Modulation of the Natriuretic Response to Atrial Natriuretic Peptide by Alterations in Peritubular Starling Forces in the Rat

Ramon E. Mendez, B. Rentz Dunn, Julia L. Troy, and Barry M. Brenner

The mechanisms underlying the natriuretic response to infusions of atrial natriuretic peptide (ANP) remain incompletely defined. By acting as renal vasodilators, atrial peptides may serve to alter peritubular capillary physical forces and favor a decrease in tubule solute reabsorption. Therefore, we studied the effects of known modifiers of intrarenal Starling forces on the natriuresis induced by infusion of intravenous hANP [4-28] (0.5 μg/kg/min) in anesthetized, euvoletic Munich–Wistar rats. In the first series of studies, infusion of ANP resulted in a significant natriuresis, diuresis, and increase in insulin clearance and in a slight fall in systemic arterial pressure, as compared to vehicle infusion. Subsequent elevation of renal perfusion pressure by superimposition of angiotensin II infusion (0.1–0.2 μg/kg/min i.v.) on continued ANP infusion resulted in marked further enhancement of natriuresis, independent of changes in glomerular filtration rate (GFR). In the second set of experiments, in which oncotic pressure in the postglomerular capillaries was elevated by hyperoncotic exchange transfusion, administration of ANP did not result in natriuresis, even though GFR increased by the same magnitude as that seen in isoncotic animals given ANP. These observations are consistent with the view that peritubular capillary hydraulic and oncotic pressures modulate the natriuretic and diuretic effects of ANP. (Circulation Research 1986;59:605–611)

SINCE the original description by deBold1 of the action of atrial extract, a constant finding in the renal response to infusion of atrial natriuretic peptide (ANP) has been a marked natriuresis and diuresis.2 The mechanisms underlying such actions remain incompletely elucidated, but several factors are believed to be important, including the rise in glomerular filtration rate (GFR), an increase in medullary blood flow, and direct inhibition of renal epithelial ion transport.3,4

An additional possible mode of action is the ability of ANP to act as a potent renal vasodilator,4,5 which may serve to alter peritubular capillary physical factors and thereby promote natriuresis and diuresis. Earlyy and coworkers6 implicated peritubular Starling forces as important determinants of the control of sodium and water excretion, based on studies in which infusions of vasodilators, such as acetylcholine or bradykinin, led to increments in sodium and water excretion. This natriuresis and diuresis was reversed by the concurrent infusion of hyperoncotic albumin and was restored by subsequent elevation of systemic arterial pressure, and presumably peritubular capillary hydraulic pressure, by infusion of angiotensin II (All).8

The present studies employed similar maneuvers in an attempt to modify possible peritubular capillary actions of ANP. In the first set of experiments, we examined the effect of increasing renal perfusion pressure with All on the renal response to ANP infusion, while in the second set of experiments ANP was administered in the setting of elevated postglomerular capillary oncotic pressure.

Materials and Methods

Studies were performed on 34 male Munich–Wistar rats weighing 233–321 g. They were allowed free access to standard rat chow (Wayne Rodent Blox #8604; Continental Grain Co., Chicago, Ill.) and tap water. Clearance experiments were carried out as follows:

Rats were anesthetized with Inactin (100 mg/kg, i.p.; Byk Gulden, Konstanz, F.R.G.) and placed on a temperature–regulated table. Following tracheostomy, the left femoral artery was catheterized with PE–50 polyethylene tubing, and a baseline collection of 140 μl of arterial blood was obtained. This arterial catheter was used for subsequent periodic blood sampling and estimation of mean arterial pressure (AP). AP was monitored with an electronic transducer (Model P23Db, Statham Instruments Division, Gould Inc., Oxnard, Calif.) connected to a direct–writing recorder (Model 7702B Hewlett–Packard Co., Palo Alto, Calif.). The left femoral vein was cannulated with PE–50 tubing, and an infusion of 7% inulin and 0.8% p-aminohippurate (PAH) in 0.9% NaCl was started at a rate of 1.2 ml/hr. PE–50 catheters were also inserted into the right and left jugular veins, and right femoral vein, as needed for infusions of plasma and solutions.
The left ureter was exposed through an abdominal incision and catheterized with PE-10 tubing.

