Acute alveolar hypoxia increases blood-to-tissue albumin transport: role of atrial natriuretic peptide

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Albert, T. S. E., V. L. Tucker, and E. M. Renkin. Acute alveolar hypoxia increases blood-to-tissue albumin transport: role of atrial natriuretic peptide. J. Appl. Physiol. 82(1): 111–117, 1997.—Plasma immunoreactive atrial natriuretic peptide (irANP) and blood-to-tissue clearance of $^{131}$I-labeled rat serum albumin ($C_{RSA}$) were examined in anesthetized rats during hypoxic ventilation ($n = 5–7$). Hypoxia (10 min) increased irANP from $211 \pm 29$ (room air) to $229 \pm 28$ (15% $O_2$; not significant), $911 \pm 205$ (10% $O_2$), and $4374 \pm 961$ pg/ml (8% $O_2$), respectively. Graded increases in $C_{RSA}$ were significant at 8% $O_2$ in fat (3.6-fold), ileum (2.2-fold), abdominal muscles (2.0-fold), kidney (1.8-fold), and jejunum (1.4-fold). $C_{RSA}$ was decreased in back skin and testes; heart, brain, and lungs were unaffected. The increases in $C_{RSA}$ were related to irANP and not to arterial $P_O_2$. Circulating plasma volume was negatively correlated with whole body $C_{RSA}$. Graded increases in extravascular water content (EVW) were found in the kidney, left heart, and cerebrum and were positively related to $C_{RSA}$ in the kidney. EVW decreased in gastrointestinal tissues; the magnitude was inversely related to $C_{RSA}$. We conclude that ANP-induced protein extravasation contributes to plasma volume contraction during acute hypoxia.

METHODS

General. Measurements of blood-tissue transport were made over the last 35 min of the 50-min gas treatment period in the four groups of rats described in the companion paper (1). Details of the experimental setup and hypoxia protocols are provided in the companion paper, with this paper reporting only those procedures related to the albumin transport. Briefly, male Wistar rats (Charles River, Kingston, NY) weighing 260–320 g were anesthetized with pentobarbital sodium (12 mg/100 g) administered subcutaneously in two depots over a 1-h period. Arterial and venous cannulas were inserted for pressure measurement, infusion of fluids and tracers, and for collection of blood samples. A tracheal cannula was inserted through a neck incision, and the opposite end was attached to a rodent ventilator for mechanical ventilation during gas treatment. Pentobarbital sodium was given intravenously as needed to maintain anesthesia.

Experimental protocols. Four groups of animals ($n = 5–7$) were studied: normoxic controls (room air) and three hypoxic (15, 10, and 8% $O_2$-balance $N_2$) groups. Gas treatment was applied via a Douglas bag attached to the ventilator.Baseline blood samples were taken 10 min before the start of the gas treatment period (time (t) = 0 min). A second set of blood samples was collected after 10 min of gas treatment (t = 10 min). Alumina evacuation was measured during the last 35 min of the gas ventilation by using a double-isotope subtraction technique (15). To start the measurement, $^{131}$I-RSA was injected intravenously as needed to maintain anesthesia. At t = 45 min (30 min after $^{131}$I-RSA injection), the clearance measurement was completed by injection of $^{125}$I-RSA (6 µCi), which served as a "reference tracer" for measurement of tissue intravascular blood volumes. Terminal blood samples were collected 5 min later at t = 50 min after the start of gas ventilation (35 min after injection of $^{131}$I-RSA). The rats were then killed with intravenous KCl, and selected tissues were collected for determination of radioactivity and water content. As described in the companion paper (1), the amounts of injected $^{131}$I-RSA and $^{125}$I-RSA were known, enabling the 20- and 50-min samples to be used to calculate circulating PV at these times. Blood samples were centrifuged, and aliquots of plasma were diluted to 1 ml and placed in count tubes for determination of $^{131}$I and $^{125}$I activities. Uniform tissue samples were rapidly dissected postmortem, blotted, and placed in covered
vials for $^{131}$I and $^{125}$I assay. Entire tissues or organs were collected in the following cases: left lateral gastrocnemius, heart (left and right ventricles), kidney, brain (cerebellum and right cerebral cortex), and testis. For back skin, abdominal muscle, visceral fat, lung (right middle lobe and left lower lobe), and intestine (jejunum, ileum, cecum, and colon), samples uniform in size and anatomic location were collected. Weights of samples varied from 0.2 to 1.3 g among the different tissues and organs but were similar within each category. After the tissue samples were weighed, 100 μl of ethanol were added to each, and the vials were placed in count tubes for determination of $^{131}$I and $^{125}$I activities. Plasma samples, tissue samples, and tracer standards were counted in $^{131}$I and $^{125}$I channels of a gamma scintillation counter (Searle Analytical, Des Plaines, IL) for 20 min. For most samples, well >10,000 counts/sample were collected. The counts were corrected for background, crossover from $^{131}$I to $^{125}$I (including effect of sample volume on crossover), and decay of $^{131}$I. Crossover of $^{131}$I to the $^{125}$I channel was measured in each experiment and ranged from 10.9 to 11.3%. After tissues were counted, they were placed in an over (95°C) and dried to constant weight (±1 mg). To estimate EVW, total water contents (wet wt − dry wt) were corrected by subtracting plasma and erythrocyte water from the total wet weight (18).

