Airway responses to esophageal acidification

Ivan M. Lang, Steven T. Haworth, Bidyut K. Medda, David L. Roerig, Hubert V. Forster, and Reza Shaker

Medical College of Wisconsin Dysphagia Institute, Divisions of Gastroenterology and Hepatology and Pulmonary Medicine, Department of Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin

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Lang IM, Haworth ST, Medda BK, Roerig DL, Forster HV, Shaker R. Airway responses to esophageal acidification. *Am J Physiol Regul Integr Comp Physiol* 294: R211–R219, 2008. The symptoms of asthma not only include airway constriction but also increased mucus production and secretion; however, no studies have examined the effects of esophageal acidification on airway mucus output. Furthermore, a major function of the airways that is impaired in asthma, is mucociliary transport, and no studies have examined the effects of esophageal acidification on airway mucociliary transport.

Therefore, the aims of this study were to quantify the effects of esophageal acidification on three of the major responses of the airways: alteration of airway size, secretion of mucus, and mucociliary transport. Moreover these studies are designed to investigate the physiological function and significance of these airway responses to esophageal acidification.

METHODS

The mucus secretion studies were performed at the Medical College of Wisconsin under an animal use protocol reviewed and approved by the IACUC of the Biomedical Resource Center. The airway diameter and other studies were performed at the Clement J. Zablocki VA Medical Center under an animal use protocol reviewed and approved by the IACUC of the Veterinary Medical Unit.

General Animal Preparation

We used 35 cats of either sex weighing from 2.4 to 4.8 kg. The cats were anesthetized with α-chloralose (50 mg/kg ip) and the femoral
vene and artery were cannulated to maintain hydration by infusion of 0.9% NaCl and to gain access to arterial blood, respectively. In all cats the esophagus was cannulated and ligated into place just distal to the upper esophageal sphincter and at the lower esophageal sphincter for perfusion of the esophagus as described below. The catheter draining the lower esophagus exited the body through a gastric fistula and the abdominal incision. The gastric fistula was also used to continuously evacuate gastric contents, thereby preventing build up of gastric secretions. The cats were prepared for recording different physiological responses as described below. Not all physiological responses were recorded from every cat. In all experiments of the effects of esophageal acidification, the esophagus was perfused at 1 ml/min first with 0.1 M PBS for at least 60 min to allow the preparation to stabilize, followed by 0.1 N HCl for 15–30 min, and, if necessary, again with 0.1 M PBS for 30–60 min. The cats were not artificially ventilated.

**Bronchial Diameter Measurement**

The lungs of cats (n = 5) were visualized using the micro-focal x-ray system described in detail below. Cats were placed supine onto the micro-focal x-ray imaging system stage, the bronchi were visualized, and the data were saved for postacquisition analysis. The trachea was intubated and the bronchi were dusted with fine tantalum powder (Sigma Aldrich 325 mesh) injected in the air inflow from a ventilator. An appropriate lung field was selected that contained airways of many different diameters and in which the tantalum had dusted the airways well enough to measure the diameters and evenly enough so that clumping of tantalum did not occur. The x-ray stage and image intensifier were then fixed at this position [source to object distance = 15.0 ± 1.0 cm (range 12 to 17 cm); source to intensifier distance = 82.9 ± 1.9 cm; magnification more than ×5, and half cone beam angle of >6°] and radiographic images of the lung field were obtained every 2 min throughout the study. The esophagus was then perfused with 0.1 M PBS for 1 h, changed to 0.1 N HCl for 15–20 min, and then returned to 0.1 M PBS for 1 h. The diameters of various airways were quantified at a later date as described below. The control values were obtained during the first 2 min of the initial 0.1 M PBS perfusion, because the preparation was well stabilized by this time.

The micro-focal x-ray system is composed of a Fein Focus-100 x-ray source (3 mm focal spot), a North American Imaging AI-5830-HP image intensifier coupled to a Silicon Mountain Design (SMD) charge-coupled device (CCD), and a New England Affiliated Technologies specimen micromanipulator stage all mounted on a precision rail with position information provided by Mitutoyo linear encoders. The geometry of the system allows magnification of the specimen to be adjusted by changing the specimen’s proximity to the x-ray source. The image data are sent from the CCD to a frame grabber board mounted in a Dell 610 workstation running Widows NT operating system.

