Monohydroxyeicosatetraenoic Acids (5-HETE and 15-HETE) Induce Pulmonary Vasoconstriction and Edema

Kenneth E. Burchop, William M. Selig, and Asrar B. Malik

5-, 15-, and 12-HETE (monohydroxyeicosatetraenoic acids) are products of the lipoxygenation of arachidonic acid. We investigated their role as possible mediators of pulmonary vasoactivity and pulmonary edema. Pulmonary artery pressure (Ppa), capillary pressure (Pcp), the change in lung wet weight (Δwt) from baseline, and capillary filtration coefficient (Kf) (as a measure of vascular permeability) were determined following an intravenous injection of each mono-HETE in lungs perfused at constant flow with either a phosphate-buffered Ringer's-albumin solution (PBR) or diluted blood. Injection of 2 μg of each compound into the pulmonary artery of lungs perfused with either PBR or diluted blood did not produce any effect. However, in PBR-perfused lungs, 4 μg 15-HETE induced increases in Ppa, Pcp, and lung wet weight (p<0.05), which were greater than the increases observed after 4 μg 5-HETE. Kf increased following both 5- and 15-HETE. The pulmonary vasoconstrictor and edemagenic responses of 5- and 15-HETE were attenuated by increasing perfusate albumin concentration from 0.5 to 1.5 g%. In contrast, 12-HETE (4 μg) had no effect on these parameters. In blood-perfused lungs, the pulmonary vascular responses to all HETE compounds (4 μg) were attenuated. In both Ringer's-albumin-perfused and blood-perfused lungs, the relative magnitude of the hemodynamic and fluid filtration responses to each mono-HETE were as follows: 15-HETE > 5-HETE > 12-HETE. In conclusion, the pulmonary vasoconstrictor and edemagenic effects of 5- and 15-HETE occur independently of blood-formed elements. 15-HETE causes greater pulmonary vasoconstriction and edema than 5-HETE. Both 5- and 15-HETE induce pulmonary edema, probably as a result of increased lung vascular permeability. The results indicate that 5- and 15-HETE are potent pulmonary inflammatory mediators.

(Circulation Research 1988;62:687–698)
fluid loss. Recirculation of perfusate (reservoir volume 250 ml) at 28 ml/min was begun after lungs had been washed with approximately 500 ml perfusate to clear the pulmonary circulation of blood.

Details of the perfusion system used in the present study have been previously described.28 Continuous pulmonary wet weight recordings were made on a three-channel Gould recorder (model 2200S, Cleveland, Ohio) calibrated so that a 0.5-g weight change resulted in a 5-cm recorder pen deflection. Pulmonary arterial and left atrial pressures were continuously monitored with transducers (Statham P50 and Gould P23ID) connected to catheters (PE90) placed in the pulmonary artery and left atrium. All monitored variables were stable, and lungs without intervention remained isogravimetric for up to 2 hours. All studies were carried out for a 70-minute period.

The perfusate used consisted of either a phosphate-buffered Ringer's solution (PBR) containing 0.5% bovine serum albumin (Fraction V, Sigma Chemical, St. Louis, Missouri), PBR containing 1.5% bovine serum albumin (Fraction V, Sigma), or blood diluted with PBR. Blood was obtained from heparinized (700 U/kg) donor guinea pigs by direct cardiac puncture. The heparinized blood was diluted (1:3 vol:vol) with PBR to a final hematocrit of 13 ± 2% and a final protein concentration of 1.5 ± 0.2%. In a separate group of experiments, the effects of varying protein concentrations in the blood on the pulmonary microvascular response to HETEs were examined because protein binding of HETEs14 may determine the magnitude of the response. For these studies, heparinized blood was obtained from donor guinea pigs in the manner described above. The whole blood was centrifuged at 1,500g at 4 °C for 10 minutes, varying amounts of the plasma were drawn off, and the blood cells were reconstituted back to the original volume with PBR containing 0.5% bovine serum albumin. This "protein poor" blood cell suspension was then diluted with PBR (1:3 vol:vol) to a final hematocrit of 15 ± 1%, and the protein concentration of this blood perfusate was determined.29 The Ringer's solution in all cases contained the following constituents (mM): NaCl 137, CaCl2, 1.8, MgCl2, 1.05, KCl 2.68, NaHCO3 0.06, NaH2PO4 0.130, Na2HPO4 0.869, and dextrose 5.55. The warmed (38 °C) perfusates were continuously gassed in the outflow reservoir with 95% O2-5% CO2, and pH, Pco2, and Po2 were periodically monitored with a Radiometer ABL2 blood gas analyzer (Copenhagen, Denmark).

