Effects of edema on small airway narrowing

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Wagner, Elizabeth M. Effects of edema on small airway narrowing. J. Appl. Physiol. 83(3): 784–791, 1997.—Numerous mediators of inflammation have been demonstrated to cause airway microvascular fluid and protein extravasation. That fluid extravasation results in airway wall edema leading to airway narrowing and enhanced reactivity has not been confirmed. In anesthetized, ventilated sheep (n = 30), airway vascular fluid extravasation was induced by infusing bradykinin (10⁻⁶ M) through a cannulated, blood-perfused bronchial artery. Airway wall edema and luminal narrowing were determined morphometrically. Airway reactivity to methacholine (MCh; 10 µg/ml, intrabronchial artery) was determined by measuring conducting airway resistance (Raw) by forced oscillation. Raw measurements were made and lung lobes were excised and quick frozen before or after a 1-h bradykinin infusion. In 10 airways per lobe (range 0.2- to 2.0-mm relaxed diameter), wall area occupied 32 ± 2% (SE) of the total normalized airway area (n = 9). Bradykinin infusion increased wall area to 42 ± 5% (P = 0.02); luminal area decreased by <5% and smooth muscle perimeter, a measure of smooth muscle constriction, was not altered (n = 5). Raw showed no change from baseline (1.4 ± 0.4 cmH₂O·l⁻¹·s⁻¹) after bradykinin infusion (n = 10). During MCh challenge, Raw increased by 3.2 ± 0.4 cmH₂O·l⁻¹·s⁻¹, and this change did not differ after administration of bradykinin. MCh challenge caused similar decreases in smooth muscle perimeter (10%) and luminal area (72 vs. 68%) before and after bradykinin infusion. However, the time constant of recovery of Raw from MCh constriction was increased from control (40 ± 3 s) to 57 ± 10 s after bradykinin infusion (P = 0.03). When lung lobes were excised at the same time after MCh challenge was terminated (n = 5), luminal area was greater before bradykinin infusion than after (86 vs. 78%; P = 0.007), as was smooth muscle perimeter. The results of this study demonstrate that airway wall edema limits relaxation after induced constriction rather than enhancing constriction.}

bradykinin; bronchial artery; inflammation; morphometry; sheep

AIRWAY WALL EDEMA, associated with inflammatory airway disease, generally is assumed to cause or promote airway narrowing. Studies have documented an extensive list of inflammatory mediators and sensory neuropeptides that cause fluid extravasation from the airway microvasculature (3). The mechanism responsible for this vascular leak is thought to involve transient formation of endothelial gaps in postcapillary venules, which allow both protein and fluid leakage (18). Although extravasated fluid may be removed from the airway through lymphatic clearance (10), vascular reabsorption, or epithelial transport mechanisms (25), little emphasis is placed on these clearance pathways. Most studies conclude that fluid leak predisposes to airways narrowing and enhanced reactivity. Wall thickening due to edema fluid accumulation theoretically could cause airway narrowing by encroaching on the airway lumen as well as by limiting the normal airway-parenchymal tethering, thereby decreasing the load against which smooth muscle contracts (23, 27). Few controlled studies have been published that quantify the degree of airway wall edema created after exposure to a substance causing fluid extravasation from the airway vasculature. Furthermore, whether airway wall edema is sufficient to cause a decrease in luminal dimensions and enhanced reactivity remains controversial. The present study was undertaken to 1) determine, morphometrically, the extent to which prolonged infusion of the inflammatory peptide bradykinin, known to cause airway vascular fluid extravasation (13), would cause airway wall edema, and 2) determine whether edematous airways are narrowed or demonstrate enhanced responsiveness to a cholinergic agonist.

