Estimate of the subepithelial hydrostatic pressure that drives inflammatory transudate into the airway lumen

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Estimate of the subepithelial hydrostatic pressure that drives inflammatory transudate into the airway lumen. J Appl Physiol 92: 1702–1708, 2002. First published December 14, 2001; 10.1152/japplphysiol.00645.2001.—Inflammatory diseases of the upper respiratory tract are characterized by the passage of plasma constituents, first through the vascular wall, and then through the airway epithelium and into the airway lumen (2). Here we refer to the passage of plasma markers through the endothelium as extravasation and trans-epithelial flux as transudation. Transudation may represent a pathological feature of a disrupted barrier, or it may be a defense mechanism allowing passage of plasma immune elements (2, 9). Several lines of indirect evidence suggest that transudation occurs by a predominantly paracellular route, with interstitial fluid being driven into the lumen by elevated subepithelial hydrostatic pressure (2, 10, 12). First, pronounced extravasation and widespread submucosal edema are present in all inflammatory airway diseases (9, 11, 19) and, in lung and other tissues, edema is associated with an elevation of interstitial hydrostatic pressure (4, 17, 18). Second, the lateral intercellular spaces (LIS) of airway epithelium are dilated in both asthma (9, 10) and neurogenic inflammation (15, 16). In vitro, pressures of as little as 10 cmH2O applied to the serosal side of gallbladder epithelium cause near maximal dilation of the LIS (21, 23, 24), and subepithelial pressures of this magnitude also markedly dilate the LIS of airway epithelium in vitro (10). Third, in asthma, the permeability of airway epithelium to large solutes is increased (8). In vitro, dilation of the LIS in itself causes increased solute and water permeability (21). However, most of the permeability increase in response to elevated subepithelial hydrostatic pressure is believed to be due to mechanical disruption of tight junctions caused by a pulling action of the widely separated lateral cell membranes (7, 27). Thus plasma transudation into the airway lumen in vivo may be effected by increases in both subepithelial hydrostatic pressure and epithelial hydraulic conductivity.

We hypothesized that, if elevated subepithelial hydrostatic pressure drives inflammatory transudation, then it should be blocked by application of an equal or greater luminal pressure. Accordingly, we measured the appearance of plasma markers into the lumen of an isolated perfused segment of rat trachea in vivo and found that stimulation of one vagal nerve caused a rapid (half-time ~5 min) and nonselective increase in the flow of markers from blood to airway lumen. Leukocyte migration also caused transudation that developed much more slowly (half-time ~2–3 h). In both cases, transudation was blocked by application of luminal hydrostatic pressures. The critical luminal pressure needed to block vagally induced transudation was ~4.5 cmH2O, and, to block epithelial transudation induced by leukocyte traffic, it was 3 cmH2O, and we conclude that these are the subepithelial pressures that drive inflammatory transudation into the airway lumen.

METHODS

Sixty male Sprague-Dawley rats weighing 350–400 g were anesthetized with nembutal (35 mg/kg ip) and tracheotomized with PE200 tubing three tracheal rings above the carina. A transverse incision was made through the ventral surface of the trachea just below the larynx, and a second...
incision was made 14–16 mm caudal to the first. L-shaped catheters (1.5 mm OD) were tightly secured at both ends of the 15- to 17-mm segment so formed, and the tracheal segment and catheters were filled with saline at 38°C. One catheter was connected to a syringe. The other remained open, with its long limb pointing vertically. Saline was flushed through the tracheal lumen with the syringe, and intraluminal pressure was set from the level in the vertical limb of the open cannula. The tracheal segment was covered with plastic sheet to prevent drying and was heated by a radiant lamp to 38–39°C. Ten milligrams per kilogram of FITC-dextran 70 (molecular mass: 70 kDa; Molecular Probes, Eugene, OR) or 25 mg/kg of Evans blue dye (Sigma Chemical, St. Louis, MO) were injected intravenously. Evans blue dye binds to plasma albumin and was used as a marker of albumin in tracheal fluid and in tissues (20). Samples of tracheal fluid were obtained at intervals of 2–10 min by flushing saline from the syringe through the lumen until 1.5 ml of saline had been collected. Each flush took ~10 s. Intervals between sampling were always the same in each group. Samples of heparinized blood were obtained simultaneously with tracheal samples. Plasma levels of tracers declined by 25–30% over the course of a typical experiment of 20- to 60-min duration. Levels of FITC-dextran were measured with an Hitachi fluorimeter (model F-2000). Evans blue was determined from the absorbance at 615 nm (Turner spectrophotometer SP-380, Barnstead, Dubuque, IA). The level of transudation was expressed in volume of plasma equivalents (PE; µl) per minute, the volume of plasma required to produce the measured concentration of marker in the tracheal perfusate (1.5 ml). PE was calculated as the product of the tracheal fluid-to-plasma ratio of marker concentration times the sample volume (1.5 ml) and divided by the time of the sampling period.