Calculation of albumin clearance. Final distribution volume of $^{131}$I- and $^{125}$I-RSA in organ and tissue samples was calculated by dividing tissue counts by counts per milliliter of final plasma. The volume of $^{125}$I-RSA in the tissues (after 5-min exposure) represents mainly intravascular (“small vessel”) PV. After 35 min of circulation, the volume of $^{131}$I-RSA in the tissues represents not only intravascular PV but also an apparent distribution volume of $^{131}$I-RSA extravasated during the experimental period. Subtraction of the $^{125}$I-RSA PV from the $^{131}$I-RSA volume yields the extravascular albumin (CRSA) over 30 min. For most tissues, interstitial fluid volumes are much larger than the extravascular distribution of tracer in 30 min (21); therefore, CRSA is taken as an estimate of unidirectional albumin transport (albumin flux per unit plasma concentration). A correction was made for a small drop in plasma $^{131}$I activity that occurred during the experimental period by dividing clearance values by the ratio of average to final $^{131}$I counts. Average $^{131}$I activity was obtained by approximate integration (trapezoidal rule) of the 5-, 15-, and 35-min counts (t = 20, 30, and 50 min of treatment, respectively). CRSA values were normalized to tissue blood-free dry weight and expressed as microliters per minute per gram (μl·min$^{-1}$·g$^{-1}$).

Materials. RSA (Cohn Fraction V, Sigma Chemical, St. Louis, MO) was iodinated with $^{131}$I- and $^{125}$I (New England Nuclear-DuPont, Wilmington, DE) by using chloramine-T and purified by anion exchange and repeated concentration-dilution with Minicon filters (Amicon, Danvers, MA). Labeled RSA (specific activity = 10–50 μCi/mg) was repurified on the day of use to reduce free $^{131}$I to <1%.

Statistics. Data are expressed as means ± SE unless otherwise indicated. Differences among treatment means were tested by using one-way analysis of variance followed by orthogonal contrasts and Duncan’s new multiple-range test for post hoc comparisons among individual means. Relationships among variables were evaluated by using linear (simple and multiple) regression analyses. Differences are reported as statistically significant when P ≤ 0.05.

RESULTS

General. Cardiovascular and blood gas data collected under baseline conditions and at different time points during gas treatment are detailed in the companion study (1) and are briefly summarized here. A subset of data collected at the beginning and end of the CRSA measurement period is recorded in Table 1. Because CRSA represents albumin extravasation averaged over the last 30 min of the treatment period, pooled averages of irANP, PV, and plasma total protein concentration were also calculated and are summarized in Table 1. Blood gas analyses were not performed on radioactive samples; hence pooled values of arterial PaO$_2$ (PaO$_2$) are not included in Table 1 (see Ref. 1 for supplemental blood gas data).

