Effect of lung inflation in vivo on airways with smooth muscle tone or edema

ROBERT H. BROWN,1,2,3 WAYNE MITZNER,1,3,4 YONCA BULUT,1 AND ELIZABETH M. WAGNER3,4

1Department of Anesthesiology and Critical Care Medicine, 2Department of Radiology, 3Division of Physiology, Department of Environmental Health Sciences, and 4Division of Pulmonary and Critical Care Medicine, Department of Medicine, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland 21205

Brown, Robert H., Wayne Mitzner, Yonca Bulut, and Elizabeth M. Wagner. Effect of lung inflation in vivo on airways with smooth muscle tone or edema. J. Appl. Physiol. 82(2): 491–499, 1997.—Fibrous attachments to the airway wall and a subpleural surrounding pressure can create an external load against which airway smooth muscle must contract. A decrease in this load has been proposed as a possible cause of increased airway narrowing in asthmatic individuals. To study the interaction between the airways and the surrounding lung parenchyma, we investigated the effect of lung inflation on relaxed airways, airways contracted with methacholine, and airways made edematous by infusion of bradykinin into the bronchial artery. Measurements were made in anesthetized sheep by using high-resolution computed tomography to visualize changes in individual airways. During methacholine infusion, airway area was decreased but increased minimally with increases in transpulmonary pressure. Bradykinin infusion caused a 50% increase in airway wall area and a small decrease in airway luminal area. In contrast to airways contracted with methacholine, the luminal area after bradykinin increased substantially with increases in transpulmonary pressure, reaching 99% of the relaxed area at total lung capacity. Thus airway edema by itself did not prevent full distension of the airway at lung volumes approaching total lung capacity. Therefore, we speculate that if a deep inspiration fails to relieve airway narrowing in vivo, this must be a manifestation of airway smooth muscle contraction and not airway wall edema.

METHODS

Our study protocol was approved by The Johns Hopkins Animal Care and Use Committee. Anesthesia was induced in 10 sheep (25–35 kg) with intramuscular ketamine (30 mg/kg) and subsequently maintained with pentobarbital sodium (20 mg·kg−1·h−1). A tracheostomy was performed, the sheep were paralyzed with pancuronium bromide (2 mg iv), and the lungs were mechanically ventilated with 100% oxygen at a rate of 15 breaths/min and a tidal volume of 12 ml/kg. A positive end-expiratory pressure of 5 cmH2O was applied. The left thorax was opened at the fifth intercostal space, and heparin (20,000 U) was administered. The esophageal and tracheal branches of the bronchoesophageal artery were ligated as previously described (29). The bronchial branch was then cannulated with an 18-gauge angiocatheter and perfused with a constant flow (0.6 ml·min−1·kg−1) of blood withdrawn from a femoral artery catheter by a variable-speed pump (Gilson Minipuls 2).

Airway imaging. High-resolution computed tomography (HRCT) scans were obtained with a Somatom Plus Scanner (Siemens, Iselin, NJ ) by using a 1-s scan time, 137 kVp, and 220 mA as previously described (4, 5). The images were reconstructed as a 256 × 256 matrix by using a maximum zoom of 4.0 (12-cm field of view). Twenty-five to 50 contiguous sections were obtained in the right middle and lower lobes by using a 1-mm table feed and a 2-mm slice thickness. The sheep were apneic with a constant controlled airway pressure for the duration of the scans (~2 min). Images were reconstructed with the use of a high-spatial frequency (resolution) algorithm that enhanced edge detection at window level of −450 Hounsfield units (HU) and window width of 1,350 HU. All airways visualized approximately perpendicular to the scan plane (long-to-short axis ratio <1:5:1) were measured. For repeated airway measurements in a given sheep within each experimental protocol, adjacent anatomic landmarks, such as airway or vascular branching points, were defined on the control-state HRCT image. After challenge, the same airways in a given animal were located from these adjacent landmarks and measured.

