Mechanism of the Serotonin Effect on Lung Transvascular Fluid and Protein Movement in Awake Sheep

By Kenneth L. Brigham and Patty Jill Owen

ABSTRACT
To see how serotonin affects filtration from lung vessels, we measured vascular pressures, lung lymph flow, lung lymph and blood plasma protein concentrations, arterial blood gases, cardiac output, and lung water content in unanesthetized sheep before and during intravenous serotonin infusions and compared serotonin effects with the effects of inflating a balloon in the left atrium in the same sheep. Serotonin caused a dose-related increase in lung lymph flow and a dose-related decrease in lymph-plasma protein concentration ratios. Steady-state 4-hour serotonin infusions at 4 µg/kg min⁻¹ caused lymph flow to increase from 5.4 ± 0.7 (sr) ml/hour to 8.3 ± 1.3 ml/hour, lymph-plasma albumin ratios to fall from 0.78 ± 0.05 to 0.72 ± 0.04, lymph-plasma globulin ratios to fall from 0.64 ± 0.02 to 0.56 ± 0.02, and pulmonary arterial and left atrial pressures to increase by 3 cm H₂O each. Lymph clearance and permeability-surface area products for eight protein fractions ranging from 36 Å to 96 Å in molecular radius during steady-state serotonin infusion studies were not significantly different from those during steady-state increased pressure studies. Four-hour infusions of serotonin at 4 µg/kg min⁻¹ caused a moderate fall in arterial Po₂ and a slight increase in arterial pH but did not affect cardiac output or cause pulmonary edema. We conclude that serotonin increases lung transvascular filtration primarily by increasing the transmural pressure gradient in exchanging vessels rather than by increasing vascular permeability.

KEY WORDS lung lymph vasoactive mediators pulmonary edema capillary permeability lung water

Increased lung vascular permeability to protein apparently plays an important pathogenetic role in a variety of human diseases in which pulmonary edema occurs without heart failure (1). Such changes in the systemic circulation are mediated by the endogenous release of vasoactive substances (2), and serotonin has been considered to be one of these mediators (3, 4). Serotonin is vasoactive in the pulmonary circulation (5-7) and may cause pulmonary edema (8), but the mechanism of its effect on lung fluid dynamics is not clear.

We measured the effects of intravenous serotonin infusions on lung vascular pressures and lung lymph flow and protein content in a chronic, unanesthetized sheep preparation. We then compared the serotonin effects with the effects of steady-state mechanical increases in lung vascular pressure in the same sheep. We found that serotonin caused a dose-related increase in lung lymph flow and lymph protein transport and that these changes were like those associated with increased left atrial and pulmonary arterial pressures.

Methods

EXPERIMENTAL PREPARATION
We prepared sheep by a series of three thoracotomies as described by Brigham et al. (9). We first eliminated abdominal contributions to a large elongated node in the posterior mediastinum (caudal mediastinal node) by resecting the tail of the node below the inferior pulmonary ligaments through a low right thoracotomy (ninth intercostal space). After 4-6 days, through a left thoracotomy (fifth intercostal space), we resected the pericardium, put a stainless steel clip at the posterior border of the left atrium, placed a silicone-elastomer-coated Foley balloon catheter (Dow Corning Corp.) in the left atrium, and inserted silicone-elastomer catheters (Dow Corning Corp.) in the left atrium and the main pulmonary artery. Finally, 4-6 days later, through a right thoracotomy (sixth intercostal space), we put a small silicone-elastomer catheter in the efferent lymph vessel emerging from the head of the caudal mediastinal node and inserted polyethylene catheters in the superior vena cava and the thoracic aorta through the neck vessels. Brigham and his associates (9) have shown that lymph collected from sheep prepared in this way responds to changes in lung vascular pressures but does not respond to changes in systemic venous pressure; it therefore represents mostly lung lymph. We waited several days after the last operation until the sheep were afebrile, the lymph flow was stable, and the lymph was free of blood before doing experiments.
EXPERIMENTAL PROTOCOLS

General.—All experiments were carried out on unanesthetized sheep standing in an experimental cage. Before beginning the studies, we located the left atrial clip fluoroscopically with the sheep standing in its experimental cage and marked this level on the skin; this mark constituted the zero reference level for all vascular pressures. During each experiment, we measured vascular pressures continuously using miniature strain gauges (Micron Instruments, Inc.) and an electronic recorder (Hewlett-Packard Co.). We measured lung lymph flow at 15-minute intervals by recording the volume drained into a graduated tube, and we measured protein concentrations in plasma from blood drawn each hour and in lymph pooled at 30-minute intervals.

