in equations in Graph Pad Prism software and were chosen on the basis of the highest R² value. Multiple linear regression was used to test the relationship between maximum airway narrowing and compliance and cartilage together. Significance was P < 0.05. Data are given as means ± SE.

RESULTS

Strain-pressure curves for control and cartilage-removed bronchi are shown in Fig. 1. Cartilage-removed airways had significantly greater strain than controls at positive luminal pressures (ANOVA). Cartilage removal increased airway compliance, as determined from the deflationary limb of the strain-pressure curve, from 0.07 ± 0.01 cmH₂O⁻¹ in controls to 0.16 ± 0.03 cmH₂O⁻¹ in cartilage-removed bronchi (P < 0.05, Student’s unpaired t-test).

Cartilage-removed airways narrowed significantly more than controls at Ptm's of 0 and 5 cmH₂O (Fig. 2). Maximum airway narrowing to EFS (on deflation) was 57 ± 3% in control airways and 74 ± 4% in cartilage-removed airways (P < 0.01, Student’s unpaired t-test). In both control and cartilage-removed airways, maximum narrowing occurred by a Ptm of 5 cmH₂O (repeat-measures ANOVA). In control airways, narrowing was maintained at higher pressures (10–20 cmH₂O), whereas in cartilage-removed airways narrowing declined (P < 0.001). Airway narrowing showed hysteresis (Fig. 2) and was significantly less during deflation in both control (P < 0.0001, Student’s paired t-test) and cartilage-removed airways (P < 0.001).

![Fig. 1. Mean strain-transmural pressure (Ptm) curves for control (n = 14) and cartilage-removed (n = 9) bronchi. *P < 0.05, **P < 0.01 compared with controls (ANOVA). Inflationary (lower) and deflationary (upper) curves are shown for each airway group. ΔCSA, change in cross-sectional area (CSA); CSA₀, CSA at 0 cmH₂O.](http://apjpphysiology.org/PDF/10.1152/jappl.00387.2001.Fig1.png)
At Ptms where narrowing was greater (0 and 5 cmH₂O) there was no significant difference in lumen perimeter between cartilage-removed and control airways (Table 1). However, when lumen perimeter is expressed as a percentage of baseline (Ptm = 0 cmH₂O), cartilage removed airways were significantly greater than controls for Ptm of 10 cmH₂O and greater. Narrowing velocity was significantly greater in cartilage-removed airways, 0.58 ± 0.04 mm/s, compared with controls, 0.38 ± 0.05 mm/s (P < 0.01, Student’s unpaired t-test). Airway smooth muscle shortening to a maximal dose of acetylcholine was also increased in cartilage-removed airways (P < 0.05, Student’s unpaired t-test). Airway smooth muscle shortening by 47 ± 2% in controls (n = 8) and by 56 ± 2% in cartilage-removed airways (n = 9) (P < 0.05).

Variation in the quantity of cartilage removed from the airway wall produced a range of compliances, and compliance and cartilage were negatively correlated (Fig. 3). Histological sections of a control and cartilage-removed airways are shown in Fig. 4. Correlation analysis also showed that maximum airway narrowing to EFS was directly proportional to deflationary compliance (P < 0.001) (Fig. 5) and indirectly proportional to airway cartilage (r = −0.64, P < 0.01). Multiple linear regression showed compliance and cartilage to be independent variables and together correlated with narrowing (r = 0.81, P < 0.001).

The effect of removing cartilage on airway smooth muscle force was assessed from luminal pressure generated in isovolumetric bronchial segments. Cartilage removal had no effect on smooth muscle force. Luminal pressure to EFS was 30 ± 2 cmH₂O in control bronchi and 28 ± 3 cmH₂O in cartilage-removed bronchi (n = 4, P > 0.05, Student’s paired t-test).

**DISCUSSION**

Our study has shown a significant relationship between maximum airway narrowing, compliance, and cartilage. The dissection of pieces of cartilage from the airway wall doubled the compliance of the airway and increased both maximum airway narrowing to EFS and the velocity of narrowing. The techniques employed...
played in this study allowed both narrowing and compliance to be determined in exactly the same part of the airway wall. To our knowledge this is the first time that the effect of bronchial compliance or cartilage on airway narrowing has been documented.

In isolated airway segments we used a two-compartment model of load in which the total load on airway smooth muscle came from the compliance of the airway wall and from the Ptm applied to the airway. Wall compliance is a sum of the elastic properties of several structural components of the airway wall, each of which may vary in their involvement in airway smooth muscle shortening. Mucosa, airway smooth muscle, and cartilage are arranged roughly concentrically in bronchi (15, 23) and are interconnected by an elastic matrix. The contribution of each of these components to airway smooth muscle load is unclear. However, a recent study by Lambert and colleagues (11) assessed for the first time the rigidity of the mucosa in bronchi and suggested that this may be a significant load on airway smooth muscle shortening. In the present study, the removal of cartilage pieces altered the strain-pressure relationship of the airway, increasing airway compliance, and this in turn was associated with increased airway narrowing. Our finding of increased narrowing in airways with reduced cartilage appears to contrast with studies in humans showing an increase in cartilage in the airways of asthmatic subjects compared with controls (2). However, in vivo, the relationship between airway cartilage and airway narrowing may be influenced by additional factors associated with the transmission of parenchymal forces to airway smooth muscle. The importance of airway cartilage to the effect of parenchymal forces on airway smooth muscle shortening in vivo has not to our knowledge been investigated.