METHODS

Experimental Preparation

Anesthesia of healthy sheep of mixed breeds was induced with ketamine (1 g im) and maintained throughout the surgical and experimental periods with pentobarbital sodium (15 mg/kg loading dose and 20 mg/kg hourly dose). Sheep were paralyzed with pancuronium bromide (2 mg iv) and treated with indomethacin (3 mg/kg iv) to block the synthesis and release of cyclooxygenase products during the experimental interventions (22). A tracheostomy was performed, and the sheep were mechanically ventilated with warmed, humidified air at a tidal volume (12 ml/kg) and rate (12–15 breaths/min) sufficient to ensure blood gases within the normal range. Supplemental oxygen was provided, and after thoracotomy was performed, all sheep were placed on 5 cmH₂O positive end-expiratory pressure. Airway pressure was measured at a sidearm of the tracheal cannula. Catheters were inserted into the femoral vein for anesthetic infusion, the femoral artery for systemic arterial pressure measurement, and the other femoral artery to supply the pump used to perfuse the bronchial artery. A midline sternotomy was performed to facilitate acquisition of lung tissue. A left lateral thoracotomy was performed between the fifth and sixth ribs to allow direct access to the bronchoesophageal artery as it exits the aorta. The thoracic tracheal and esophageal branches of the bronchoesophageal artery were ligated. After the anticoagulant heparin was given (20,000 US Pharmacopoeia units), the artery was cannulated with an 18-gauge catheter inserted directly into the vessel. The catheter was secured in place, and the bronchial branch of the bronchoesophageal artery was perfused with autologous blood from the femoral artery at a flow controlled by a calibrated roller pump (Gilson) and set initially at a flow of 0.6 ml·min⁻¹·kg⁻¹, a value within the reported normal range for sheep (9). Bronchial artery pressure was measured at a side port of the inflow cannula. Airway and vascular pharmacological agonists were infused through a side port of the perfusion circuit immediately proximal to the pressure port. Measurements of hemodynamic variables were made with Gould transducers and recorded on a Grass recorder. A 30-min stabilization period followed surgery.
Airway Morphometry

The right middle or left middle lobe was double clamped at functional residual capacity (FRC) with two long curved abdominal clamps. The clamped lobe was excised quickly by cutting between the clamps and then was immersed in liquid nitrogen. The time from clamping to immersion was < 30 s. The frozen lobe was then sliced into 1-cm slabs with a band saw in a cold room, and the slabs of lung were placed in acetic acid precooled to −70°C for freeze substitution. After 24 h in acetic acid, the samples were brought to room temperature, and small (<0.5-cm²) blocks of parenchyma were cut with a scalpel. According to standard procedures, blocks were embedded in J-B-4 plastic (Polysciences), cut into 3-µm sections, and treated with toluidine blue and diamobenzidine. Airway dimensions were quantitatively assessed in histological sections with techniques established by James et al. (14). Airways were analyzed only if they were cut in cross section having a maximum/minimum diameter < 1.5 and were located in regions free of atelectasis. Cross sections of 10 airways per lobe were analyzed microscopically with a quantitative image analyzer (Zeiss microscope and NIH Image). Internal perimeter (defined as the length of the epithelial basement membrane), smooth muscle perimeter, and external airway perimeter (defined as the external border with the lung parenchyma) were measured. Luminal and total airway wall areas were calculated as follows: %lumen area = lumen area/(wall area + maximum lumen area) · 100; %wall area = wall area/(wall area + maximum lumen area) · 100. Smooth muscle perimeter was normalized to maximal airway diameter.

Airway Resistance Measurements

Conducting airway resistance measurements was done by using forced oscillation (12), a method that has been previously used and validated in sheep (20). With this method, a gas volume of 30 ml was oscillated at end expiration for 1.5 s at a frequency of 9 Hz. Airway pressure was measured at a sidearm of the tracheal cannula, and a flow signal was obtained from a pneumotachograph positioned upstream from the cannula. Oscillatory signals were analyzed with an on-line computer that measured and averaged pressures at points of peak flow over 8–10 oscillatory cycles.

Protocol

Morphometric and physiological measurements were made in the following series of experiments.