**Pulmonary Capillary Pressure and Segmental Vascular Resistance**

Pulmonary capillary pressure (Pcap) was estimated using the double-occlusion technique of Linehan et al.30 The double-occlusion technique consisted of a brief (2-3-second) simultaneous occlusion of both arterial inflow and venous outflow during which the arterial pressure (Pao) decreased and venous pressure (Pv) increased to an equilibrium pressure that approximated Pcap.31 Pcap was used to partition resistance into upstream, or arterial (Rao), and downstream, or venous (Rv), components from the following equations where Q equals flow:

![Diagram](http://circres.ahajournals.org/)

**Figure 1.** Pathways and products of the arachidonic acid cascade. Included are structures of 5-, 15-, and 12-hydroxyeicosatetraenoic acids (HETEs).
Capillary Filtration Coefficient

The capillary filtration coefficient (Kc) was measured at specific intervals in all experimental groups under Zone III conditions. Following an isogravimetric period, the outflow pressure was rapidly elevated by 3 cm H2O for 5 minutes. P_v was measured immediately before the increase in P_v and at the end of the 5-minute elevation of P_v to obtain the change. The resulting increase in lung weight corresponds to a two-compartment model: a rapid component attributed to vascular filling and a slower component representing an increase in the interstitial volume attributed to transvascular fluid filtration. Kc was determined according to the method of Drake et al. The rate of lung weight gain was calculated for each minute following the rise in P_v and was expressed as a semilogarithmic function over time. The slow component of weight gain (corresponding to the increase in interstitial volume) was extrapolated to time 0 to obtain an estimate of the fluid filtration rate, which was divided by the change in P_v to obtain K_c. At the end of each experiment, nonpulmonary tissue was dissected from the lungs, and the lungs were dried to a constant weight at 70°C. K_c was expressed in units of ml/(min-cm H2O·g dry lung wt).

Experimental Protocols

A bolus injection of either 2 or 4 μg of each mono-HETE (5-, 15-, and 12-HETE) was made directly into the pulmonary artery of either PBR- or blood-perfused lungs following the baseline measurements. All parameters were then monitored for up to 70 minutes post-HETE injection. In control studies, hemodynamic or fluid filtration parameters did not change significantly for up to 2 hours following injection of saline in control lungs (n = 3). 15-HETE (obtained from The Upjohn Company, Kalamazoo, Michigan) was dissolved in hexane:ethanol (3:1) to a final concentration of 1.0 mg/ml, 5-HETE (U-68687, Upjohn) was dissolved in 25% EtoAc/hexane to a final concentration of 5.0 mg/ml, and 12-HETE (Biomol, Philadelphia, Pennsylvania) was dissolved in ethanol to a final concentration of 80.0 μg/ml. All the mono-HETEs used in these studies were routinely examined by reverse phase high-pressure liquid chromatography (HPLC) against appropriate standards prior to and after each series of experiments to ensure purity of the compounds.

Statistical Analysis

Data are expressed as mean±SEM. Statistical analysis in the perfusion experiments was performed using repeated measures analysis of variance followed by multiple comparisons testing using the Bonferroni t test. Statistical significance was accepted at p < 0.05.
Results

15-HETE

As seen in Figure 2, the injection of 4 μg 15-HETE into isolated guinea pig lungs perfused with PBR containing 0.5% albumin increased (p < 0.05) Pw (top) and Paw (bottom) within 20 minutes postinjection. Pw and Paw continued to rise and reached maximum values (mean ± SEM) of 15.2 ± 2.3 and 9.4 ± 0.9 cm H2O, respectively, at the end of the 70-minute experimental period. 15-HETE produced increases (p < 0.05) in both Rl (Figure 3; top) and Rv (Figure 3; bottom). The increases in Rl and Rv were similar in magnitude and paralleled the rises in Pw and Paw. Lung wet weight gradually increased over time following injection of 15-HETE (Figure 4) and reached a maximum value (mean ± SEM) of 2.3 ± 0.8 g over baseline (control guinea pig lung wet weight at baseline, 3.0 ± 0.1 g) at the end of the 70-minute experimental period. The final lung wet/dry weight ratio (mean ± SEM) was 10.1 ± 1.0. As seen in Figure 5, Kf increased (p < 0.05) approximately threefold over baseline at 70 minutes postinjection of 15-HETE. The increase in Kf closely paralleled the rise in lung wet weight. In two of the experiments (data not included in Figure 5), the lung wet weight increased at such a rapid rate by 70 minutes postinjection of 15-HETE that Kf determinations were not possible.

Injection of the same volume of the 15-HETE vehicle (i.e., 4 μL hexane:ethanol diluted 3:1) into control lungs (n = 6) produced relatively small changes (compared with injection of 15-HETE) in all parameters measured (Table 1). The final lung wet/dry weight ratio (mean ± SEM) was 7.8 ± 0.5. The magnitude of the changes in the vehicle control group were similar to the saline control group, and the values at 70 minutes postvehi-
were less than the 15-HETE challenged group (p<0.05).