Ventilation with either 15 or 10% O$_2$ for 10 min resulted in a graded reduction in PaO$_2$ from 80 ± 3 to 32 ± 1 Torr, whereas no further reduction in PaO$_2$ was observed during ventilation with 8% O$_2$. Plasma irANP, on the other hand, increased in a dose-dependent fashion in all groups, with an average 20-fold elevation observed in rats ventilated with 8% O$_2$. The irANP response developed gradually in rats ventilated with 15% O$_2$ (PaO$_2$ values between 42 and 50 Torr). A more rapid response was observed with more severe hypoxia (PaO$_2$ values <40 Torr), with large increases in irANP occurring within 10 min and persisting throughout the CRSA measurement period. The irANP response to hypoxia was also associated with small but significant decreases in circulating PV. Although increases in plasma total protein concentration were also suggested, they were proportionally smaller than the decrements in PV and were not statistically different from control values. Taken together, these data indi-

<p>| Table 1. PaO$_2$, irANP levels, PV, and plasma TP concentrations during normoxic and hypoxic ventilation |
|----------------|----------------|---------|-------|----------|---------|</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>PaO$_2$, Torr</th>
<th>irANP, pg/ml</th>
<th>PV, μl/g</th>
<th>TP, mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>10 min$^*$</td>
<td>20 min$^*$</td>
<td>50 min$^*$</td>
</tr>
<tr>
<td>Room air</td>
<td>7</td>
<td>80 ± 3</td>
<td>211 ± 29</td>
<td>199 ± 28</td>
</tr>
<tr>
<td>15% O$_2$</td>
<td>6</td>
<td>46 ± 1</td>
<td>229 ± 28</td>
<td>328 ± 44</td>
</tr>
<tr>
<td>10% O$_2$</td>
<td>5</td>
<td>32 ± 1*</td>
<td>911 ± 205*</td>
<td>1,168 ± 416*</td>
</tr>
<tr>
<td>8% O$_2$</td>
<td>6</td>
<td>35 ± 1*</td>
<td>4,374 ± 961*</td>
<td>2,104 ± 634*</td>
</tr>
</tbody>
</table>

Values are means ± SE, n. No. of rats. PaO$_2$, arterial PaO$_2$; irANP, immunoreactive atrial natriuretic peptide; PV, plasma volume; TP, total protein. *Significantly different from room air control, P = 0.05. † Values for each time period were pooled before averaging. ‡ PV values are missing in 1 rat due to a technical error, and irANP values from a separate rat were omitted because the 50-min value was an outlier ( >3 SD from group mean). $^*$Time from start of gas treatment (clearance of $^{131}$I-labeled rat serum albumin was measured between 15 and 50 min).
cate that both fluid and protein were extravasated in the most severely hypoxic groups.

**Effect of hypoxia on CRSA.** Under normoxic conditions, 131I-RSA extravasation rates into individual tissues were similar to our previous measurements (15, 18). The various tissues and organs represented a 140-fold range of basal CRSA, from 0.029 ± 0.004 µl·min⁻¹·g⁻¹ for fat to 4.05 ± 0.79 µl·min⁻¹·g⁻¹ for lung. The mean effects of graded hypoxic ventilation on CRSA in individual tissues are shown in Fig. 1. One rat from the 15% O₂ group has been omitted from Fig. 1 because its response behavior clearly deviated (>3 SD) from the remainder of the group. This rat exhibited a large increase in plasma irANP at 50 min (2,976 pg/ml), and CRSA was elevated two times or more basal values in several tissues. The remaining five rats ventilated with 15% O₂ showed small but significant increases in irANP (+43%) by 50 min with no detectable change in CRSA in any tissue. Four out of five rats ventilated with 10% O₂ showed variable increases in CRSA in one or more tissues; however, group averages were not significantly greater than in controls. In contrast, all six rats ventilated with 8% O₂ exhibited elevated CRSA in multiple tissues, with the average CRSA being significantly higher than room air controls in visceral fat (3.6-fold), ileum (2.2-fold), abdominal muscle (2.0-fold), kidney (1.8-fold), and jejunum (1.4-fold). Higher CRSA values were also noted in the lateral gastrocnemius, cecum, and colon; these differences did not reach statistical significance. In back skin and testis, CRSA was significantly reduced in both 10 and 8% O₂ groups. Brain, heart, and lung clearances did not differ from control values.