Lung volume measurements. Standard-resolution computed tomography (CT) scans were obtained with a Somatom
Plus Scanner (Siemens, Iselin, NJ) by using a 1-s scan time, 137 kVp, and 210 mA. The images were reconstructed as a 256 × 256 matrix by using a maximum zoom of 2.0. Twenty-five to 50 contiguous sections were obtained, starting at the apex of the lungs and moving caudally to the bases by using an 8-mm table feed and an 8-mm slice thickness. Images were reconstructed with the use of a standard lung algorithm at the above window settings, and lung volume was measured at each inflation pressure. The area of the right lung on each CT scan was defined as the area within the pleural border excluding the heart and diaphragm. The total right lung volume was calculated as the sum of the lung areas of each CT slice times the slice thickness.

Image analysis. The HRCT images were analyzed using the airway analysis module of the Volumetric Image and Display Analysis image-analysis software package (University of Iowa, Iowa City, IA) as previously described and validated (1, 4). Because adjacent vascular structures make the outer boundary of airways difficult to define, we measured airway wall area by first measuring mean wall thickness in each airway. To measure airway wall thickness, the operator drew two to five lines through the airway wall. The program automatically displayed a histogram of the pixel intensity along that line. The inflection points of increased intensity along the line that represent the inner and outer edges of the airway wall were selected. These points were identified as those that changed 10% of the intensity range between the center of the wall and the lumen or parenchyma, respectively. The program then automatically measured the distance between the two points. Because the software we use is capable of measuring fractions of a pixel when the rays are drawn oblique to the side of a pixel, the measurements of wall thickness were not necessarily quantized to multiples of the pixel dimension. From the measured luminal area and wall thickness, we could then calculate the wall area and outer airway area by simply assuming that the average measured wall thickness was uniform around the airway. The total airway area inside the outer airway perimeter then equals \( \pi (T + (A_{lum}/\pi))^2 \), where \( A_{lum} \) is luminal area and \( T \) is wall thickness. A few of the walls of the smallest airways could not be measured with this approach at all pressures.

Protocol. Each sheep served as its own control. The sheep were anesthetized and ventilated as described above. Nine sheep received 0.2 mg/kg atropine iv, a dose previously shown to completely block vagal tone in dogs (9, 14). In all sheep, scans were acquired at static transpulmonary pressure (Ptp) values of 1, 4, 9, and 20 cmH2O in random order. To standardize lung volume history, before each measurement, the Ptp was increased to 30 cmH2O, held for 5 s, and then decreased and maintained at the designated Ptp for the duration of the scans (~2 min). This maneuver was repeated for each of the four airway pressures during the acquisition of the HRCT and the standard CT scans. After each experimental condition, the animals were ventilated normally.

Because results from the first series of sheep suggested that the dose of atropine used did not completely eliminate airway muscle tone, we compared the results with atropine with those after vagotomy in four of the sheep. In these four animals, scans were initially acquired at a slightly broader range of static Ptp values (at 1, 4, 9, 20, and 30 cmH2O), then bilateral vagotomies were performed, and repeat scans were acquired at the same static Ptp values.

To test the effects of increased airway tone on the response to lung inflation, four sheep received a continuous infusion of 10 µg/min methacholine (MCh) through the bronchial artery, a dose previously shown to cause airway constriction resulting in an approximately fivefold increase in airway resistance (27). After 10 min of MCh infusion, scans were acquired (each after inflation to 30 cmH2O) at static Ptp values of 1, 4, 9, and 20 cmH2O in random order.

To test the effects of airway inflammation and edema on airways with smooth muscle tone abolished by vagotomy, four sheep received a continuous infusion of bradykinin through the bronchial artery at an initial rate of 6 µg/min for 10 min followed by a maintenance dose of 2 µg/min, a dose previously shown to cause a significant increase in airway wall thickness (28). During the maintenance infusion, scans were acquired at static Ptp values of 1, 4, 9, and 20 cmH2O as above.

Analysis. In each sheep, 9–16 airways (range 1.4–21.8 mm in diameter) were identified and measured under the various conditions. Figure 1 shows the distribution of the relaxed airways studied in all sheep. Airway area at 20 cmH2O Ptp in the relaxed state after atropine administration or vagotomy was defined as 100% (maximal area). Data are expressed as a percentage of maximal area. The change in mean airway area (as a percentage of the relaxed state at 20 cmH2O Ptp) for a given change in Ptp and the initial airway size (defined as the relaxed airway area at 4 cmH2O Ptp) were separately analyzed by linear regression for the atropine, vagotomy, MCh, and bradykinin challenges; significance of the slopes of the regression lines, being different from zero, were tested.