Since the plasma volume of rats prepared similarly for micropuncture is reduced by approximately 20%, the following protocol for maintaining the euvoilemic state was used. After insertion of jugular catheters, isoncotic rat plasma was infused over 60 minutes in a total amount equal to 1% of body weight, followed by a reduction in infusion rate to 0.58 ml/hr for the remainder of each experiment, except where noted. After allowing 30 minutes for equilibration, timed urine collections and blood samples of 140 µl, taken at the midpoint of each collection, were obtained for determination of inulin and PAH clearances and urinary electrolyte excretion in a baseline period. Values for all study periods represent mean values obtained during two consecutive 10 to 15 minute periods. Infusions were then started according to the following protocols, as summarized in Figure 1.

**GROUP 1. ANP ALONE (n = 6).** Synthetic human ANP [4-28] (Wyeth Laboratories, King of Prussia, Pa.) was administered intravenously as a priming dose (4 µg/kg body weight) over 2 minutes, followed by a sustaining i.v. infusion at the rate of 0.5 µg/kg/min (1.56 ml/hr). After an equilibration period of 3 minutes, by which time a diuresis was clearly established, measurements for the first and second study periods were taken. The plasma infusion rate was increased to 0.026 ml/min concurrently with ANP infusion in an attempt to maintain the euvoilemic state. This rate was found in preliminary studies to maintain a constant hematocrit during infusion of ANP.

**GROUP 2. VEHICLE ALONE (n = 6).** Rats in this group were given 0.9% saline vehicle (VEH) in volumes and rates equivalent to those in group 1.

**GROUP 3. ANP + AII (n = 6).** These animals received ANP in the same manner as those in group 1, but with the superimposition during the second study period of AII (Sigma Chemical Co., St. Louis, Mo.) infused i.v. at the rate of 0.1-0.2 µg/kg/min (0.58 ml/hr).

**GROUP 4. VEHICLE + AII (n = 6).** These rats were given 0.9% saline vehicle during both study periods, but with the addition of an i.v. AII infusion at the rate of 0.1-0.2 µg/kg/min (0.58 ml/hr) during the second study period.

The second set of experiments employed a similar experimental preparation as previously described. These animals (n = 6) were rendered hyperoncotic in the baseline state by isovolemic exchange of 25% albumin for native plasma. Exchange transfusion was chosen in this study to minimize the resulting volume expansion and hemodilution induced by intravenous infusions of hyperoncotic albumin. For these purposes, homologous rat blood was collected from littersmates on the morning of study into lightly heparinized polyethylene syringes and was centrifuged at 4000 rpm for 10 minutes. The red blood cells were separated and suspended in 25% human albumin (Buminate, Travenol Laboratories Inc., Glendale, Calif.) in a volume equal to the amount of plasma removed. A volume of this hyperoncotic blood, approximately equal to 3% of body weight, was then infused intravenously over a 10-minute period in exchange for an identical volume of whole blood simultaneously withdrawn from the femoral artery catheter. This was followed by a sustaining i.v. infusion of 25% albumin at the rate of 1.13 ml/hr designed to keep plasma protein concentration constant. After 30 minutes for equilibration, baseline clearances were performed and ANP infusion was given as described above.

Since the percentage of plasma protein represented by albumin increases with hyperoncotic albumin infusion, this percentage was determined in a separate group of four rats. After a baseline euvoilemic isoncotic period, the animals were rendered hyperoncotic and then given ANP according to the protocol described above. Arterial blood samples of 150 µl were obtained for serum protein electrophoresis during the baseline, hyperoncotic, and hyperoncotic + ANP periods.

**Chemical Analysis and Calculations**

Inulin concentration in plasma and urine was determined by the macro-anthrone method of Führ et al. PAH concentration was determined by a colorimetric technique. Plasma and urinary sodium and potassium concentrations were determined by flame photometry. Total plasma protein concentration was measured by refractometry. Serum protein electrophoresis was done using agarose gels. They were stained and the percentage albumin fraction quantitated by densitometry.