In 30 rats, one of the vagal nerves was carefully isolated from the carotid artery and, after a baseline period, was stimulated with two platinum electrodes (10 V, 20 pulses per second, 0.2-ms duration) using a Grass S-44 stimulator (Grass Instruments, West Warwick, RI). The effectiveness of vagal stimulation was assessed from changes in heart rate and blood pressure measured via a catheter in the carotid artery connected to a Baxter pressure transducer (PX260), a

Table 1. Hemodynamics, transudation, and extravasation before and during vagal stimulation in rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Vagal Stimulation Pressure, 0 cmH₂O</th>
<th>Vagal Stimulation Pressure, 10 cmH₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>440 ± 40</td>
<td>250 ± 30&lt;sup&gt;§&lt;/sup&gt;</td>
<td>260 ± 40&lt;sup&gt;§&lt;/sup&gt;</td>
</tr>
<tr>
<td>Systemic arterial pressure, mmHg</td>
<td>125 ± 15</td>
<td>90 ± 10&lt;sup&gt;§&lt;/sup&gt;</td>
<td>85 ± 10&lt;sup&gt;§&lt;/sup&gt;</td>
</tr>
<tr>
<td>Transudation of Evans blue-albumin</td>
<td>0.03 ± 0.35</td>
<td>0.22 ± 0.11&lt;sup&gt;§&lt;/sup&gt;</td>
<td>0.01 ± 0.003†</td>
</tr>
<tr>
<td>Transudation of dextran 70</td>
<td>0.05 ± 0.1</td>
<td>0.18 ± 0.06&lt;sup&gt;§&lt;/sup&gt;</td>
<td>0.03 ± 0.03†</td>
</tr>
<tr>
<td>Tissue Evans blue-albumin, µl/µg</td>
<td>0.06 ± 0.01</td>
<td>0.12 ± 0.2&lt;sup&gt;§&lt;/sup&gt;</td>
<td>0.12 ± 0.2&lt;sup&gt;§&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. baseline levels by nonpaired t-test; †P < 0.05 vs. 0 cmH₂O pressure group.
Grass amplifier P122, and MacLab 4s data-acquisition system. On average, vagal stimulation reduced heart rate from 440 to 250 beats/min and arterial pressure by 30–40 mmHg. One hour after surgery, markers were injected, and their appearance was measured in the trachea every 5 or 10 min for 1 h (n = 24). In six control experiments, at the end of baseline, the luminal pressure was increased in intervals to 3, 6, and 10 cmH2O for 10 min each interval, and epithelial transudation was measured. In these and other experiments, the vagal nerve was then stimulated for 5 min under constant luminal pressure in separate groups, with luminal pressure of 0, 3, 6, or 10 cmH2O (n = 6 in each group). Six additional rats were used for morphological studies and analyses of albumin accumulation in tracheal tissue.

In 20 rats, leukocyte migration was induced by 0.25 × 10−7 M formyl-methionyl-leucyl-phenylalanine (fMLP) (Sigma Chemical, St. Louis, MO), which was added to the tracheal perfusate after the baseline period. One hour after surgery, Evans blue dye (25 mg/kg) was infused into the bloodstream, and samples of plasma and tracheal fluid (sample volume, 1.5 ml) were taken every 15 min during a baseline period (1 h). For 1 more hour, variable luminal pressures (0, 3, 6, 10 cmH2O) were applied for 15-min intervals, and epithelial transudation was measured (control). After that, fMLP was introduced into the airway lumen, and leukocyte migration and plasma transudation were measured at 15-min intervals every 1 h for 3 h. After the third hour, variable luminal pressures (0, 3, 6, 10 cmH2O) in three groups (n = 6 in each) were applied for 15-min intervals, and transudation was measured as described above. Migrating leukocytes were counted with a hemocytometer.

