Effect of cardiogenic and noncardiogenic pulmonary edema on histamine responsiveness in sheep

James R. Snapper, Peter L. Lefferts, Weixuan Lu, Young Sil Hwang, and Jonathan D. Plitman. Effect of cardiogenic and noncardiogenic pulmonary edema on histamine responsiveness in sheep. J. Appl. Physiol. 85(5): 1635–1642, 1998.—We compared the effects of cardiogenic pulmonary edema, brief pulmonary vascular congestion without frank edema, and noncardiogenic pulmonary edema on responsiveness to inhaled histamine in chronically instrumented awake sheep. Histamine responsiveness was measured before and after 1) cardiogenic pulmonary edema induced by raising left atrial pressure to 35 cmH2O (Pla) for 3.5 h by partial obstruction of flow across the mitral valve, 2) brief cardiogenic congestion via Pla for 0.5 h, 3) noncardiogenic pulmonary edema induced by 25 mg/kg intravenous perilla ketone (PK), and 4) 3.5 h of monitoring without Pla or PK (controls). Treatment for 3.5 h with Pla (n = 9) and PK (n = 11) each significantly lessened the histamine dose required to cause a fall to 65% of baseline dynamic lung compliance (ED65Cdyn), i.e., increased responsiveness. Sheep treated for 0.5 h with Pla (n = 7) and controls (n = 5) showed no significant change in ED65Cdyn. Intravenous atropine (0.1 mg/kg) before the second histamine challenge altered neither the reduction of ED65Cdyn in Pla (n = 8) and PK (n = 9) sheep nor the ED65Cdyn level of controls (n = 9). These data imply that the local effects of edema, rather than bronchial vascular hemodynamics, cholinergic reflexes, and permeability changes, are germane to lung hyperresponsiveness during pulmonary edema in sheep.

bronchi; bronchial hyperreactivity; lung

Hyperresponsiveness to nonantigenic bronchial provocation occurs in humans during pulmonary vascular congestion and cardiogenic pulmonary edema (3, 14, 18–20). Hyperresponsiveness is also seen in animals during noncardiogenic pulmonary edema (8). Although it has not been documented per se in humans with the adult respiratory distress syndrome, such patients do have increased airway resistance which can be reduced by bronchodilators (27), and increased airway responsiveness has been demonstrated in some survivors of adult respiratory distress syndrome (10, 22). Because lung hyperresponsiveness may contribute to the symptoms (e.g., cardiac asthma) and abnormal lung mechanics characteristic of pulmonary edema, an understanding of the mechanisms underlying pulmonary edema-induced lung hyperresponsiveness is potentially important.

The present experiments compare the lung responsiveness of chronically instrumented awake sheep to histamine inhaled during cardiogenic pulmonary edema, brief pulmonary vascular congestion, and noncardiogenic pulmonary edema. Cardiogenic pulmonary edema and brief pulmonary vascular congestion both share increased hydrostatic pressure and vascular congestion, whereas altered hydrostatic pressure and vascular congestion are not observed in noncardiogenic pulmonary edema. Pulmonary edema formation is observed in both cardiogenic and noncardiogenic pulmonary edema but is not significant during brief periods of vascular congestion. Noncardiogenic pulmonary edema is associated with increased pulmonary microvascular permeability, the influx of inflammatory cells into the lungs, and the release of a variety of mediators which potentially could mediate alterations in lung responsiveness. Similar changes are not observed in cardiogenic pulmonary edema or during brief periods of vascular congestion. These comparisons thus potentially allow us to discern the relative import of specific mechanisms in pulmonary edema-related increased lung hyperresponsiveness (15). Some of these mechanisms may also be relevant to asthma. Because cholinergic mechanisms have been proposed as contributing to altered airway responsiveness in a dog pulmonary congestion model (9), we also studied the effects of atropine on alterations in lung responsiveness in our ovine models of cardiogenic and noncardiogenic pulmonary edema.