Whole kidney filtration fraction (FF) was calculated as the ratio of glomerular filtration rate (GFR) to the renal plasma flow rate (RPF)

$$FF = \frac{GFR}{RPF}$$

Protein concentration in postglomerular plasma (C_p) was derived from simultaneous determination of FF and systemic plasma protein concentration (C_s) by

$$C_p = C_s \times \left(1 - FF\right).$$

Postglomerular plasma oncotic pressure (π_p) was calculated by use of the Landis-Pappenheimer equation modified according to the percentage albumin of total plasma proteins.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>BASELINE PERIOD</th>
<th>FIRST STUDY PERIOD</th>
<th>EQUIV.</th>
<th>SECOND STUDY PERIOD</th>
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<tbody>
<tr>
<td>ANP (n=6)</td>
<td></td>
<td>hANP, 4µg/kg prime + 0.5µg/kg/min</td>
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<tr>
<td>VEH (n=6)</td>
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<td>VEHICLE</td>
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<tr>
<td>ANP+AH (n=6)</td>
<td></td>
<td>hANP, 4µg/kg prime + 0.5µg/kg/min</td>
<td>AII 0.1-0.2µg/kg/min</td>
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<tr>
<td>VEH+AH (n=6)</td>
<td></td>
<td>VEHICLE</td>
<td>AII 0.1-0.2µg/kg/min</td>
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**Figure 1. Protocol for infusions.**
Statistical Analysis

All results are expressed as means ± SEM. Statistical analyses were performed by analysis of variance with the Bonferroni modification for multiple comparisons, and by paired or Student’s t tests, where appropriate. In the studies with AII, statistical comparisons were applied to the differences between values obtained in the baseline and first study period, and between the first and second study periods. This approach was used to preserve the sequential design of superimposing infusions. Statistical significance was defined as \( p < 0.05 \).

Results

Studies With Angiotensin II

**Baseline Period.** Mean values for the parameters studied in this initial period were similar in all four groups.

**First Study Period.** In this period the effects of ANP infusion were examined as compared to vehicle infusion. Since the responses to ANP infusion in groups 1 and 3 were indistinguishable, the results were pooled for comparison with the vehicle groups (2 and 4), the results of which were also virtually identical. As depicted in Figure 2, infusion of ANP in groups 1 and 3 resulted in an average 10-fold rise in urinary sodium excretion (\( U_{NaV} \)), from a baseline of 0.52 ± 0.20 to 5.16 ± 0.84 \( \mu \)Eq/min, which was significantly higher (\( p < 0.001 \)) than the small rise seen with vehicle infusion alone in groups 2 and 4 (0.52 ± 0.15 to 0.76 ± 0.21 \( \mu \)Eq/min). This increased \( U_{NaV} \) in groups 1 and 3 resulted from increments in both urine flow rate (\( V \), from 4.45 ± 0.47 to 20.15 ± 2.96 \( \mu \)l/min), and urinary sodium concentration (\( U_{Na} \), from 95.4 ± 25.2 to 249.0 ± 16.2 mEq/l), both being significantly higher (\( p < 0.001 \) and \( p < 0.01 \) for \( V \) and \( U_{Na} \), respectively) than the changes occurring during vehicle infusion (4.42 ± 0.67 to 4.35 ± 0.54 \( \mu \)l/min and 96.9 ± 15.7 to 147.5 ± 26.2 mEq/l, \( V \) and \( U_{Na} \), respectively). Similarly, there was a sharp increase in fractional sodium excretion (\( FE_{Na} \)), which rose 9-fold over the baseline of 0.23 ± 0.08 to 2.09 ± 0.34%.