Group 1: Dose response (n = 10 sheep). After the stabilization period, a control lobe was removed for later histological processing. Bradykinin was then infused (1 ml/min) directly into the bronchial artery at a sideport of the perfusion tubing for 1 h at concentrations of 10⁻⁷ M (n = 5) and 10⁻⁶ M (n = 5). Because of the vasodilating properties of bradykinin (24), the bronchial artery perfusion pump speed was increased to maintain bronchial artery infusion pressure at the prevasoconstriction level. A second lung lobe was removed after the completion of the bradykinin infusion when control bronchial blood flow and pressure were reestablished (3–4 min after bradykinin infusion was complete). Additional sheep were studied to serve as time controls (n = 5). Changes in airway resistance induced by intrabronchial artery infusion of methacholine (10 µg/ml; 1 ml/min) were determined before and after bradykinin infusion. This methacholine concentration was selected on the basis of preliminary histological studies that demonstrated significant and reproducible small airway narrowing. After complete recovery from the initial methacholine challenge, bradykinin (10⁻⁷ M, n = 5; 10⁻⁶ M, n = 5) was infused as described for group 1 studies. After bronchial blood flow and pressure were restored to the pre-bradykinin level, methacholine challenge was repeated. Maximum changes in airway resistance and the time course of recovery from maximum bronchoconstriction were monitored. Airway resistance values during recovery were approximated by a single exponent, and the time constant of recovery was calculated from the slope of the logarithmic decay curve.

Additional sheep were studied to serve as time controls for methacholine reactivity (n = 3). Changes in airway resistance induced by intrabronchial artery infusion of methacholine (10 µg/ml; 1 ml/min) were determined before and after a 1-h period of control perfusion.

Group 3: Morphometric assessment of airways constriction (n = 5 sheep). After the normal stabilization period, methacholine was infused directly into the bronchial artery (10 µg/ml; 1 ml/min). After a steady-state constriction was achieved (~2 min), on the basis of a constant peak inspiratory pressure, a lung lobe was removed for histological processing. Methacholine infusion was terminated, and airway and bronchial artery pressures were allowed to return to baseline levels. Bradykinin (10⁻⁶ M) was then infused as described for groups 1 and 2. After control bronchial blood flow conditions were reestablished, methacholine challenge was repeated. A lung lobe was removed after steady-state constriction was achieved.

Group 4: Morphometric determination of recovery from constriction (n = 5 sheep). Lung lobes were excised during the recovery period after a sustained constriction induced with methacholine (10 µg/ml; 1 ml/min) before and after bradykinin infusion (10⁻⁶ M). After the sustained response was achieved, methacholine infusion was terminated, resistance measurements were acquired for 1 min, the sheep was allowed five tidal breaths, and the lung lobe was excised at FRC. This lung acquisition time was selected on the basis of the results from group 2 studies.

Statistical Analysis

An estimate of the average airway dimensions during each experimental condition was obtained for each animal on the basis of sampling 10 airways per lobe. Data were obtained before and after bradykinin administration in each sheep, so each animal served as its own control. Statistical significance was determined by Student’s t-test for paired observations. Differences were considered significant when P < 0.05. Values are reported as means ± SE.

RESULTS

A total of 30 sheep (30.8 ± 0.9 kg body wt) were studied in the four experimental protocols. After the 30-min surgical recovery period, baseline mean aortic pressure averaged 99 ± 2 mmHg, airway resistance was 1.4 ± 0.1 cmH₂O·l⁻¹·s, and peak inspiratory pressure was 16.0 ± 0.4 cmH₂O. Control bronchial blood flow averaged 18.4 ± 0.5 ml/min and resulted in a
bronchial artery infusion pressure of $127 \pm 5$ mmHg. To maintain an approximately equivalent bronchial artery inflow pressure during bradykinin infusion, bronchial blood flow was increased to $48.3 \pm 2.2$ ml/min.

Morphometric determination of airway dimensions from group 1 animals was carried out on membranous airways ranging from 0.2 to 2.0 mm in diameter. Figure 1 shows representative histological sections of control, edematous, and constricted, edematous airways. The airways sampled from lobes treated with the two bradykinin concentrations did not differ in size from control lobes when the average maximal diameters were compared (controls: $0.92 \pm 0.06$ mm, $n = 9$; $10^{-6}$ M bradykinin: $0.68 \pm 0.05$ mm, $n = 5$, $P = 0.3187$; and $10^{-5}$ M bradykinin: $1.12 \pm 0.10$ mm, $n = 5$, $P = 0.4238$).