The diameters of the airways were determined in an objective fashion. One of two methods was used depending on the size and location of the airway. For small and intermediate diameter airways (<2.1 mm), we used the following model (Eq. 1) developed previously to estimate blood vessel diameter using angiography (5).

\[
\lambda(x) = \begin{cases} 
\frac{m(x - a) + b}{2\mu_0 \sqrt{r_0^2 - (x - a)^2} + m(x - a) + b}, & \text{for } x > a \\
\frac{2\mu_0 \sqrt{r_0^2 - (x - a)^2} - \sqrt{r_1^2 - (x - a)^2}}{2\mu_1 \sqrt{r_1^2 - (x - a)^2} + m(x - a) + b}, & \text{for } x < a
\end{cases}
\]

The absorbance across the airway cross section was measured and fit to the above equation where \(\lambda(x)\) is the x-ray absorbance measured at a radial distance \(x\) from the airway axis; \(\mu_0\) and \(\mu_1\) are the attenuation coefficients of the tantalum powder contrast on the vessel wall and within, respectively; \(r_0\) and \(r_1\) are the outside wall and internal airway radius; \(x\); establishes the location of the airway axis within the region of interest; and \(m\) and \(b\) are, respectively, the slope and intercept of a linear baseline correction used to compensate for spatial variation in background absorption. A modified Gauss-Newton procedure was used to fit the above equation to the absorbance data to estimate \(\mu_0\), \(\mu_1\), \(r_0\), \(r_1\), \(a\), \(b\), and \(m\) as previously described for the blood-contrast medium mixture within pulmonary vascular vessels (5). A cylindrical airway may actually present an elliptical cross section as the result of not being aligned perpendicular to the x-ray beam. That does not alter the form of Eq. 1. It only alters the physical interpretation of \(\mu_0\) and \(\mu_1\), which is irrelevant to the estimation of diameter.

The above method is optimal when there is sufficient distance between airway segments of interest and there is a region of background on both sides of the airway. This is commonly the case for the intermediate and smaller airway. For large (>2.1 mm) airways the diameter can be measured by manually placing a line region on interest perpendicular to the axis from edge-to-edge of the airway.

The radiographic image is a two-dimensional projection of a three-dimensional object, i.e., the lungs; and objects closer to the x-ray source appear larger in the image. Therefore, each measurement of diameter in the radiographic image was calibrated separately. Distance within the plane of the radiographic image was calibrated by moving the cat a known distance perpendicular to the x-ray beam while recording the image. The distance moved in pixels of a shadow of an airway of interest in the image per micrometer of movement was the distance calibration factor for that airway.

Selection of airways for quantification was determined by the quality of the dusting of the airway and its size. We selected airways in which the lumen was dusted with tantalum heavy enough for the radiological technique to delineate both sides of the projected image of the lumen and light enough such that there was no clumping of tantalum. The optimum dusting resulted in a continuous thin line of tantalum on each side of the projected image. Also we attempted to quantify changes in a variety of airways; therefore, we selected a range of airway diameters.

**Tracheal Mucociliary Transport**

Cats were placed onto the micro-focal x-ray imaging system stage and a small hole was made in a distal tracheal cartilage ring using a cautery. Care was taken to avoid bleeding into the trachea as bleeding retarded mucociliary transport. Small discs of tantalum (1 mm diameter, 0.3 μg) were injected through a 14-gauge needle inserted into the tracheal hole before each experimental procedure and the velocity of movement was recorded. Between injections of discs a saline-soaked gauze pad was placed over the hole in the tracheal cartilage. After transiting through the trachea, the tantalum discs deposited on the hard palate. An endotracheal tube was not placed in these studies to disturb the trachea as little as possible.

Tantalum discs were placed into the trachea one at a time in cats (n = 4), and their position was captured and recorded at intervals during their movement oral while the esophagus was perfused with
0.1 M PBS. The perfusate was changed to 0.1 N HCl and, after 10 min, tantalum discs were again placed into the trachea one at a time and their rate of movement recorded over 20 min post HCl. We did not quantify tantalum disc movement after esophageal neutralization using 0.1 M PBS, because in preliminary experiments we found that the mucociliary transport rate did not begin to return to control levels for at least 1 h after esophageal neutralization.