Increased Pressure Studies.—Once in each of six sheep we measured responses to increased lung vascular pressure. After at least 2 hours of base-line observations of pressure, lymph flow, and lymph and plasma protein concentrations, we inflated the left atrial balloon enough to increase left atrial pressure by 15–20 cm H₂O and kept this pressure stable for 4 hours. As Brigham and co-workers have reported (9), a steady state was reached about 2 hours after the balloon was inflated, and overt pulmonary edema did not occur.

Serotonin Studies.—We studied serotonin by two different protocols. We investigated (1) steady-state intravenous serotonin infusions and (2) serotonin dose-response relationships.

We did nine steady-state serotonin experiments in the same six sheep in which the increased pressure studies had been done. After at least 2 hours of base-line observations of pressures, lymph flow, and lymph and plasma protein concentrations, we infused serotonin creatinine sulfate (Calbiochem) dissolved in 0.89% NaCl solution through the superior vena caval catheter at 4 μg/kg min⁻¹ for 4 hours using a constant-rate infusion pump (Harvard Apparatus Co., Inc.). We chose this dose because it produced a substantial response without causing the preparation to become unstable.

Serotonin dose-response relationships were investigated after at least 2 hours of base-line observations of pressures and lymph flow by infusing serotonin intravenously at increasing rates. We started with 1–2 μg/kg min⁻¹, waited for a plateau in lymph flow and pressure responses, increased the infusion rate to 3–4 μg/kg min⁻¹, waited for a plateau, and again increased the infusion rate to 5–6 μg/kg min⁻¹, continuing the infusion until a response plateau was defined.

OTHER METHODS

Protein Analyses.—We measured total protein concentrations in lymph and blood plasma with an automated system (AutoAnalyzer, Technicon Instruments Corp.) by a modified Biuret method (10) and separated protein fractions into steady-state base-line and steady-state experimental lymph and blood plasma samples by polyacrylamide gradient gel electrophoresis. We used 4–30% polyacrylamide gradient gel slabs (Pharmacia Fine Chemicals) and Tris-barbital buffer at pH 8.0 and ionic strength 0.06. We performed electrophoresis for 16.5 hours at 125 v constant voltage, stained with 0.5% Ponceau S in 7.5% trichloroacetic acid, destained electrophoretically in 7% acetic acid, and scanned the gels spectrophotometrically at 510 nm. Using the measured total protein concentrations, we calculated the concentrations of each of eight protein fractions consistently identified in lymph and plasma samples. To estimate the effective molecular radius for each of the eight fractions, we ran gel slabs with both lymph and plasma samples and five proteins of known molecular weights and free diffusion coefficients. Using the Einstein-Stokes equation (11), we calculated the effective molecular radius for each of the five known proteins, plotted these values as a function of migration distance, and estimated the effective molecular radius of the eight plasma and lymph protein fractions from this standard curve. Figure 1 is a plot of the curve showing the known proteins and the location of the eight plasma and lymph fractions on the curve. Fraction I is albumin. In the summary tables, we give total globulin concentrations as the sum of fractions II–VIII.

Indicator-Dilution Lung Water Measurements.—We measured extravascular lung water in vivo during steady-state base-line and experimental periods by indicator-dilution techniques. We used both ⁴¹Cr-labeled erythrocytes and ¹²⁵I-labeled albumin as intravascular indicators to avoid errors due to red cell–plasma transit time differences (12, 13). We labeled red cells from the animal by incubating a blood sample for 1 hour with ⁴¹Cr-sodium chromate at room temperature and washed the cells three times with 0.89% NaCl solution. For each study, we injected a mixture of 10μc of ⁴¹Cr-erythrocytes, 10μc of ¹²⁵I-albumin, and 30μc of ³H-water as a bolus through the superior vena caval catheter and took arterial blood samples at 1.0-second intervals by allowing blood to flow from the aortic catheter into heparinized tubes on a precisely timed rotating disk collector. We measured radioactivity in 0.5-ml samples of (1) each arterial blood sample and (2) the injected mixture diluted 1 to 51 in the sheep’s own blood drawn before the study. We measured ⁴¹Cr and ¹²⁵I activity in a gamma spectrometer (Auto Gamma model 3002, Packard Instrument Co., Inc.) and ³H activity in a liquid scin-
SEROTONIN EFFECTS ON LUNG FLUID BALANCE