Airway wall area as a percentage of total airway area [wall area/(wall area + maximum lumen area)·100] during control conditions comprised 32% of the total airway area (Table 1). Bradykinin delivered directly to the bronchial artery at $10^{-7}$ M was slightly increased, no significant difference was discerned compared with paired controls ($P = 0.11$). As shown in Fig. 2, the increase in wall area was the result of a significant increase in the region external to airway smooth muscle in the $10^{-6}$ M bradykinin group ($P = 0.05$), but this increase did not reach statistical significance for the $10^{-7}$ M bradykinin group ($P = 0.07$). The inner wall area in all three groups occupied 5–7% of the total airway and did not increase with either $10^{-7}$ M bradykinin ($P = 0.40$) or $10^{-6}$ M bradykinin ($P = 0.09$). The corresponding percent lumen area normalized to the maximal airway area for these three groups of airways is presented in Fig. 3A. Lumen area during control conditions averaged $88 \pm 1$% of the maximal size. After treatment with $10^{-7}$ M bradykinin, lumen area was unchanged from control ($85 \pm 3$%; $P = 0.25$) and treatment with $10^{-6}$ M bradykinin resulted in a luminal area of $84 \pm 2$% ($P = 0.02$). Under control conditions, smooth muscle perimeter normalized to maximal airway diameter was $3.24 \pm 0.01$ and was not altered by bradykinin administration ($10^{-7}$ M: $3.22 \pm 0.02$, $P = 0.38$; $10^{-6}$ M: $3.22 \pm 0.02$, $P = 0.20$). This

Table 1. Airway dimensions before and after $10^{-6}$ M bradykinin

<table>
<thead>
<tr>
<th></th>
<th>Pre-Bradykinin</th>
<th>Post-Bradykinin</th>
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<tr>
<td></td>
<td>Control</td>
<td>MCh</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Diameter, mm</td>
<td>0.93 ± 0.08</td>
<td>0.76 ± 0.07</td>
</tr>
<tr>
<td>%Wall area</td>
<td>32 ± 2</td>
<td>39 ± 3</td>
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<tr>
<td>%Inner wall area</td>
<td>6.5 ± 0.4</td>
<td>5.9 ± 0.3</td>
</tr>
<tr>
<td>%Outer wall area</td>
<td>26.7 ± 1.2</td>
<td>32.9 ± 3.1</td>
</tr>
<tr>
<td>%Lumen area</td>
<td>88 ± 1</td>
<td>72 ± 4</td>
</tr>
<tr>
<td>Muscle perimeter</td>
<td>3.24 ± 0.01</td>
<td>2.92 ± 0.06</td>
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Values are means ± SE; n, no. of lobes. %Wall area = (wall area/wall area + maximum lumen area) · 100; %inner wall area = (inner wall area/total wall area + maximum lumen area) · 100; %outer wall area = (outer wall area/total wall area + maximum lumen area) · 100; %lumen area = (lumen area/maximum lumen area) · 100; muscle perimeter = smooth muscle perimeter/airway diameter. *$P \leq 0.05$ from pre-bradykinin.
observation demonstrates that there was no bradykinin-induced smooth muscle constriction. Time controls (n = 3) in which lung tissue was acquired before and after a 1-h control perfusion showed lumen area remained constant (84 vs. 86%; $P = 0.315$) over this time period.

Physiological measurements obtained from studies in group 2 experiments are shown in Figs. 4 and 5. Airway resistance measured before administration of $10^{-7}$ M bradykinin averaged $1.3 \pm 0.3$ cmH$_2$O·l$^{-1}$·s and was unchanged after bradykinin infusion, averaging $1.2 \pm 0.2$ cmH$_2$O·l$^{-1}$·s ($P = 0.37$). Similarly, baseline airway resistance was unaltered by the administration of $10^{-6}$ M bradykinin (preadministration: $1.4 \pm 0.4$ cmH$_2$O·l$^{-1}$·s and postadministration: $1.3 \pm 0.5$ cmH$_2$O·l$^{-1}$·s; $P = 0.35$). As shown in Fig. 4, methacholine-induced increases in airway resistance were not altered by the administration of either bradykinin concentration. In sheep treated with $10^{-7}$ M bradykinin, methacholine challenge caused a $4.1 \pm 1.1$ cmH$_2$O·l$^{-1}$·s (385%) increase in airway resistance before bradykinin administration and a $4.7 \pm 2.1$ cmH$_2$O·l$^{-1}$·s (358%) increase after bradykinin ($P = 0.30$). For the $10^{-6}$ M bradykinin group, methacholine caused a $2.3 \pm 0.7$ cmH$_2$O·l$^{-1}$·s (205%) increase before and $2.4 \pm 0.5$ cmH$_2$O·l$^{-1}$·s (234%) after bradykinin administration ($P = 0.43$). To confirm that methacholine reactivity was not altered by time alone, three additional control experiments were performed. Baseline resistance did not change over the course of 1 h of control perfusion. Methacholine challenge caused airway resistance to increase by $2.2 \pm 0.4$ cmH$_2$O·l$^{-1}$·s (149%) initially. After 1 h of control perfusion, the airway resistance change was not altered, increasing $2.2 \pm 0.5$ cmH$_2$O·l$^{-1}$·s (162%; $P = 0.63$).