Tracheal mucociliary transport rate was quantified by acquiring images of the trachea while the injected tantalum disc traversed the field of view. We calculated the transport rate by allowing the disc to traverse as much of the field of view as possible and determining the time and distance traveled. We only used those discs that traveled at least one-half the length of the observed trachea (3–4 cm). We calibrated the distance by viewing a tantalum marker sewn onto the skin while moving the x-ray stage a known distance. Due to slight bleeding at the tracheal cartilage hole some tantalum discs were initially retarded in their transport by clotted blood at the site. In these cases we waited, usually 10–20 min, until the disc began to move at a steady rate. Images were saved in 20-s epochs (512 × 512 image frames at 30 frames/s) as the disc traversed the entire tracheal field of view. The average velocity was calculated as the slope of the regression line of the distance vs. time of travel of each disc. The velocity of at least three discs for each experimental condition was determined and averaged to obtain the representative average value for that experimental condition.

Tracheobronchial Mucus Secretion

A Y-shaped cannula was placed into the most proximal portion of the trachea. A catheter (PE-50 tubing) was inserted into one arm of the Y-shaped tracheal cannula and advanced into the airway slowly until resistance was met. The catheter was then removed ~0.5 cm from the airway and taped into place onto the tracheal cannula. The end of the catheter was ~12 cm from the larynx. The cats were then placed at 30° angle with the legs elevated to allow gravity to drain the airways of fluid. The catheter was infused with 0.9% NaCl, and fluid draining the airway was collected from the dependent arm of the Y-shaped tracheal cannula. The effect of esophageal acidification on airway secretions was determined as described below. This technique allowed the quantification of airway secretions with minimal surgical intervention or disruption of the airways. This technique did not stimulate coughing or cause respiratory distress.

The airway of cats (n = 7) was infused with 0.9% NaCl at 0.19 ml/min and allowed to equilibrate for 30 min. Perfusates were then collected over ice every 10 min for the duration of the study. For the first 60 min, the esophagus was perfused with 0.1 M PBS, this was followed by 0.1 N HCl for 30 min, and then 0.1 M PBS for another hour. The volumes of the samples were quantified, and the hexosamine concentrations were determined using a modification of a colorimetric technique described previously (27, 56).

We quantified mucus secretion based on concentration of a major constituent of mucus rather then volume because in preliminary experiments we found that the volume changed little during the experiments. Hexosamines are major constituents of mucus, and hexosamine concentration of the perfusates was determined using a modification of a technique we developed previously (27, 56): 1) equal volumes of 4 N HCl were added to the collected perfusate samples and heated over boiling water in 50-ml glass centrifuge tubes for 6 h; 2) the pH of the samples was adjusted to pH 6.5 using 4 N NaOH then filtered through Whatman no. 42 filter paper; 3) 1 ml of the sample was combined with 1 ml of acetaldehyde, heated over boiling water in 50-ml glass centrifuge tubes for 15 min, and then rapidly cooled for 10 min in ice water; 4) 10 ml of Ehrlich’s reagent (56) was added to the sample and placed in the dark for 30 min; 5) absorbance of the samples was determined at 540 nm. Known concentrations of glucosamine diluted to 50% using deionized distilled water were processed similarly starting with step 3 to determine a standard curve of absorbance vs. concentration. A standard curve was determined for each sample group analyzed, and the known and unknown samples were processed simultaneously under the same conditions. The concentration of the unknown samples was determined by comparison of absorbance measurements to the standard curve.

Measurement of Lung Function

Total lung resistance (Rt) and dynamic compliance (Cdyn) were calculated from recordings of airflow and pleural pressure. We recorded airflow in cats using a pneumotachometer attached to the endotracheal tube and intrapleural pressure using a pressure transducer attached to a saline (0.9% NaCl)-filled PE-240 tube with a side port and beveled tip placed in the chest cavity. Signals from the intrapleural pressure transducer (Ppl) and pneumotachometer (V) were fed into a Grass polygraph (model 7). The flow signal (V) was integrated using a Grass integrator (model 7P10) to calculate corresponding changes in lung volume (Vt). Ppl, V, and Vt signal outputs were digitized and stored on computer using an analog-to-digital data-acquisition system (CODAS, Dataq Instruments, Akron, OH). The Rt was calculated using Rt = aPpl/AV. The resistance associated with the tracheal tubing was not significant and therefore was set to equal zero. The dynamic pulmonary compliance (Cdyn) was calculated using Cdyn = ∆V/∆Ppl when V = 0 obtained at end inspiration or end expiration. Rt and Cdyn were calculated from values taken from 10–12 consecutive breaths.

