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

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 125I-labeled rat serum albumin (CRSA) were examined in anesthetized rats during hypoxic ventilation (n = 5–7/group). Hypoxia (10 min) increased irANP from 211 ± 29 (room air) to 229 ± 28 (15% O2, not significant), 911 ± 205 (10% O2), and 4,374 ± 961 pg/ml (8% O2), respectively. Graded increases in CRSA were significant at 8% O2 in fat (3.6-fold), ileum (2.2-fold), abdominal muscles (2.0-fold), kidney (1.8-fold), and jejunum (1.4-fold). CRSA was decreased in back skin and testes; heart, brain, and lungs were unaffected. The increases in CRSA were related to irANP and not to arterial PO2. Circulating plasma volume was negatively correlated with whole body CRSA. Graded increases in extravascular water content (EVW) were found in the kidney, left heart, and cerebrum and were positively related to CRSA in the kidney. EVW decreased in gastrointestinal tissues; the magnitude was inversely related to CRSA. We conclude that ANP-induced protein extravasation contributes to plasma volume contraction during acute hypoxia.

plasma volume; capillary permeability; albumin clearance; edema

IN A COMPANION PAPER (1), we described the changes in plasma immunoreactive atrial natriuretic peptide concentration (irANP) produced by exposure of anesthetized rats to graded alveolar hypoxia. We showed that within 10 min of ventilation with 10% or 8% O2, there was a large increase in plasma irANP that was sustained throughout a 50-min period of hypoxia. There was also an early decrement in plasma volume (PV) and a corresponding increase in hematocrit, with little change in plasma protein concentration. Similar reductions in intravascular volume are produced by intravenous administration of ANP, suggesting that this peptide acts at the capillary membrane to increase permeability to fluid and plasma proteins (2, 19). Subsequent studies utilizing direct measurements of plasma protein transport in intact rats (18, 24) and in single capillaries of frog mesentery (6) have corroborated this view.

The purpose of the present study was to evaluate the role of ANP in mediating increases in protein and fluid extravasation during acute hypoxia. These are the same experiments as reported in the companion paper (1); however, here we focus on events occurring at the tissue level. Blood-to-tissue albumin transport was measured in multiple tissues by using a sensitive double-isotope technique. PV and extravascular water (EVW) contents of individual tissues were also assessed. Our main finding was that ventilation hypoxia increased albumin extravasation into abdominal muscles, visceral fat, kidney, and gastrointestinal tissues in a dose-dependent manner. Of all the parameters measured, irANP was the best predictor of albumin extravasation in these tissues. The importance of hypoxia-induced protein extravasation to fluid shifts occurring at whole-animal and tissue levels is discussed.

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% O2-balance N2) 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). Albumin extravasation was measured during the last 35 min of the gas ventilation by using a double-isotope subtraction technique (15). To start the measurement, 131I-labeled rat serum albumin (131I-RSA, 6 µCi) was injected intravenously at t = 15 min. Blood samples (100 µl) were collected 20 and 30 min after the start of gas treatment and 5 and 15 min after isotope infusion for measurement of plasma 131I activity. These blood samples were replaced with similar volumes of lactated Ringer solution. At t = 45 min (30 min after 131I-RSA injection), the clearance measurement was completed by injection of 125I-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 131I-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 131I-RSA and 125I-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 131I and 125I 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 oven (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 volumes of $^{131}$I- and $^{125}$I-RSA in organ and tissue samples were calculated by dividing tissue count rates 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 $^{125}$I-RSA extravasated during the experimental period. Subtraction of the $^{125}$I-RSA PV from the $^{131}$I-RSA volume yields the extravascular albumin accumulation, measured as a plasma clearance of albumin (C$_{RSA}$) over 30 min. For most tissues, interstitial fluid volumes are much larger than the extravascular distributions of tracer in 30 min (21); therefore, C$_{RSA}$ 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 calculated by dividing counts per milliliter of final plasma by the number of counts/sample. The clearance values were normalized to PaO$_2$, irANP levels, PV, and plasma TP concentrations during normoxic and hypoxic ventilation (Table 1).