**Relationship between plasma irANP and CRSA.** Figure 2 illustrates changes in CRSA plotted as a function of either PaO₂ (A) or the logarithm of average plasma irANP concentrations (B). All data points from control and hypoxic groups are included in these graphs, including the aberrant 15% O₂ rat in which irANP was elevated. PaO₂ levels were similar between 8 and 10% O₂ groups, yet irANP and CRSA reached their highest levels in the 8% O₂ animals. The P values obtained from single regression analyses suggest that irANP was a better predictor of the CRSA response to hypoxia. Furthermore, when both factors were analyzed simulta-

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**Fig. 1.** Effects of room air (control) and graded hypoxic gas ventilation of anesthetized rats on extravasation of tracer albumin [131I-rat serum albumin (RSA) clearance (CRSA)] into various tissues. Clearances were measured over last 30 min of a 50-min period of gas treatment. Tissues are arranged in increasing order of control clearances. R, right; L, left; Visc, visceral; Ab, abdominal; Lat Gas, lateral gastrocnemius; Bk, back. *Significantly different from control, P ≤ 0.05.
neously by multiple regression, irANP alone (at constant $P_{A02}$) was a significant factor in the $C_{RSA}$ response to hypoxia, whereas $P_{A02}$ was not. In comparison, the decreases in $C_{RSA}$ observed in back skin and testis were not as strongly related to either irANP ($P = 0.06$ for both tissues) or $P_{A02}$ ($P > 0.5$) when both factors were included in the regression analysis.

The tissue specificity of the $C_{RSA}$ response to hypoxia in this study was strikingly similar to previous observations in rats given exogenous ANP (18, 22, 24). In our own experiments (18), infusion of synthetic ANP (400 ng·kg$^{-1}$·min$^{-1}$) in rats given supplemental fluids to maintain PV increased clearance of $^{131}$I-BSA into gastrointestinal tissues (2.9–4.6-fold), kidney (2-fold), fat (1.9-fold), skeletal muscles (1.6-fold) and left ventricle (1.5-fold) but not in skin or lungs (brain and testis were not sampled). However, the magnitude of $C_{RSA}$ response for a given increment in irANP was greater in these previous experiments, particularly for gastrointestinal tissues. Regression slope coefficients relating $C_{RSA}$ to log irANP obtained in normovolemic rats given variable doses of ANP have been included in Fig. 2B (dashed lines) to illustrate this difference. [From Tucker et al. (18).]

PV and EVW. If elevated protein extravasation was a factor in the observed PV reductions during hypoxia, we would expect to find 1) a negative correlation between whole body $C_{RSA}$ and circulating PV and 2) a positive correlation between $C_{RSA}$ and water content of individual tissues. The former relationship is illustrated in Fig. 3, in which changes in PV during the clearance period are plotted as a function of whole body $C_{RSA}$, which was obtained by subtracting the $^{131}$I-RSA (35-min) distribution volume from the $^{125}$I-RSA (5-min) distribution volume. These data indicate that even though most of the hypoxia-induced PV reduction occurred before the clearance measurement (see Table 1), there was a definite linear relationship between decreases in PV and increments in $C_{RSA}$ during the clearance measurement period ($P = 0.0001$, $r^2 = 0.7132$).

Small-vessel PV, measured at the termination of the experiment as the tissue $^{125}$I-RSA (5-min) distribution

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**Fig. 2.** Correlation of $C_{RSA}$ with arterial $P_{O2}$ ($P_{A02}$) and plasma immunoreactive atrial natriuretic peptide (irANP) during graded hypoxia in individual rats. Inspired $O2$ levels are indicated as follows: room air ( ), 15% ( ), 10% ( ), 8% ( ). A: $C_{RSA}$ vs. $P_{A02}$. $P_{A02}$ was measured at beginning of clearance period (10 min after start of gas treatment). B: $C_{RSA}$ vs. log irANP. irANP values were obtained by pooling measurements collected at beginning and end of clearance period. Solid lines, regression slopes (with model P values). Dashed lines, regression slopes for same tissues (Lat Gas substituted for Ab muscle) in anesthetized rats infused with exogenous ANP representing concentration-response relations under baseline conditions in normoxic rats. [From Tucker et al. (18).]
Tissue PV and EVW during normoxic and hypoxic ventilation

