PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **185**, 62–68 (1987)

# The Effects of Atrial Natriuretic Peptide on Whole-Kidney and Proximal Straight Tubular Function in the Rabbit (42517)

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*Abstract.* The mechanisms by which atrial natriuretic peptide (ANP) produces a diuresis and natriuresis remain unclear. It has been suggested that the major if not sole mediator of ANP's renal effects is a hemodynamically induced increase in glomerular filtration rate (GFR). Data from clearance studies in anesthetized rabbits demonstrate that ANP administration can produce a significant increase in absolute and percentage sodium excretion (42.0 ± 5.9 → 64.6 ± 10.2 μeq/min, $P < 0.01$, and 1.97 ± 0.28 → 3.12 ± 0.35%, $P < 0.001$, respectively) without increasing GFR (16.8 ± 2.1 → 16.1 ± 2.5 cc/min, $P > 0.30$). The natriuresis occurred despite a fall in renal plasma flow (RPF) (56.7 ± 7.0 → 44.5 ± 9.4 cc/min, $P < 0.01$), a rise in filtration fraction (0.33 ± 0.01 → 0.46 ± 0.05, $P < 0.01$), and an unchanged filtered load of sodium (2.28 ± 0.27 → 2.16 ± 0.32 μeq/min, $P > 0.10$). Isolated tubular microperfusion studies demonstrated that ANP, present as a $10^{-9}$ $M$ concentration in the solution bathing perfused proximal straight tubules (PST), did not affect fluid flux ($J_v$) (0.38 ± 0.07 → 0.41 ± 0.07 nl/mm/min, $P > 0.30$) or phosphate reabsorption ($J_p$) (1.50 ± 0.5 → 1.38 ± 0.36 pmole/mm/min, $P > 0.50$). When ANP was infused into rabbits prior to harvesting the PSTs for isolated tubular microperfusion and the results were compared to tubules taken from control animals, there was again no effect on $J_v$ (0.37 ± 0.05 vs 0.42 ± 0.05 nl/mm/min, $P > 0.50$) or $J_p$ (2.41 ± 0.27 vs 2.42 ± 0.44 pmole/mm/min, $P > 0.90$). These findings suggest that ANP can inhibit sodium transport without increasing whole-kidney GFR or RPF, but does not directly inhibit transport in the proximal straight tubule. 

The infusion of synthetic atrial natriuretic peptide (ANP) induces a fall in mean arterial pressure as well as a diuresis and natriuresis (1–4). The mechanism by which the renal effects are produced remains unclear. There is usually, although not universally, a fall in renal vascular resistance (5–8), associated with a concomitant fall in renal blood flow (5–7), and often a rise in glomerular filtration rate (GFR) (5–8). Many investigators have suggested that the natriuresis is secondary to a hemodynamically mediated increase in GFR (4, 9–11). Some investigators have presented data suggesting an inhibition of sodium transport in proximal (6, 11–13) or distal (14–16) segments of the nephron. Other studies have demonstrated that ANP does not inhibit proximal or distal sodium transport (9, 17, 18). We attempted in this study to determine whether ANP alters sodium and phosphate transport by virtue of its effects on glomerular function, tubular function, or both. Accordingly, whole-kidney function and isolated tubular transport were examined utilizing the techniques of classical clearance methodology and isolated tubular perfusion. The rabbit was chosen as the animal model because this mammalian species provides the investigator with the opportunity to compare data from tubular transport measurements *in vitro* (16) with data from renal function studies *in vivo*. The results indicate that ANP can cause a natriuresis through nonhemodynamic means. Direct tubular inhibition of sodium transport in the proximal straight tubule (PST), however, does not occur.

**Materials and Methods.** *Animals.* Female New Zealand White rabbits were used in both the clearance and microperfusion studies. Animals were kept in the animal holding facility for at least 3 days prior to the experiment and had free access to both tap water and standard laboratory rabbit chow.