### METHODS

Sheep preparation. Yearling sheep of either sex weighing 30–40 kg were instrumented for measurement of vascular pressures and lung mechanics as previously described (8, 25). After anesthesia was induced with intravenous thiamylal and general endotracheal anesthesia was induced with halothane, catheters were placed directly into the left atrium and pulmonary artery through a left thoracotomy so pressure measurements could be made. An additional balloon-tipped catheter (18-Fr Foley) was positioned in the mitral valve orifice through the left atrial wall. Inflation of the balloon produced partial mitral valve obstruction and increased left atrial pressure (Ppl). An envelope made from 0.01-in. thick silicone sheeting (Specialty Manufacturing, Saginaw, MI), measuring 4 × 3 cm, with Silastic catheters (0.157-in. ID) extending from within the envelope, was positioned within the pleural space for measurement of intrapleural pressure (Ppl). Through an incision on the neck, catheters were placed into the aorta via the carotid artery and into the superior vena cava via the external jugular vein. A tracheostomy was performed, and a no. 10 cuffed tracheostomy tube (Shiley, Irvine, CA) was inserted. The sheep were allowed 5–7 days to recover from the operation. Free access to food and water was allowed during this period. All surgery and experimentation was performed in compliance with US Department of Agricul-
Physiological measurements. Awake sheep were studied while they were standing in a specially constructed, pressure-compensated, integrated-flow, whole body plethysmograph connected to an external valve via flexible noncollapsible tubing (8). A constant bias flow of humidified air was used to reduce the effective dead space of the tubing. Tidal volume (VT) was measured by pressure-compensating the integrated volume signal. Airway opening pressure (Pao) was measured in the trachea by a multiple-side hole catheter positioned 2 cm beyond the distal end of the tracheostomy tube. Ppl was obtained from the pleural envelope, and transpulmonary pressure (Ptp) was measured as the pressure difference between Ppl and Pao. The pressures from the plethysmograph, catheters, and Silastic pleural envelope were measured by using similar differential transducers (model MP-45; Validyne Engineering, Northridge, CA), and the pressure signals were tuned to 20 Hz to eliminate phasic distortion.

Before each set of lung mechanics measurements was made, the sheep's lungs were inflated to 40 cmH2O Pao by using the bias flow and occluding the expiratory limb of the tubing. Simultaneous VT/V and VT/Ptp curves were recorded during spontaneous respiration on a dual-beam storage oscilloscope (Tektronix, Wilsonville, OR) and photographed for calculation of dynamic lung compliance (Cdyn) and resistance to airflow across the lungs (RL). Cdyn was calculated as VT divided by Ptp at points of zero flow and expressed in liters per centimeters H2O at BTPS. RL was calculated by dividing Ptp by flow at mid-VT and expressed as centimeters H2O per liter per second at BTPS. The external valve was obstructed at end expiration to allow calculation of thoracic gas volume (TGV) at functional residual capacity by the modified Boyle's law technique.

Lung responsiveness to aerosol histamine was determined using solutions of histamine diphosphate (Sigma Chemical, St. Louis, MO) in 0.9% saline. Concentrations are expressed as milligrams of histamine base per milliliter. Aerosols were generated by a Calibrex nebulizer (BGI, Waltham, MA) using 100% O2-producing output particles of 2- to 4-µm median diameter. Aerosols (0.0, 0.003, 0.01, 0.03, 0.1, 3.0, 10.0, and 30.0 mg/ml histamine) were administered by inflating the lungs with the output of the nebulizer five times to a Pao of 40 cmH2O. VT, VT/Ptp, and VT/Pt curves were continuously monitored, and Cdyn, RL, and TGV were calculated at the time of maximal change (~1 min after the last inflation). The concentration of histamine was increased in stepwise fashion until Cdyn decreased to <65% of its baseline value, a level of response that all sheep included in this study reached at a histamine concentration of ~30.0 µg/ml. The effective dose of histamine that would have caused a reduction of Cdyn to 65% of its baseline value (ED50Cdyn) was calculated by interpolation, assuming a linear dose-response relationship between the last two histamine doses. If RL doubled or TGV increased by ≥25%, the respective effective doses (ED25RL and ED35TGV) were also calculated.

Pulmonary artery pressure, Pla, and aortic blood pressure were continuously measured by using saline-filled pressure transducers (model 1208C, Hewlett-Packard, Andover, MA).