Results

INCREASED PRESSURE STUDIES

We carried out steady-state increased pressure experiments once in each of six sheep. The effects of a 4-hour pressure increase on cardiac output, blood gases, hematocrit, and indicator-dilution lung water are summarized in Table 1. By the end of the 4 hours, cardiac output had decreased, and pH and lung water had increased slightly. Vascular pressures, lymph flow, and lymph and plasma protein concentrations for the steady-state increased pressure studies are summarized in Table 2. The results are like those reported by Brigham and his associates (9). For an average increase in left atrial pressure of 14 cm H₂O, lymph flow doubled, lymph albumin and globulin concentrations fell, plasma albumin and globulin concentrations did not change, and lymph-plasma ratios for both albumin and globulin decreased.

SEROTONIN STUDIES

Steady-State Serotonin Infusions.—We performed steady-state serotonin infusions nine times in the same six sheep in which the increased pressure studies had been done. Figure 2 shows the effects of a 4-hour intravenous serotonin infusion at 4 µg/kg min⁻¹ on lung vascular pressures, lymph-plasma protein concentration ratios, and lung lymph flow in one representative experiment. Sero-

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1 Nine measurements in seven sheep gave values of 0.84 ± 0.02 (SD) for the whole blood fractional water content and 0.92 ± 0.01 (SD) for the plasma fractional water content. (Brigham, Woolverton, and Staub, unpublished data.)

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TABLE 1
Summary of Steady-State Hematocrits, Blood Gases, Cardiac Outputs, and Lung Water

<table>
<thead>
<tr>
<th></th>
<th>Base line</th>
<th>Increased left atrial pressure</th>
<th>Serotonin (4 μg/kg min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial blood hematocrit</td>
<td>0.28 ± 0.01</td>
<td>0.31 ± 0.02*</td>
<td>0.27 ± 0.01</td>
</tr>
<tr>
<td>Arterial blood P_{\text{O}_2} (torr)</td>
<td>80 ± 1</td>
<td>83 ± 2</td>
<td>70 ± 2*</td>
</tr>
<tr>
<td>Arterial blood P_{\text{CO}_2} (torr)</td>
<td>38 ± 1</td>
<td>39 ± 3</td>
<td>40 ± 1</td>
</tr>
<tr>
<td>Arterial blood pH</td>
<td>7.51 ± 0.01</td>
<td>7.55 ± 0.02</td>
<td>7.54 ± 0.01*</td>
</tr>
<tr>
<td>Cardiac output (ml/min kg⁻¹)</td>
<td>129 ± 5</td>
<td>102 ± 7*</td>
<td>128 ± 10</td>
</tr>
<tr>
<td>Indicator-dilution extravascular lung water (ml/kg)</td>
<td>5.4 ± 0.2</td>
<td>6.1 ± 0.3*</td>
<td>5.0 ± 0.4</td>
</tr>
</tbody>
</table>

All values are means ± se. The number of observations over the number of sheep is given in parenthesis below each value.

* Significantly different from base line (P < 0.05).

Serotonin caused vascular pressures and lymph flow to increase, but the protein ratio fell.

As shown in Table 1, 4-hour intravenous infusions of serotonin creatinine sulfate at 4 μg/kg min⁻¹ did not affect cardiac output, lung water, hematocrit, or arterial P_{\text{CO}_2} significantly. Serotonin caused a slight increase in arterial blood pH and a moderate fall in arterial blood P_{\text{O}_2}.