This increment was significantly higher (\( p < 0.001 \)) than the minimal change seen in the vehicle groups (0.24 ± 0.01 to 0.32 ± 0.09%). AP fell slightly but significantly during ANP infusion (Figure 3) from 111 ± 2 to 105 ± 2 mm Hg, whereas groups 2 and 4 demonstrated a near-constancy of AP (115 ± 2 and 117 ± 3 mm Hg during baseline and first study period, respectively). Despite this fall in AP, the GFR rose by 16% over baseline values during ANP infusion (Figure 4, from 1.39 ± 0.07 to 1.66 ± 0.07 ml/min), a significant increment (\( p < 0.01 \)) as contrasted with the essentially unaltered GFR during vehicle infusion (1.42 ± 0.05 and 1.40 ± 0.06 ml/min, baseline and first study period, respectively). RPF, as estimated from the PAH clearance (\( C_{PAH} \)), remained constant (\( p > 0.5 \)) during baseline and first study periods when either ANP (4.61 ± 0.20 to 4.88 ± 0.23 ml/min) or vehicle (4.38 ± 0.25 to 4.47 ± 0.31 ml/min) was administered. The \( C_{PAH} \) has been shown to be a reliable indicator of RPF during ANP infusion, with no detectable change in extraction ratio.

Since the GFR rose during ANP infusion but the RPF remained stable, FF was significantly increased (\( p < 0.05 \)) in groups 1 and 3 from 0.31 ± 0.02 in the baseline to 0.34 ± 0.02, as contrasted with the near-constancy observed in groups 2 and 4 (0.33 ± 0.1 and 0.32 ± 0.1, baseline and first study period, respectively). Attendant with the natriuresis, there was a near doubling of urinary potassium...
excretion (U\(_A\)V, from 1.2 ± 0.1 to 2.1 ± 0.1 μEq/min) in the ANP groups, whereas U\(_V\) remained stable in the vehicle groups at 1.1 ± 0.1 μEq/min (p<0.001, groups 1 and 2 vs. groups 3 and 4).

Second study period. In this period, renal perfusion pressure was increased by superimposing All infusion on ANP infusion (group 3) or on vehicle (group 4). The resulting effects were also compared to continued ANP infusion alone (group 1) or vehicle alone (group 2). As shown in Figure 2, U\(_A\)V was markedly enhanced over its already elevated level when All was superimposed on ANP infusion (from 5.83 ± 1.0 to 13.5 ± 1.5 μEq/min). This was significantly higher (p<0.005) than the minimal increase seen in group 4 (from 0.36 ± 0.14 to 0.81 ± 0.14 μEq/min), and the constancy of U\(_A\)V seen in group 1 (p<0.005). FE\(_Na\) was also significantly increased in group 3 (from 2.45 ± 0.44 to 7.73 ± 1.99%), as compared to the modest rise in group 4 (from 0.36 ± 0.14 to 0.81 ± 0.14 %, p<0.01) and group 2 (from 1.74 ± 0.50 to 2.01 ± 0.34 %, p<0.005). All infusion caused an elevation of AP in groups 3 and 4 when superimposed on ANP or vehicle (Figure 3, from 104 ± 2 to 121 ± 6 mm Hg and from 117 ± 4 to 136 ± 5 mm Hg, respectively), whereas AP tended to fall slightly in groups 1 and 2 (from 106 ± 3 to 102 ± 3 mm Hg and from 117 ± 4 to 114 ± 3 mm Hg, respectively). The changes in GFR during this period are shown in Figure 4. All groups had similar decrements in GFR during the second study period so that the fall in GFR occurring during All infusion in group 3 was indistinguishable from that seen with ANP alone in group 1. There was an attendant fall in RPF in the groups receiving All (from 4.35 ± 0.23 to 3.36 ± 0.17 and from 4.96 ± 0.41 to 3.41 ± 0.29 ml/min, groups 3 and 4, respectively), which reached statistical significance in group 4 (p<0.02), when compared with the slight increase observed with vehicle alone (from 3.98 ± 0.38 to 4.13 ± 0.41 ml/min). As a result, FF remained unaltered during both study periods. The U\(_A\)V was unaffected by concomitant All infusion and did not change in the other groups as well.

Plasma sodium (P\(_Na\)) and potassium (P\(_K\)) were similar in the baseline period in all four groups and remained unaltered during both study periods.