Fig. 3. Extravasation is not altered by luminal pressure. The serosal surface of trachea 5 min after Evans blue injection with zero luminal pressure (A), 5 min after vagal stimulation with zero pressure (B), 5 min after Evans blue injection (C), and 5 min after vagal stimulation (D). Both C and D are at 10-cmH2O luminal pressure. Vagi were stimulated 10 min after injection of Evans blue dye.

To document extravasation of plasma markers, the exposed abluminal surface of the tracheal segment was filmed using a dissecting microscope (Leika Wild M4; Heerbrugg, Germany) coupled to a digital image recording system in four rats (two controls and two vagally stimulated). To quantify the transvascular flux of plasma components (extravasation), we measured accumulation of Evans blue in whole tracheas 20 min after 5-min vagal stimulation with luminal pressures of 10 (n = 4) or 0 cmH2O (n = 5). The total amount of Evans blue in the tracheal segment was determined fluorometrically after extraction with formamide, as described (20). The amount of tissue Evans blue albumin was determined from tissue weight and plasma amount of Evans blue albumin and expressed as microliters of PE per microgram of tissue. At the end of some experiments, samples of trachea were fixed in 4% glutaraldehyde and processed for scanning electron microscopy, as described elsewhere (29). Six rats were used as sham-operated controls.

ANOVA was used to assess statistical differences between means, with P < 0.05 being taken as significant. Data are given as means ± SE.

RESULTS

Transudation of albumin and 70-kDa dextran was determined simultaneously. Baseline transudation for albumin was 0.03 ± 0.01 µl PE/min and was 0.05 ± 0.01 µl PE/min for dextran (n = 10). As shown in a typical experiment (Fig. 1) in the absence of luminal hydrostatic pressure, vagal stimulation caused a rapid simultaneous increase in albumin and dextran appear-
ance in the airway lumen. The maximal increase in Evans blue transudation was to \(0.22 \pm 0.11\ \mu l/PE/\text{min}\) \((P < 0.001)\) and in dextran transudation was to \(0.18 \pm 0.06\ \mu l/PE/\text{min}\) \((P < 0.01)\), values that are not statistically different from one another (paired \(t\)-test). Furthermore, between animals, the vagally induced increases in the rates of appearance of the two markers were significantly correlated \((r = 0.9)\). After stimulation, transudation returned to baseline with a halftime of \(-10\ \text{min}\). The summary data for vagally induced transudation and hemodynamic variables are given in Table 1.

When the vagus was stimulated in the presence of a \(10\-cmH_2O\) luminal pressure head, no increase in transudation was seen until the pressure was released (Fig. 2A). Similarly, transient application of a \(10\-cmH_2O\) pressure head after transudation had been increased by vagal stimulation also resulted in block (Fig. 2B). Although luminal pressure completely blocked vagally induced transudation, it had no effect on vagally induced transvascular flux of plasma components, as judged visually from the leakage of Evans blue (Fig. 3). Furthermore, the total amount of Evans blue-albumin in tracheas from vagally stimulated rats was not influenced by luminal hydrostatic pressure: it was \(0.12 \pm 0.03\ \mu l/PE/\mu g\) tissue in tracheas with zero luminal pressure \((n = 4)\) vs. \(0.12 \pm 0.02\ \mu l/PE/\mu g\) tissue in the presence of \(10\-cmH_2O\) luminal pressure. In rats without vagal stimulation, the amount of tissue Evans blue-albumin was \(0.06 \pm 0.01\ \mu l/PE/\mu g\) tissue \((n = 4)\) at the end of the same time period.