Table 3 summarizes steady-state vascular pressures, lymph flow, and lymph and plasma protein concentrations for all of the serotonin studies. The serotonin infusion rate we used caused a small but significant increase in both left atrial and pulmonary arterial pressures. Lymph flow increased in every experiment. The average increase was 50%, but the degree of increase was variable among the sheep. Both albumin and globulin concentrations in lymph decreased, but plasma concentrations were not changed. Therefore, as in the increased pressure studies, lymph-plasma albumin and globulin concentration ratios declined.

Figure 3 shows lymph-plasma total protein concentration ratios as a function of lung lymph flow for all of the steady-state observations. Lymph-plasma protein ratios decreased with increasing lymph flow in the base-line and increased pressure studies. The same relationship between these two variables occurred during serotonin infusion.

Table 4 summarizes the steady-state lymph-plasma ratios for eight protein fractions during the base-line studies, the increased pressure studies, and the serotonin infusion studies. Although there was some variability, the ratios tended to decrease with increasing molecular size in all three studies. Mean ratios for all eight fractions were lower during the increased pressure studies than they were during base-line studies. Mean ratios for six of the eight fractions were lower during the serotonin infusion studies than they were during base-line studies.

Figure 4 shows the steady-state lymph protein clearance (lymph flow × lymph-plasma concentration ratios) for each of the eight protein fractions during base-line, increased pressure, and serotonin infusion studies. In all three studies, clearance declined with increasing molecular size. Clearance was significantly higher than it was under base-line conditions for five of the fractions during the increased pressure studies and for seven of the fractions during the serotonin infusion studies. The differences between increased pressure and serotonin values were not significant for any of the eight protein fractions.
### Summary of Steady-State Data for Increased Pressure Studies

**Table 2**

<table>
<thead>
<tr>
<th>Sheep</th>
<th>Body weight (kg)</th>
<th>Sex</th>
<th>Condition</th>
<th>(P_{pa}) (cm H(_2)O)</th>
<th>(P_{la}) (cm H(_2)O)</th>
<th>(\dot{Q}_{lym}) (ml/hour)</th>
<th>Protein concentration (g/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lymph</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Albumin</td>
</tr>
<tr>
<td>1</td>
<td>32</td>
<td>F</td>
<td>Base line</td>
<td>23</td>
<td>6</td>
<td>2.6</td>
<td>2.31</td>
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<tr>
<td></td>
<td></td>
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<td>Increased</td>
<td>42</td>
<td>24</td>
<td>5.8</td>
<td>1.46</td>
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<tr>
<td>2</td>
<td>42</td>
<td>F</td>
<td>Base line</td>
<td>19</td>
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<td>5.1</td>
<td>2.79</td>
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<td>18</td>
<td>8.2</td>
<td>2.05</td>
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<td>3</td>
<td>45</td>
<td>F</td>
<td>Base line</td>
<td>19</td>
<td>5</td>
<td>4.9</td>
<td>2.10</td>
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<td></td>
<td></td>
<td></td>
<td>Increased</td>
<td>30</td>
<td>19</td>
<td>7.0</td>
<td>2.24</td>
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<td>4</td>
<td>49</td>
<td>F</td>
<td>Base line</td>
<td>21</td>
<td>10</td>
<td>8.3</td>
<td>1.03</td>
</tr>
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<td></td>
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<td>Increased</td>
<td>30</td>
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<td>15.5</td>
<td>0.70</td>
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<tr>
<td>5</td>
<td>45</td>
<td>F</td>
<td>Base line</td>
<td>19</td>
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<td>6.9</td>
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<td>Increased</td>
<td>29</td>
<td>22</td>
<td>16.7</td>
<td>1.79</td>
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<td>37</td>
<td>F</td>
<td>Base line</td>
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<td>1</td>
<td>2.3</td>
<td>2.63</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Increased</td>
<td>31</td>
<td>19</td>
<td>5.0</td>
<td>2.01</td>
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<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>Base line</td>
<td>20 ± 1</td>
<td>6 ± 1</td>
<td>5.0 ± 1.0</td>
<td>2.17 ± 0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Increased</td>
<td>32 ± 1*</td>
<td>20 ± 1*</td>
<td>9.7 ± 2.1*</td>
<td>1.71 ± 0.23*</td>
</tr>
</tbody>
</table>

\(P_{pa}\) and \(P_{la}\) are pulmonary arterial and left atrial pressures, respectively, referred to the posterior border of the left atrium. \(\dot{Q}_{lym}\) is lung lymph flow. Globulin includes all proteins except albumin.

