Interaction between airway edema and lung inflation on responsiveness of individual airways in vivo

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Brown, Robert H., Wayne Mitzner, and Elizabeth M. Wagner. Interaction between airway edema and lung inflation on responsiveness of individual airways in vivo. J. Appl. Physiol. 83(2): 366–370, 1997.—Inflammatory changes and airway wall thickening are suggested to cause increased airway responsiveness in patients with asthma. In five sheep, the dose-response relationships of individual airways were measured at different lung volumes to methacholine (MCh) before and after wall thickening caused by the inflammatory mediator bradykinin via the bronchial artery. At 4 cmH2O transpulmonary pressure (Ptp), 5 µg/ml MCh constricted the airways to a maximum of 18 ± 3%. At 30 cmH2O Ptp, MCh resulted in less constriction (to 31 ± 5%). Bradykinin increased airway wall area at 4 and 30 cmH2O Ptp (159 ± 6 and 152 ± 4%, respectively; P < 0.001). At 4 cmH2O Ptp, bradykinin decreased airway luminal area (13 ± 2%; P < 0.01), and the dose-response curve was significantly lower (P = 0.02). At 30 cmH2O, postbradykinin, the maximal airway narrowing was not significantly different (26 ± 5%; P = 0.76). Bradykinin produced substantial airway wall thickening and slight potentiation of the MCh-induced airway constriction at low lung volume. At high lung volume, bradykinin increased wall thickness but had no effect on the MCh-induced airway constriction. We conclude that inflammatory fluid leakage in the airways cannot be a primary cause of airway hyperresponsiveness.

INFLAMMATORY CHANGES in the airway wall are commonly suggested as a primary cause of the increased airway responsiveness seen in patients with asthma (4, 16). Mechanically, this could occur if the inflammation included sufficient fluid leakage from the bronchial vasculature to substantially thicken the airway wall. With a thicker wall, a given degree of smooth muscle shortening could lead to exaggerated luminal narrowing (15, 22). Although such a scenario seems intuitively reasonable, there are little published data on which to base quantitative estimates of this mechanism. In previous work in a canine model in which airway edema was induced with systemic saline volume loading, it was shown not only that there was a maximal limit to the increase in wall thickness (~50%) but also that at this limit the airway luminal area was decreased by only ~30% (8). One concern with these experiments, however, was that the mechanism causing the edema might be quite different from what occurs with local inflammatory swelling of the airway wall. With inflammation, chemicals are released that can cause the airway vascular permeability to increase, with subsequent protein and plasma exudation (20). This inflammatory exudate might be greater and more localized to the airway wall than edema elicited with acute systemic saline volume loading. More recent studies in a sheep model addressed this concern by generating airway edema with the inflammatory mediator bradykinin (6, 29). In these latter studies, a substantial increase in airway wall area was also observed, but this increased thickness still caused only minor decreases in luminal area.

It thus would seem that edema per se cannot lead to a substantial degree of airway obstruction. The next logical question, whether this increased wall thickness affects the airway responsiveness to agonist challenge, has not been systematically studied. Although there are theoretical reasons and some experimental evidence suggesting that airway responsiveness is increased with airway wall thickening (7), the influence of lung volume and agonist dose is unknown. In the present study, we attempted to address these issues by measuring the dose-response relationship to methacholine (MCh) of individual airways in vivo before and after wall thickening caused by bradykinin at different lung volumes. Results show a surprisingly small effect of airway wall edema on airway responsiveness.

METHODS

Our study protocol was approved by The Johns Hopkins Animal Care and Use Committee. Anesthesia was induced in five 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 thoracic tracheal branches of the bronchoscopical artery were cut as previously described (32). The bronchial branch was then cannulated with an 18-gauge angiocatheter and perfused with a constant flow (0.6 mg·min−1·kg−1) of blood withdrawn from a femoral artery catheter by a variable-speed pump (Gilon, Villiers-les-Bel, France). The vagus nerves were ligated bilaterally to prevent reflex-mediated airway responses (31).

Airway imaging. High-resolution computed tomography (HRCT) scans were obtained with a Somatom Plus Scanner (Siemens, Iselin, NJ) as previously described (5, 7). 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 anesthetized with a constant airway pressure (controlled with an underwater overflow) for
the duration of the scans (~2 min). HRCT images were reconstructed by using methods previously described (5, 7).