We tested the effects of esophageal acidification or tantalum on airway function. In six cats we measured lung function continuously while the esophagus was perfused with 0.1 M PBS for 60 min, followed by 0.1 N HCl for 30 min. Total lung resistance and dynamic compliance were calculated at the end of each perfusion period. In five cats we measured lung function before and 15 min after dusting the airways with tantalum as described above.

Measurement of Blood Gases

We examined the effects of esophageal acidification on blood gases in two ways. The esophagus of cats (n = 8) was perfused with 0.1 M PBS for 60 min followed by 0.1 M HCl for 30 min. Arterial blood was sampled at the end of each perfusion period and analyzed for pH, PO2, and Pco2. In addition, in six cats the esophagus was infused at a rate of 1.02 ml/min for 30 min using 0.1 M PBS then 30 min using 0.1 N HCl. The perfusates were collected during each period and the flow rates of fluid out of the esophagus were calculated. In a separate set of cats (n = 6) we tested whether the injection over 5 s of twice the volume of 0.1 N HCl lost during perfusion of the esophagus for 5 min altered blood gases.

Statistics

For studies in which an experimental group was compared with its own control, a paired t-test was used. For studies in which the control group differed from the experimental group, an unpaired t-test was used. When multiple comparisons were made, an ANOVA was used to identify whether a difference occurred, and if the multiple comparison involved repeated measures of the same variable, a repeated measures one-way ANOVA was used. Tukey’s test was used to determine which groups differed in a multiple comparison. In all cases, a P value of 0.05 or less was considered statistically significant.

RESULTS

The Effects of Esophageal Acidification on Lung Function

Bronchial diameter. Perfusion of the esophagus with 0.1 N HCl reduced the diameter of all airways examined. Figure 1 shows a radiographic example of the effects of esophageal acidification and subsequent neutralization on the diameter of three different sized airways in the same animal at end expiration. The administration of 0.1 N HCl caused all three
airways to constrict and, after 15 min of neutralization, the airways diameters returned to near pre-HCl levels.

Figure 2 shows the composite data from five different animals of six different size ranges of airways: 0.20–0.40 mm (mean of 0.26 mm), 0.53–0.86 mm (mean of 0.70 mm), 1.02–1.42 mm (mean of 1.23 mm), 1.77–1.96 mm (mean of 1.85 mm), 2.12–2.75 mm (mean of 2.42 mm), 3.40–4.30 mm (mean of 3.95 mm). All airways constricted after 15 min of HCl and returned to pre-HCl levels within 15 min. The average reduction in airway diameter ranged from 10 to 22%. When we grouped the airways into two groups based on size, the smaller airways (<1.42 mm) constricted to a greater degree (P < 0.003, t-test) than the larger airways (>1.77 mm).

Figure 3 depicts the time course of changes in diameters of airways due to esophageal acidification and subsequent neutralization. This figure depicts the changes in the average reduction in diameter in four different sized (0.73, 1.49, 1.65, and 3.45 mm) airways in one animal after up to 20 min of esophageal perfusion with HCl. These results showed that the decrease in airway diameters due to esophageal acidification began within 5 min and was significantly reduced within 10 min. The diameters returned to preacidification levels within 5 min of neutralization.

**Mucociliary transport.** Perfusion of the esophagus with 0.1 N HCl for 10 min reduced the rate of orad transport of a 0.3-μg disc of tantalum through the trachea (Figs. 4 and 5) by a mean of 53% (Fig. 6). Examples of tantalum disc movement are depicted radiographically in Fig. 4 and graphically in Fig. 5. Figure 6 is a graph of the composite results from all studied animals (n = 4).
Tracheobronchial mucus secretion. The hexosamine output from the tracheobronchial tree remained fairly constant during the first hour of collection during perfusion of the esophagus with 0.1 M PBS (Fig. 7). When the esophageal perfusate was changed to 0.1 N HCl, the hexosamine output increased significantly (3.4 ± 0.7 vs. 8.4 ± 2.6 mg; *P < 0.05) during the first 10-min collection period only (Fig. 7). The volume of fluid output from the airways did not change significantly over the recording period (Fig. 8).

RL and dynamic compliance. We found that esophageal acidification, tantalum, or both had no significant effects on RL and Cdyn (Table 1).