Table 2.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>PV, µl/g</th>
<th></th>
<th></th>
<th></th>
<th>EVW, µl/g</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Room Air</td>
<td>15% O₂</td>
<td>10% O₂</td>
<td>8% O₂</td>
<td>Room Air</td>
<td>15% O₂</td>
<td>10% O₂</td>
</tr>
<tr>
<td></td>
<td>(n = 7)</td>
<td>(n = 6)</td>
<td>(n = 5)</td>
<td>(n = 6)</td>
<td>(n = 7)</td>
<td>(n = 6)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td>Visceral fat</td>
<td>11.2 ± 1.4</td>
<td>10.9 ± 1.2</td>
<td>11.0 ± 1.8</td>
<td>8.5 ± 0.9</td>
<td>112 ± 8</td>
<td>114 ± 10</td>
<td>114 ± 7</td>
</tr>
<tr>
<td>Abdominal muscle</td>
<td>16.9 ± 1.6</td>
<td>17.3 ± 2.6</td>
<td>13.5 ± 0.7</td>
<td>16.8 ± 1.8</td>
<td>1,066 ± 40</td>
<td>3,134 ± 45</td>
<td>3,035 ± 64</td>
</tr>
<tr>
<td>Lateral gastrocnemius</td>
<td>24.9 ± 1.7</td>
<td>23.3 ± 1.0</td>
<td>20.7 ± 0.9</td>
<td>24.6 ± 1.4</td>
<td>3,264 ± 26</td>
<td>3,233 ± 18</td>
<td>3,235 ± 34</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>46.6 ± 3.8</td>
<td>42.5 ± 7.9</td>
<td>41.5 ± 5.8</td>
<td>51.4 ± 4.3</td>
<td>3,858 ± 60</td>
<td>3,918 ± 104</td>
<td>3,771 ± 67</td>
</tr>
<tr>
<td>Right cerebrum</td>
<td>31.1 ± 4.0</td>
<td>31.8 ± 3.8</td>
<td>22.6 ± 2.1</td>
<td>30.6 ± 4.2</td>
<td>3,911 ± 75</td>
<td>3,996 ± 66</td>
<td>4,018 ± 43</td>
</tr>
<tr>
<td>Back skin</td>
<td>17.1 ± 2.5</td>
<td>15.1 ± 1.9</td>
<td>11.7 ± 2.6</td>
<td>11.1 ± 0.7</td>
<td>1,851 ± 30</td>
<td>1,848 ± 59</td>
<td>1,937 ± 34</td>
</tr>
<tr>
<td>Colon</td>
<td>64.1 ± 6.8</td>
<td>59.9 ± 5.1</td>
<td>62.4 ± 6.4</td>
<td>65.8 ± 7.5</td>
<td>3,530 ± 108</td>
<td>3,355 ± 136</td>
<td>3,249 ± 63</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>301 ± 15</td>
<td>329 ± 25</td>
<td>293 ± 19</td>
<td>235 ± 30</td>
<td>2,746 ± 75</td>
<td>2,842 ± 59</td>
<td>2,742 ± 102</td>
</tr>
<tr>
<td>Ileum</td>
<td>74.0 ± 4.4</td>
<td>62.5 ± 5.5</td>
<td>68.1 ± 6.2</td>
<td>68.2 ± 7.6</td>
<td>2,900 ± 80</td>
<td>2,938 ± 72</td>
<td>3,122 ± 25</td>
</tr>
<tr>
<td>Cecum</td>
<td>89.5 ± 6.2</td>
<td>84.8 ± 6.1</td>
<td>73.4 ± 6.2</td>
<td>87.4 ± 5.6</td>
<td>3,578 ± 98</td>
<td>3,471 ± 90</td>
<td>3,414 ± 149</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>186 ± 10</td>
<td>181 ± 19</td>
<td>173 ± 11</td>
<td>141 ± 11†</td>
<td>2,733 ± 64</td>
<td>2,806 ± 39</td>
<td>2,750 ± 119</td>
</tr>
<tr>
<td>Jejunum</td>
<td>89.8 ± 10</td>
<td>82.9 ± 7.4</td>
<td>73.7 ± 5.7</td>
<td>85.9 ± 5.1</td>
<td>3,131 ± 125</td>
<td>3,133 ± 48</td>
<td>3,109 ± 43</td>
</tr>
<tr>
<td>Testis</td>
<td>45.9 ± 1.3</td>
<td>39.3 ± 4.0</td>
<td>33.0 ± 3.3†</td>
<td>32.9 ± 2.6†</td>
<td>7,108 ± 58</td>
<td>6,979 ± 42</td>
<td>6,956 ± 70</td>
</tr>
<tr>
<td>Kidney</td>
<td>570 ± 29</td>
<td>535 ± 35</td>
<td>516 ± 29</td>
<td>519 ± 30</td>
<td>2,763 ± 36</td>
<td>2,908 ± 63</td>
<td>2,972 ± 98*</td>
</tr>
<tr>
<td>Lungs</td>
<td>876 ± 92</td>
<td>1,010 ± 87</td>
<td>1,089 ± 188</td>
<td>885 ± 84</td>
<td>3,688 ± 188</td>
<td>4,060 ± 116</td>
<td>4,309 ± 288</td>
</tr>
</tbody>
</table>