Hyperoncotic Studies

Rats which had been rendered hyperoncotic by isovolemic exchange transfusion of 25% albumin for native plasma had a significantly increased (p<0.001) systemic plasma protein concentration (C\(_A\), 7.3 ± 0.1 g/dl), as compared to the isoncotic euvoletic state (6.1 ± 0.1 g/dl). Despite this substantially elevated C\(_A\), the postglomerular protein concentration (C\(_E\)), calculated from C\(_A\) and the whole kidney FF, was found to be unchanged from euvoletic levels (8.9 ± 0.2 and 9.0 ± 0.3 g/dl, respectively). This constancy of C\(_E\) was due to a lower FF (0.18 ± 0.03) as a consequence of a higher RPF (5.55 ± 0.38 ml/min) in the hyperoncotic state. However, the percentage of plasma protein represented by albumin increased significantly (p<0.001) from 52.1 ± 1.3% in the isoncotic state to 81.2 ± 0.7% in the hyperoncotic state, as measured by gel electrophoresis. Since albumin exerts a greater oncotic pressure than globulin for any given protein concentration, the increased albumin to globulin ratio achieved with hyperoncotic plasma exchange resulted in a significantly higher (p<0.05) postglomerular plasma oncotic pressure (π\(_E\)) of 44.6 ± 2.4 mm Hg, as compared to 37.7 ± 1.2 mm Hg in the isoncotic state. Infusion of ANP in these rats with elevated π\(_E\) resulted in a significant rise in GFR (from 1.13 ± 0.20 to 1.36 ± 0.25 ml/min, p<0.05), an increment identical in magnitude to that observed in isoncotic rats given ANP (Figure 5a). Despite this similar increase in GFR, however, the natriuretic response to ANP, shown in Figure 5b, was completely prevented in the hyperoncotic animals (0.38 ± 0.13 and 0.45 ± 0.21 μEq/min during hyperoncotic baseline and ANP infusion periods, respectively). ANP infusion did not produce further changes in C\(_A\), C\(_E\), or π\(_E\). The percentage of albumin remained essentially unchanged at 82.7 ± 1.5% during administration of ANP, as did all other parameters, including AP, RPF, U\(_Na\), V, FE\(_Na\), P\(_Na\), and P\(_K\).

Discussion

Several studies have documented significant increases in GFR resulting from administration of either atrial extracts or synthetic ANP in a variety of experimental animal preparations. The resulting increase in filtered load of sodium contributes to the natriuresis and diuresis but cannot completely account for it since several studies have reported dissociations between increments in GFR and natriuresis, particularly at low infusion rates of ANP. Furthermore, the moderate increases in distal delivery cannot account for the large increases in urinary sodium. A direct inhibitory action on epithelial ion transport has also been postulated. Inhibition of proximal reabsorption has been suggested from increased fractional lithium and phosphate excretions. It seems, however...
er, that these results were most likely a consequence of increased tubule flow rates and filtered loads, since a direct effect of ANP on volume absorption in rabbit proximal straight and convoluted tubules in vitro has not been observed. In addition, micropuncture studies during ANP infusion showed increased absolute proximal and loop solute and fluid reabsorption rates with decreases only in fractional reabsorption. In contrast, an inhibitory action on solute reabsorption in the medullary collecting duct is likely, as has been suggested from studies using a microcatheterization technique. Indeed, studies by Zeidel et al indicate that ANP inhibits sodium transport in suspensions of papillary collecting duct cells. A further contribution to the diuresis might arise from inhibition of vasopressin-stimulated water transport by ANP, as has been demonstrated in rabbit cortical collecting tubule. The contribution of this direct inhibition to the overall magnitude of natriuresis, however, appears to be minimal if hemodynamic actions of ANP are blocked, as by aortic constriction.

ANP has been found to cause preferential increase in inner medullary blood flow, leading Maack and co-workers to suggest “washout” of the hypertonic medullary interstitium as a contributing factor. If such a mechanism were operative, it would not be expected to lead to the hypernatriemic urine seen regularly with ANP infusion.

