IN ADDITION to its potent diuretic, natriuretic, and vasodilator properties, atrial natriuretic peptide (ANP) also causes an increase in hematocrit not fully accounted for by urinary fluid loss (4). This rise in hematocrit is due to a decrease in plasma volume resulting from a transfer of plasma fluid and proteins from the intravascular to the interstitial compartment (1, 6, 27, 30–32, 35, 40). Such an effect of ANP may result from an increase in filtration pressure, filtration surface area, and/or permeability at the capillary level. Although the mechanism of this effect is still not fully understood, it requires the presence of angiotensin II (ANG II) (27). Moreover, we recently reported that the hemoconcentrating but not the hypotensive activity of ANP was blunted in rats with experimental diabetes mellitus (32), a model associated with resistance to the renal actions of ANP (24) as well as with alterations in the renin-angiotensin system (12).

Both clinical and experimental nephrotic syndromes are associated with altered fluid balance and blood volume homeostasis (9). Two models of experimental nephrosis have been particularly useful in exploring nephrotic sodium metabolism. They result from the intravenous injection of the anthracyclins, adriamycin, which induces a glomerular lesion similar to minimal change nephropathy in humans (3), and from the intraperitoneal administration of anti-glomerular epithelial cell (anti-Fx1A) antibodies (23) to induce passive Heymann nephritis (PHN), a model of membranous nephropathy in humans. Both models are characterized by no change or an increase in the plasma concentration of ANP (9, 28, 33) and blunted renal responsiveness to exogenously administered ANP (8, 14, 19) and/or to endogenously secreted ANP elicited by volume expansion (2, 5, 28, 33). In addition, patients with nephrotic syndrome have altered renal responses to changes in plasma ANP induced by either water immersion or isotonic volume expansion (11, 20, 21). However, to date no information is available regarding the extrarenal effect of ANP on transcapillary shift of plasma fluid toward the interstitium in the nephrotic state.

In the present studies, we found that rats with nephrotic syndrome resulting from PHN exhibited blunted hemoconcentration in response to ANP infusion despite a normal hypotensive response. The blunted extrarenal response to ANP was restored by the simultaneous infusion of a subpressor dose of ANG II. In contrast, rats with adriamycin nephrosis had both hypotensive and hemoconcentrating responses to infused ANP that did not differ from those in control rats.

**METHODS**

Animal model. We carried out studies in male Sprague-Dawley rats (Bantin-Kingman, Fremont, CA) housed in climate-controlled conditions and provided standard rat laboratory diet and water ad libitum. Two models of nephrotic syndrome were studied. Adriamycin nephrosis was induced with a single intravenous injection of adriamycin (doxorubicin, Sigma Chemical, St. Louis, MO; 7–8 mg/kg at a concentration of 10 mg/ml) dissolved in normal saline to seven rats
weighing 140–160 g (3, 28). PHN was induced by the intraperitoneal injection of anti-Fx1A antiserum (5 ml/kg body wt) to 17 rats weighing 140–200 g (13, 23, 33). Appropriate control rats, matched for age and weight at the time of the adriamycin or anti-Fx1A administration, received an equal volume of normal saline (n = 31). Rats were studied 21–28 and 9–14 days after the injection of adriamycin or the anti-Fx1A antiserum, respectively. To determine 24-h water intake, urine volume, and daily urinary excretion of sodium, potassium, and proteins, rats were placed in metabolic cages on the last day of the experiment.