Preparation of 3-furyl ketones (perilla ketone). 1-(3-Furyl)-4-methyl-1-pentanone (perilla ketone or PK) is a toxic product of the purple mint plant Perilla frutescens. It causes increased pulmonary microvascular permeability and pulmonary edema without acute changes in pulmonary hemodynamics (4). It was prepared from 3-furaldehyde (Sigma Chemical) by the method of Garst and Wilson (7). PK (1 g/ml) was then mixed 1:1 (vol/vol) with dimethyl sulfoxide (Sigma Chemical).

Experimental protocols. Pla of 35 cmH2O and 25 mg/kg of intravenous PK were used as models of cardiogenic and noncardiogenic pulmonary edema, respectively. We have observed that these regimens cause similar linear increases in fluid transit across the lung (measured as lung lymph flow) and in lung edema shown on chest radiograph over a 4-h monitoring period (2). Twelve sheep were studied for the effects of PK. After 1 h of stable baseline measurements, histamine responsiveness (initial value) was determined. PK was then infused intravenously over 20 min. The animals were monitored for 3.5 h after the initiation of PK infusion, at which point histamine responsiveness was determined again (final value). Because PK causes progressive, irreversible respiratory failure, the sheep that received PK were killed with an intravenous overdose of barbiturate at the end of the protocol. Sixteen sheep were studied in the Pla experiments. After 1 h of stable baseline measurements, initial histamine responsiveness was determined. The left atrial balloon was then slowly inflated over 10–15 min so as to cause Pla to increase to 35 cmH2O. This pressure was maintained, and animals were monitored for either 0.5 h (n = 7) or 3.5 h (n = 9), at which point final histamine responsiveness was determined. At the end of the Pla experiments, the mitral valve balloon was deflated, allowing the Pla to return to normal. To assess the reproducibility of the response to elevated Pla, two 3.5-h Pla experiments were performed 3 wk apart on an individual sheep. Similarly, two 0.5-h Pla studies were performed on another individual sheep.

Five of the surgically prepared sheep served as controls, receiving two determinations of inflated histamine responsiveness separated by 3.5 h, but they received neither PK nor Pla. Twenty-six additional sheep were studied in control, PK, or 3.5-h Pla protocols with the addition of the intravenous infusion of 0.1 mg/kg of atropine 30 min before the final determination of histamine responsiveness (n = 8 for 3.5-h Pla, n = 9 for PK, n = 9 for controls receiving neither PK nor PK). We have previously documented that this dose of atropine causes dilated pupils that are unreactive to light in the awake sheep and abolishes the effect of electrical stimulation of the vagus (5 V, 20 Hz, 20 s) on lung mechanics in the anesthetized sheep (15).

If an animal showed distress or significant respiratory failure during an experiment, the experiment was terminated and the sheep was either killed with intravenous barbiturate (PK trial) or the left atrial balloon was deflated (Pla trial).

Statistics. Data were analyzed by a combination of parametric and distribution-free statistics (Statistica/W, StatSoft, Tulsa, OK; and SigmaStat, Jandel Scientific, San Rafael, CA). After analysis of variance, multiple comparisons were performed by the Newman-Keuls test, by Dunn's test, or by the Wilcoxon or Mann-Whitney tests, with Bonferroni correction of the resultant P values. The Bonferroni correction was also applied to the instances of repetitive distribution-free correlation analysis. The null hypothesis was rejected for either P < 0.05.