On the other hand, an action by ANP as a renal vasodilator may serve to alter peritubular capillary physical forces and thereby promote natriuresis and diuresis. Studies done by Earley and colleagues using whole kidney clearance techniques in the dog concluded that tubule reabsorption of sodium is determined to a great extent by the prevailing vasomotor tone of the renal resistance vessels and that the mechanism of vasodilator-induced natriuresis was due to increased transmission of hydraulic pressure to peritubular capillaries. Indeed, ANP has been shown to be a potent renal vasodilator, reducing preglomerular tone and thereby resulting in elevated hydraulic pressures within glomerular capillaries. Furthermore, ANP has been demonstrated to be a potent antagonist of vasoconstrictor influences. For example, ANP antagonizes the constrictor effects of All and norepinephrine in isolated perfused rat kidney and aortic ring preparations and can dilate precontracted ring preparations of rat renal arcuate and interlobar arteries.

Therefore, the present experiments were designed to examine the effect of known modifiers of peritubular capillary Starling forces on the natriuresis induced by ANP. In the first set of these studies, the effect of increasing renal perfusion pressure on ANP-induced natriuresis was investigated. In the first study period, infusion of ANP resulted in natriuresis, diuresis, increased GFR, and a slight fall in AP. Subsequent infusion of All in group 3 rats resulted in marked further enhancement of natriuresis and diuresis, independent of changes in GFR. ANP-induced natriuresis could thus be a result of the reduced preglomerular tone which then allows transmission of a greater fraction of systemic arterial pressure to glomerular, as well as peritubular capillaries. The increased peritubular capillary hydraulic pressure then impedes tubule fluid reabsorption and predisposes to natriuresis and diuresis. In the presence of ANP, an acute pressor response with infusion of All in group 3 animals appears to have led to transmission of even greater hydraulic pressure to peritubular capillaries and thereby further augmented natriuresis and diuresis. In contrast, combined infusions of All and vehicle in group 4 resulted in only a modest rise in UNa/V despite markedly elevated renal perfusion pressure.

All has been shown to exert dose-dependent effects on sodium excretion. At low infusion rates (generally less than 0.1 µg/kg/min), All has an antinatriuretic effect, probably due to direct stimulation of sodium transport in the proximal tubule, whereas at higher doses natriuresis occurs. Studies by Olsen et al have shown that this natriuretic effect is dependent on the increase in renal perfusion pressure, since natriuresis was prevented despite increasing All dosage when renal perfusion pressure was maintained at normotensive levels. Other maneuvers which increase renal perfusion pressure acutely, such as epinephrine infusion or carotid occlusion and vagotomy, also cause a natriuresis, but no natriuresis is observed if renal perfusion pressure is kept constant. In other experimental settings where renal perfusion pressure is elevated, such as the spontaneously hypertensive rat, infusion of ANP results in enhanced natriuresis as compared to normotensive animals. It thus seems clear that the enhanced natriuresis in the present experiments is secondary to the All-induced rise in renal perfusion pressure. A direct interaction between ANP and All is unlikely since All does not compete for ANP receptor sites.

In the present studies a rise in peritubular capillary hydraulic pressure during ANP infusion was inferred. These pressures have actually been measured by Dunn et al in the vascular and tubule elements of the exposed renal papilla, who demonstrated consistent in-
increases in vasa recta pressures of greater magnitude than loop of Henle or collecting duct pressures within minutes of ANP infusion. This increase in vasa recta pressures would tip the balance of Starling forces towards a decrease in local capillary fluid uptake. Fluid would then accumulate in the papillary interstitial space, thereby favoring movement back into the tubule lumen, possibly through opened intercellular channels.

If this interpretation is correct, it follows that other maneuvers that result in an acute increase in oncotic pressure within peritubular capillaries should effectively neutralize this rise in capillary hydraulic pressure and thus restore peritubular Starling forces more nearly to normal. Indeed, studies by Earley and co-workers have shown that infusion of hyperoncotic albumin reversed the natriuretic effect of vasodilatation in renal vasculature and of volume expansion with saline. According to this issue was addressed in the second set of experiments where ANP was administered in the setting of elevated postglomerular plasma oncotic pressure. In this situation, ANP infusion failed to augment sodium excretion despite a substantial increment in GFR.

Taken together, these observations support a role for alterations in peritubular Starling forces in modulating the natriuretic response to ANP infusion.

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