The Effects of Esophageal Acidification on Blood Gases

Fluid loss from the esophagus. We found that when the esophagus is perfused at 1.02 ml/min, 0.990 ± 0.002 ml/min (n = 4) is recovered over 30-min periods whether the perfusate is 0.1 M PBS or 0.1 N HCl and there was no significant difference (P > 0.05) between PBS and HCl. Thus the amount of fluid, PBS, or HCl not recovered was 0.03 ml/min or 0.15 ml over 5 min of HCl perfusion. We found that the bolus intra-

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Fig. 4. Effect of 0.1 N HCl in esophagus on tracheal mucociliary transport. This figure is a composite of radiographic images of the trachea of a cat taken at various times after 2 tantalum discs were inserted into the trachea. The 0-s frame is at the top and time progresses down the figure and the long vertical arrow points to the position of the disc at time 0. Each disc was inserted after 10 min of the indicated esophageal perfusate: 0.1 M PBS or 0.1 N HCl. The short vertical arrow points to the disc at the end of the recording period. Note that the disc took 59 s to travel 7.5 mm during PBS perfusion; but 124 s to travel 5.8 mm during HCl perfusion. Therefore HCl perfusion decreased the tracheal mucociliary transport rate by ~63% in this animal.

Fig. 5. Effect of esophageal acidification on mucociliary transport. This graph depicts the transport of 2 tantalum discs in the same animal; the first (•) during 0.1 M PBS perfusion of the esophagus and the second (○) during 0.1 N HCl perfusion. The locations, relative to the starting point in the trachea, at various times during their travel are noted by circles. Note, that in this animal the rate of movement of the discs is ~46% slower when the esophagus is perfused with HCl.

Fig. 6. Effect of esophageal acidification on mucociliary transport. This graph depicts the difference in mucociliary transport rates between perfusing the esophagus with 0.1 M PBS or 0.1 N HCl (n = 4). Perfusion of the esophagus with HCl significantly (*P < 0.05) decreased tracheal mucociliary transport by ~53%.

Fig. 7. Effect of esophageal acidification on mucous output from the tracheobronchial tree. This is a graph of the hexosamine output of tracheobronchial perfusates collected every 10 min (n = 7) as the esophageal perfusate is changed from 0.1 M PBS to 0.1 N HCl and back to 0.1 M PBS. Note that hexosamine output only increased during the first 10-min collection period after esophageal acidification. Hexosamine was used as a measure of mucus secretion. *P = 0.008 for repeated-measures ANOVA for a difference among groups, and *P < 0.05 for a difference between groups using Tukey’s multiple comparison test. The group at 70 min differed significantly from all other groups and no other significant differences between groups were found.
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Table 1. The effects of tantalum and esophageal acidification on total lung resistance and dynamic compliance

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Values are mean ± SE (n = 6). There were no significant effects (P > 0.05) of HCl given intravenously on blood gases when the blood was sampled 5 min after administration.

out at physiological concentrations of HCl, i.e., 0.1 N HCl, indicating that these changes may occur under physiological conditions like the reflux of acid gastric contents.

We found that esophageal acidification had a greater effect on smaller airways than the larger airways. A similar preferential effect on the smaller airways was found after vagal stimulation in dogs (3) or microembolism in cats (4). While the mechanism of this differential effect on the smaller airways is unknown, its effect on airflow is clear. The larger airways, i.e., >3 mm, have a much greater effect on airway resistance than the smaller airways (4, 31), therefore, this response to esophageal acidification acts to minimize rather than maximize airway resistance. The consequence of this differential effect was confirmed when we found that esophageal acidification had no effect on RL. These findings suggest that perhaps increasing airway resistance is not the function of this physiological response to esophageal acidification.

Our inability to demonstrate any change in lung mechanics following esophageal acidification is unlikely to be a consequence of our anesthetic protocol or our use of tantalum. Chloralose is routinely used in studies of airway smooth muscle control (8, 36) and digestive tract reflexes (26) in cats. Tantalum dusting (37) has been found to be chemically inert, causing no inflammation of the airways or lungs and no significant changes in blood gases or measures of lung function. Also, we found that lung mechanics did not change upon esophageal acidification whether or not the animals had first been exposed to tantalum.