In lungs perfused with diluted blood (final protein concentration 1.5 g%), the changes in hemodynamics and fluid filtration induced by injection of 4 μg 15-HETE (Figures 6, 7, and 8) were attenuated compared with the responses in lungs perfused with PBR (0.5% albumin). Following injection of 15-HETE, $P_{pa}$ increased from a baseline value of 9.4 ± 0.5 to 11.1 ± 0.4 cm H2O within 3 minutes and remained elevated (p<0.05) (2–3 cm H2O greater than baseline) for the duration of the study. $P_{pc}$ also increased from a baseline of 6.0 ± 0.2 to a maximum value of 8.7 ± 0.6 cm H2O at the end of the study (p<0.05). There was no change in $R_v$ following the 15-HETE injection, while there was an immediate increase (p<0.05) in $R_v$ (Figure 7). In blood-perfused lungs, injection of 15-HETE caused a small increase in lung wet weight of 0.43 ±0.21 g by the end of the 70-minute experimental period (Figure 8), but there was no change in $K_f$ from baseline (Figure 5). The vehicle alone did not produce any significant change in the measured parameters.

The pulmonary hemodynamic and fluid filtration responses to injection of 4 μg 15-HETE into lungs perfused with PBR containing 1.5 g% albumin (i.e., protein concentration the same as the concentration of the diluted blood perfusate and three times the

![Plot](Plot.png)

**Table 1.** Response to Injection of 4 μg 15-HETE Vehicle (Hexane/ETOH; 3:1) Into Ringer's-Albumin (0.5%)-Perfused Isolated Lungs

<table>
<thead>
<tr>
<th>Time postinjection (minutes)</th>
<th>Baseline</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>50</th>
<th>70</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{pa}$</td>
<td>6.9±0.4</td>
<td>7.3±0.7</td>
<td>7.4±0.5</td>
<td>7.7±0.6</td>
<td>7.7±0.5</td>
<td>7.7±0.4</td>
<td>7.8±0.5*</td>
<td>8.5±0.8*</td>
<td>8.9±0.8*</td>
</tr>
<tr>
<td>$P_{pc}$</td>
<td>4.6±0.3</td>
<td>—</td>
<td>5.0±0.3</td>
<td>5.2±0.4</td>
<td>5.3±0.3</td>
<td>5.2±0.3</td>
<td>5.2±0.3</td>
<td>5.8±0.4*</td>
<td>6.2±0.5*</td>
</tr>
<tr>
<td>$R_v$</td>
<td>0.08±0.02</td>
<td>—</td>
<td>0.08±0.02</td>
<td>0.09±0.01</td>
<td>0.08±0.02</td>
<td>0.09±0.02</td>
<td>0.09±0.02</td>
<td>0.10±0.02</td>
<td>0.10±0.02</td>
</tr>
<tr>
<td>$R_{pa}$</td>
<td>0.09±0.02</td>
<td>—</td>
<td>0.10±0.02</td>
<td>0.10±0.02</td>
<td>0.10±0.02</td>
<td>0.10±0.01</td>
<td>0.10±0.01</td>
<td>0.12±0.02</td>
<td>0.14±0.02*</td>
</tr>
<tr>
<td>$\Delta Wt$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.02±0.02</td>
<td>0.07±0.04</td>
<td>0.09±0.05</td>
<td>0.19±0.09*</td>
</tr>
<tr>
<td>$K_f$</td>
<td>3.74±0.33</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4.73±0.94</td>
<td>3.78±0.77</td>
<td>4.41±0.55</td>
<td>4.82±0.37</td>
<td>5.05±0.42</td>
</tr>
</tbody>
</table>

$P_{pa}$, pulmonary arterial pressure (cm H2O); $P_{pc}$, pulmonary capillary pressure (cm H2O); $R_{pa}$, pulmonary arterial resistance (cm H2O/ml/min); $R_v$, pulmonary venous resistance (cm H2O/ml/min); $\Delta Wt$, change in lung wet weight from baseline (g); $K_f$, capillary filtration coefficient (ml/min-cm H2O·g lung dry wt) x 10^-5; HETE, monohydroxyeicosatetraenoic acid. Values are mean±SEM; n=3.

*Different (p<0.05) from baseline.
PBR-0.5% albumin discussed above) are summarized in Table 2. The relative magnitude of changes in each hemodynamic parameter was similar to that seen in blood-perfused lungs. The change in lung wet weight from baseline (0.35 ± 0.049 g) at the end of the experiment was also similar to those observed in blood-perfused lungs given 15-HETE (0.43 ± 0.219 g), and there also was no change in $K_f$.

In those lungs in which the blood perfusate contained varying concentrations of protein (the perfusate protein concentration ranged from 0.5 to 0.8 g/100 ml), the...
injection of 4 µg 15-HETE produced increases in pressures and resistances similar to those in lungs perfused with diluted blood containing 1.5 g% protein (Table 3). However, the increases in lung wet weight and Kf produced by 15-HETE challenge were greater at the lower protein concentration (Table 3). At a protein concentration of 0.8 g/100 ml, the increases in weight and Kf were abolished to the same degree as in the lungs perfused with diluted blood (1.5 g% protein concentration), while the responses were evident at a protein concentration of 0.5 g% (Table 3).