General procedure. On the day of the acute experiment, animals were anesthetized with an intraperitoneal injection of 100 mg/kg of Inactin (Andrew Lockwood and Associates, Sturtevant, WI) and placed on a heated table to maintain rectal temperature at 37 ± 0.5°C. Animals underwent tracheostomy and breathed spontaneously; they were prepared for acute experimentation as previously described (27, 29–32). Briefly, catheters were inserted into a femoral artery and vein and the right carotid artery for sampling blood, infusing fluids and drugs, and continuous measurement of arterial pressure via a Statham pressure transducer (model P23 ID, Gould Instruments, Oxnard, CA) connected to a Grass polygraph (model 7D, Grass Instrument, Quincy, MA). Both kidneys were then removed through retroperitoneal flank incisions. During the surgical preparation, rats received a constant intravenous infusion of plasma substitute (Hespan, 6% hetastarch in 0.9% sodium chloride, Du Pont Pharmaceuticals, Wilmington, DE) at a rate of 40 µl/min via a syringe pump (Harvard Apparatus, S. Natick, MA) until a total volume of 0.5% body weight was administered, to replace estimated fluid losses. Thereafter, the infusion rate was reduced to 10 µl/min for the duration of the studies. Experiments were started 30–45 min after completion of surgical procedures. After a 45-min control period, control rats received either rat ANP (1–28) (Peninsula Laboratories, Belmont, CA; n = 15) at a dose of 1 µg·kg⁻¹·min⁻¹ in normal saline or vehicle alone (n = 10). Nephrotic rats received the ANP infusion. The infusion of vehicle or ANP was at a rate of 10 µl/min for 45 min. In additional experiments, the influence of ANG II on the responses to ANP was evaluated in six control rats, and eight rats with PHN. For this purpose, a subpressor dose of ANG II (2.5 ng·kg⁻¹·min⁻¹; Sigma) was infused throughout the experiment, starting at the beginning of the control period as previously described (27). These animals received the ANP infusion, and the total infusion rate was limited to 10 µl/min to maintain the administered volume at a constant level. Three 50-µl samples of blood were taken 15, 30, and 45 min after the start of the control and experimental periods for determination of hematocrit and plasma protein concentration. Hematocrit was measured in duplicate on each blood sample by spinning blood at 12,000 rpm in a Microfuge (Clay Adams, Parsippany, NJ) for 3 min.

Plasma protein concentration was estimated in duplicate by refractometry (National Instrument, Baltimore, MD). Plasma volume was measured by the dilution principle using the Evans blue dye technique (32). For this purpose, 50 µl of Evans blue (Sigma; 5 mg/ml dissolved in normal saline) was injected intravenously, and the tubing was flushed with 200 µl of normal saline 5 min before the end of the 45-min infusion period. At the end of the experimental period, 5 ml of arterial blood were withdrawn and rapidly transferred into an ice-cold tube containing the following protease inhibitors: 1 ml of EDTA, 500 kallikrein inhibitor units of aprotinin, 10 µg of pepstatin A, and 100 µg of phenylmethylsulfonyl fluoride per milliliter of blood. Blood was immediately centrifuged (4,000 rpm) at 4°C for 15 min, and plasma was kept frozen at −70°C until subsequently analyzed for Evans blue dye concentration and immunoreactive ANP and guanosine 3',5'-cyclic monophosphate (cGMP).

Analytical techniques, calculations, and statistical evaluation. Analytical methods used in the laboratory have previously been described (28–33). Briefly, urine sodium and potassium concentrations were measured by flame photometry (model 943, Instrument Laboratories, Lexington, MA) and plasma Evans blue dye concentration in a spectrophotometer at 620 nm (Titrtek Multiskan Plus, Labsystem and Flow Laboratories, Finland). Urinary protein concentration was determined by the Coomassie blue method.

Plasma concentration of immunoreactive ANP was determined after extraction on a Sep-Pak C 18 column (Waters Chromatography Div., Millipore, Milford, MA) preequilibrated with 0.1% trifluoroacetic acid. After elution with 75% methanol in 0.1% trifluoroacetic acid and evaporation to dryness under a stream of nitrogen, the residue was reconstituted in assay buffer and measured for ANP immunoreactivity with a commercially available kit (Peninsula). Plasma concentration of cGMP was determined after extraction with water-saturated ethyl ether. After evaporation to dryness under a stream of air, the residue was reconstituted in assay buffer and measured for cGMP immunoreactivity with a commercially available kit (Du Pont).