We found that esophageal acidification at physiological concentrations significantly increased the output of mucus from the tracheobronchial tree by 147% and decreased tracheal mucociliary transport rate by 53%. Prior studies have found that while a basal level of mucus is needed for proper ciliary function (40, 44, 50), increased mucus secretion and viscosity retard mucociliary transport rate by reduction of ciliary beat frequency and efficiency (40, 44, 50). Therefore, our findings of decreased mucociliary transport and increased mucus secretion in different experiments are consistent with the known relationship between these variables. Contrary to the changes

Table 2. Effect of 0.3 ml of 0.1 N HCl bolus IV on blood gases

![Graph showing the effect of esophageal acidification on volume of output from the tracheobronchial tree. This is a graph of the volume of collected perfusates from the tracheobronchial tree as the esophageal perfusate is changed from 0.1 M PBS to 0.1 N HCl and back to 0.1 M PBS. Note that esophageal acification had no effect on the volume of fluid collected from the airways.](http://ajpregu.physiology.org/)

**DISCUSSION**

Numerous prior studies have found that esophageal acidification caused a variety of changes in the airways (1, 2, 7, 13, 17, 23, 32, 47, 53, 59). These include increased tracheal pressure (2, 23) or airway resistance (decreased compliance; Refs. 1, 13, 17, 47, 53, 59) and increased microvascular leakage (7, 17, 30). In all cases, the magnitudes of the responses were small in both an absolute sense and compared to responses to other appropriate stimuli like acidification of the larynx (23) or trachea (53). Compounding this problem was the fact that some studies (7, 13, 17, 30) used high nonphysiological concentrations of HCl, e.g., over 0.2 N HCl, to stimulate this response; and one study (30) found no response. We were unable to demonstrate any change in lung mechanics following esophageal acidification unless the acid was given at very low concentrations (over 0.1 M PBS and not over 0.1 N HCl). This is a graph of the volume of collected perfusates from the tracheobronchial tree as the esophageal perfusate is changed from 0.1 M PBS to 0.1 N HCl and back to 0.1 M PBS. Note that esophageal acidification had no effect on the volume of fluid collected from the airways.

Table 3. The effect of esophageal acidification on blood gases

![Graph showing the effect of esophageal acidification on volume of output from the tracheobronchial tree. This is a graph of the volume of collected perfusates from the tracheobronchial tree as the esophageal perfusate is changed from 0.1 M PBS to 0.1 N HCl and back to 0.1 M PBS. Note that esophageal acidification had no effect on the volume of fluid collected from the airways.](http://ajpregu.physiology.org/)

**Fig. 8.** Effect of esophageal acidification on volume of output from the tracheobronchial tree. This is a graph of the volume of collected perfusates from the tracheobronchial tree (n = 7) as the esophageal perfusate is changed from 0.1 M PBS to 0.1 N HCl and back to 0.1 M PBS. Note that esophageal acidification had no effect on the volume of fluid collected from the airways.

**Table 1.** The effects of tantalum and esophageal acidification on total lung resistance and dynamic compliance

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Values are mean ± SE (n = 6). There were no significant effects (P > 0.05) of HCl given intravenously on blood gases when the blood was sampled 5 min after administration.
in airway diameter, this change in mucus output and subsequent change in mucociliary transport are substantial and may serve an important physiological function. Mucus is an excellent buffer for acid (12, 16, 19) and its output into the airway would have a significant protective role against the aspiration of gastroesophageal refluxate. In addition, airway mucus is eventually expelled to the larynx and pharynx where it can also neutralize gastric reflux that reached supraesophageal levels.

The ability of digestive tract stimulation to cause airway mucus secretion has been observed previously (14). It was found that repeated distension of the stomach increased tracheal mucus secretion, which was mediated by the vagus nerves and cholinergic receptors. However, these studies did not control for gastric acid secretion and gastroesophageal reflux. It is well documented that gastric distension causes an increase in gastric secretion of HCl (38), an increase in the number of transient relaxations of the lower esophageal sphincter (20, 21), and an increase in gastroesophageal reflux (21, 24). Therefore, it is possible that the observed increase in tracheal mucus secretion was due to acid stimulation of the esophagus rather than gastric distension.