Values are means ± SE. n. No. of rats; EVW, extravascular water. PV are 125I-RSA 5-min distribution volumes normalized to body wt (g). EVW values are expressed as volume per gram tissue blood-free dry weight. PV and EVW were measured after 50 min of room air or hypoxic ventilation. *Significantly different from room air, P < 0.05. †Significant linear trend shown by regression analysis relative to level of hypoxia, P < 0.05.
present study. However, a negative relationship between whole body CRSA and circulating PV was clearly present in tissues previously found to be “ANP insensitive” (e.g., back skin). Furthermore, the presence of factors that blunt the action of released ANP on albumin and fluid extravasation in the hypoxic state could explain the leveling off of PV reduction and hematocrit increase after their initial changes in the rats exposed to 8 and 10% \( \text{O}_2 \). In hypoxic states where tissue perfusion and hydrostatic are better maintained (i.e., in conscious models), elevated \( C_{\text{RSA}} \) would likely have a proportionally greater effect on PV redistribution into extravascular spaces.

Transfer of protein from the circulation to the interstitial compartment acts to augment transfer of fluid volume, potentiating the loss of PV and accumulation of interstitial fluid (16). Of what advantage to a hypoxic animal is increased extravasation of fluid and protein? Loss of circulating PV could certainly be considered detrimental to systemic circulatory function at a time when increased blood supply is needed to compensate for decreased arterial \( \text{O}_2 \) content. However, there is considerable evidence that hypoxia-induced ANP release benefits cardiopulmonary function by reducing hypoxia-induced pulmonary hypertension (3, 8, 10). The antihypertensive action of ANP on the pulmonary circulation could occur directly, by relaxing pulmonary arterioles (7), or indirectly, by reducing cardiac output in the face of increased pulmonary vascular resistance (11, 19). The relationship between \( \text{irANP} \) and \( C_{\text{RSA}} \) observed in the present study suggests that an additional effect of ANP could be to reduce cardiac output and filling pressures by increasing microvascular fluid filtration in peripheral tissues. In contrast to the minimal diuresis observed in our hypotensive pentobarbital sodium-anesthetized rats, renal excretory function is usually enhanced during acute hypoxia in conscious humans and animals (5). Under these conditions, a transvascular shift of protein into peripheral tissues would have the important consequence of minimizing the rise in plasma colloid osmotic pressure during diuresis, thus favoring PV reduction over the loss of interstitial fluid.

In humans exposed to 3–5 h of a simulated altitude of 4,500 m (\( \text{PO}_2 \approx 40 \) Torr), Parving (14) observed a small increase in the transcapillary escape rate of \( ^{131}\text{I}-\text{albumin} \) from 5.6 to 6.5%, which did not reach statistical significance. This led to the conclusion that increased albumin extravasation was unlikely to have contributed to the 7% reduction in PV observed in these subjects. Average increases in whole body \( C_{\text{RSA}} \) in hypoxia groups were also small and insignificant in the present study. However, a negative relationship between whole body \( C_{\text{RSA}} \) and circulating PV was clearly evident and statistically significant (Fig. 3). This finding suggests that on a whole body basis, the albumin extravasation rate is an insensitive index to changes in the driving force for fluid filtration. This can be at least partially explained by the nonlinear osmotic properties of serum albumin, which dictate disproportionately larger deviations in osmotic pressure per unit change in albumin concentration. Another problem is that whole body extravasation is dominated by visceral tissues that comprise only a small fraction of the total extracellular fluid volume. Taking these factors into account, we conclude that the modest increases in local extravasation rates in the present study resulted in the observed redistribution of extracellular fluid.