The time constant of recovery of airway resistance from methacholine challenge before bradykinin infusion averaged for both concentration groups was $40 \pm 3$ s. The changes in the recovery time can be seen in Fig. 5. After administration of $10^{-6}$ M bradykinin, recovery of airway resistance was significantly slower than before bradykinin infusion. The average time constant of recovery of resistance was increased by 32% ($P = 0.03$). Administration of $10^{-7}$ M bradykinin did not affect significantly the time constant of recovery of airway resistance from methacholine-induced constriction ($P = 0.24$).

Morphometric indexes of group 3 airways (range 0.3- to 1.6-mm maximal diameter) obtained during steady-state contraction with infused methacholine are presented in Table 1 and Fig. 3B. Despite the significant increase in wall area demonstrated in group 1 experiments, no enhancement of airway narrowing during induced constriction was observed in the group of lungs after bradykinin administration. Normalized luminal area decreased to a similar extent during methacholine challenge to $72 \pm 4$% before and $68 \pm 5$% after bradykinin infusion ($P = 0.24$). Normalized smooth muscle perimeter decreased comparably before and after bradykinin by $\sim10$% ($P = 0.34$). Wall area during constriction averaged $39 \pm 3$% of the total maximally dilated airway area and increased to $45 \pm 2$% after bradykinin infusion ($P = 0.02$). Again, wall area in-

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**Fig. 2.** Bradykinin dose-response effects on average (means ± SE) inner and outer airway wall area as percentage of total airway area [%wall area = (inner or outer wall area/total wall area + maximum lumen area) · 100]. Control: n = 9 lobes; $10^{-7}$ M: n = 5 lobes; $10^{-6}$ M: n = 5 lobes. *$P = 0.05$.

**Fig. 3.** A: Bradykinin dose-response effects on average (means ± SE) lumen area as percentage of maximum. B: average lumen area as percentage of maximum during infusion of methacholine (MCh; 10 µg/ml) directly into bronchial artery before and after bradykinin treatment. Control: n = 9 lobes; $10^{-7}$ M: n = 5 lobes; $10^{-6}$ M: n = 5 lobes; MCh: n = 5 lobes; MCh + $10^{-6}$ M bradykinin: n = 5 lobes. *$P = 0.02$. 

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creases were confined to the outer region of the airway wall, external to the smooth muscle (P = 0.02; inner wall area: P = 0.23).

Results from group 4 experiments, which were performed to determine whether edematous airways recovered from methacholine constriction more slowly than did normal airways, are presented in Table 1. The airways sampled in this protocol before and after bradykinin were not different, averaging 0.82 ± 0.02 mm and 0.84 ± 0.06 mm relaxed maximal diameter (P = 0.59). During recovery from methacholine challenge and at the same time point after the cessation of methacholine infusion, airway luminal area was significantly greater before bradykinin infusion (86%) than after this treatment (78%; P = 0.007). Furthermore, the smooth muscle perimeter was significantly less during recovery from methacholine challenge after bradykinin treatment (P = 0.003). These morphometric observations were consistent with the physiological result reported for group 2 experiments showing an increased time constant of recovery from resistance changes after bradykinin infusion. Wall area in this transitional, partially constricted condition was significantly greater after bradykinin infusion compared with wall area before infusion (P = 0.05). This change in wall area was confined to the region external to airway smooth muscle (P = 0.003).