Injection of 2 µg 15-HETE (n = 4) did not produce significant changes in the measured parameters with any of the different perfusates used (i.e., PBR or diluted blood).

5-HETE

Injection of 4 µg 5-HETE into lungs perfused with PBR containing 0.5% albumin caused small but significant increases in Ppa and Pexp by the end of the 70-minute experimental period (Figure 2). Ppa increased from a baseline of 7.1 ± 0.3 to 9.0 ± 0.6 cm H2O (mean ± SEM) at 70 minutes postinjection, while Pexp increased from a baseline of 5.2 ± 0.3 to 7.1 ± 0.4 cm H2O at 70 minutes postinjection. 5-HETE did not cause any change in R, but did increase Rv from a baseline of 0.11 ± 0.01 to 0.18 ± 0.02 cm H2O/ml/min by the end of the study (Figure 3). As seen in Figure 4, the lung wet weight increased within 50 minutes following injection of 5-HETE and reached a maximum value of 1.30 ± 0.43 g over baseline (baseline lung wet weight, 3.0 ± 0.1 g) by 70 minutes postinjection. The lung wet/dry weight ratio (mean ± SEM) was 9.5 ± 0.5. As seen in Figure 5, Kf increased (p < 0.05) within 10 minutes postinjection of 5-HETE and reached its maximum value (3.5 times baseline) at 70 minutes postinjection. Figure 9 shows a tracing from a representative experiment illustrating a Kf determination for control (baseline) and a Kf measurement made at 50 minutes postchallenge with 4 µg 5-HETE.

Injection of an equal volume of the vehicle in which the 5-HETE was dissolved did not produce significant changes in any measured parameter: Ppa was 7.0 ± 0.7 cm H2O at baseline, and the value at 70 minutes after the vehicle was 7.9 ± 0.3 cm H2O; the lung wet weight increased only 0.10 ± 0.07 g; and baseline Pexp was 4.7 ± 0.4 cm H2O compared with the 70 minutes postvehicle value of 5.7 ± 0.8 cm H2O.

In lungs perfused with diluted blood (protein concentration 1.5 g%), 5-HETE produced small but significant (p < 0.05) increases in Ppa from a baseline of 10.6 ± 0.3 to a 70-minute value of 12.8 ± 0.5 cm H2O and in Pexp from a baseline value of 6.9 ± 0.3 to a 70-minute value of 8.7 ± 0.5 cm H2O (Figure 6). There was no change in Rv following injection of 5-HETE, but there was a small (p < 0.05) increase in Rv (Figure 7) within 50 minutes postinjection of 5-HETE. As seen in Figure 8, lungs perfused with diluted blood remained

**Table 2. Effects of 15-HETE (4 µg) in Lungs Perfused With Ringer's-Albumin (1.5 g%) Solution**

<table>
<thead>
<tr>
<th>Time postinjection (minutes)</th>
<th>Baseline</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>50</th>
<th>70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ppa</td>
<td>6.5 ± 0.7</td>
<td>7.0 ± 0.7</td>
<td>7.1 ± 0.7</td>
<td>7.2 ± 0.7</td>
<td>7.1 ± 0.6</td>
<td>7.0 ± 0.6</td>
<td>7.1 ± 0.5</td>
<td>7.6 ± 0.5</td>
<td>8.2 ± 0.5*</td>
</tr>
<tr>
<td>Pexp</td>
<td>4.4 ± 0.3</td>
<td>---</td>
<td>4.8 ± 0.3</td>
<td>4.9 ± 0.3</td>
<td>4.9 ± 0.3</td>
<td>4.6 ± 0.2</td>
<td>4.6 ± 0.2</td>
<td>5.1 ± 0.2</td>
<td>5.7 ± 0.4*</td>
</tr>
<tr>
<td>Rv</td>
<td>0.07 ± 0.01</td>
<td>---</td>
<td>0.08 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.08 ± 0.02</td>
<td>0.09 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.09 ± 0.02</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>∆Wt</td>
<td>0.10 ± 0.02</td>
<td>0.09 ± 0.01</td>
<td>0.10 ± 0.02</td>
<td>0.10 ± 0.02</td>
<td>0.09 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.11 ± 0.01*</td>
<td>0.13 ± 0.01*</td>
</tr>
<tr>
<td>Kf</td>
<td>3.9 ± 0.66</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>5.14 ± 0.65</td>
<td>4.20 ± 0.89</td>
<td>3.00 ± 0.23</td>
<td>5.55 ± 0.90</td>
</tr>
</tbody>
</table>

Ppa, pulmonary arterial pressure (cm H2O); Pexp, pulmonary capillary pressure (cm H2O); Rv, pulmonary arterial resistance (cm H2O/ml/min); Rv, pulmonary venous resistance (cm H2O/ml/min); ∆Wt, change in lung wet weight from baseline (g); Kf, capillary filtration coefficient (ml/min-cm H2O-g lung dry wt) × 10^-2; HETE, monohydroxyeicosatetraenoic acid. Values are mean ± SEM, n = 3.