The percent increase in the airway wall area and the total airway area and the percent decrease in airway luminal area after bradykinin compared with vagotomy alone were analyzed by one-way analysis of variance with Bonferroni correction for multiple pairwise comparisons. To test whether the decreased luminal area could be accounted for by the increased airway wall area, paired t-tests were used to compare the increase in airway wall area (mm2) with the decrease in airway luminal area (mm2) for the airways during bradykinin infusion at each Ptp.

To test for differences in lung volume, the right lung volume (upper, middle, and lower lobes) during atropine, MCh, and bradykinin was analyzed by one-way analysis of variance with Bonferroni correction for multiple pairwise comparisons. Significance was accepted at \( P < 0.05 \).
mean airway areas in individual sheep as a function of Ptp are shown in Fig. 2. In each animal this relationship was nonlinear, showing a very compliant region below 4 cmH\textsubscript{2}O and a much stiffer region at pressures above 9 cmH\textsubscript{2}O. For each 1-cmH\textsubscript{2}O increase in Ptp between Ptp values of 1 and 4 cmH\textsubscript{2}O, the mean airway luminal area increased 5.7 ± 0.6%, and between 9 and 20 cmH\textsubscript{2}O the area increased only 1.1 ± 0.1%.

In the four sheep with subsequent vagotomy, the range of Ptp was extended to 30 cmH\textsubscript{2}O. Vagotomy resulted in significant increases in airway areas at all Ptp values (Fig. 3). The increase in luminal area averaged ∼16% at the different Ptp values. Specifically, airway area after vagotomy increased 21 ± 2, 18 ± 2, 19 ± 2, 13 ± 2, and 11 ± 2% for Ptp values of 1, 4, 9, 20, and 30 cmH\textsubscript{2}O, respectively. For comparison, airway areas in Fig. 3 are all normalized to the values with atropine, with the area at 20 cmH\textsubscript{2}O being considered 100%. After vagotomy, the mean airway lumen increased 5.0 ± 1.1% for each 1-cmH\textsubscript{2}O increase in Ptp between Ptp values of 1 and 4 cmH\textsubscript{2}O (P < 0.001), which was comparable to the distensibility in this pressure range found after atropine. However, between 9 and 20 cmH\textsubscript{2}O, the airway distensibility was much decreased, with mean airway area increasing only 0.6 ± 0.3% for each 1-cmH\textsubscript{2}O increase in Ptp (P = 0.03).

In the range above 20 cmH\textsubscript{2}O in these four sheep, there was still some distension after only atropine, with airway area increasing 0.5 ± 0.2% for each 1-cmH\textsubscript{2}O increase in Ptp (P = 0.015). However, after vagotomy, the 0.4 ± 0.3% increase per centimeter of H\textsubscript{2}O in this high-pressure range was not statistically significant (P = 0.24).

Figure 4 shows individual relaxed and contracted airways from one sheep at two Ptp values. After MCh one can see clear contraction of the airways at both low and high pressures. Contraction of the airway with MCh had a profound effect on the pressure-area relationship (Fig. 5). The mean luminal areas across sheep during MCh infusion decreased by 65 ± 1, 72 ± 1, 72 ± 2, and 67 ± 2% relative to the relaxed state at Ptp values of 1, 4, 9, and 20 cmH\textsubscript{2}O respectively. During MCh infusion, airway area increased minimally with increases in Ptp. Even at the highest pressures there was no tendency for the curves to turn upward. Over the entire Ptp range between 1 and 20 cmH\textsubscript{2}O, the mean airway area increased only 0.7 ± 0.1% for each 1-cmH\textsubscript{2}O increase in Ptp (P = 0.0001). Thus at a Ptp of 20 cmH\textsubscript{2}O the mean airway area remained at only 33 ± 2% of maximal size.