Image analysis. The airway luminal areas were analyzed by using the airway analysis module of the Volumetric Image and Display Analysis software package (University of Iowa) as previously described and validated (1, 5). Because adjacent vascular structures sometimes make the outer boundary of airways difficult to define, we measured airway wall area as described previously (7) by first measuring mean wall thickness in each airway. Three lines were randomly drawn through the airway wall. The program automatically displayed a histogram of the pixel intensity along each line. The inflection points of increased intensity along the line that represents the inner and outer edges of the airway wall were selected, and the three measurements were averaged. From the measured luminal area and wall thickness, we then calculated the total airway area, comprising the area inside the outer airway perimeter, then equals \( \pi [T + \sqrt{(A_i/\pi)}]^2 \), where \( A_i \) is luminal area and \( T \) is wall thickness. A few of the walls of the smallest airways could not be measured with this approach.

Protocol. Each sheep served as its own control. The sheep were anesthetized and ventilated as described above. In all sheep, scans were acquired at static transpulmonary pressure (Ptp) values of 4 and 30 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 maintained at the designated Ptp for the duration of the scans (~2 min). After each experimental condition, the animals were ventilated normally.

The effects of lung inflation on methacholine (MCh)-induced decreases in airway size were determined first. To eliminate potential reflex-mediated effects of MCh (31), we studied the direct effects of MCh in vagotomized sheep. An initial set of baseline scans were acquired (baseline 1). Sheep then received a continuous infusion of MCh in increasing concentrations of 0.125, 0.5, 1.5, and 5 \( \mu \)g/ml at 2 ml/min through the bronchial artery. With a nominal bronchial artery perfusion rate of 20 ml/min, this delivery rate resulted in molar concentrations of \( 6 \times 10^{-8} \), \( 2.5 \times 10^{-7} \), \( 7.6 \times 10^{-7} \), and \( 2.5 \times 10^{-6} \) MCh, respectively. After 10 min of MCh infusion, scans were acquired at static Ptp values of 4 and 30 cmH2O in random order. The subsequent MCh concentration was then administered.

After scans were acquired at the highest agonist dose, the MCh infusion was stopped and the airways were allowed to recover (~30 min). A second set of baseline images (baseline 2) were obtained. To determine whether airway wall edema altered the degree of MCh-induced constriction, sheep were administered a continuous infusion of bradykinin (10^{-6} M) through the bronchial artery at an initial rate of 2 ml/min for 10 min followed by a maintenance dose of 1 ml/min, a dose previously shown to cause a significant increase in airway wall thickness (6, 30). We have shown that this dose of bradykinin has no direct airway smooth muscle contractile effects in our sheep model (29).

During this infusion, scans were again acquired at static Ptp values of 4 and 30 cmH2O as described above. Subsequently, the sheep were administered MCh in the same doses as described above, and scans were again acquired at static Ptp values of 4 and 30 cmH2O.

Analysis. Airway luminal area at 30 cmH2O Ptp in the relaxed state after vagotomy was defined as 100% (maximal area). Data are expressed as a percentage of maximal area.

The mean airway luminal areas for all airways in all the sheep (as percentage of maximum) at initial baseline (baseline 1) and after recovery from MCh (baseline 2) were compared by paired \( t \)-test. Paired \( t \)-tests were also used to compare the increases in wall area after bradykinin at each Ptp, the effective dose that caused a 75% decrease in luminal area from maximum (ED75), and the maximal constriction at the highest MCh dose before and after bradykinin administration.

To compare the mean airway area after MCh with and without bradykinin, generalized analysis of variance was used to control for repeated measurements of the airways within each sheep and for repeated measurements between sheep. The generalized analysis of variance was performed separately for each lung volume studied, with the mean airway luminal area (as a percentage of maximum) the dependent variable and with dose, bradykinin, the different sheep, and the multiple airways measured per sheep the independent variables.

RESULTS

In each sheep, 12–16 airways were identified and measured under the various conditions. The airways studied ranged in size from 2.5 to 12.8 mm in internal diameter after vagotomy at 4 cmH2O Ptp. There was no difference in airway luminal area as a percentage of maximum between the first and second baseline measurement at either 4 or 30 cmH2O Ptp (P = 0.19 and P = 0.23, respectively). The decrease in Ptp from 30 to 4 cmH2O caused an ~20% reduction in luminal area.

Bradykinin caused a significant increase in baseline airway wall area at both 4 and 30 cmH2O Ptp. At 4 cmH2O Ptp, wall area was 159 ± 6% of baseline (P < 0.0001), and at 30 cmH2O Ptp, it was 152 ± 4% of baseline (P < 0.0001). There was no significant change in wall area with increased Ptp (P = 0.25). Figure 1 shows the effect of bradykinin on the relationship between wall area and relaxed airway luminal area at Ptp of 4 cmH2O (A) and 30 cmH2O (B). These plots show that the increase in wall area with bradykinin administration occurred over the entire range of airways studied.