*Different (p < 0.05) from baseline.
### TABLE 3. Effects of 15-HETE (4 μg) in Lungs Perfused With Diluted Blood With Varying Concentrations of Proteins

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Protein</th>
<th>Baseline</th>
<th>70 minutes postinjection</th>
<th>Protein</th>
<th>Baseline</th>
<th>70 minutes postinjection</th>
<th>Protein</th>
<th>Baseline</th>
<th>70 minutes postinjection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>12.0</td>
<td>6.9</td>
<td>0.6</td>
<td>15.4</td>
<td>10.8</td>
<td>0.8</td>
<td>18.0</td>
<td>13.7</td>
</tr>
<tr>
<td>2</td>
<td>0.6</td>
<td>6.7</td>
<td>0.12</td>
<td>0.7</td>
<td>13.7</td>
<td>0.15</td>
<td>0.8</td>
<td>6.7</td>
<td>0.13</td>
</tr>
<tr>
<td>3</td>
<td>0.8</td>
<td>0.12</td>
<td>0.13</td>
<td>0.3</td>
<td>0.38</td>
<td>0.12</td>
<td>0.19</td>
<td>0.12</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Whole guinea pig blood was centrifuged, varying amounts of plasma were drawn off, and cells were diluted to a prescribed volume with a phosphate-buffered Ringer's solution containing 0.5% bovine serum albumin.

Protein, protein concentration of isolated lung perfusate (g/100 ml); \( P_{\text{pa}} \), pulmonary arterial pressure (cm H\(_2\)O); \( P_{\text{cap}} \), pulmonary capillary pressure (cm H\(_2\)O); \( R_{\text{p}} \), pulmonary arterial resistance (cm H\(_2\)O/ml/min); \( R_{\text{v}} \), pulmonary venous resistance (cm H\(_2\)O/ml/min); \( \Delta W_t \), change in lung wet weight from baseline (g); \( K_f \), capillary filtration coefficient (ml/min-cm H\(_2\)O-g lung dry wt) \( \times 10^{-2} \); HETE, monohydroxyeicosatetraenoic acid.

All values represent individual data points collected either during baseline or at 70 minutes postinjection of a 4-μg bolus of 15-HETE in each individual experiment.

### Notes
- The pulmonary hemodynamic and fluid filtration responses to injection of 4 μg 5-HETE into lungs perfused with PBR containing 1.5% albumin are summarized in Table 4. The relative magnitude of change over time in each hemodynamic parameter was isogravimetric for the entire 70-minute experimental period following injection of 4 μg 5-HETE, and there also was no change in \( K_f \) (Figure 8).

### Figure 9
Typical responses of an isolated guinea pig lung (perfused with phosphate-buffered Ringer's solution containing 0.5 g% albumin) at baseline (Panel A) and at 50 minutes postinjection of 4 μg 5-hydroxyeicosatetraenoic acid (HETE) (Panel B). Data for change in weight (\( \Delta W_t \), g) produced during capillary filtration coefficient (\( K_f \)) determination, mean pulmonary arterial pressure (\( P_{\text{pa}} \), cm H\(_2\)O), mean pulmonary venous pressure (\( P_{\text{v}} \), cm H\(_2\)O), and capillary pressure (\( P_{\text{cap}} \), cm H\(_2\)O) as estimated by double-occlusion technique are shown. Paper speed of recorder was transiently increased during \( P_{\text{cap}} \) measurements to aid in analysis. Evident is increase in \( K_f \) (increased slope of weight gain) and, thus, change in vascular permeability to water produced by 5-HETE.
very similar to that seen in the blood-perfused lungs containing 1.5 g% protein. There was also no significant change from baseline in either $K_t$ or lung wet weight at the end of the experiment.

In those lungs in which the perfusate contained diluted blood with concentrations of protein varying from 0.5 to 0.7 g%, the injection of 4 $\mu$g 5-HETE produced increases in pulmonary hemodynamics that were similar to those in lungs perfused with blood containing 1.5 g% protein (Table 5). As seen in Table 5, in the range of concentrations of protein examined, the increases in lung wet weight produced by injection of 5-HETE were not affected by the concentration of protein present. Injection of 2 $\mu$g 5-HETE ($n=3$) in either blood-perfused or PBR-albumin-perfused lungs did not cause any significant change in any measured parameter.

**12-HETE**

As seen in Figures 2, 3, and 4, the injection of 4 $\mu$g 12-HETE into lungs perfused with PBR-albumin did not cause any significant change in any measured parameter. 12-HETE also did not alter the lung wet/dry weight ratio and $K_t$ (Figures 2, 3, 4, and 5). Injection of 12-HETE (4 $\mu$g) into lungs perfused with diluted blood also did not produce any change in any measured parameter (Figures 5, 6, 7, and 8).