*Clearance studies.* Animals were anesthetized with intravenous pentobarbital (35 mg/kg). The jugular vein and carotid artery were exposed and cannulated as an infusion access and for blood pressure monitoring and blood sampling, respectively. A urinary bladder catheter was inserted. Paraaminohippurate



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(PAH) and inulin were given as a 0.8-cc bolus of 10% inulin and 5% PAH followed by a constant infusion at 2.34 cc/hr. A 90-min equilibration period followed. During the equilibration period and the subsequent control and experimental periods, urine output was matched with a normal saline infusion. The first period consisted of three 20-min control urine collections. During the second period, control animals received the ANP vehicle alone. Experimental animals were given rat ANP 8-33 (Merck, Sharp, & Dohme Research Laboratories) (20) suspended in saline as a 1.0 μg/kg bolus and a 0.1 μg/kg/min infusion. The total volume of saline administered as the vehicle for ANP was less than 1.5 cc. Four 20-min urines were collected during the infusion.

*Isolated tubular perfusion studies.* The technique of *in vitro* isolated tubular perfusion as originally described by Burg *et al.* (19) and as modified in this laboratory (21) was employed. Rabbits were sacrificed and a kidney was removed, decapsulated, and cut into 1- to 2-mm coronal slices. Individual PSTs were isolated and placed in a perfusion chamber. The solution bathing the tubule contained (m*M*) sodium, 150; chloride, 112; bicarbonate, 25; potassium, 5; phosphate, 2; ionized calcium, 1.5; sulfate, 1.0; magnesium, 1.0; glucose, 5.5; acetate, 10; alanine, 5; and fatty acid-free bovine serum albumin, 6 g/dl. The solution was gassed with a mixture of 95% $O_2$ and 5% $CO_2$. The temperature was maintained at 37.0°C. The pH of the solution ranged between 7.40 and 7.50. The bathing solution was changed intermittently throughout the experiment. The tubules were perfused via concentric glass pipettes with a solution similar to that described above except that the concentration of magnesium was 0.7 m*M*, there was no albumin present, and $NaH_2{}^{33}PO_4$, 5 to 7 μCi/ml, and [$^3$H]methoxyinulin, 15 μCi/ml, were added. The osmolalities of the bathing and perfusing solutions were equal.

The tubules were allowed to equilibrate for 30–40 min before collection of the perfusate began. In the *in vitro* exposure experiments, five or six control collections were obtained. Thereafter, ANP ($10^{-9}$ *M*) was introduced into the bath, and six experimental collections were obtained (Group I). Control tubules (Group II) were exposed to the vehicle alone during the experimental period.

Five rabbits (Group III) received an infusion of ANP in a dose of 0.1 μg/kg/min for 20 min following a 1.0 μg/kg bolus injection. Tubules taken from these animals were then perfused, and the values obtained for $J_v$ and $J_p$ were compared to those from six control rabbits (Group IV). The latter animals were handled in exactly the same manner as those in Group III, except that they received only the vehicle used for the ANP (saline) in equivalent volumes. The total volume of saline administered during the infusion was less than 1.5 cc. The collections of perfusate in the Groups III and IV tubules were divided into two periods to evaluate the stability of the preparation.

*Measurements and calculations.* The methodology employed for the measurement of urine and plasma electrolytes, inulin, and PAH has been described previously by this laboratory (22). Relative renal vascular resistance was calculated as the ratio of mean arterial pressure to renal plasma flow as measured by PAH. The quantities of $^3$H and $^{33}$P in the postperfusion perfusate collections and in the samples of the perfusate prior to perfusion were measured in a liquid scintillation counter. Correction for spillover of $^{33}$P into the $^3$H channel was carried out. $J_v$ was calculated as the amount of fluid absorbed across the tubular epithelium per minute time divided by the length of the tubule. Absolute fluid absorption was determined by the difference in the tritiated inulin concentration before and after the perfusate transited the tubule. $J_p$ was calculated as the quantity of phosphate removed from the perfusate per minute time divided by the tubule's length. Absolute phosphate transport was determined from the change in the concentration of $^{33}$P in the perfusate effected by passage through the tubule. The independent effect of fluid flux on phosphate concentration was included in this calculation. The rate at which the perfusate traversed the tubule ($P_r$) was controlled by the height of the perfusate reservoir above the perfusion chamber. The specific formulas employed for calculation of $J_v$, $J_p$, and $P_r$ have been described in detail previously (23). Student's *t* test for dependent variables was used to compare the data between control and experimental periods in the clearance and perfusion experiments. Data for each period was the mean of all the values obtained within that

period. A $t$ test for nonpaired variables was used to compare the results of Groups III and IV.