Dose-response curves to MCh are shown in Fig. 2. At 4 cmH2O Ptp (A), increasing doses of MCh resulted in a constriction at maximal dose to 18 ± 3% of the relaxed airway luminal area. At 30 cmH2O Ptp (B), increasing doses of MCh resulted in less of a constriction (to 31 ± 5% of maximum) at the same maximal dose of 5 \( \mu \)g/ml. At 4 cmH2O Ptp, bradykinin administration caused a small decrease (13 % 2%) in baseline airway luminal area (P < 0.01). This caused the airway luminal area with MCh to be significantly lower at this low level of inflation during bradykinin administration, reaching a minimal luminal area of 13 ± 3% (P = 0.02). At 30 cmH2O, bradykinin administration had no effect on airway luminal area (P = 0.75); the maximal MCh dose caused a decrease in luminal area to 26 ± 5%, which was not significantly different from the comparable pre-bradykinin constriction (P = 0.76). A comparison of the average ED75 values showed no significant difference with and without bradykinin at Ptp of 30 cmH2O (2.53 ± 10^{-6} vs. 2.09 ± 10^{-6}, respectively; P = 0.36) or a
Ptp of 4 cmH\(_2\)O (9.75 \times 10^{-7} \) vs. 5.12 \times 10^{-7}, respectively; \( P = 0.37 \)).

DISCUSSION

Our results demonstrate the presence of substantial airway wall edema elicited by infusion of the inflammatory mediator bradykinin directly into the bronchial artery in vivo. Bradykinin caused an \( \sim 50\% \) increase in wall area that was not altered by increasing Ptp to 30 cmH\(_2\)O. That the wall area was not affected by lung inflation suggests that airway wall edema is neither relocated nor reabsorbed with lung inflation. Because lung inflation causes a decrease in interstitial pressure \((28)\), this observation 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 \((\approx 10 \text{ min})\).

We also found that this magnitude of airway wall edema had only a slight effect on the luminal area at low lung volume. At low lung volume, this \( 50\% \) increase in wall area caused a \( 13\% \) decrease in airway luminal area, and at high lung volume, luminal area was not affected by the edema. These observations are consistent with previous work examining the effects of lung volume on edematous airways by using HRCT \((6)\). It was shown in this previous work that airway narrowing caused by wall edema at low lung volume could be reversed completely by lung expansion. This lung volume dependence of the effect of wall thickening may help explain some of the variability in previously published morphological work. Postmortem histological studies have generally found no significant changes in airway luminal dimensions with edema \((2, 14, 21)\). However, because lungs are normally fixed in the fully inflated state, these negative results would be predicted from our present findings.

Another potential confounder regarding wall edema and luminal area may be airway size. In a morphometric study of small airways \((<3 \text{ mm diameter})\) that had been fixed at 5 cmH\(_2\)O Ptp, we showed that for a similar exposure to bradykinin to that used in the present study, there was a trivial effect on luminal narrowing \((5\%)\) \((29)\). Edema fluid accumulated primarily external to the airway smooth muscle in these small airways. Thus the site of fluid accumulation as well as lung volume and airway size may impact whether wall thickening will alter baseline airway luminal area.

The major focus of the present study was to determine whether airway wall edema altered MCh-induced airway narrowing at different lung volumes. Our results showed only a minor effect of edema on the airway responsiveness to MCh. Only at a Ptp of 4 cmH\(_2\)O was responsiveness to MCh slightly augmented, whereas at 30 cmH\(_2\)O Ptp responsiveness was unchanged. To assess airway responsiveness it is customary to calculate an effective dose that caused a \( 50\% \) decrease in airway luminal area \((\text{ED}_{50})\). We could not do this in the present study because at the lower lung volumes the lowest
MCh dose already decreased airway luminal area by >60%. We thus calculated an ED75 value. On the basis of this value, there was no appreciable difference in responsiveness to MCh before and after bradykinin infusion at either lung volume.