* Significantly different from base line (\(P \leq 0.05\)).
### TABLE 3

#### Summary of Steady-State Data for Serotonin Studies

<table>
<thead>
<tr>
<th>Sheep</th>
<th>Condition</th>
<th>Base line</th>
<th>Serotonin</th>
<th>Base line</th>
<th>Serotonin</th>
<th>Base line</th>
<th>Serotonin</th>
<th>Base line</th>
<th>Serotonin</th>
<th>Base line</th>
<th>Serotonin</th>
<th>Base line</th>
<th>Serotonin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Base line</td>
<td>22</td>
<td>5</td>
<td>2.6</td>
<td>2.24</td>
<td>2.76</td>
<td>2.74</td>
<td>4.21</td>
<td>0.82</td>
<td>0.66</td>
<td>0.82</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Base line</td>
<td>19</td>
<td>7</td>
<td>3.4</td>
<td>1.85</td>
<td>2.55</td>
<td>2.38</td>
<td>3.90</td>
<td>0.78</td>
<td>0.65</td>
<td>0.77</td>
<td>0.59</td>
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<td>Base line</td>
<td>21</td>
<td>4</td>
<td>5.6</td>
<td>2.10</td>
<td>2.63</td>
<td>2.43</td>
<td>4.22</td>
<td>0.86</td>
<td>0.62</td>
<td>0.76</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Base line</td>
<td>22</td>
<td>4</td>
<td>7.2</td>
<td>1.65</td>
<td>2.50</td>
<td>2.07</td>
<td>3.29</td>
<td>0.80</td>
<td>0.76</td>
<td>0.64</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Base line</td>
<td>22</td>
<td>4</td>
<td>6.9</td>
<td>1.68</td>
<td>1.88</td>
<td>2.62</td>
<td>3.58</td>
<td>0.64</td>
<td>0.52</td>
<td>0.36</td>
<td>0.29</td>
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<td>6</td>
<td>Base line</td>
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<td>9</td>
<td>6.8</td>
<td>1.35</td>
<td>2.35</td>
<td>2.49</td>
<td>4.03</td>
<td>0.54</td>
<td>0.48</td>
<td>0.12</td>
<td>0.08</td>
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<tr>
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<td>Base line</td>
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<td>2.35</td>
<td>2.07</td>
<td>3.20</td>
<td>3.30</td>
<td>0.73</td>
<td>0.63</td>
<td>0.46</td>
<td>0.32</td>
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<tr>
<td></td>
<td>Base line</td>
<td>24</td>
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<td>8.1</td>
<td>1.43</td>
<td>2.49</td>
<td>2.15</td>
<td>3.93</td>
<td>0.66</td>
<td>0.63</td>
<td>0.47</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>Base line</td>
<td>20 ± 0.4</td>
<td>5 ± 1</td>
<td>5.4 ± 0.7</td>
<td>1.97 ± 0.18</td>
<td>2.48 ± 0.09</td>
<td>2.51 ± 0.13</td>
<td>3.91 ± 0.12</td>
<td>0.78 ± 0.05</td>
<td>0.64 ± 0.02</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serotonin</td>
<td>23 ± 1*</td>
<td>8 ± 1*</td>
<td>8.3 ± 1.3*</td>
<td>1.70 ± 0.13*</td>
<td>2.16 ± 0.01*</td>
<td>2.35 ± 0.08</td>
<td>3.84 ± 0.06</td>
<td>0.72 ± 0.04</td>
<td>0.56 ± 0.02*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

See Table 2 for explanation of abbreviations.

* Significantly different from base line (P < 0.05).