**Results.** *Clearance studies.* The clearance data are summarized in Table I. All parameters measured were stable during the control and experimental periods in the control animals except for a very minor fall in serum sodium concentration. The administration of ANP resulted in a fall in mean arterial pressure (MAP) and renal plasma flow as measured by the clearance of PAH. Relative renal vascular resistance did not change. Glomerular filtration rate (GFR), as measured by the clearance of inulin, was maintained at control levels. This combination of hemodynamic alterations resulted in an almost 40% increase in filtration fraction. A modest but significant diuresis and natriuresis nonetheless resulted. The fractional and absolute excretion of phosphate also rose. To verify the stability of the clearance and infusion parameters, a time course analysis of MAP, GFR, and percentage excretion of sodium ($\%E_{Na}$) was performed (Fig. 1). In none of the individual urine collection periods was the GFR level statistically different from the mean control value, while the values for MAP and $\%E_{Na}$ were consistently and significantly altered throughout the infusion.

*Isolated tubular perfusion studies.* In the isolated tubular perfusion studies, PSTs exposed to ANP in the surrounding bath (Fig. 2) demonstrated no difference between control and experimental periods for $J_v$ ($0.38 \pm 0.07 \rightarrow 0.41 \pm 0.07$ nl/mm/min, $P > 0.20$), for $J_p$ ($1.50 \pm 0.50 \rightarrow 1.38 \pm 0.36$ pmole/mm/min, $P > 0.50$), or for $P_r$ ($13.66 \pm 0.34 \rightarrow 13.79 \pm 0.31$ nl/min, $P > 0.20$). The values in control tubules were unchanged between periods 1 and 2 for $J_v$ ($0.62 \pm 0.10 \rightarrow 0.66 \pm 0.08$, $P > 0.10$), for $J_p$ ($1.85 \pm 0.25 \rightarrow 1.98 \pm 0.30$, $P > 0.30$), and for $P_r$ ($12.45 \pm 0.27 \rightarrow 13.24 \pm 0.49$, $P > 0.05$), verifying that the tubules were both stable and viable. PSTs dissected from ANP-infused rabbits (Group III) compared to tubules from control animals (Group IV) were without differences in mean $J_v$ ($0.37 \pm 0.05$ vs $0.42 \pm 0.05$ nl/mm/min, $P > 0.50$), in mean $J_p$ ($2.41 \pm 0.27$ vs $2.42 \pm 0.44$ pmole/mm/min, $P > 0.90$), or in $P_r$ ($11.90 \pm 0.20$ vs $11.95 \pm 0.16$ nl/min, $P > 0.80$). Analyses of the data from early and late collection periods in Groups III and IV were calculated to verify the stability of each preparation (Fig. 3). There were no differences between periods in Groups III or IV for $J_v$ ($0.38 \pm 0.04 \rightarrow 0.45 \pm 0.07$ nl/mm/min, $P > 0.30$, and $0.36 \pm 0.05 \rightarrow 0.39 \pm 0.06$ nl/

TABLE I. THE EFFECT OF ANP ADMINISTRATION ON RABBIT CLEARANCE PARAMETERS[a]