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 C$_{RSA}$ measurement period is recorded in Table 1. Because C$_{RSA}$ 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 Po$_2$ (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 C$_{RSA}$ 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>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. *No. of rats. PaO$_2$, arterial PO$_2$; 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 $C_{\text{RSA}}$. Under normoxic conditions, $^{131}\text{I-RSA}$ 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 $C_{\text{RSA}}$, 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 $C_{\text{RSA}}$ in individual tissues are shown in Fig. 1. One rat from the 15% O$_2$ 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 $C_{\text{RSA}}$ was elevated two times or more basal values in several tissues. The remaining five rats ventilated with 15% O$_2$ exhibited small but significant increases in irANP (+43%) by 50 min with no detectable change in $C_{\text{RSA}}$ in any tissue. Four out of five rats ventilated with 10% O$_2$ showed variable increases in $C_{\text{RSA}}$ in one or more tissues; however, group averages were not significantly greater than in controls. In contrast, all six rats ventilated with 8% O$_2$ exhibited elevated $C_{\text{RSA}}$ in multiple tissues, with the average $C_{\text{RSA}}$ 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 $C_{\text{RSA}}$ values were also noted in the lateral gastrocnemius, cecum, and colon; these differences did not reach statistical significance. In back skin and testis, $C_{\text{RSA}}$ was significantly reduced in both 10 and 8% O$_2$ groups. Brain, heart, and lung clearances did not differ from control values.

Relationship between plasma irANP and $C_{\text{RSA}}$. Figure 2 illustrates changes in $C_{\text{RSA}}$ plotted as a function of either $\text{PaO}_2$ (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% O$_2$ rat in which irANP was elevated. $\text{PaO}_2$ levels were similar between 8 and 10% O$_2$ groups, yet irANP and $C_{\text{RSA}}$ reached their highest levels in the 8% O$_2$ animals. The $P$ values obtained from single regression analyses suggest that irANP was a better predictor of the $C_{\text{RSA}}$ response to hypoxia. Furthermore, when both factors were analyzed simulta-

![Fig. 1. Effects of room air (control) and graded hypoxic gas ventilation of anesthetized rats on extravasation of tracer albumin ($^{131}\text{I-rat serum albumin (RSA) clearance (C_{RSA})}$ 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, $P \leq 0.05$.](http://jap.physiology.org/10.1152/jappl.1998.82.5.113)
neously by multiple regression, irANP alone (at constant PaO₂) was a significant factor in the CRSA response to hypoxia, whereas PaO₂ was not. In comparison, the decreases in CRSA observed in back skin and testis were not as strongly related to either irANP (P = 0.06 for both tissues) or PaO₂ (P > 0.5) when both factors were included in the regression analysis.

The tissuespecificity of the CRSA 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 131I-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 CRSA response for a given increment in irANP was greater in these previous experiments, particularly for gastrointestinal tissues. Regression slope coefficients relating CRSA 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 CRSA and circulating PV and 2) a positive correlation between CRSA 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 CRSA, which was obtained by subtracting the 131I-RSA (35-min) distribution volume from the 125I-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 CRSA during the clearance measurement period (P = 0.0001, r² = 0.7132).

Small-vessel PV, measured at the termination of the experiment as the tissue 125I-RSA (5-min) distribution
volume, and tissue EVW content evaluated by desiccation of the tissue samples are recorded in Table 2 for each treatment group. In testis, PV in rats ventilated with 10 or 8% O$_2$ were significantly lower than in controls. For back skin and left ventricle, average PV were not different between groups by mean comparisons; however, linear-regression analyses indicated significant positive correlations between PV and the fraction of inspired O$_2$, suggesting that hypoxia decreased PV in these tissues as well. Differences in EVW between control and hypoxia groups were insignificant for most tissues, including several of the tissues where C$_{RSA}$ was elevated. The jejunum, cecum, and colon all exhibited a trend toward lower EVW with increasing severity of hypoxia, and mean EVW in 8% O$_2$ rats was significantly decreased in both jejunum (−10%) and colon (−13%) compared with that in controls. Of all the tissues with elevated C$_{RSA}$ during hypoxia, increased EVW was manifest only in the kidney. Water content was also increased in the left ventricle and right cerebrum; however, these changes appeared to be unrelated to changes in C$_{RSA}$.