Our results suggest that the total amount of acute airway wall thickening per se is not a primary factor in the degree of agonist-induced luminal narrowing. We suggest that the potentiation of airway responsiveness by wall edema is dependent primarily on the extent to which the luminal area is decreased before agonist challenge. Therefore, any intervention that thickens the airway wall inside the airway smooth muscle or otherwise leads to a decreased lumen size would potentiate agonist-induced airway narrowing (15). In our present study, at high lung volume, despite an increase in wall area, there was no change in luminal area, and airway responsiveness to MCh was unaltered. Similarly, in a morphometric study of smaller airways in sheep exposed to bradykinin, despite increased wall area (30%) primarily external to smooth muscle layer, no changes in luminal area or airway reactivity were discerned (29). Hydrostatic edema in the airway wall of sheep induced by a prolonged period of hyperperfusion of the bronchial vasculature resulted in an increase in wall area external to airway smooth muscle. In that study, despite a 19% increase in airway wall area, no changes in luminal area, airway resistance, or airway reactivity were observed (3). Contrary to this theory that potentiation of airway responsiveness depends primarily on the extent to which the luminal area is decreased before agonist challenge, our results suggest that this is not the case.

In the canine study, peripheral infusion of saline sufficient to increase airway wall area by 16% not only caused a concomitant decrease in airway luminal area by 22% (7) but also caused a potentiation of histamine-induced airway reactivity. Reasons for this difference are not clear, but there were several methodological differences. Airway edema in the dog study was caused by acute systemic volume loading, a maneuver that necessarily caused pulmonary hypertension, possibly activating neural reflexes that can enhance the constrictor response (9). This is in contrast to the sheep studies where we selectively infused a mediator known to cause fluid extravasation in the airway blood vessels. Furthermore, the sheep were vagotomized to eliminate any potential reflex responses.

To quantify how the magnitude of wall swelling might alter the extent of luminal narrowing for a given degree of muscle shortening, we performed a geometric analysis on a nominal airway 5 mm in diameter with and an airway wall 1 mm thick, similar to what was done in a previous analysis of canine airways (7). The present analysis was done for two doses of MCh before and after bradykinin at 4 cmH2O Ptp. We assumed that the edema was relatively uniformly distributed in the airway wall, an assumption consistent with results from our previous analysis (7). With no edema, we found that the smallest dose of MCh decreased luminal areas to 50% of relaxed area. To achieve this degree of constriction required 24% muscle shortening. At the largest dose of MCh we found luminal areas to be 22% of relaxed area, and to achieve this degree of constriction required 41% muscle shortening. After the airways were made edematous by bradykinin, there was a luminal reduction to 87% of baseline, and with this reduced lumen the smallest dose of MCh caused a further decrease in luminal area to 47%. To achieve this increased degree of constriction required smooth muscle shortening of 20%, i.e., almost the same as before the bradykinin. At the largest dose of MCh after edema, the luminal area was decreased to 17% of relaxed area. To achieve this increased degree of constriction now required smooth muscle shortening of 40%, i.e., again almost the same as before the bradykinin. This simple geometric analysis thus provides additional support for the conclusion that the degree of smooth muscle shortening with MCh challenge was the same before and after the bradykinin-induced edema.

In this study we found that lung inflation was able to completely reverse bradykinin-induced airway luminal narrowing while having limited effectiveness at reversing MCh-induced airway narrowing, a finding consistent with previous work in sheep (6). How airways respond to distending forces via lung inflation depends on several factors, especially the extent to which the airway smooth muscle is contracted. There is clear evidence that given a sufficiently large contraction, some airway smooth muscle is very difficult to stretch. Previous work using HRCT to measure airway area showed increased Ptp had a minimal effect on the airway area of sheep when the smooth muscle was significantly constricted (6). Olsen et al. (24) 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. (12) similarly reported minimal distension in MCh-contracted canine airways with lung inflation in dogs. Murtagh et al. (23) showed that, with a large aerosol dose of MCh, sufficient to contract in situ canine airways to ~25% of maximal caliber, pressures up to 96 cmH2O were required to pull open the airways.

In clinical asthma, the intrinsic forces of contraction may also be greater than that which lung inflation can overcome. This conjecture is supported by many observations showing that a deep inspiration does not relieve bronchoconstriction in asthmatic patients (10, 11, 25–27). 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 and potentiate airway constriction (13, 18, 19). Our present findings in sheep do not support this mechanism. We found that, with an ~50% increase in airway wall area, the airway response to MCh was only minimally enhanced at low lung volume. And at high lung volume there was no effect at all of this substantial airway wall thickening. Because it is unlikely that airways can be acutely thickened much more than 50% (8) it would seem unlikely that edematous wall thickening per se could prevent a deep inspiration from distending airways of asthmatic individuals.

In summary, we found that the inflammatory mediator bradykinin produced a substantial airway wall thickening and a slight potentiation of the MCh-induced airway constriction at low lung volume. At high lung volume, bradykinin caused a similar increase in wall thickness but had no effect on the MCh-induced airway constriction.

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