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.

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 pentobarbital (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 SES17111D) 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. The images recorded on tape were played back and analyzed off-line with the Video Pro analysis system. The video image was calibrated by using a probe of known diameter inserted into the lumen (2). The images recorded on tape were played back through a Video Pro analysis system. The video image was calibrated by using a probe of known diameter inserted into the airway (19). At the above Ptm, the bronchial lumen was circular (19), and airway narrowing was determined from the EFS-induced change in lumen cross-sectional area (CSA), 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 five 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 R^{2} > 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 R^{2} 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 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://jap.physiology.org/)
Table 1. Effect of varying \( \text{Ptm} \) on airway lumen

<table>
<thead>
<tr>
<th>( \text{Ptm} ) (cmH(_2)O)</th>
<th>Lumen Perimeter, mm</th>
<th>%Lumen Perimeter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Cartilage-removed</td>
</tr>
<tr>
<td>20</td>
<td>14.7 ± 0.6</td>
<td>15.0 ± 0.9</td>
</tr>
<tr>
<td>15</td>
<td>14.5 ± 0.6</td>
<td>14.8 ± 1.0</td>
</tr>
<tr>
<td>10</td>
<td>14.3 ± 0.6</td>
<td>14.6 ± 1.0</td>
</tr>
<tr>
<td>5</td>
<td>13.4 ± 0.5†</td>
<td>13.5 ± 0.9*</td>
</tr>
<tr>
<td>0</td>
<td>11.6 ± 0.4†</td>
<td>10.5 ± 0.8†</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 14 \) control and 9 cartilage-removed bronchi. Lumen perimeter measured on deflation to different transmural pressures (\( \text{Ptm} \)); *\( P < 0.05 \), †\( P < 0.001 \) repeated-measures ANOVA, compared with the preceding higher pressure. %Lumen perimeter is the perimeter of the lumen expressed as a percentage of baseline (\( \text{Ptm} = 0 \) cmH\(_2\)O); ‡\( P < 0.05 \) compared to control.

Fig. 2. Electrical field stimulation (EFS)-induced airway narrowing in control (\( n = 14 \)) and cartilage-removed (\( n = 9 \)) bronchi. Narrowing was recorded at different \( \text{Ptm} \) on inflation and deflation (shown by arrows). Airway narrowing was measured from changes in CSA of the lumen. *\( P < 0.01 \), †\( P < 0.001 \) compared to controls (ANOVA).

At \( \text{Ptm} \)s where narrowing was greater (0 and 5 cmH\(_2\)O) 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 (\( \text{Ptm} = 0 \) cmH\(_2\)O), cartilage removed airways were significantly greater than controls for \( \text{Ptm} \) of 10 cmH\(_2\)O 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 bronchi 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 cmH\(_2\)O in control bronchi and 28 ± 3 cmH\(_2\)O 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 em-
rowing may be in relationship between airway cartilage and airway nar-
jects compared with controls (2). However, in vivo, the increase in cartilage in the airways of asthmatic sub-
appears to contrast with studies in humans showing an increased narrowing in airways with reduced cartilage
with increased airway narrowing. Our
airway compliance, and this in turn was associated
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 dissec-
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 preload 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, assum-
ing 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 signifi-
cantly 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-

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conditions, 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 smooth muscle shortening [an estimated 30 g/cm elastic load halfed bronchial narrowing whereas 10 g/cm halved airway 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 smooth muscle shortening [an estimated 30 g/cm elastic load halfed bronchial narrowing whereas 10 g/cm halved airway smooth muscle shortening (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