**DISCUSSION**

In the companion paper (1), it was shown that plasma ANP levels were more closely related to the fraction of inspired O$_2$ (“alveolar hypoxia”) than to PaO$_2$ in arterial blood (“arterial hypoxemia”). This distinction allows us to evaluate the contribution of these factors to the observed changes in blood-to-tissue albumin transport. In tissues where increased C$_{RSA}$ was observed, these changes were better explained by elevations in plasma irANP as opposed to decrements in PaO$_2$. The increases in C$_{RSA}$ were greater in 8% O$_2$-treated animals, which had higher irANP levels, even though average PaO$_2$ values were not different from animals ventilated with 10% O$_2$.

Although changes in irANP and C$_{RSA}$ during hypoxia were clearly related, some of the tissues (most notably the jejunum and ileum) responded in a weaker manner compared with our own studies in nonhypoxic rats infused with exogenous ANP (see Fig. 2). It seems reasonable to attribute this decreased sensitivity to known compensatory hormonal and hemodynamic changes produced by hypoxia. Increases in plasma vasopressin (12), sympathoadrenal activity (9), and chemoreceptor stimulation (13) during hypoxic ventilation could decrease microvascular pressures and/or surface area for exchange via selective vasoconstriction of peripheral tissues. Other investigations (23) reported selective gastrointestinal vasoconstriction in dogs given intravenous ANP in the presence of autonomic blockade. Thus it is possible that decreases in jejunum, cecum, and colon EVW observed in the present study resulted from ANP-mediated vasoconstriction. Hypoxia-induced reductions in tissue perfusion were also sug-

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**Table 2. Tissue PV and EVW during normoxic and hypoxic ventilation**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Room Air (n=7)</th>
<th>15% O$_2$ (n=6)</th>
<th>10% O$_2$ (n=5)</th>
<th>8% O$_2$ (n=6)</th>
</tr>
</thead>
<tbody>
<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>
</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>
</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>
</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>
</tr>
<tr>
<td>Right cerebrum</td>
<td>31.1 ± 4.0</td>
<td>31.8 ± 3.0</td>
<td>22.6 ± 2.1</td>
<td>30.6 ± 4.1</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>
</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>
</tr>
<tr>
<td>Right ventricle</td>
<td>301 ± 15</td>
<td>329 ± 25</td>
<td>293 ± 19</td>
<td>235 ± 30</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>86.2 ± 7.6</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>
</tr>
<tr>
<td>Left ventricle</td>
<td>186 ± 10</td>
<td>181 ± 19</td>
<td>173 ± 11</td>
<td>141 ± 13</td>
</tr>
<tr>
<td>Jejunum</td>
<td>88.8 ± 10.0</td>
<td>82.9 ± 7.4</td>
<td>73.7 ± 5.7</td>
<td>85.9 ± 5.1</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>
</tr>
<tr>
<td>Kidney</td>
<td>570 ± 29</td>
<td>535 ± 35</td>
<td>516 ± 29</td>
<td>519 ± 30</td>
</tr>
<tr>
<td>Lungs</td>
<td>876 ± 92</td>
<td>1,010 ± 87</td>
<td>1,089 ± 188</td>
<td>885 ± 84</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Room Air (n=7)</th>
<th>15% O$_2$ (n=6)</th>
<th>10% O$_2$ (n=5)</th>
<th>8% O$_2$ (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EVW, µl/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visceral fat</td>
<td>112 ± 8</td>
<td>114 ± 10</td>
<td>114 ± 7</td>
<td>101 ± 5</td>
</tr>
<tr>
<td>Abdominal muscle</td>
<td>3,066 ± 40</td>
<td>3,134 ± 45</td>
<td>3,035 ± 64</td>
<td>3,011 ± 33</td>
</tr>
<tr>
<td>Lateral gastrocnemius</td>
<td>3,264 ± 26</td>
<td>3,233 ± 18</td>
<td>3,235 ± 34</td>
<td>3,265 ± 14</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>3,858 ± 60</td>
<td>3,918 ± 104</td>
<td>3,771 ± 67</td>
<td>3,629 ± 110</td>
</tr>
<tr>
<td>Right cerebrum</td>
<td>3,911 ± 75</td>
<td>3,996 ± 66</td>
<td>4,018 ± 43</td>
<td>4,116 ± 66†</td>
</tr>
<tr>
<td>Back skin</td>
<td>1,851 ± 30</td>
<td>1,848 ± 59</td>
<td>1,937 ± 34</td>
<td>1,850 ± 47</td>
</tr>
<tr>
<td>Colon</td>
<td>3,503 ± 108</td>
<td>3,355 ± 136</td>
<td>3,249 ± 63</td>
<td>3,064 ± 107†</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>2,746 ± 75</td>
<td>2,842 ± 59</td>
<td>2,742 ± 102</td>
<td>2,851 ± 88</td>
</tr>
<tr>
<td>Ileum</td>
<td>2,900 ± 80</td>
<td>2,938 ± 72</td>
<td>3,122 ± 25</td>
<td>2,994 ± 83</td>
</tr>
<tr>
<td>Cecum</td>
<td>3,578 ± 98</td>
<td>3,471 ± 90</td>
<td>3,414 ± 149</td>
<td>3,382 ± 86</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>2,733 ± 64</td>
<td>2,806 ± 39</td>
<td>2,750 ± 119</td>
<td>3,060 ± 78††</td>
</tr>
<tr>
<td>Jejunum</td>
<td>3,131 ± 125</td>
<td>3,133 ± 48</td>
<td>3,109 ± 43</td>
<td>2,817 ± 74††</td>
</tr>
<tr>
<td>Testis</td>
<td>7,108 ± 58</td>
<td>6,979 ± 42</td>
<td>6,956 ± 70</td>
<td>6,959 ± 56</td>
</tr>
<tr>
<td>Kidney</td>
<td>7,263 ± 36</td>
<td>7,290 ± 63</td>
<td>2,972 ± 98*</td>
<td>3,003 ± 74††</td>
</tr>
<tr>
<td>Lungs</td>
<td>3,688 ± 188</td>
<td>4,060 ± 116</td>
<td>4,309 ± 288</td>
<td>3,794 ± 269</td>
</tr>
</tbody>
</table>