### TABLE 4

#### Steady-State Lymph-Plasma Concentration Ratios for Eight Protein Fractions

<table>
<thead>
<tr>
<th>Protein fraction</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective molecular radius (Å)</td>
<td>36</td>
<td>42</td>
<td>45</td>
<td>48</td>
<td>62</td>
<td>72</td>
<td>82</td>
<td>96</td>
</tr>
<tr>
<td>Base-line ratio (N = 15)</td>
<td>0.80 ± 0.04</td>
<td>0.76 ± 0.03</td>
<td>0.72 ± 0.03</td>
<td>0.74 ± 0.03</td>
<td>0.63 ± 0.03</td>
<td>0.60 ± 0.03</td>
<td>0.46 ± 0.04</td>
<td>0.49 ± 0.03</td>
</tr>
<tr>
<td>Increased pressure ratio (N = 6)</td>
<td>0.64 ± 0.05</td>
<td>0.64 ± 0.06</td>
<td>0.63 ± 0.05</td>
<td>0.55 ± 0.03</td>
<td>0.49 ± 0.09</td>
<td>0.52 ± 0.07</td>
<td>0.31 ± 0.04</td>
<td>0.28 ± 0.07</td>
</tr>
<tr>
<td>Serotonin ratio (N = 9)</td>
<td>0.72 ± 0.04</td>
<td>0.69 ± 0.04</td>
<td>0.65 ± 0.04</td>
<td>0.64 ± 0.03</td>
<td>0.51 ± 0.04</td>
<td>0.50 ± 0.03</td>
<td>0.47 ± 0.03</td>
<td>0.43 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± se.
Steady-state lymph flow for eight protein fractions as a function of effective molecular radius for base-line, increased pressure, and serotonin studies. The broken line was drawn empirically through the base-line data.

Renkin (17) has suggested that if transvascular protein movement is largely by diffusion then the permeability-surface area product \((PS)\) for a protein can be calculated by the equation:

\[
PS = \frac{LR}{1 - R} \tag{1}
\]

where \(L\) is lymph flow and \(R\) is the lymph-plasma concentration ratio for the protein. Several workers have suggested that a substantial fraction of transvascular protein movement is convective rather than diffusive (9, 18, 19). However, for comparison with published data, \(PS\) values calculated by Eq. 1 for the eight protein fractions that we measured are shown in Figure 5. \(PS\) declined with increasing molecular size, and none of the values in either the increased pressure or the serotonin studies was significantly different from the base-line value.

The ratio of the postmortem extravascular lung water to the dry weight of the bloodless lung in six sheep killed at the end of a 4-hour intravenous serotonin infusion at 4 \(\mu\)g/kg min\(^{-1}\) averaged 4.38 ± 0.86 (sd). The same value for six sheep prepared identically and killed under base-line conditions averaged 4.36 ± 0.16. The difference between the groups was not significant.

Serotonin Dose-Response Relationships.—Figure 6 illustrates the effects of increasing the serotonin infusion rate on vascular pressures, lung lymph flow, and lymph-plasma protein concentration ratios in one experiment. Each increase in serotonin caused a further increase in pressures and lymph flow, and a further decrease in the lymph-plasma protein concentration ratio. The lymph flow and the lymph-plasma protein ratio response during seven serotonin experiments in five sheep like the one illustrated in Figure 6 are shown in Figure 7. Lymph flow always increased as the serotonin infusion rate was increased. The lymph-plasma protein ratios declined with increasing serotonin infusion rates, and the decline continued to the highest infusion rates tested.

Discussion

According to the Starling equation (20), there are two basic mechanisms by which microvascular fluid filtration may increase: (1) an increase in the net transmural pressure gradient (hydrostatic gradient–osmotic gradient) or (2) an increase in the permeability-surface area product for the filtering membrane. Under steady-state conditions, lymph flow represents the net microvascular fluid filtration rate. In addition, the lymph protein concentration does not change in transit through lymph

\[
PS = \frac{LR}{1 - R} \tag{1}
\]
nodes (20) or peripheral lymphatics (18, 21) and therefore appears to represent the protein concentration in the microvascular filtrate. Several workers have used lymph measurements to study microvascular filtration in the lung (22, 23) and other organs (18, 24, 25).

We found that intravenous serotonin infusions in unanesthetized sheep caused a dose-related increase in lung lymph flow. Although serotonin also increased pulmonary arterial and left atrial pressures significantly, these pressure changes were small. Based on the studies of the responses to mechanically increased lung vascular pressure in the same sheep, the pulmonary arterial and left atrial pressure effects of serotonin seemed insufficient to account for the increase in lymph flow. However, serotonin has been shown to cause pulmonary venous constriction (6, 26), and such an increase in postmicrovascular resistance could increase the pressure in exchanging vessels out of proportion to changes in upstream and downstream pressures.