| | Controls ($n$ = 6) | | | ANP ($n$ = 12) | | |
|---|---|---|---|---|---|---|
| | C | E | $P$ | C | E | $P$ |
| MAP (mm Hg) | 86.2 ±2.3 | 85.7 ±1.2 | NS | 92.3 ±2.1 | 82.3 ± 1.7 | <0.001 |
| $C_{IN}$ (ml/min) | 15.8 ±1.1 | 14.3 ±0.9 | NS | 16.8 ±2.1 | 17.1 ± 2.5 | NS |
| $C_{PAH}$ (ml/min) | 61.1 ±6.8 | 62.0 ±9.0 | NS | 56.7 ±7.0 | 49.0 ± 9.4 | <0.05 |
| RVR (mm Hg/ml/min) | 1.55 ± 0.27 | 1.56 ± 0.25 | NS | 1.75 ± 0.19 | 1.99 ± 0.30 | NS |
| FF | 0.27 ± 0.02 | 0.25 ± 0.03 | NS | 0.33 ± 0.01 | 0.47 ± 0.07 | <0.01 |
| $V$ (ml/min) | 0.24 ± 0.07 | 0.19 ± 0.05 | NS | 0.22 ± 0.04 | 0.33 ± 0.05 | <0.05 |
| $\%E_{Na}$ | 2.18 ± 0.51 | 1.96 ± 0.54 | NS | 1.97 ± 0.28 | 3.04 ± 0.34 | <0.005 |
| $U_{Na}V$ (μeq/min) | 45.2 ±6.7 | 35.6 ±7.5 | NS | 42.0 ±5.9 | 66.6 ±10.5 | <0.02 |
| $P_{Na}$ (meq/liter) | 139.3 ±1.0 | 138.7 ±1.1 | <0.05 | 136.7 ±0.8 | 135.4 ± 0.9 | <0.001 |
| $F_{Na}$ (meq/min) | 2.21 ± 0.16 | 1.98 ± 0.13 | NS | 2.21 ± 0.24 | 2.41 ± 0.33 | NS |
| $\%E_{PO_4}$ | 2.9 ±1.2 | 4.1 ±1.2 | NS | 9.9 ±1.3 | 13.1 ± 2.0 | <0.02 |
| $U_{PO_4}V$ (pmole/min) | 0.86 ± 0.45 | 1.1 ±0.4 | NS | 2.4 ±0.3 | 3.2 ± 0.4 | <0.05 |
| $P_{PO_4}$ (m$M$) | 1.7 ±0.1 | 1.7 ±0.1 | NS | 1.6 ±0.1 | 1.6 ± 0.1 | NS |

*Note.* Abbreviations: C, control period; E, experimental period; MAP, mean arterial pressure; $C_{IN}$, clearance of inulin; $C_{PAH}$, clearance of paraaminohippurate; RVR, relative renal vascular resistance ($MAP/C_{PAH}$); FF, filtration fraction ($C_{IN}/C_{PAH}$); $V$, urine flow; $\%E_{Na}$, percentage excretion of sodium; $U_{Na}V$, absolute rate of sodium excretion; $P_{Na}$, serum sodium concentration; $F_{Na}$, filtered load of sodium ($C_{In} \times P_{Na}$); $\%E_{PO_4}$, percentage excretion rate of phosphate; $U_{PO_4}V$, absolute rate of phosphate excretion; $P_{PO_4}$, plasma phosphate level.

[a] All data are means ± SE.

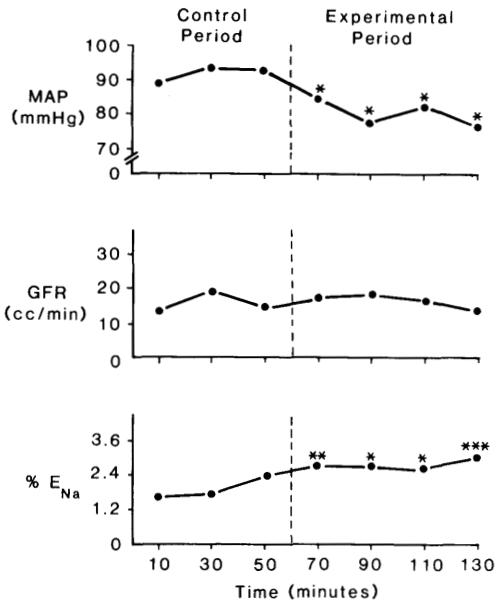


FIG. 1. Time course graph of the mean values for arterial pressure (MAP), glomerular filtration rate (GFR, as estimated by inulin clearance), and the fractional excretion of sodium ($\%E_{Na}$). During the experimental period, ANP was administered as a 1.0 μg/kg bolus and 0.1 μg/kg/min infusion. $P$ values are calculated for each experimental value versus the mean control value. $P$ values: * < 0.01, ** < 0.05, *** < 0.1.

mm/min, $P > 0.70$, respectively), $J_p$ (2.51 ± 0.47 → 2.32 ± 0.42 pmole/mm/min, $P > 0.70$, and 2.51 ± 0.26 → 2.32 ± 0.33 pmole/mm/min, $P > 0.60$, respectively), or $P_r$ (12.1 ± 0.19 → 11.8 ± 0.27 nl/min, $P > 0.40$, and 11.9 ± 0.18 → 11.9 ± 0.28 nl/min, $P > 0.80$, respectively).