Estimated changes in plasma volume were calculated according to the following formula: \( \Delta V = (100/H_1 - 100/H_2) \times (100 \times (1 - H_1)/H_2) \), where \( \Delta V \) is the percent change in plasma volume, and \( H_1 \) and \( H_2 \) are the initial and final hematocrits, respectively. Data are expressed as means ± SE. Analysis of variance with or without repeated measures followed by Dunnett’s test was used to assess significance among and between groups, respectively (Statview, Brain Power, Calabasas, CA). Relationships between variables were assessed by logarithmic regression analysis. \( P < 0.05 \) was considered the minimal level of significance.

RESULTS

Group characteristics. Results from the metabolic cage study indicated that nephrotic rats had similar water intake, urine volume, and urinary excretion of sodium and potassium compared with controls (Table 1). Urinary protein excretion was significantly elevated in both adriamycin-treated rats and rats with PHN compared with controls and was greater in adriamycin-treated rats than in rats with PHN (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Adriamycin</th>
<th>PHN</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>31</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>BW, g</td>
<td>315 ± 6</td>
<td>268 ± 6</td>
<td>275 ± 9</td>
</tr>
<tr>
<td>WI, ml/24 h</td>
<td>36.0 ± 2</td>
<td>34.2 ± 1.7</td>
<td>37.1 ± 1.8</td>
</tr>
<tr>
<td>UV, ml/24 h</td>
<td>17.7 ± 0.9</td>
<td>18.1 ± 1.2</td>
<td>24.4 ± 1.7</td>
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<tr>
<td>U_{Na}, mmol/24 h</td>
<td>2.04 ± 0.07</td>
<td>5.02 ± 0.08</td>
<td>2.05 ± 0.18</td>
</tr>
<tr>
<td>U_{K}, mmol/24 h</td>
<td>5.03 ± 0.37</td>
<td>1.07 ± 0.18</td>
<td>5.43 ± 0.18</td>
</tr>
<tr>
<td>U_{Na}, mg/24 h</td>
<td>13.7 ± 2.3</td>
<td>528.9 ± 45.6*</td>
<td>218.6 ± 36.5*‡</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW</td>
<td>Body weight</td>
</tr>
<tr>
<td>WI</td>
<td>Water intake</td>
</tr>
<tr>
<td>UV</td>
<td>Urine flow rate</td>
</tr>
<tr>
<td>U_{Na}</td>
<td>Urine excretion of sodium</td>
</tr>
<tr>
<td>U_{K}</td>
<td>Potassium</td>
</tr>
<tr>
<td>U_{Prot}</td>
<td>Proteins</td>
</tr>
</tbody>
</table>