**Discussion**

In isolated guinea pig lungs perfused with PBR containing 0.5% albumin, injections of either 5-HETE or 15-HETE (4 $\mu$g) produced pulmonary vasoconstriction and edema. The edema may be the result of the rise in the vascular permeability in these lungs as evidenced by the increase in $K_t$, a measure of the lung vascular permeability to water. These changes were dose-dependent because a 2-$\mu$g injection of 5- or 15-HETE did not have an effect. The increased pulmonary vascular permeability and lung water content observed with 5- and 15-HETE in contrast to the effects of other lipoxygenase metabolites such as leukotrienes C and D (LTC and LTD), which do not result in an increase in vascular permeability and edema. Also, in contrast to 5- and 15-HETE, 12-HETE did not produce changes in pulmonary hemodynamics or lung fluid filtration. Based on magnitude of increases in $R_v$, the mono-HETEs may be ranked in order of potency as follows: 15-HETE > 5-HETE > 12-HETE.

### Table 4. Effects of 5-HETE (4 $\mu$g) in Lungs Perfused With Ringer's-Albumin (1.5 g%) Solution

<table>
<thead>
<tr>
<th>Time postinjection (minutes)</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>50</th>
<th>70</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{pa}$</td>
<td>7.1±0.9</td>
<td>7.6±0.7</td>
<td>7.8±0.6</td>
<td>7.8±0.8</td>
<td>7.6±0.7</td>
<td>7.6±0.6</td>
<td>7.8±0.1</td>
<td>8.2±0.6</td>
</tr>
<tr>
<td>$P_{exp}$</td>
<td>4.8±0.1</td>
<td>5.2±0.1</td>
<td>5.1±0.1</td>
<td>5.2±0.2</td>
<td>5.2±0.3</td>
<td>6.0±0.7*</td>
<td>6.2±0.8*</td>
<td></td>
</tr>
<tr>
<td>$R_p$</td>
<td>0.08±0.03</td>
<td>0.09±0.03</td>
<td>0.09±0.03</td>
<td>0.08±0.02</td>
<td>0.09±0.03</td>
<td>0.09±0.01</td>
<td>0.08±0.01</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>$R_v$</td>
<td>0.06±0.01</td>
<td>0.08±0.01</td>
<td>0.08±0.01</td>
<td>0.08±0.01</td>
<td>0.06±0.01</td>
<td>0.08±0.01</td>
<td>0.10±0.02*</td>
<td>0.11±0.03*</td>
</tr>
<tr>
<td>$\Delta W_t$</td>
<td>3.34±0.10</td>
<td>3.57±0.56</td>
<td>3.16±0.87</td>
<td>2.65±0.15</td>
<td>4.07±0.68</td>
<td>3.34±0.32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$P_{pa}$, pulmonary arterial pressure (cm H$_2$O); $P_{exp}$, pulmonary capillary pressure (cm H$_2$O); $R_p$, pulmonary arterial resistance (cm H$_2$O/ml/min); $R_v$, pulmonary venous resistance (cm H$_2$O/ml/min); $\Delta W_t$, change in lung wet weight from baseline (g); $K_t$, capillary filtration coefficient (ml/min-cm H$_2$O-g lung dry wt) x 10$^{-2}$; HETE, monohydroxyeicosatetraenoic acid. Values are mean ± SEM; $n=3$.

*Different (p<0.05) from baseline.

### Table 5. Effects of 5-HETE (4 $\mu$g) in Lungs Perfused With Diluted Blood With Varying Concentrations of Protein

<table>
<thead>
<tr>
<th>Protein</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>70 minutes postinjection</td>
<td>Baseline</td>
<td>70 minutes postinjection</td>
</tr>
<tr>
<td>Protein</td>
<td>0.5</td>
<td>10.0</td>
<td>6.6</td>
</tr>
<tr>
<td>$P_{pa}$</td>
<td>5.0</td>
<td>8.3</td>
<td>5.2</td>
</tr>
<tr>
<td>$P_{exp}$</td>
<td>6.6</td>
<td>8.1</td>
<td>0.10</td>
</tr>
<tr>
<td>$R_p$</td>
<td>0.12</td>
<td>0.11</td>
<td>0.08</td>
</tr>
<tr>
<td>$R_v$</td>
<td>0.14</td>
<td>0.10</td>
<td>0.24</td>
</tr>
<tr>
<td>$\Delta W_t$</td>
<td>0</td>
<td>0.76</td>
<td>0</td>
</tr>
</tbody>
</table>

Whole guinea pig blood was centrifuged, varying amounts of plasma were drawn off, and cells were diluted to a prescribed volume with a phosphate-buffered Ringer's solution containing 0.5% bovine serum albumin.