**Discussion.** The natriuresis in the clearance experiments reported here cannot be explained by the hemodynamic alterations observed. In the current study, the filtration fraction rose. As an isolated event, this should have decreased rather than increased sodium excretion by enhancing sodium reabsorption in the proximal tubule (24–26). The filtered load of sodium and the GFR did not change, thereby eliminating these two potential factors as causes for the natriuresis observed (27–29). Some authors (3) have attributed the ANP-induced natriuresis to "medullary washout." In a study which indirectly evaluated medullary washout, the circulating level of antidiuretic hormone (ADH) was not controlled or measured. Therefore the suppression of ADH could not be completely excluded as a factor in the diuresis observed (5). Borenstein *et al.* (30) measured an increase in medullary blood flow following the administration of an atrial extract accompanied by a rise in renal plasma flow. In the data presented here, renal plasma flow fell, suggesting that medullary washout probably did not occur.

Another potential explanation is that the natriuresis was caused by a preferential perfusion of superficial cortical nephrons which transport sodium less avidly than their juxtamedullary counterparts. Our data do not directly address this possibility. However, recent micropuncture observations from Huang *et al.* (17) demonstrated that doses of ANP associated with enhanced whole-kidney GFR did not preferentially increase the single nephron GFR in superficial nephrons. The accumulated data thus suggest that inhibition of sodium transport at the tubular level best explains the natriuresis that we observed. Potentially, even a slight inhibition of tubular sodium transport would cause a significant natriuresis at the higher tubular flow rates associated with an increased GFR. Thus, the natriuresis seen with higher doses of ANP, or

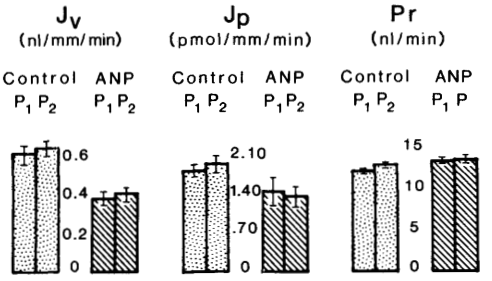


FIG. 2. Isolated proximal straight tubule microperfusion transport parameters from time course control tubules (Control) are compared to values obtained in tubules exposed to atrial natriuretic peptide (ANP) *in vitro*. The composition of the bathing solution was the same for the first and second periods ($P_1$ and $P_2$) of the control tubules and for the first period in the ANP tubules. In period 2 of the ANP tubules, the peptide was added in a concentration of $10^{-9}$ *M*. $J_v$ is a measure of the lumen to bath fluid flux; $J_p$ is a measure of lumen to path phosphate transport. $P_r$ is the rate at which the perfusion solution traversed the tubule. Control: $n = 6$. ANP: $n = 8$. There were no statistically significant differences between $P_1$ and $P_2$ values within either group for all parameters studied.

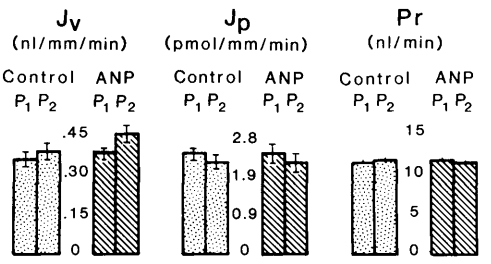


FIG. 3. Mean transport values resulting from isolated tubular perfusion studies obtained in proximal straight tubules harvested from control animals ($n = 6$) and animals given ANP *in vivo* (1.0 μg/kg bolus and 0.1 μg/kg/min infusion; $n = 5$). Two successive collection periods ($P_1$ and $P_2$) were obtained to examine any effects of continued perfusion. $J_v$, $J_p$, and $P_r$ as in Fig. 2. There were no statistically significant differences between $P_1$ and $P_2$ values within either group, nor between control and ANP tubules, for any parameter studied.

in animals more sensitive to its effects, may be secondary to a combination of an enhanced GFR and an inhibition of sodium transport.