*Values are means ± SE of variables obtained from metabolic cage during 24 h preceding acute experiment. PHN, passive Heymann nephritis; BW, body weight; WI, water intake; UV, urine flow rate, U_{Na}, U_{K}, and U_{Prot}, urinary excretion of sodium, potassium, and proteins. †* < 0.05 vs. control rats. ‡* < 0.05 between nephrotic rats (all other values were not significant).
Effect of ANP infusion on systemic hemodynamics, hematocrit, plasma protein concentration, and plasma volume in control rats. Infusion of ANP induced a decrease in mean arterial pressure (MAP) of $8.2\pm 1.0\%$ ($P < 0.05$ vs. both baseline and vehicle-infused control rats) from a basal value of $120 \pm 3$ mmHg (Fig. 1). Heart rate was not affected by ANP (356 $\pm 11$ vs. 359 $\pm 11$ beats/min; NS). As depicted in Fig. 2A, ANP infusion led to a progressive rise in hematocrit of $5.6 \pm 0.6$, $8.3 \pm 0.8$, and $9.5 \pm 0.6\%$ at 15, 30, and 45 min, respectively, from a basal value of $46.0 \pm 0.9\%$ (all $P < 0.05$ vs. both baseline and vehicle-infused control rats). The maximal increase in plasma protein concentration (PPC) resulting from the 45-min ANP infusion ($3.9 \pm 0.7\%$), from a basal value of $4.97 \pm 0.05$ g/dL; $P < 0.05$ vs. both baseline and vehicle-infused control rats) was of smaller magnitude than the corresponding change in hematocrit (Fig. 2B). The decrease in plasma volume calculated from the change in hematocrit amounted to $16.1 \pm 0.9\%$ for ANP. This calculated change was confirmed by the direct measurement of plasma volume at the end of the experiment using Evans blue dye. As shown in Fig. 3A, plasma volume was significantly lower in ANP-infused compared with vehicle-infused control rats ($21.3 \pm 1.4$ vs. $25.2 \pm 1.1$ ml/kg; $P < 0.05$ between groups). Such a decrease in plasma volume should have increased PPC by ~20%, much greater than the observed increase of only 3.9%, indicating that some loss of plasma protein had occurred in response to ANP infusion. In the vehicle group, no significant change in MAP (Fig. 1) or heart rate ($318 \pm 10$ vs. $312 \pm 11$ beats/min) was noted over the course of the experiment, whereas hematocrit and PPC decreased slightly ($-1.4 \pm 0.6$ and $-0.8 \pm 0.5\%$, respectively, both $P < 0.05$ vs. baseline; Fig. 2).

Effect of ANP infusion in nephrotic rats. Baseline values for MAP and hematocrit were significantly lower in nephrotic rats compared with control animals, whereas neither heart rate nor PPC differed significantly between groups (Table 2). The decrease in MAP observed in both adriamycin-treated animals and rats with PHN ($9.4 \pm 2.3$ and $9.0 \pm 2.0\%$, both $P < 0.05$ vs. baseline; Fig. 1) was similar in magnitude to that observed in control animals infused with ANP. As shown in Fig. 2, the ANP-induced increase in hematocrit was markedly blunted in rats with PHN ($1.9 \pm 0.8$, $2.8 \pm 1.2$, and $2.4 \pm 1.3\%$ at 15, 30, and 45 min, respectively; all $P < 0.05$ vs. ANP infusion in control animals), and the ANP-induced increase in PPC did not occur. These responses to ANP infusion were preserved in adriamycin-treated rats and rats with PHN.
both ANP- and vehicle-infused control rats. Adriamycin-treated rats infused with ANP had plasma volume similar to that measured in ANP-infused control animals.

Effect of ANP infusion on plasma concentrations of ANP and cGMP. Infusion of ANP for 45 min elevated plasma immunoreactive ANP concentration to the same extent in control, adriamycin-treated rats, and rats with PHN (Fig. 3B). In contrast, plasma immunoreactive cGMP concentration, measured at the conclusion of the experiment, was significantly lower in rats with PHN (141 ± 25 pmol/ml; P < 0.05) compared with control (281 ± 18 pmol/ml) and adriamycin-treated rats (290 ± 49 pmol/ml; Fig. 3C). When values from the four groups of animals were pooled together, a positive correlation existed between the maximal change in hematocrit and plasma concentration of cGMP (Fig. 4; r = 0.79, P < 0.0001), whereas no correlation was observed between changes in hematocrit and plasma concentration of ANP.

Influence of ANG II infusion on responses to ANP in rats with PHN. Baseline MAP did not differ significantly in animals infused with ANG II (2.5 ng·kg⁻¹·min⁻¹) compared with their respective controls (Table 2). MAP in response to ANP infusion decreased similarly in control rats and rats with PHN infused with ANG II (by 8.8 ± 2.8 and 10.0 ± 3.3%, respectively; both P < 0.05 vs. baseline and vs. vehicle-infused control rats) and to the same extent as in controls not infused with ANG II (Fig. 1). Acute infusion with ANG II did not affect the hemococoncentrating response to ANP in control rats but restored to normal the ANP-induced increase in hematocrit and PPC in rats with PHN (Fig. 5).