Bradykinin infusion caused an increase in wall area and a decrease in airway luminal area at low lung volume. Figure 6 shows relaxed and edematous airways from one sheep at two Ptp values. After bradykinin, the airway lumen can be seen to be smaller with a thicker airway wall at both high and low Ptp. Figure 7 shows the percent increase in wall area (compared with vagotomy alone) as a function of Ptp. Bradykinin administration increased airway wall area at each Ptp value, but there were no significant differences (P = 0.07; wall area increased 54 ± 5, 51 ± 5, 68 ± 5, and 61 ± 4% at pressures of 1, 4, 9, and 20 cmH\textsubscript{2}O respectively).

The mean airway pressure-area curves for each sheep after vagotomy and bradykinin administration are shown in Figure 8. At 1 cmH\textsubscript{2}O the luminal area was decreased significantly in all sheep, but the magnitude of this decrease varied from 9 to 53% in the different sheep. In contrast to the pressure-area curves of airways narrowed by smooth muscle contraction with MCh, Fig. 8 shows that airways with edema could be easily dilated with increasing Ptp. There was variability among the sheep in the sensitivity of airway size...
to increasing Ptp, with some sheep requiring Ptp values of 20 cmH₂O to reach the relaxed size and others requiring only 4 cmH₂O. Reasons for this variability in the response to bradykinin may relate to how the individual sheep handle excess leakage from the bronchial circulation. In addition, the variable response to lung inflation may be related to the variable decrease in luminal area after bradykinin administration.

Bradykinin infusion also caused an increase in the total or outer airway area. The total airway area increased 12 ± 2 (21 ± 3%), 12 ± 2 (19 ± 3%), 20 ± 2 (29 ± 2%), and 23 ± 2 (29 ± 2%) mm² compared with after vagotomy alone at Ptp values of 1, 4, 9, and 20 cmH₂O, respectively (P < 0.0001). These increases in the total airway area occurred simultaneously with mean decreases in luminal area of 7 ± 1 (24 ± 3%), 6 ± 1 (21 ± 3%), 4 ± 1 (11 ± 2%), and 1 ± 2 (1 ± 1%) mm² at Ptp values of 1, 4, 9, and 20 cmH₂O, respectively. Thus, during bradykinin infusion, the increases in wall area were significantly greater than the decreases in luminal areas at all Ptp values (P < 0.0001).

The pressure-volume curves of the lung for relaxed (atropine), MCh, and bradykinin conditions are shown in Fig. 9. There were minimal differences between the mean curves in the three groups, with the only difference occurring at Ptp of 9 and 20 cmH₂O during MCh infusion, when lung volumes were slightly decreased by 7 and 6% (P = 0.02), respectively. The distensibility of the lung was compared with that of the airways by comparing matched pressure-area curves. For this purpose, lung area was calculated as lung volume to the two-thirds power for each of the three conditions.

Figure 10 shows the airway area (averaged among all sheep) plotted against this calculated parenchymal area.

To determine whether there was an effect of initial airway size on the responses to MCh or bradykinin, we partitioned the airways into three groups, small (<4 mm), medium (4–8 mm), and large (>8 mm in diameter). Whereas change in airway size was significantly related to the change in pressure, the pressure-area curves for three partitioned sizes were similar (data not shown). When the change in pressure was taken into account, the initial size of the airway was not a factor in the response to atropine (P = 0.86), vagotomy (P = 0.37), MCh (P = 0.85), or bradykinin (P = 0.54).

**DISCUSSION**

Our results demonstrate that, when sheep airways are contracted with an exogenously administered smooth muscle agonist, baseline size is greatly reduced, and airways become minimally responsive to increasing Ptp. However, when the airway caliber is decreased because of airway wall edema, the airways can readily be distended by increasing Ptp. In the interpretation of our results, we have assumed that bradykinin acted to cause bronchial vascular fluid flux and had no direct airway smooth muscle contractile effects (25). Had there been any direct effects on the smooth muscle, it would have only served to lessen the differences observed between the effects of MCh and bradykinin.