The effect of removing cartilage on the strain-pressure relationship could vary in different species in which there are differing amounts of cartilage or in which the architecture of the cartilage pieces is different. Mid-sized bronchi in pigs and humans have a similar cartilage area per millimeter of basement membrane (~0.35 mm in humans compared with 0.5 mm in pigs) (Ref. 2; present study). Human and pig bronchi also have similar compliances (15), possibly as a result of their similar cartilage content. In some species, including pig and cat, cartilage pieces overlap and may slide across each other or separate when subjected to compressive pressures or bronchoconstriction (16, 17, 23; present study). This may influence the effect of cartilage on the strain-pressure relationship of the airway. However, our results define a relationship between the compliance of the airway wall from all sources and airway narrowing. These findings, coupled with our observation that airway smooth muscle shortening (measured morphometrically) was increased in cartilage removed airways, indicates that load-bearing elements in the cartilage layer restrict airway narrowing in vitro.

Removal of cartilage in the test airways also resulted in the severing of fibrous attachments between pieces of cartilage, which together increased the airway compliance. Dissection of cartilage may also have altered airway smooth muscle responses and hence airway narrowing as a result of damaging the airway smooth muscle. However, this is unlikely because the dissection of cartilage did not change active pressure to EFS in bronchial segments when they were studied under isovolumic conditions.

The effect of cartilage removal on airway narrowing was observed at various Ptm’s. Changes in airway Ptm are accompanied by simultaneous changes in airway smooth muscle pre-load as well as in the afterload. The preload sets the resting length of the airway smooth muscle and hence its maximum force production when it is stimulated. By utilizing a range of Ptm’s, each airway is able to narrow at its optimum preload, assuming that this falls in the range of pressures tested. The increased narrowing in cartilage-removed bronchi appeared to result from a reduced load from the airway wall and not from any difference in preload because the lumen perimeter was the same in the two airway groups at Ptm’s in which narrowing was found to be increased.

Airway narrowing varied only slightly over the different Ptm’s used in the study. Narrowing in both airway groups was greatest at Ptm of 5 cmH2O, suggesting that the airway smooth muscle may have been at its optimum length at that pressure or above. As the Ptm increased ≥10 cmH2O, narrowing was significantly reduced in cartilage-removed airways but less so in controls. At these pressures, airway smooth muscle in the cartilage-removed group may be stretched beyond its optimum operating length, reducing airway narrowing. The strain-pressure curves and to a lesser extent the percent change in lumen perimeters support this possibility. At Ptm ≥10 cmH2O airway narrowing to EFS was very similar in the two groups. The two-compartment model of load (total load = Ptm + wall compliance) indicates that, at high Ptm, load from the airway wall constitutes a smaller proportion of the total load on airway smooth muscle. Under these con-
ditions, the differences in wall compliance of the cartilage-removed airway will be a less important factor in airway narrowing, which may account for the similar responses to EFS.

As shown in this and previous studies (17, 21), porcine airways do not appear to narrow to complete closure. Other species such as dog show greater narrowing or even airway closure (5). Dog bronchi are more compliant than pig (14, 15), which, as shown in this present study, may facilitate airway narrowing in vitro and may therefore account for greater narrowing in that species compared with pig. Airway smooth muscle shortening in canine muscle strips is increased by removing pieces of cartilage (9), possibly as a result of reducing the load on the muscle. This agrees with our porcine data and provides indirect evidence for a relationship between compliance and airway narrowing in dog. Little is known about airway narrowing in the human isolated airway.

This study complements and extends previous findings in tissue strips (7, 9). We assessed whether loads associated with airway smooth muscle in situ in the present study equated to those loads shown to impede tracheal smooth muscle shortening (7). A mathematical transformation of airway wall compliance to linear elastic load (data not shown) suggested that bronchial segments in our study were subject to slightly greater loads than those shown to restrict airway smooth muscle shortening [an estimated 30 g/cm elastic load (7)]. However, this transformation from bulk to linear elastic moduli is subject to several assumptions because of anisotropic properties of the airway wall in bronchi as well as the geometrical differences between airway segments and strips. The lesser load apparently needed to restrict smooth muscle shortening in strips, compared with airway narrowing in tubes, suggests that total wall compliance overestimates the load actually exerted on the airway smooth muscle within the bronchial wall. Such an overestimation could occur in the presence of additional sources of load more compliant than cartilage. One such source could be the fibrous tissue network connecting airway smooth muscle to cartilage, which has been shown to stretch out during contraction of the airway smooth muscle (17, 18, 23, 24).

These data provide the first demonstration of a relationship between the compliance of the isolated airway wall and airway narrowing. Although the structural basis of load-bearing parts of the airway wall may vary between airways and species, these findings may have general application to human airways both in health when the compliance of an airway is known or in disease when compliance is altered (1).

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REFERENCES