DISCUSSION

The present studies, performed in bilaterally nephrectomized rats, have demonstrated that the extrarenal effects of ANP are preserved in adriamycin-treated nephrotic rats. In contrast, the ANP-induced hemoconcentration resulting from a reduction in plasma volume is blunted in rats with PHN, but its hypotensive action is preserved. Acute infusion of a subpressor dose of ANG II in rats with PHN restored the ANP-mediated increase in hematocrit to normal. Although the reported changes in some cases were small and not

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Table 2. Baseline values for systemic hemodynamic variables, hematocrit, and plasma protein concentration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>Hematocrit, %</th>
<th>PPC, g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25</td>
<td>120 ± 3</td>
<td>341 ± 8</td>
<td>46.5 ± 0.9</td>
<td>4.82 ± 0.06</td>
</tr>
<tr>
<td>Adriamycin nephrosis</td>
<td>7</td>
<td>102 ± 13*</td>
<td>373 ± 17</td>
<td>40.1 ± 1.1*</td>
<td>5.03 ± 0.14</td>
</tr>
<tr>
<td>PHN</td>
<td>9</td>
<td>107 ± 4*</td>
<td>344 ± 14</td>
<td>40.1 ± 2.7*</td>
<td>5.04 ± 0.30</td>
</tr>
<tr>
<td>Control + ANG II</td>
<td>6</td>
<td>117 ± 4</td>
<td>407 ± 13</td>
<td>48.6 ± 0.8</td>
<td>5.76 ± 0.16</td>
</tr>
<tr>
<td>PHN + ANG II</td>
<td>8</td>
<td>98 ± 8†</td>
<td>358 ± 24</td>
<td>35.5 ± 0.9†</td>
<td>6.50 ± 0.21</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; HR, heart rate; PPC, plasma protein concentration. ANG II was infused acutely for 90 min at dose of 2.5 ng·kg⁻¹·min⁻¹. *P < 0.05 vs. control rats. †P < 0.05 vs. control rats infused with ANG II.
outside the likely variability in measurement, they nevertheless describe a cogent picture of extrarenal ANP action in these models of experimental nephrosis. In control rats, infusion of ANP at a pharmacological but mildly hypotensive dose (1 µg·kg\(^{-2}\)·min\(^{-1}\)) resulted in a roughly 8–10% increase in hematocrit and a decrease in plasma volume of around 14–16% as measured by the Evans blue dye method. These results are similar to those that we (27, 30–32) and others (1, 6, 35, 40) have previously reported. Because renal fluid losses were absent in these bilaterally nephrectomized rats, the decrease in plasma volume resulted from a shift of fluid from the vascular space to the interstitial compartment. A similar increase in hematocrit occurs at lower doses of infused ANP (33, 35, 40) as well as when a more modest increase in plasma concentration of ANP (4- to 6-fold) is induced by endothelin infusion (29). Thus, although the plasma concentration of ANP achieved by our infusion is well out of the physiological range, the differences that we have observed may also take place at more physiologically relevant concentrations. In this regard, we have observed that ANP-mediated effects on hematocrit and plasma volume are most pronounced at mild to moderate plasma concentrations of ANP, with values greater than fivefold over baseline having little further effect to increase hematocrit (29).