RESULTS

Primary studies. The pattern of changes in lung mechanics induced by aerosol histamine, in both the absence and the presence of pulmonary edema, was similar to those previously reported in sheep (24). Because approximately two-thirds of the sheep studied failed to double RL, even with the highest concentra-
tions of histamine, and >90% of sheep failed to increase TGV by 25%, alterations in lung responsiveness are presented in terms of Cdyn. Figure 1 contains the $ED_{65\text{Cdyn}}$ data from the individual control sheep before and after a 3.5-h monitoring period. There was no significant change in $ED_{65\text{Cdyn}}$ over this period. Figure 1 also shows $ED_{65\text{Cdyn}}$ values for individual sheep before and after either PK, 3.5 h of $\uparrow$Pla, or 0.5 h of $\downarrow$Pla. $ED_{65\text{Cdyn}}$ was significantly reduced after PK and after 3.5 h of $\uparrow$Pla, but 0.5 h of $\uparrow$Pla caused no significant change. The magnitude of increase in histamine responsiveness of the individual animals (measured as $\log_{10}$ of final $ED_{65\text{Cdyn}}$ $- \log_{10}$ of initial $ED_{65\text{Cdyn}}$) did not differ significantly between the PK and 3.5-h $\uparrow$Pla groups. The PK and 3.5-h $\uparrow$Pla animals characteristically showed signs that were consistent with pulmonary edema (tachypnea, frothy liquid coughed from tracheostomy tube) that increased over the duration of the monitoring period. The control and 0.5-h $\uparrow$Pla animals did not. The $ED_{65\text{Cdyn}}$ data for the four groups are summarized numerically in Table 1. There were no significant differences among the groups at baseline.

Figure 2 shows the results of within-subject repetition of the 3.5-h and 0.5-h $\uparrow$Pla protocols. The first study on each animal (solid symbols) is part of the data displayed in Fig. 1 and summarized in Table 1. The responses in the repeat studies (open symbols) are similar in magnitude and direction to those in the first studies.

Figures 3–6 contain the complete initial and final histamine dose-response curves for the individual sheep in the four experimental groups. These data are shown primarily for comparison of the baseline Cdyn values (abscissa = S (saline inhalation)) at the beginning of the initial and final dose-response curves. Note that, in those groups shown to have significantly reduced final $ED_{65\text{Cdyn}}$ (i.e., PK and 3.5-h $\uparrow$Pla), there appears to be considerable downward shift in Cdyn at baseline. The magnitude of the shift in Cdyn of the individual animals (measured as percent change from baseline) did not differ significantly between the PK and 3.5-h $\uparrow$Pla groups. Table 2 summarizes the Cdyn data for the four groups immediately before the initial and final inhaled-histamine challenges. There were no significant differences among the initial baselines. The PK and 3.5-h $\uparrow$Pla groups had significantly reduced baseline Cdyn values before the final histamine challenge, whereas the control and 0.5-h $\uparrow$Pla groups did not.

Figure 7 contains the baseline Cdyn values measured before the initial and final histamine challenges in each

Table 1. Initial and final values of $ED_{65\text{Cdyn}}$

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Initial $ED_{65\text{Cdyn}}$</th>
<th>Final $ED_{65\text{Cdyn}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>5.27 (2.26)</td>
<td>5.14 (2.48)*</td>
</tr>
<tr>
<td>PK</td>
<td>11</td>
<td>5.98 (11.36)</td>
<td>0.85 (1.37)†</td>
</tr>
<tr>
<td>$\uparrow$Pla 3.5 h</td>
<td>9</td>
<td>8.61 (5.26)</td>
<td>0.49 (0.62)†</td>
</tr>
<tr>
<td>$\uparrow$Pla 0.5 h</td>
<td>7</td>
<td>4.66 (3.12)</td>
<td>4.18 (1.82)*</td>
</tr>
</tbody>
</table>

Values are expressed as medians (with quartile range in parentheses) in milligrams histamine per milliliter. n, No. of sheep. $ED_{65\text{Cdyn}}$, effective dose to achieve 65% baseline dynamic compliance; PK, perilla ketone; $\uparrow$Pla, increase in left atrial pressure; *P < 0.05 vs. initial value; †P < 0.05 vs. final value for controls.
of the four experimental groups. Each datum is plotted vs. its associated ED$_{65}$C$_{dyn}$ value to allow investigation of the possibility of a correlation between a value of C$_{dyn}$ and the ED$_{65}$C$_{dyn}$ measured immediately thereafter. Table 3 contains the various correlation coefficients obtained. The n values in this table reflect not the number of animals but the number of pairs of individual C$_{dyn}$ and ED$_{65}$C$_{dyn}$ values used in the correlation calculation. The division of the data into Control, 0.5-h $\uparrow$Pla and PK 1 3.5-h $\uparrow$Pla separates those groups which did not have a significant reduction of C$_{dyn}$ and ED$_{65}$C$_{dyn}$ over the course of the experiments from those which did. Significant correlations occur only when groups with and without significant reduction of these values are analyzed simultaneously.