Values are means ± SE. n. No. of rats; EVW, extravascular water. PV are $^{125}$I-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.
gested by our observation of significantly lower \( C_{RSA} \) and tissue PV values 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 hematoctrit increase after their initial changes in the rats exposed to 8 and 10% \( O_2 \). In hypoxic states where tissue perfusion and hydrostatic are better maintained (i.e., in conscious models), elevated \( C_{RSA} \) 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 circulation function at a time when increased blood supply is needed to compensate for decreased arterial \( O_2 \) 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 irANP and \( C_{RSA} \) 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 pentobartital 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 \((P_O_2 = 40 \text{ Torr})\), Parving (14) observed a small increase in the transcapillary escape rate of \(^{131}I\) 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 \( C_{RSA} \) in hypoxia groups were also small and insignificant in the present study. However, a negative relationship between whole body \( C_{RSA} \) 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 \( C_{RSA} \) 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 pressure 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% \( O_2 \) (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 of \( \Delta EVW \) appeared to be inversely related to the magnitude of \( C_{RSA} \) elevation \((\Delta C_{RSA}) \) in these tissues, with ileum showing the smallest \( \Delta EVW \) \((\Delta C_{RSA} = 2.2\text{-fold}) \) and colon showing the largest \( \Delta EVW \) \((\Delta C_{RSA} = 1.3\text{-fold}) \). These data suggest that increases in \( C_{RSA} \) 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 \( C_{RSA} \) was unaffected by hypoxia, although EVW tended to increase in rats ventilated with 15 and 10% \( O_2 \) (\( P = 0.09 \)). 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% \( O_2 \) (i.e., those with the greatest 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% \( O_2 \) 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 \( O_2 \) from alveolar gas (20). This is not likely to be of consequence under conditions where reductions of alveolar \( P_O_2 \) 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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REFERENCES