Synthetic ANP infusion is frequently associated with an increase in GFR (5–8). Some investigators have reported a linear correlation between the increment in GFR and the increase in sodium excretion and have argued that the enhancement of GFR is the major, if not sole, mechanism for the observed natriuresis (5, 8, 9). Other studies have demonstrated that ANP can induce a natriuresis without increasing GFR (24–26). While there are species and technique differences that prevent direct comparisons between experiments, the data indicate that the enhancement of GFR may be a dose-related phenomenon, occurring only at higher doses of ANP. Yukimura *et al.* (25) have reported that ANP infused at a rate of 0.2 ng/min for 15 min caused a threefold increase in urine flow rate without affecting GFR. Under similar experimental conditions, however, a 1.0 ng/min infusion increased GFR significantly while eliciting a fivefold increase in urine flow rate. However, these experiments do not include a suitable control for comparison. Seymour *et al.* (24), using increasing doses of ANP, demonstrated an increment in absolute sodium excretion from 24 ± 6 to 184 ± 50 μeq/min without any significant change in GFR. The combined data from our clearance study and that available in the literature suggest that mechanisms other than an enhancement of GFR are at least partially responsible for the natriuresis associated with exogenous ANP administration.

Because of the development of a phosphaturia, the proximal tubule was initially felt to be the probable locus of ANP's renal effects (11, 13). This was further suggested by the peptide's ability to block lithium reabsorption, a proximal tubular activity (6). ANP administration has also been shown to inhibit sodium-dependent transport in brush border membrane vesicles from proximal tubules (12). However, evidence is accruing which suggests that ANP has no direct proximal tubular effects. Baum and Toto have recently reported that $J_v$ was not affected by ANP present in the bath of perfused proximal convoluted tubules (PCT) or PSTs, nor did ANP alter transport in the PCT when it was included in the perfusate (18). Huang *et al.*, using a dose of ANP ten times that employed in the studies reported here, demonstrated a natriuresis and a rise in GFR that was associated with an increase in absolute volume reabsorption in the proximal tubule (17). This finding was compatible with preserved glomerulotubular balance; i.e., there was no inhibition of the enhanced absolute rate of fluid reabsorption typically seen with an increase in glomerular filtration rate.

Our studies in the proximal straight tubule are in agreement with the data of Baum and Toto (18). There was no effect on $J_v$ or $J_p$ when $10^{-9}$ *M* ANF was present in the bath surrounding the perfused tubules. We did not add ANP to the solution perfusing the tubules because current data suggest a basolateral receptor for the ANP molecule (31). Furthermore, the *in vivo* infusion of ANP at a dose shown to cause a natriuresis (Group III) guaranteed tubular receptor activation. The study by Hammond *et al.* (13) verified that the *in vivo* administration of ANP can cause effects that persist long enough for subsequent *in vitro* studies to be performed. These data suggest that while the effective half-life of ANP appears to be rather short (3, 6), if the ANP-induced natriuresis was associated with a change in sodium transport in the PST, our isolated tubular perfusion studies (Group III) should have been able to document this effect. There-

fore, the data presented here, added to the observations available in the literature (17, 18), strongly suggest that ANP does not inhibit sodium reabsorption in the proximal tubule.

In summary, clearance data are presented which demonstrate that ANP can cause a significant natriuresis without affecting GFR. These observations, in conjunction with the data available in the literature (24, 25, 30), suggest that the natriuresis is, at least in part, secondary to a nonhemodynamic inhibition of sodium transport at the renal tubular level. Micropuncture and perfusion data indicate that this transport inhibition does not take place in the proximal tubule.

This work was supported, in part, by the Veterans' Administration. The authors thank Dr. Edward Blaine (Merck Institute for Therapeutic Research) for the generous supply of atrial peptide.

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Received May 12, 1986. P.S.E.B.M. 1987, Vol. 185.
Accepted January 21, 1987.