DISCUSSION

Airway wall edema, a hallmark of inflammatory airway disease, is generally assumed to cause both airway narrowing and enhanced reactivity (23). The mechanism by which fluid accumulation in the airway wall could contribute to airway narrowing is either through encroaching on the lumen or by limiting the normal airway-parenchymal tethering (23, 27). However, direct evidence to confirm that either of these proposed mechanisms are consequences of airway wall edema is limited. In this study in an animal model, airway wall edema was generated by exposure to an inflammatory peptide previously shown to cause fluid extravasation (13) and confirmed morphometrically. Despite a 30% increase in the normalized airway wall area, only a trivial change in lumen area (<5%) could be discerned. Physiological confirmation of this result was obtained as evidenced by the maintenance of airway resistance at baseline levels after bradykinin administration.

That airway wall edema does not alter airway luminal area or lung resistance has been demonstrated previously (5, 14). In the studies of Kimura et al. (16) and Blosser et al. (5), as well as the present study, fluid accumulated only external to the airway smooth muscle and may account for the lack of effect on airway luminal dimensions. Yet if airway-parenchymal interdependence is important for airway stability, airway narrowing might have been predicted. When airway edema is accompanied by pulmonary vascular engorgement and edema elicited by massive volume loading with saline, significant luminal narrowing has been demonstrated (8). In the study by Brown and colleagues (8), airway wall edema, measured with high-resolution computed tomography (HRCT), acted only inwardly and decreased luminal area but had no effect on the external airway perimeter. Together, these studies suggest that the site of fluid accumulation may predict whether luminal area is decreased. Furthermore, these results are in accord with those of Lai-Fook (17), who demonstrated that at FRC, airway-parenchymal interdepen-
ence would not be expected to play an important role in determining airway size. It is also possible that other forms of adventitial thickening may interact with free edema fluid to alter the airway coupling with the parenchyma to allow airway narrowing to take place. Chronic inflammatory cell influx may alter the wall structure, thereby altering the effectiveness of interdependent forces.

In the present study when airways were challenged with a cholinergic agonist, no amplification of constriction was seen either of small airways, which were measured morphometrically, or of larger airways, which were measured with standard resistance measurements (forced oscillation). The only substantive effect that the induced airway wall edema had on airway luminal area was the prolongation of constriction after methacholine challenge. In vivo, this was demonstrated by the increased time constant of recovery of airway resistance from constriction of airways exposed to methacholine after bradykinin treatment compared with the same challenge before bradykinin administration. This observation was confirmed morphometrically in airways of lungs from other animals in which the extent of luminal narrowing was significantly greater after bradykinin treatment at a fixed time point after methacholine challenge than before bradykinin treatment (group 4). The mechanism responsible for this observation can only be speculated on at this time. However, the more hydrated edematous wall may impact on intrinsic properties of smooth muscle relaxation, cholinergic-receptor binding, or drug diffusion and uptake by the vasculature. Recovery from cholinergic constriction has been shown to be perfusion limited, and edema fluid may limit agonist uptake and removal (26). To eliminate this potential confounder, in the present study, bronchial blood flow was controlled and set at the same level during methacholine challenge before and after bradykinin administration. Whatever the mechanism is, the results of this study suggest that bradykinin-induced airway wall edema limits relaxation after induced constriction rather than enhancing constriction.

The results of this study are in direct contrast to those of Kimura and colleagues (16), who used a similar experimental approach. Significantly enhanced reactivity to acetylcholine was demonstrated after airways were made edematous with bradykinin. In their study, airways of exsanguinated cats studied in situ showed a greater maximal response to acetylcholine after a 30-min exposure to $10^{-7}$ M bradykinin. Wall area increases in their study were also confined to the region external to airway smooth muscle, and bradykinin alone caused no change in lung resistance. However, control wall areas occupied ~45% of the normalized airway. In the present study, control wall area occupied ~30% of the normalized airway. In the study of James et al. (14), airway wall area of human control and asthmatic, membranous airways of a size comparable to those studied by Kimura et al. (16) and used in the present study occupied ~20% and 30% of the normalized airway, respectively. It is somewhat surprising that the airway wall area of cat airways should be so much greater than that of human or sheep airways. A possible explanation for the enhanced reactivity observed in the feline airways is that the control airways, perfused with artificial perfusate, were already significantly thickened compared with an in vivo situation. Thus in the study of Kimura et al., further fluid extravasation induced during bradykinin administration resulted in a grossly edematous airway wall area of ~60%. It remains to be determined whether a significantly thicker sheep airway wall would have demonstrated enhanced reactivity.