These results strongly suggest that, during vagal stimulation, elevated subepithelial pressure drives vascular transudation into the airway lumen and that the driving pressure is \(10\ \text{cmH}_2\text{O}\). To obtain a precise estimate of the driving pressure, the vagi were continuously stimulated, and the luminal pressure head was reduced or elevated from 0 to \(10\ \text{cmH}_2\text{O}\). A control, we first applied the same pressure steps during the baseline period. Summary results are shown in Fig. 4. For the control experiments \((n = 6)\), luminal pressure steadily decreased the appearance of plasma markers in the tracheal fluid. The minimal hydrostatic pressure needed to block epithelial transudation (or reduce it to baseline) during vagal stimulation was \(4.5 \pm 1\ \text{cmH}_2\text{O}\) \((n = 6)\).

By scanning electron microscopy, we were unable to detect any effects of vagal stimulation on the ultrastructure of the tracheal epithelium (Fig. 5).

Fig. 4. Estimates of driving pressure required for epithelial transudation. The rate of epithelial transudation during baseline, vagal stimulation, and application of formyl-methionyl-leucyl-phenylalanine (fMLP) is shown, plotted against applied intraluminal hydrostatic pressure. Transudation was measured at 4 pressures. The point at which the relationship between pressure and flow intersects with the value for baseline flow provides an estimate of driving pressure. Values are means ± SE; \(n = 6\) observations for each group. *\(p < 0.05\) by nonpaired \(t\)-test between groups.

Fig. 5. Scanning electron microscopy of the tracheal epithelial surface. A: control (no stimulation). B: after vagal stimulation with zero luminal hydrostatic pressure. C: 3 h after leukocyte migration induced by fMLP. Groups of leukocytes, erythrocytes, and epithelial desquamation are noticeable.
As an alternative means of inducing transudation, we perfused the tracheal lumen with fMLP and sampled the luminal contents hourly. Leukocyte transmigration increased from $10 \pm 16$ cells $\mu l^{-1} h^{-1}$ during the first hour to $190 \pm 70$ cells $\mu l^{-1} h^{-1}$ during the third hour ($P < 0.05$). For the same two periods, epithelial transudation increased from $0.04 \pm 0.02$ to $0.10 \pm 0.04 \mu l$ PE/min ($P < 0.05, n = 6$). By scanning electron microscopy, we observed erythrocytes and leukocytes on the tracheal surface with occasional regions of epithelial desquamation (Fig. 5). When a luminal pressure of $10 \text{ cmH}_2\text{O}$ was applied continuously during the third hour of fMLP perfusion, epithelial transudation was significantly decreased, whereas the number of leukocytes appearing in the tracheal lumen did not change significantly (Fig. 6). The pressure needed to block transudation during leukocyte migration was $3 \pm 1 \text{ cmH}_2\text{O} (n = 6; \text{see Fig. 4})$.

**DISCUSSION**

Here we show that inflammatory transudation into the tracheal lumen is blocked by application of a luminal hydrostatic pressure. Furthermore, the critical pressure needed to block transepithelial flux was $4.5 \text{ cmH}_2\text{O}$ when transudation was induced by neurogenic inflammation and was $3 \text{ cmH}_2\text{O}$ for transudation associated with leukocyte migration. Thus our experiments provide strong evidence for a causal role of elevated subepithelial pressure in inflammatory transudation and provide an estimate for that pressure.

We induced transudation acutely by vagal stimulation and subacutely by induction of leukocyte migration. In the rat, vagal stimulation causes a well-characterized condition known as neurogenic inflammation (15, 16), in which release of substance P from the vagal nerve terminals causes rapid and marked extravasation followed by further inflammatory changes (20). Confirming the results of others (16, 20), we saw, on stimulation of the vagal nerve, almost instantaneous extravasation of albumin (labeled with Evans blue). This transvascular flux of plasma components was associated with rapid transudation into the airway lumen. As our laboratory has previously shown, in vitro transudation, driven by application of serosal pressures of 10 or 20 cmH$_2$O, occurs without molecular sieving (3, 10). In the gall bladder in vitro, the flows driven by elevated subepithelial hydrostatic pressure have been proposed to occur at large “pores” in the epithelium (1), very likely occurring at a small fraction of the sites where tight junctions meet, the so-called “tricellular junctions” (27). Such tricellular junctions have also been implicated as points of increased permeability in other epithelia (14, 22, 28). However, scanning electron microscopy did not reveal any discernible changes in the ultrastructure of the tracheal epithelium during vagally induced transudation, presumably because only a small number of tricellular junctions are involved, and these are obscured by cilia.