In the studies reported in the present paper and elsewhere (9), when lung vascular pressures were increased mechanically, lymph flow increased and the lymph-plasma protein concentration ratios fell. Similarly, the increased lymph flow produced by serotonin was accompanied by a fall in the lymph-plasma protein ratios. When lymph-plasma protein ratios as a function of lymph flow for steady-state serotonin and increased pressure studies were compared, they were indistinguishable. Thus, for a given lymph flow, serotonin caused the lymph-plasma protein ratios to decrease to a degree similar to that caused by mechanically increased pressure.

Lymph clearance for eight protein fractions ranging in molecular radius from 36 Å (albumin) to 96 Å during steady-state serotonin infusions was similar to that during steady-state increased pressure studies. Because the lymph flow increased relatively more than the lymph-plasma ratio decreased, clearance for several proteins increased significantly from the base-line level in both increased pressure and serotonin studies. However, this change does not mean that permeability was increased, since several theoretical models of microvascular exchange predict an increase in clearance with increasing lymph flow even when the permeability–surface area product is constant (9, 17). In fact, permeability–surface area products calculated by a diffusion model (17) were not significantly different from base line for any of the eight protein fractions we studied in either increased pressure or serotonin experiments. The effects of serotonin on lung lymph flow in our preparation apparently resulted from an increase in transmural pressure in exchanging vessels rather than an increase in vascular permeability. Although it is possible that higher doses of serotonin could affect permeability, the continued decline in lymph-plasma protein ratios which we saw with infusion rates up to 6 μg/kg min⁻¹ (Fig. 7) suggests that this possibility is unlikely.

Electron microscopic studies indicate that serotonin, like histamine, causes large (5000–8000 Å radius) gaps to appear between venular endothelial cells (3, 4). On the basis of these morphological changes, serotonin has been thought to increase vascular permeability; it has been termed a histamine-type mediator. However, the relevance of the morphological changes to serotonin effects on transvascular exchange has been questioned. Although Joyner et al. (27) and Carter et al. (28) saw increased dog leg lymph flow in response to serotonin, the effects on lymph-plasma protein ratios and blood-to-lymph dextran transport were like the effects of vasodilatory agents. They concluded that
SEROTONIN EFFECTS ON LUNG FLUID BALANCE

Serotonin does not increase permeability in the dog leg.

Serotonin could have caused the lung transmural pressure gradient to increase in our experiments either by increasing resistance on the venous side more than on the arterial side or by causing perimicrovascular pressure to decrease. Experimental data from anesthetized dogs and isolated, perfused dog lungs suggest that serotonin increases pulmonary vascular resistance (5, 6) and causes small pulmonary veins to constrict (6, 26). However, serotonin also causes pulmonary arteriolar resistance to increase, and several investigators have suggested that arteriolar resistance increases relatively more than does venous resistance (6, 26). From our data, it is impossible to tell whether serotonin increased pulmonary venous resistance more than arterial resistance, but it is possible that effects in the anesthetized sheep are different than those in the anesthetized dog or isolated, perfused lung preparations. Serotonin also causes airway constriction (5), reflected in our experiments by a moderate fall in arterial Po2 without significant pulmonary edema. It is possible that increased airway resistance caused intrapleural pressure to fall, resulting in a decrease in pressure around exchanging vessels. Such a change is highly speculative both because we did not measure airway resistance or intrapleural pressure and because it is not clear that decreases in intrapleural pressure cause pressure around exchanging vessels to decrease (29). Comparisons of the effects of serotonin with the effects of mechanically increased vascular pressure on lung fluid and protein filtration indicate that the serotonin effect is primarily a pressure effect, but our data do not permit conclusions about how transmural pressure in exchanging vessels is increased.

Even though serotonin does not appear to cause lung vascular permeability to increase, it may still be an important mediator in disorders in which pulmonary edema occurs without heart failure (1). It is likely that several endogenous vasoactive substances are released simultaneously (2); if vascular permeability is increased by another mediator, then the effects of serotonin on filtration will be greatly magnified.

References


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Mechanism of the serotonin effect on lung transvascular fluid and protein movement in awake sheep.
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