Whether wall edema plays a role in airway hyperreactivity has been further questioned in recent studies in human subjects. Boulet et al. (6), using HRCT to measure wall thickness, demonstrated no difference in the ratio of bronchial thickness to airway diameter of controls, patients with stable asthma, and patients with fixed airflow obstruction despite a wide range of methacholine responsiveness (6). Only within the group of subjects with fixed obstruction was there a correlation between wall thickness and reactivity. Berman and colleagues (4) recently demonstrated that protein extravasation as measured by bronchoalveolar lavage was similar between control subjects and asthmatic subjects challenged with bradykinin. But only the asthmatic subjects demonstrated a significant dose-dependent increase in peripheral airway resistance. If lavaged protein accurately reflects airway vascular extravasation, this study suggests that wall edema and extravasation play little role in the mechanism of airways reactivity.

Several aspects of the experimental design of this study and the data analysis require further comment. The acquisition of two lung lobes per animal allowed paired comparisons to be made of mean airway dimensions before and after bradykinin treatment in each animal. However, the order of sampling could not be randomized because of the uncertainty regarding whether and after what duration of time complete recovery occurs from bradykinin-induced airway edema. The time control experiments demonstrated that neither lumen area nor reactivity changed over the course of 1 h of normal perfusion of the bronchial artery. Because the same airway could not be studied under both conditions, a representative value for the airway dimensions of interest was obtained for each lobe based on the average of 10 airways. The range of airway size studied, defined by maximum internal diameter, was small (0.2–2.3 mm) and the groups were not significantly different in diameter, so the assumption was made that the mean value was adequately representative of this size range of airways.

The selection of bradykinin as the inflammatory mediator used to generate airway wall edema was based on several factors. Bradykinin is a peptide formed from plasma precursors and has been shown to cause airway microvascular leakage mediated through B2-receptor binding on endothelium (13). Although bradykinin has been shown to cause airway smooth muscle contraction in several species, it has been shown to be...
lacking any bronchoconstrictive effect in normal sheep (24). Only sensitized, allergic sheep respond to bradykinin with an increase in airway resistance (1). The exact dose and time response required for fluid leak in the sheep bronchial vasculature was unknown. Evidence exists to suggest that the opening of endothelial gaps is a rapid event and may become tachyphylactic within minutes of stimulation (18). The results of this study with a 60-min administration of bradykinin may represent the recovery from the first minutes of bradykinin administration. Hence, the amount of wall thickening measured may represent a minimum level. However, this seems unlikely because in another study, a 10-min infusion of the same bradykinin concentration in sheep resulted in a roughly equivalent increase in the wall area of larger airways measured with HRCT (7).

Although bradykinin is known to cause plasma extravasation (13), and increased airway wall area was documented in this study, this peptide may have had other effects in this model. Bradykinin is known to stimulate C fibers (15), regulate submucosal gland secretion (21), and cause both endothelial and epithelial release of nitric oxide (11, 19), which all might affect subsequent methacholine responsiveness.

The observation that wall area increased only external to the airway smooth muscle was somewhat unexpected. Because it has been demonstrated histologically that the smallest postcapillary venules have the greatest number of sites where endothelial gap formation occurs (16), it was anticipated that the mucosal region, where postcapillary venules are most abundant, would have shown an increase in area. This was not the case, suggesting either that fluid had cleared from the mucosa or that the bronchial vessels most sensitive to bradykinin and prone to leak are located in the region external to airway smooth muscle. With the application of similar histological methods in sheep, hydrostatic edema of the airway wall was shown to collect exclusively external to airway smooth muscle also (5).

In summary, the results of this study demonstrate that significant airway wall edema is generated after infusion of an inflammatory mediator known to cause plasma extravasation. However, despite a 30% increase in the outer airway wall area, no change in baseline airway resistance and a trivial decrease in luminal area were observed. Furthermore, the increase in airway resistance elicited with intravascular methacholine challenge was not amplified after wall thickening with bradykinin. However, recovery from methacholine challenge was significantly prolonged in edematous airways. Thus, in this model, airway wall edema limited relaxation after induced constriction rather than enhancing constriction.

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