Atropine studies. Figure 8 shows the histamine responsiveness data from the three groups of animals given intravenous atropine before the final histamine challenge. In the presence of atropine, the PK and 3.5-h $\uparrow$Pla sheep showed a significant decrease in ED$_{65}$C$_{dyn}$ at the end of the monitoring period. The magnitude of the increase in responsiveness of the individual animals (measured as log$_{10}$ of final ED$_{65}$C$_{dyn}$ – log$_{10}$ of initial ED$_{65}$C$_{dyn}$) did not significantly differ between the PK and PK+atropine groups nor between the 3.5-h $\uparrow$Pla and 3.5-h $\uparrow$Pla+atropine groups. Atropine also failed to alter the histamine responsiveness of the control animals.

**DISCUSSION**

Lung edema may alter lung responsiveness through a variety of mechanisms. Several reflect local effects of edema fluid. These are potentially pertinent irrespec-
tive of whether the edema is due to increased permeability or to increased hydrostatic pressure, in that the pattern of accumulation of both types of edema appears to be similar (26).

Edema can stimulate vagal afferents (13). Both rapidly adapting receptors and unmyelinated C fibers (Paintal's "J receptors") are activated in this way (16, 17). Cholinergic efferent tone could then reflexly increase, and airway hyperresponsiveness could result from enhanced bronchomotor activity, increased secretion of intraluminal liquid, and also from bronchial vasodilation (see below). Antidromic stimulation of sensory nerve fibers in the lung might also result, causing the local release by axon reflex of proinflammatory neuropeptides such as substance P and neurokinin A. These could have responsiveness-enhancing effects including increased vascular permeability, which could accelerate edema formation, alteration of airway smooth muscle tone, and vasodilation.

Other potential mechanisms of hyperresponsiveness relate primarily to states of increased permeability or of increased hydrostatic pressure. Bronchial hemodynamic abnormalities, for example, pertain principally to cardiogenic pulmonary edema. The circulation to the intrapulmonary airways of the sheep drains via the pulmonary vessels. Thus increased Pla may congest and distend the bronchial vasculature. Pla is not affected by PK. It has been shown, in an exsanguinated perfused model, that hydrostatic overload of the bronchial circulation does distend the submucosal bronchial plexus of sheep intrapulmonary airways (11).

Increased lung permeability, a hallmark of noncardiogenic pulmonary edema, could facilitate the access of agonists to their sites of action, thus increasing lung...
responsiveness. Increased permeability can also occur during cardiogenic edema; disruption of the blood-gas barrier has been observed in rabbit lungs after acute hydrostatic overload of the pulmonary vasculature (1). The increase in lung permeability in sheep acutely exposed to Pla of 35 cmH₂O is modest, particularly compared with that observed after PK (2). Pulmonary microvascular permeability seems to be decreased, possibly adaptively, in humans with mitral stenosis or chronic left heart failure (6).

In the present studies, both PK (a model of noncardiogenic pulmonary edema) and 3.5-h Pla (a model of cardiogenic pulmonary edema) similarly and significantly increase lung responsiveness to inhaled histamine. This finding is unaffected by intravenous atropine, and it does not occur when the exposure to Pla is limited to 0.5 h. Based on the classification of mechanisms outlined above, what can be inferred?

First, the lack of hyperresponsiveness in the 0.5-h Pla model tends to deny the importance of bronchial hemodynamics in the genesis of hyperresponsiveness in the sheep while emphasizing the importance of the effects of edema fluid. When Pla is abruptly raised, the effect on the bronchial circulation is quite rapid—a significant fall in bronchial blood flow can be measured within a few minutes after elevation of Pla in sheep. On the other hand, we have radiographically observed minimal or no accumulation of lung edema after only the other hand, we have radiographically observed within a few minutes after elevation of Pla in sheep. On significant fall in bronchial blood flow can be measured.