Protein, protein concentration of isolated lung perfusate (g/100 ml); $P_{pa}$, pulmonary arterial pressure (cm H$_2$O); $P_{exp}$, pulmonary capillary pressure (cm H$_2$O); $R_p$, pulmonary arterial resistance (cm H$_2$O/ml/min); $R_v$, pulmonary venous resistance (cm H$_2$O/ml/min); $\Delta W_t$, change in lung wet weight from baseline (g); $K_t$, capillary filtration coefficient (ml/min-cm H$_2$O-g lung dry wt) x 10$^{-2}$; HETE, monohydroxyeicosatetraenoic acid.

All values represent individual data points collected either during baseline or at 70 minutes postinjection of a 4-$\mu$g bolus of 5-HETE in each individual experiment.
The greater increases in \( P_a \) and \( P_{op} \) with 15-HETE than with 5-HETE corresponded to greater increases in both \( R_a \) and \( R_v \). The constrictor effects of 5-HETE were confined to the pulmonary venous segment of the vasculature. The increases in vascular resistance observed with 5- and 15-HETE were independent of changes in the circulating concentrations of either thromboxane or prostacyclin over time (unpublished observation), which is in contrast to pulmonary vasoconstriction induced by LTC4 and LTD4, whose pulmonary vasoactive effects are mediated in some species by the release of thromboxane A2.35

15-HETE produced a greater increase in lung wet weight than 5-HETE. The greater rise in \( P_{op} \) induced by 15-HETE may partially explain the greater transvascular fluid filtration (as assessed by lung wet weight gain over time); however, both 15- and 5-HETE caused significant increases in \( K_f \). The greater increase in lung wet weight over time produced by 15-HETE may be the result of both increased pulmonary vessel wall permeability to water and capillary hydrostatic pressure, while 5-HETE appears to cause pulmonary edema primarily through an increase in vascular permeability. Both compounds produced significant increases in wet/dry lung weight compared with lungs challenged with either 12-HETE or vehicle controls.

The mechanism by which 5- and 15-HETE increase pulmonary vascular permeability is not apparent from these studies. It is known that 12-HETE (the major platelet lipooxygenase metabolite), 5-HETE (the major neutrophil lipooxygenase monohydroxy fatty acid metabolite), and 15-HETE (a hydroxylated fatty acid product produced primarily by neutrophils) are rapidly incorporated into the phospholipids and triglycerides of human neutrophils,48 mouse peritoneal macrophages,57 mouse macrophage-like tumor cells,60 cultured bovine aortic endothelial and smooth muscle cells,77 and human umbilical vein endothelial cells.88

The time required for maximal uptake of mono-HETEs by cells is between 15 and 30 minutes following exposure.13,36,37 The increases in pulmonary vascular pressures and lung wet weight produced by either 5- or 15-HETE in the present study also reached significant levels between 15 and 30 minutes following injection. The incorporation and substitution of these monohydroxy fatty acid products of leukocytes and platelets could alter the functional properties and characteristics of cell membranes,14,17,37 such as membrane fluidity,25 as well as induce protein kinase C activation,39 which may explain the increase in vascular permeability to water. Another result of incorporation of these monoHETEs into cellular membranes may be their removal from the circulation17,26-28 and their metabolism to other polar products.17,20

The results of the experiments using blood as the lung perfusate demonstrated that blood-formed elements do not amplify the pulmonary vasoactive and microvascular fluid filtration responses to injection of 5-, 15-, and 12-HETE. When the lungs were perfused with blood containing 1.5 g% protein rather than with the Ringer's solution containing 0.5 g% protein, the pulmonary hemodynamic changes produced by the mono-HETEs were markedly attenuated and the increases in lung wet weight and \( K_f \) were abolished. These experiments suggest that the reduction of the pulmonary vascular response to the mono-HETEs in blood-perfused lungs may be related to protein binding of the HETEs.

Two separate experiments were performed to examine if the pulmonary vascular response to the HETEs was reduced by the perfusate protein concentration (i.e., albumin binding) or metabolism of HETEs by blood-formed elements. The first experiment used PBR containing 1.5 g% (versus 0.5 g%) albumin. This protein concentration approximated the mean protein concentration of the blood perfusate. Injection of the same dosage of each HETE (4 \( \mu \)g into lungs perfused with this higher concentration of albumin produced responses similar to those observed in blood-perfused lungs. Pulmonary vascular resistance as well as the lung wet weight and \( K_f \) values were attenuated to the same degree as with the blood perfusate. Administration of 12 \( \mu \)g 5- and 15-HETE at the end of two experiments (a threefold increase in dosage from 4 to 12 \( \mu \)g correlating with the similar threefold increase in albumin concentration from 0.5 to 1.5 g%) produced a response similar to that seen in lungs perfused with the lower 0.5 g% albumin concentration (i.e., lung wet weight and vascular pressures increased) (unpublished observations). The second experiment involved reducing the protein concentration of the blood perfusate. We accomplished this by centrifuging the whole blood, removing the plasma, and adding varying concentrations of albumin plus PBR to the blood cells. The increases in lung wet weight and \( K_f \) produced by 15-HETE were attenuated as the protein concentration increased (threshold concentration was approximately 0.7 g%). At a protein concentration of 0.5 g% in the blood perfusate, the increases in lung weight and \( K_f \) with 15-HETE were similar to those seen in the lungs perfused with PBR containing 0.5% albumin. On the other hand, injection of 4 \( \mu \)g 5-HETE into lungs perfused with blood (protein concentrations ranging from 0.5 to 0.7 g%) resulted in responses similar to lungs perfused with blood having a higher protein concentration. In both blood-perfused experiments, the increases in lung wet weight were less than those seen in lungs perfused with the cell-free PBR solution containing 0.5% albumin, and there was no significant change in \( K_f \) from baseline. Therefore, the magnitude of the pulmonary vascular permeability response to 15-HETE is limited primarily by the amount of protein present in the perfusate, and the response to 5-HETE is limited by both the perfusate protein concentration (i.e., albumin binding) and by the removal of 5-HETE by blood-formed elements.