Although the mechanism or mechanisms by which ANP alters fluid distribution are not known, the accumulation of plasma proteins in susceptible vascular beds (30, 35, 40) suggests that increased capillary permeability may be an important component. The abnormal response of rats with PHN suggests that this component is reversibly altered in this experimental model of nephrotic syndrome, whereas it remains intact in rats with adriamycin nephrosis. The blunted hemoconcentration that we observed in rats with PHN after ANP infusion was accompanied by an increased plasma volume compared with control animals infused with either vehicle or ANP. There are several possible explanations for this abnormal response to ANP. Enhanced catabolism of the infused peptide seems unlikely in view of the nearly identical plasma concentrations measured in the three experimental groups. Moreover, as discussed above, the concentrations were in any case much higher than those required to elicit a maximal increase in vascular permeability in control rats based on our previous studies (29). Rats with PHN had lower resting arterial pressure than normal rats, and it is possible that this lower pressure in some way rendered them resistant to the infused ANP. The impact of this lower blood pressure on ANP-induced fluid partition is probably minimal if any. Trippodo and Barbee (26) found that spinal cord transection, a maneuver which produced a more substantial lowering of arterial pressure, did not influence the effect of ANP on fluid distribution. Moreover, adriamycin-treated rats had a preserved hemoconcentrating response to ANP despite significantly lower baseline blood pressure compared with control rats. Furthermore, the hemoconcentrating activity of ANP was restored in rats with PHN during simultaneous infusion of a subpressor dose of ANG II. In addition, despite lower baseline blood pressure, nephrotic animals had a quantitatively similar hypotensive response to infused ANP as seen in the control animals, indicating that this action of ANP is preserved in nephrosis. Interestingly, a blunted hypotensive response to a bolus injection of brain natriuretic peptide has recently been reported in adriamycin nephrosis (38), suggesting the possibility of differential responsiveness to the vascular actions of members of the natriuretic peptide family.

Clinical and experimental nephrotic syndromes have been associated with either normal or elevated resting plasma ANP concentration (9, 28, 33). One consequence of chronic elevation of plasma ANP concentration could be a downregulation of biologically active ANP receptors. However, we could not find any differences in either density or affinity of ANP renal receptors in both adriamycin-treated rats (28) and rats with PHN (33). These observations are in agreement with those of Perico et al. (18), reporting that the binding of ANP to inner medullary collecting duct cells did not differ between controls and nephrotics. Thus renal resistance to ANP in vivo is unlikely to result from reduced binding of the peptide to its receptors. Whether these findings regarding normal renal ANP receptor characteristics in experimental nephrosis can be extended to extrarenal ANP receptors is not known at the present time. Our data indicate that responsiveness of the vasculature with respect to blood pressure is preserved in the nephrotic state, implying that the receptors mediating vasorelaxation are functionally intact. These observations make it likely that the characteristics of the natriuretic peptide receptors responsible for ANP-related increases in permeability, presumably on capil-
lary endothelial cells, are also normal in rats with PHN, particularly since the low-dose ANG II infusion was able to restore responsiveness to normal.

A fourth possible explanation for this abnormal responsiveness to infused ANP that we observed is impaired cell signaling in response to the binding of ANP to its biologically active receptors. We observed that the plasma concentration of cGMP in rats with PHN after ANP infusion was only half the value measured in both control and adriamycin-treated rats. Although the source of circulating cGMP is not known with certainty, it probably originates in vascular tissue including endothelial cells (4, 39). The lower plasma cGMP level in rats with PHN could result from impaired generation or enhanced degradation of intracellular cGMP, currently the only recognized intracellular messenger of ANP (4, 36). We recently reported that enhanced cGMP phosphodiesterase activity could contribute to abnormal cGMP metabolism and account for the resistance to the natriuretic actions of ANP observed in both adriamycin nephrosis and PHN (28, 33). Moreover, because the hypotensive action of ANP was preserved in rats with PHN, the defect may be a selective one, found in the kidney (33) and possibly the endothelium, but not in vascular smooth muscle. A reduced plasma cGMP concentration could result from diminished ANP binding to its receptors, impaired cell signaling after binding, or enhanced phosphodiesterase activity in these selective tissues manifesting ANP resistance, and it is not possible to distinguish among these possibilities at present.