Second, the effect of atropine in the present experiments implies that the cholinergic vagal reflex effects described above are relatively unimportant in this situation in the sheep, analogous to our previous findings on the lung hyperresponsiveness observed in the ovine endotoxemia model of acute lung injury (8). This inference can probably be extended to some degree to include the local release of neuropeptides, the effects of which seem to be partially mediated by acetylcholine release in some species, including sheep (5). Our findings seem at odds with those of Kikuchi et al. (9), who observed vagally mediated synergy between the effects of inhaled histamine and brief periods of pulmonary congestion on lung mechanics in dogs. Their study was designed differently from ours, however. In addition to the difference in species used and the use of vagotomy rather than muscarinic blockade, their experiments consisted of increasing doses of inhaled histamine (with or without prior vagotomy) followed by 1-min periods of Pla. Thus their protocol seems to test responsiveness to Pla after altering lung mechanics with histamine.

Table 2. Initial and final baseline values of Cdyn

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Initial Cdyn</th>
<th>Final Cdyn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>0.093 ± 0.005</td>
<td>0.095 ± 0.009*</td>
</tr>
<tr>
<td>PK</td>
<td>11</td>
<td>0.085 ± 0.009</td>
<td>0.056 ± 0.006†</td>
</tr>
<tr>
<td>Pla 3.5 h</td>
<td>9</td>
<td>0.085 ± 0.009</td>
<td>0.043 ± 0.008†</td>
</tr>
<tr>
<td>Pla 0.5 h</td>
<td>7</td>
<td>0.083 ± 0.005</td>
<td>0.068 ± 0.005*</td>
</tr>
</tbody>
</table>

Values are means ± SE in liters per cmH₂O n. No. of sheep. Cdyn, dynamic compliance. *P < 0.05 vs. initial value; †P < 0.05 vs. final value for controls.

Table 3. Correlation of Cdyn and log₁₀ ED₆₅Cdyn

<table>
<thead>
<tr>
<th>Group and Sequence</th>
<th>Values</th>
<th>n</th>
<th>Spearman ρ</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All groups</td>
<td>64</td>
<td>0.9160</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>All groups</td>
<td>Initial</td>
<td>32</td>
<td>-0.1305</td>
<td>NS</td>
</tr>
<tr>
<td>All groups</td>
<td>Final</td>
<td>32</td>
<td>0.5435</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Control + 0.5 h Pla</td>
<td>All</td>
<td>24</td>
<td>0.2102</td>
<td>NS</td>
</tr>
<tr>
<td>Control + 0.5 h Pla</td>
<td>Initial</td>
<td>12</td>
<td>0.3439</td>
<td>NS</td>
</tr>
<tr>
<td>Control + 0.5 h Pla</td>
<td>Final</td>
<td>12</td>
<td>0.0912</td>
<td>NS</td>
</tr>
<tr>
<td>PK + 3.5 h Pla</td>
<td>All</td>
<td>40</td>
<td>0.4781</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PK + 3.5 h Pla</td>
<td>Initial</td>
<td>20</td>
<td>-0.1662</td>
<td>NS</td>
</tr>
<tr>
<td>PK + 3.5 h Pla</td>
<td>Final</td>
<td>20</td>
<td>0.2970</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, not significant. n, Pairs of individual Cdyn and ED₆₅Cdyn values used in correlation calculation.

Figure 7. Cdyn vs. ED₆₅Cdyn (log₁₀ scale) by experimental group and sequence. See Table 3 for corresponding correlation coefficients.

Figure 8. Responsiveness to inhaled histamine of sheep before and after exposure to PK (n = 9), 3.5 h of Pla (n = 8), and a 3.5-h control monitoring period (n = 9). Intravenous atropine (0.1 mg/kg) was given 0.5 h before the second histamine challenge. Each pair of connected data symbols represents a single animal. Ordinate displays histamine concentrations on a log₁₀ scale. A reduced ED₆₅Cdyn implies increased responsiveness.
rather than responsiveness to histamine after periods of $\text{P}1\text{a}$.