12-HETE did not have any effects on hemodynamics or fluid balance in lungs perfused with either PBR containing albumin or blood. This lower level of potency of 12-HETE compared with other mono-HETEs has also been reported for other properties of 12-HETE such as 12-HETE's chemotactic ability for
neutrophils\textsuperscript{15,16} and eosinophils.\textsuperscript{21} In contrast to both 5- and 15-HETEs, which are both hydroxylated fatty acids produced primarily by polymorphonuclear leukocytes (PMNs), 12-HETE is produced primarily by platelets and is the major lipoxygenase metabolite of platelets. This finding is consistent with observations that lung vascular injury and subsequent edema formation in a variety of models involves PMN-endothelial interactions, and not platelets.\textsuperscript{35}

In conclusion, the present study indicates that both 5- and 15-HETE are pulmonary vasoconstrictors (15-HETE > 5-HETE) and that both mono-HETEs increase lung vascular permeability. The pulmonary vascular effects of 5- and 15-HETE are not amplified by the presence of blood-formed elements, but rather, the effects are attenuated in blood-perfused lungs. The attenuation following 15-HETE appears to be the result of albumin binding, while the attenuation of the response to 5-HETE challenge in blood may be due to both albumin binding and metabolism of 5-HETE by blood-formed elements. The increased pulmonary vascular permeability and lung water content observed with 5- and 15-HETE are marked, and these changes contrast with other lipoxygenase metabolites such as LTC\textsubscript{4} and LTD\textsubscript{4}, which do not increase vascular permeability and lung water content.\textsuperscript{11} 12-HETE, in contrast to 5- and 15-HETEs, does not directly increase pulmonary vascular resistance or lung fluid filtration.

The mono-HETEs are generated in sufficient quantities to be detected in vivo in animal models of acute lung injury\textsuperscript{22-28} and attain maximum detectable concentrations coincident\textsuperscript{22,25} with increased vascular permeability. These lipoxygenation products are locally generated by inflammatory cells such as neutrophils, and their microvascular effects may be modulated by the local concentrations of albumin and blood-formed elements in the microvessel. The present study indicates that both 5-HETE and 15-HETE are pulmonary inflammatory mediators and may be involved in the neutrophil-dependent pulmonary vascular injury and edema.

Acknowledgments

The authors thank Dr. Herbert Johnson at The Upjohn Company for providing the 5- and 15-HETEs used in these experiments and Dr. William Jubi at the Albany Veterans Administration Hospital for HPLC analysis of the HETEs.

References

8. O'Flaherty JT: Neutrophil degranulation: Evidence pertaining to its mediation by the combined effects of leukotriene B\textsubscript{4}, platelet-activating factor, and 5-HETE. J Cell Physiol 1985;122:229-239
25. Perlman MB, Jubiz WA, Blumenstock FA, Malik AB: Generation of lipoxynase products in pulmonary lymph after thrombin induced microembolism: Relationship to increased...
36. Stenson WF, Parker CW: 12-L-hydroxy-5,8,10,14-eicosatetraenoic acid, a chemoattractant fatty acid, is incorporated into neutrophil phospholipids and triglycerides. Prostaglandins 1979;18:285–292
38. Frawley CL, Johnson AR, Campbell WB: Specific incorporation of 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) into phosphatidylcholine (PC) in human endothelial cells (abstract). Fed Proc 1984;43:757

Key Words • monohydroxyeicosatetraenoic acids • isolated guinea pig lungs • lipoygenase metabolites • pulmonary vasoconstriction • pulmonary hemodynamics • capillary filtration coefficient • albumin-Ringer's perfusate • blood perfusate • hematocrit
Monohydroxyeicosatetraenoic acids (5-HETE and 15-HETE) induce pulmonary vasoconstriction and edema.
K E Burhop, W M Selig and A B Malik

doi: 10.1161/01.RES.62.4.687

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/62/4/687

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/