Unlike many other visceral structures, the trachea and conductive airways have skeleton-bronchial cartilage rings, which support the tissue layers of the tracheal wall. When luminal pressure is applied, tissue between and behind the rings is not compressed. Also, it is likely that the epithelium hydraulic conductivity is less than that of either connective tissue or endothelium. Thus, when luminal pressure is applied, the main pressure drop is across the epithelium, and extravasation is not affected, a hypothesis that we confirmed experimentally.

It is well established in vitro that leukocyte migration across cultured epithelial cell sheets compromises barrier function as revealed by increases in electrical conductance and in the flux of paracellular markers (6). In vivo, perfusion of the rat tracheal lumen with the chemotactic agent fMLP induced leukocyte migration that was accompanied by increased epithelial transudation. By scanning electron microscopy, we saw large numbers of leukocytes on the epithelial surface and focal areas of damaged epithelium. Thus epithelial
transudation during leukocyte traffic is likely to occur by a different route than with vagal stimulation through portions of denuded or ruptured epithelia, as seen in Fig. 5.

Our results provide direct evidence for a central role of elevated interstitial hydrostatic pressure in neurogenic inflammation in the trachea. Indirect evidence, reviewed in the introduction, suggests that any maneuver that causes extravasation causes an increase in interstitial pressure that disrupts tight junctions, thereby permitting bulk flow down the pressure gradient into the lumen. Here we have shown that application of a luminal pressure head blocks transudation induced by either of two mechanisms. Because this blockade is essentially complete, it is unlikely that transcytosis actively participates in transudation in these models.

For vagally mediated transudation, we estimated the value of the interstitial hydrostatic pressure driving transudation by determining the minimal luminal pressure head required to stop transudation. The value obtained, 4.5 cmH2O, is very close to values obtained by direct micropuncture (4–6 cmH2O) in pulmonary edema (4, 17, 18). From this we conclude not only that elevated subepithelial pressure plays a causal role in transudation induced by vagal stimulation, but also that the maximal value of this pressure averages +4.5 cmH2O. The interstitial pressure generated by subepithelial edema cannot exceed the pressure within the microvessels, no matter how leaky the latter may become. It is reassuring, therefore, that our estimate of 4.5 cmH2O (~4 mmHg) is less than the hydrostatic pressure of 5–12 mmHg found within airway venules.

We also estimated the minimal luminal pressure needed to block transudation associated with leukocyte transmigration, which was substantially lower than pressure in vagal stimulation. We suggest that the lower pressure is because the rate of extravasation is much lower during leukocyte migration than during vagal stimulation.

Our finding that transudation is driven by a serosa-to-mucosa hydrostatic pressure gradient provides an explanation of the beneficial effects of positive pressure ventilation in prevention and treatment of lung edema (30). Because airways in vivo are covered with a thin but continuous lining of airway surface liquid, pressure applied to the surface of this liquid should be readily transferred to the epithelium itself. We believe that this is why our results are relevant to gas pressure in the airways, although the flow of gas certainly provides some shear stress on the airway surface liquid. Thus if the pressures in the airway lumen during positive pressure ventilation are greater than those that we have shown to drive transudation, they will block plasma flow into the airway lumen.

In vitro, increases in hydraulic conductivity of epithelia induced by serosa-to-mucosa pressure gradients are believed to involve disruption of tight junctions (22, 25). Similarly, the increase in transudation in vivo during either vagal stimulation or leukocyte migration probably reflects an increased permeability of the same route (14). Whether the increase in tight junctional permeability represents a simple mechanical tearing or is a regulated event involving stretch-activated signaling pathways is presently unknown (5, 14, 26).

In conclusion, our experiments provide strong evidence for a causal role of elevated subepithelial pressure in neurogenic inflammatory transudation and provide an estimate for that pressure during neurogenic inflammation of ~5 and 3 cmH2O during transudation associated with leukocyte traffic.

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REFERENCES