These inferences emphasize the potential importance of the local mechanical effects of edema liquid in the genesis of pulmonary edema-related lung hyperresponsiveness. The present experiments do not directly address the potential involvement of permeability, but this mechanism would not seem to be intimately involved because 3.5-h $\text{P}1\text{a}$ and $\text{PK}$ cause similar degrees of hyperresponsiveness. Furthermore, studies in persons with asthma and in smokers have not suggested a strong relationship between epithelial permeability (measured as $^{99}$Tc-diethylene triamine pentaacetate clearance) and airway responsiveness (12).

Alterations in lung responsiveness were calculated from histamine-induced changes in $\text{Cdyn}$. Histamine causes changes in the peripheral lungs (2-mm airways and smaller (23)) and does not, in sheep, consistently cause changes in $\text{Rl}$ or $\text{TGV}$ (24). In our models, neither cardiogenic nor noncardiogenic pulmonary edema facilitated histamine-induced changes in $\text{Rl}$ or $\text{TGV}$. Frequency dependence of compliance was not noted in these spontaneously breathing sheep, in which the respiratory rate varied from $\sim 4$ breaths/min to $< 1$ breaths/s. In the presence of pulmonary edema, factors such as inhomogeneity of airflow, airway closure, and changes in tissue resistance may contribute to the observed alteration in histamine responsiveness.

It is important to recognize that those groups of sheep with significant reductions in $\text{ED}_{65}\text{Cdyn}$ also had significant reductions of $\text{Cdyn}$ at the time of the final histamine challenge. This is a potentially confounding factor in the interpretation of the data, in that the conditions of the experiment alter lung mechanics in a way not explicit in the normalized principal outcome variable, $\text{ED}_{65}\text{Cdyn}$. It could be claimed, furthermore, that the reduction of $\text{ED}_{65}\text{Cdyn}$ merely reflects the reduction in $\text{Cdyn}$, which could result from reduced lung volume, reduced airway caliber, and/or altered aerosol deposition. If this were true, one would expect there to be a significant correlation between $\text{Cdyn}$ and $\text{ED}_{65}\text{Cdyn}$. In the ovine endotoxia model of acute lung injury, we have observed no correlation between reductions in $\text{ED}_{65}\text{Cdyn}$ and reductions in either $\text{Cdyn}$ or functional residual capacity (8), although in the present study (Table 3), significant correlations between $\text{Cdyn}$ and $\text{ED}_{65}\text{Cdyn}$ are found. We feel that these correlations are potentially misleading in that they occur only with the simultaneous analysis of data that include clusters of animals both with and without altered $\text{Cdyn}$ and $\text{ED}_{65}\text{Cdyn}$. In other words, the "significant" correlations may be artifacts of superimposition of heterogeneous groups, which places one "cloud" of points in the right upper quadrant of the graph (Fig. 7) and another cloud in the lower left, and a line is then drawn between the two. If one looks for significant correlations within a single cloud (e.g., all control and 0.5-h $\text{P}1\text{a}$ animals or the final values of all $\text{PK}$ and 3.5-h $\text{P}1\text{a}$ animals (pulmonary edema)), there are none. We cannot exclude the possibility that more meaningful correlations might emerge with larger groups. We would argue that even if $\text{ED}_{65}\text{Cdyn}$ were in some instances an indirect reflection of $\text{Cdyn}$, the animals with significantly reduced $\text{ED}_{65}\text{Cdyn}$ would still, by definition, be hyperresponsive to bronchial provocation. One's ability to draw parallels between this hyperresponsive state and the hyperresponsive state of human pulmonary edema (in which, as in the sheep, lung compliance is reduced (21)) is not impaired by the potential involvement of $\text{Cdyn}$.

In summary, we have shown that responsiveness to bronchial provocation with inhaled histamine in sheep is increased during cardiogenic and noncardiogenic pulmonary edema and that this increase is not antagonized by intravenous atropine. Brief pulmonary-bronchial vascular congestion does not cause hyperresponsiveness to histamine. From these findings, we infer that the local structural effects of edema fluid, rather than bronchial hemodynamics or vagal reflexes, are the principal factors in the genesis of the hyperresponsiveness.

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