In the airways relaxed with atropine or vagotomy, the distensibility was comparable to that of the lung...
Parenchyma except at low Ptp (Fig. 10). Thus above 4 cmH₂O, the airways stiffness (assessed by the slope of the curves) matches that of the surrounding lung parenchyma. This finding contrasts with a previous study of airway distensibility in dogs (4), in which most airways became completely nondistensible (i.e., much stiffer than the lung parenchyma) above ~7 cmH₂O. Thus sheep airways appear to behave differently from those in the dog. Sheep airways also had a different response to atropine. In the dog, we found that the airways were completely relaxed with the large dose of atropine used, but with the same weight-adjusted dose of atropine in the sheep, we found evidence that the airways were not completely relaxed. This led to the additional experiments in which the vagus nerves were cut. With vagotomy, the airway areas reached values at high Ptp that were ~16% larger than those reached after atropine. These results suggest the presence of noncholinergic airway tone being carried in the vagal trunk. However, even after vagotomy, the sheep airways were still not as rigid as those in the dog, suggesting intrinsic structural differences between these two species.

At low Ptp, results in Fig. 10 also show that the relaxed airways appear to be more distensible than the lung itself. A similar observation was also made in the canine lung (4). An intuitive explanation was offered in that study based on structural considerations, but no quantitative predictions have been made. The finding suggests that the forces of interdependence that distend the airway at high Ptp may be much less important at lung volumes below functional residual capacity (FRC). During MCh infusion (Fig. 10) the airways are clearly much stiffer than the lung over the entire pressure range. With the edematous airway walls, Fig. 10 shows the airways are also more distensible than the parenchyma over the entire pressure range. This increased distensibility results from a combination of the decreased size at the lowest pressure and the inability of wall edema to prevent distension of the airway with lung inflation.

With the dose of MCh given, lung inflation to a Ptp of 20–30 cmH₂O caused minimal dilation of the airways. How airways respond to lung inflation depends on several factors, especially how contracted smooth muscle responds to distending forces. With regard to our present study, there is clear evidence that, given a sufficiently large contraction, some airway smooth muscle is very difficult to stretch. For example, Olsen et al. (22) showed very stiff pressure-area curves of isolated canine bronchi contracted with a high dose of acetylcholine (15 µg/ml). Even with a transmural pressure of 30 cmH₂O, there was only minimal distension. Gunst et al. (15) similarly reported minimal distension in MCh contracted canine airways with lung inflation in dogs. Murtagh et al. (21) showed that, with a large
aerosol dose of MCh, sufficient to constrict in situ canine airways to ~25% of maximal caliber, pressures up to 96 cmH2O were required to pull open the airways. Dose level may also account for the different response to lung inflation of contracted canine airways (4). In the dog, there was considerable variability among animals in the extent of dilation of MCh contracted airways as Ptp was increased from FRC; some animals’ airways showed substantial immediate dilation, whereas others required Ptp to be increased to 15 cmH2O. However, at 20 cmH2O, airways in all animals showed substantial dilation to near maximal size. In the present study in sheep, the MCh was delivered directly to the airways by giving a continuous infusion of agonist to the cannulated bronchial artery. With a MCh infusion rate of 10 µg/min and a bronchial artery flow of 20 ml/min, the MCh concentration in the perfusate was 0.5 µg/ml. In the dogs we infused MCh into a peripheral vein at a rate of 67 µg/min, so with a nominal cardiac output of 2,000 ml/min, the MCh concentration in the blood was ~0.033 µg/ml. Thus a 20-fold higher vascular concentration was given in the sheep. This difference was manifested by the sheep airways having been narrowed by 72% at FRC (Fig. 5), whereas in the dogs the degree of contraction at FRC was only ~40% (4).

Changes in lung volume can have effects on airway size independent of Ptp (18, 20). We found that the lung pressure-volume curve was shifted slightly downward at the higher pressures during MCh infusion. Although such a decreased lung volume would be expected to contribute to the decreased airway size at the higher Ptp values of 9 and 20 cmH2O, the decreases in lung volume were far too small (6–7%) to account for either the 67% difference in airway area at maximal Ptp or the inability of lung inflation to pull out the constriction.