The hemoconcentrating activity of ANP in rats with PHN was restored by the simultaneous infusion of ANG II at a low, subpressor dose. We previously demonstrated the importance of ANG II in ANP-induced hemoconcentration: chronic pretreatment of rats with the angiotensin-converting enzyme (ACE) inhibitor, captopril, or acute pretreatment with the ACE inhibitor, enalaprilat, or the ANG II type I receptor antagonist, losartan, abolished the ANP-induced increase in hematocrit (27). Furthermore, the effect of ANP on hematocrit was restored in ACE inhibitor-treated rats by the simultaneous infusion of a subpressor dose of ANG II (i.e., 2.5 ng·kg\(^{-1}\)·min\(^{-1}\)). In the context of the present studies, rats with PHN have depressed 1) plasma renin activity and concentration (37, 2) plasma ANG II concentration (25), and 3) renal renin mRNA (10). Human membranous nephropathy has also been characterized as a low-renin, plasma volume-expanded state (9, 16). In contrast, adriamycin-treated rats have high levels of both tissue and blood ACE (34) and similar glomerular renin staining (22) compared with control animals. Taken together, these results suggest that the renin-angiotensin system is either normal or enhanced in adriamycin nephrosis but is depressed in rats with PHN. Although we did not measure plasma ANG II levels in our experiments, it is conceivable that they were insufficient in rats with PHN to allow ANP to increase hematocrit. The mechanism of such an effect has previously been discussed (27) and could be explained by changes in local hydrostatic forces. By analogy with the renal microcirculation, it is conceivable that ANP-induced precapillary vasodilatation is detectable only in vessels previously partially constricted with ANG II. A reduction in the preferential postcapillary constrictor effect of ANG II, as expected in rats with PHN, would then prevent the increase in the transcapillary pressure gradient and thus fluid movement toward the interstitial compartment induced by ANP. Alternatively, ANP and ANG II may interact at the endothelial level, where ANP receptors have been located (15) and contrasting effects of ANP and ANG II on electrolyte transport have been observed (7, 17). Because bilateral nephrectomy would have removed the major source of circulating renin in both groups, the preserved response to infused ANP in rats with adriamycin nephrosis indicates that sufficient residual ANG II, whether generated from circulating renin or from other tissue sources, was present in this model but not in rats with PHN.

In summary, adriamycin nephrosis in the rat is characterized by normal responsiveness to the hypotensive and hemoconcentrating actions of exogenous ANP, whereas rats with PHN, a form of experimental nephrotic syndrome resembling human membranous nephropathy, exhibit a blunted response to ANP-induced increases in hematocrit and protein extravasation while showing a normal decrease in blood pressure. PHN induces a selective impaired responsiveness to ANP that may relate to an interaction with ANG II at target cells governing vascular permeability, since simultaneous infusion of a subpressor dose of ANG II restored the responsiveness to infused ANP.

**Perspectives**

Analysis of the mechanisms involved in nephrotic sodium retention has led to two competing hypotheses (9). In one, the underfill model, sodium retention is initiated by a diminished plasma volume brought on by a reduced plasma onotic pressure from low albumin concentration, whereas in the other, the overflow model, sodium retention occurs despite an expanded plasma volume. Renal resistance to ANP is a regular occurrence in both experimental and clinical nephrotic syndrome (9, 19) and has been linked to sodium retention. The present experiments demonstrate that selective resistance to the extrarenal actions of ANP also occurs in an immunologically mediated form of experimental nephrotic syndrome (PHN) but not in a toxic model (adriamycin nephrosis). In each case, the hypotensive action of infused ANP was preserved, whereas the hemoconcentrating effect, which is due to a reduction in plasma volume, was normal in adriamycin nephrosis but markedly blunted in rats with PHN. These rats also exhibited expanded plasma volume, suggesting that this extrarenal resistance to ANP is a determinant of the expanded plasma volume. PHN is known to have suppression of the renin-angiotensin system, and infusion of a low dose of ANG II restored the hemoconcentrating effect of infused ANP to normal. These observations will allow analysis of the relationship between plasma volume and sodium retention in nephrotic
syndrome to take place in a new context and suggest that interactions between ANG II and ANP may provide a basis to account for divergent measurements of plasma volume in this condition, helping to reconcile the controversy between underfill and overflow mechanisms of nephrotic edema.

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