The lack of a detectable increase in EVW in tissues where \( C_{\text{RSA}} \) was elevated is not surprising for the following reasons. First, given that the total EVW is much larger than the plasma space, a PV loss of the magnitude observed in these experiments (=15%) would have a relatively small impact on EVW, particularly if it were distributed over several tissues. Second, it is unlikely that either capillary hydrostatic pressures or exchange surface areas were static in these experiments. Hypoxic ventilation resulted in prominent hypotension in all groups and reduced cardiac output in rats ventilated with 8% \( \text{O}_2 \) (see Ref. 1). Thus it is possible that decrements in capillary hydrostatic pressure and flow opposed increases in filtration induced by albumin extravasation. This might also explain why EVW decreased during hypoxia in most of the gastrointestinal samples. Interestingly, the change in EVW (\( \Delta \text{EVW} \)) appeared to be inversely related to the magnitude of \( C_{\text{RSA}} \) elevation (\( \Delta C_{\text{RSA}} \)) in these tissues, with ileum showing the smallest \( \Delta \text{EVW} \) (\( \Delta C_{\text{RSA}} = 2.2\text{-fold} \)) and colon showing the largest \( \Delta \text{EVW} \) (\( \Delta C_{\text{RSA}} = 1.3\text{-fold} \)). These data suggest that increases in \( C_{\text{RSA}} \) antagonized absorptive forces in these tissues.

In light of the known permeability actions of ANP (6, 18, 24), it has been suggested that ANP contributes to hypoxia-induced pulmonary edema (4). In the present study, lung \( C_{\text{RSA}} \) was unaffected by hypoxia, although EVW tended to increase in rats ventilated with 15 and 10% \( \text{O}_2 \) (\( P = 0.09 \)). The variability of EVW data was higher in the lungs than in other tissues; therefore, the possibility of a Type II error (i.e., the probability of failing to detect a real difference) cannot be ruled out. Interestingly, rats given 8% \( \text{O}_2 \) (i.e., those with the greatest \( \text{irANP} \) response) had mean EVW values that were more similar to control. In this case, it is possible that a reduction in cardiac output, as was observed in a group of identically treated rats (1), could have contributed to a lower EVW via a reduction in pulmonary microvascular pressure. When regression analysis was performed on all data except those from the 8% \( \text{O}_2 \) group, we found no significant correlation between average ANP levels and lung EVW (\( r^2 = 0.12 \)). Taken together, these observations do not support increased permeability as a mechanism linking ANP to pulmonary edema. However, given the high variability in lung EVW and the possibility that cardiac output was altered in the most severely hypoxic animals, a definitive relationship between ANP and lung fluid balance, whether it be positive or negative, cannot be ascertained from this study.

Increased pulmonary capillary transit time subsequent to reduction of cardiac output might favor uptake of \( \text{O}_2 \) from alveolar gas (20). This is not likely to be of consequence under conditions where reductions of alveolar \( \text{Po}_2 \) occur in the presence of normal alveolar mem-
branes but could be beneficial when arterial hypoxemia is the result of pulmonary edema or fibrosis of the alveolar-blood interface. In this regard, ANP-induced PV reduction could improve arterial blood oxygenation by reducing pulmonary blood flow and minimizing pulmonary edema. This interpretation is supported by our finding of similar (if not higher) PaO2, during constant ventilation with 8% O2 compared with 10% O2.

Summary. In anesthetized rats ventilated with 10% or 8% O2, plasma ANP concentrations were elevated, and there was loss of fluid and protein from the plasma. In animals exposed to 8% O2, transport of tracer albumin was elevated in several tissues previously shown to be most sensitive to the action of exogenous ANP (abdominal muscle, visceral fat, kidney, jejunum, ileum) but not in ANP-insensitive tissues (lungs, heart, skin). In individual tissues, the increases in albumin transport showed significant correlation with plasma concentrations of iANP but less so with levels of PaO2. A notable depression in iANP sensitivity compared with previously observed effects of exogenous ANP was attributed to systemic vasoconstriction in the hypoxic rats, with subsequent reduction of functional capillary surface area. Loss of protein into peripheral tissues was associated with reductions of circulating PV during acute hypoxia. In intestinal tissues, decreases in EVW during 8% O2 were inversely related to the magnitude of C RSA elevation. We conclude that ANP-induced protein extravasation antagonizes reabsorptive forces, thus favoring PV contraction during acute hypoxia. ANP-induced volume contraction could act in compensatory fashion by either improving arterial oxygenation or antagonizing pulmonary hypertension during acute hypoxia. Whether such a mechanism contributes importantly to the physiology or pathophysiology of hypoxia in the conscious state remains to be determined.

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