Lung inflation had minimal effect on the total area of the relaxed airway wall. Administration of the inflammatory mediator bradykinin caused a substantial (~50%) increase in wall area at all Ptp values. That the wall area was not affected by the level of lung inflation suggests that airway wall edema does not move much with lung inflation. Because lung inflation is known to lead to a decreased interstitial pressure, this observa-
tion would suggest that either there is a uniform decrease in interstitial pressure or the resistance to fluid movement in the interstitium is sufficiently high that minimal movement can occur over the course of each experimental pressure change (~10 min).

In clinical asthma, the intrinsic forces of contraction may be greater than that which lung inflation can overcome. This would be consistent with several observations showing that a deep inspiration does not relieve bronchoconstriction in asthmatic patients (2, 3, 11–13, 23, 24). Several authors have proposed that, in asthma, edema fluid from inflammation and cellular infiltrate collects between the airway smooth muscle and the surrounding lung parenchyma and that this fluid should attenuate the forces of radial traction produced by increased lung volume (16, 17, 19). Our present findings in the sheep do not support this mechanism. We found that, with an ~50% increase in airway wall area, lung inflation could readily distend airways that were narrowed by the edema at low lung volume. Because it is unlikely that airways can be acutely thickened much more than 50% (6), it would seem unlikely that edematous wall thickening per se could prevent a deep inspiration from distending asthmatic airways. If these considerations apply in the human lung, then we suggest that the lack of effect of a deep inspiration in asthmatic subjects must result from smooth muscle tone. In the present study, lung inflation up to 20 cmH₂O Ptp caused only minimal dilation of the airway under contracted conditions. Although we did not investigate the effect of different MCh doses, it is clear that, if airways are stimulated to constrict at low lung volume, it may be very difficult to distend them in vivo. In this regard, Skloot et al. (26) recently showed when normal human subjects were challenged with MCh and then prevented from taking a deep breath, they were subsequently unable to dilate their airways with a deep inspiration. Apparently, the prolonged time at low lung volume after aerosol challenge with a contractile agonist led to a stronger and a more durable airway contraction that was less sensitive to lung inflation.

The effect of lung volume per se on MCh reactivity in humans was investigated by Ding et al (10). They found that reduced lung volume caused an increased response to MCh. Our study addressed this question of lung volume on MCh reactivity of individual airways. The present results in sheep are not in agreement with Ding et al. because we saw minimal effect of lung volume on the size of the MCh contracted airways. The reason for this discrepancy may be related to those factors discussed above to account for the differences observed between sheep and dogs. There also may be species differences in the structural components of

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**Fig. 8.** Airway pressure-area curves for all airways in each of 4 vagotomized sheep with (solid lines) and without (dashed lines) bradykinin administration. Values are means ± SE. All airway areas are normalized to their respective areas at Ptp = 20 cmH₂O after vagotomy.
airways and parenchyma that contribute to the effectiveness of the mechanical interaction.

To limit the duration of apnea required for the scans, we measured airways only in the right middle and lower lobes where the maximal number of airway are perpendicular to the plane of the scanner. For our lung volume measurements, we are able to separately measure the right and left lungs, but we are not able to differentiate upper, middle, and lower lobes. Therefore, our lung volume measurements include the right upper lobe. Because the right upper lobe is not perfused by the bronchoesophageal artery (8), the airways and the...
parenchyma in the right upper lobe would not have been affected by the infusions of MCh or bradykinin. Thus, to the extent that these interventions can alter lung volume, the absolute lung volume changes would be in error. However, because all of our calculations are relative to the maximal lung volume in the relaxed state after atropine, this would minimize the effect of such an error. Although we did not attempt to quantify the magnitude of this error, we think it small because the right upper lobe comprises only ~25% of the right lung.

In summary, when sheep airways have high airway smooth muscle tone, their baseline luminal area is greatly reduced and they become extremely unresponsive to increases in Ptp. However, when baseline luminal area is reduced by inflammatory thickening of the airways, the airways can be more readily distended. Therefore, we speculate that an inability of a deep inspiration to relieve airway constriction in vivo is a manifestation of airway smooth muscle contraction and not airway wall edema.

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Address for reprint requests: R. H. Brown, The Johns Hopkins School of Hygiene and Public Health, Div. of Physiology, Rm. 7006, 615 North Wolfe St., Baltimore, MD 21205.

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