The relationship between airway contraction and mucus output is unclear. Some studies suggest that contraction of the airways causes can cause mucus output (43, 55), whereas others found no such relationship (9, 39a, 56, 60). Increased tracheal mucus output can occur without tracheal contractions (9, 39a) and increased tracheal contractions are not always associated with increased mucus output (56, 60). A similar dichotomy in the relationship of motility of the surrounding musculature with mucus secretion from other types of exocrine glands has also been found (15, 27, 45, 46, 51). A possible explanation for this dichotomy is that exocrine glands like the tracheal submucosal mucus secreting glands may occur by two mechanisms: apocrine secretion and exocytosis. Each of these mechanisms may be controlled differently such that only one may be related to contractions of the surrounding musculature. There is experimental support for this dichotomy as the intestinal submucosal mucous secreting glands exhibit both types of secretory mechanisms (27, 28). It is also possible that the motor response to esophageal acidification extends to the myoepithelial cells as they are under neural control (48) and their contraction has been associated with mucus secretion (49). However, more studies are needed to confirm this conclusion with regard to the airway submucosal mucus secreting glands.

It is also possible that mucus secretion caused airway constriction. One prior study (6) noted that in anesthetized dogs when large amounts of tracheal secretions, stimulated by atrial injection of capsaicin, collected in the larynx this reflexly caused prolonged and irregular tracheal contractions. Therefore, this study could imply that in our study tracheobronchial secretions stimulated by esophageal acidification may have reflexly caused airway constriction. This is unlikely because in our study tracheobronchial secretions were not allowed to collect anywhere as there was always free flow of airway secretions and the functioning mucociliary clearance apparatus. The amount of mucus secreted by the animals in our study was relatively small as the stimulus was at physiological levels, and we used no pharmacological agents to strongly stimulate mucus secretion. In addition, it has been found by numerous studies that the larynx is the most sensitive airway to mechanical stimulation (41, 57). Although the lower airway also constricts to mechanical stimulation of its mucosa (57), there is no evidence that the levels of mucus output that we observed can stimulate this response.

Bronchial constriction in response to esophageal acidification may serve other functions. The narrowing of airways increases the velocity of airflow and, therefore, airway constriction may facilitate the expulsion of the secreted mucus and refluxed materials by coughing (34). This function would not only assist in clearing the airway, but it would also provide a more rapid delivery of a significant buffer, i.e., airway mucus, to the larynx and pharynx. Both effects of contraction of airway smooth muscle along with increased mucus output would have beneficial effects in counteracting the effects of reflux of gastric acid to the pharynx, larynx, and airways. Other functions of airway constriction have been proposed (35), e.g., optimizing anatomical dead space (57) or V/Q matching (35), but none of them would have any beneficial effects in response to esophageal acidification and most have been discounted as having no real function.

The mechanism by which esophageal acidification stimulates the airways is unknown. Tachykinins have been found to be released into the airway by esophageal acidification (17, 25), and tachykinins can contract airway smooth muscle and cause tracheal mucus secretion (13, 49, 55), but the release of tachykinins in the airways by esophageal acidification has been attributed to a neural rather than a humoral mechanism (17, 25). Changes in blood gases could affect the airways but we found that perfusion of the esophagus with 0.1 N HCl for 30 min or bolus intravenous injection of 0.3 ml of 0.1 N HCl (more than the amount lost during 10 min of perfusion of the esophagus) had no affect on blood gases. Therefore, since the airway response to esophageal acidification occurs within 10 min it is unlikely that esophageal acidification affected the airway by changing blood gases or pH. Also, there is no known absorptive mechanism or ionic pump mechanism of the esophageal mucosa that functions in this capacity. A vascular shunt from the esophagus to airways is possible, but there is no experimental evidence for such a pathway and we found that the absorptive capacity of the esophagus to any fluid is minimal. Therefore, a humoral mechanism is unlikely.

A neural mechanism accounting for the airway responses to esophageal acidification is possible, but due to limitations in our techniques no firm conclusions can be drawn. We chose to optimize the experimental techniques toward minimizing uncontrollable variables rather than precisely measuring stimulus-response time delays, therefore, our studies are inconclusive in this regard. However, given the surgical preparation and experimental protocol, the observed stimulus-response delay of 5–10 min is too rapid to be accounted for by a humoral mechanism.

**Perspectives and Significance**

Our studies provided evidence for a new physiological response. The reflux of acidic gastric contents to the esophagus occurs normally in humans and our studies suggest that the airways may respond to this acid by the release of mucus from the airways. The threshold for this response has not yet been defined, but the response is appropriate to the stimulus as mucus is a good buffer of acid. The airway mucus could buffer...
REFERENCES