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Four-Hour Atrial Natriuretic Peptide Infusion in Conscious Rats: Effects on Urinary Volume, Sodium, and Cyclic GMP (42813)

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Abstract. Atrial natriuretic peptide (ANP) is released from the cardiac atria in response to acute volume loads; when infused acutely ANP causes diuresis and natriuresis. Cyclic GMP (cGMP) appears to be the second messenger for ANP in the kidney. The role that ANP plays in the long-term regulation of salt and water balance is unclear, however, since resistance to ANP's natriuretic and diuretic activity develops during prolonged administration. The purpose of the present study is to examine the relationship between the rate of cGMP excretion in response to ANP and the development of resistance to ANP's diuretic and natriuretic activity. Following a 30-min baseline period of infusion of Ringer's solution conscious rats received ANP at 15 μg/kg/hr (n = 6) or Ringer's alone (n = 5) for 240 min. ANP-infused rats had a significant diuresis and natriuresis during the first hour of infusion; urinary cGMP excretion also increased compared to baseline. By 120 min after initiating the infusion in ANP-rats urinary volume and sodium excretion had declined to values not significantly different from those of baseline or control. In contrast, urinary cGMP excretion remained elevated for the duration of the ANP infusion, whether compared to baseline values or the control group. Resistance to the diuretic and natriuretic activity of ANP is not a result of mechanisms that involve cGMP generation.

Atrial natriuretic peptide (ANP) is released from the cardiac atria (1) and appears to be important in regulating the excretion of acutely administered volume loads (2, 3). Cyclic GMP (cGMP) appears to be the second messenger for the acute action of ANP in the kidney (2, 4). Several investigators have demonstrated that ANP stimulates cGMP production by the kidney (2, 4–6) and that the diuretic and natriuretic effects of ANP can be mimicked *in vivo* by infusion of cGMP (7).

The role of ANP in the long-term regulation of salt and water balance is unclear, however, since resistance develops to the diuretic and natriuretic actions of ANP after relatively brief periods of ANP infusion (8–11) and in some chronic edematous states in which positive sodium balance persists despite elevated plasma levels of ANP (12–14).

The purpose of this study is to examine the relationship between the development of resistance to the natriuretic and diuretic effects of ANP and ANP's effect on urinary cGMP excretion. Specifically, we tested the hypothesis that the phenomenon of resistance occurs because of loss or reduction in renal synthesis of cGMP during a 4-hr ANP infusion in rats. Our data demonstrate a dissociation between ANP's diuretic and natriuretic effects and ANP's effect on the rate of urinary cGMP excretion.

Methods. Female Sprague–Dawley rats (Harlan Industries, Madison, WI) weighing 200–250 g were housed in individual cages for one week prior to the experiments and allowed free access to water and standard chow (Wayne Rodent Bloc, Continental Grain Co., Chicago, IL). One or two days before study the animals were anesthetized with ketamine hydrochloride (60 mg/kg intramuscularly) and pentobarbital (20 mg/kg intraperitoneally). Polyethylene catheters (PE 10, Clay Adams, New York, NY) were placed in the left carotid artery and the right jugular vein and exteriorized through an incision in the skin of the nape of the neck. A larger catheter (PE 240) with its proximal end heat-flared was placed in the urinary bladder and exteriorized through an incision in the abdominal wall. Following catheter placement the rats were allowed to recover and returned to their cages.

All rats were deprived of food for 18 hr

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before study but were allowed free access to tap water. The rats were divided into two groups: group I, control rats (n = 5), and group II, ANP-infused rats (n = 6). After being weighed each rat was placed in a small enclosure that limited movement and allowed collection of urine from the bladder catheter and fluid administration and blood sampling from the vascular catheters. The rats were conscious during the entire experimental period. An infusion of modified Ringer's solution (sodium 147 meq/liter, potassium 3.6 meq/liter, chloride 141 meq/liter) was begun through the venous catheter at 6 ml/kg/hr and continued throughout the experiment. This infusion was used for the administration of inulin (Sigma Chemical Co., St. Louis, MO; 25 mg/kg prime followed by 22.5 mg/kg/hr) and PAH (Merck, Sharpe and Dohme, West Point, PA; 8 mg/kg prime followed by 18 mg/kg/hr). The arterial catheter was connected to a pressure transducer (Bell and Howell, Pasadena, CA) and recorder (Lafayette Instruments, Lafayette, IN) for measurement of arterial blood pressure and heart rate.

Following 60 min for equilibration a 30-min baseline period for urine collection and heart rate and blood pressure measurement was begun. At the midpoint of this baseline period 750 μl of blood was obtained from the arterial catheter for determination of hematocrit and plasma levels of inulin, PAH, sodium, and potassium. The volume of blood removed was replaced with an equal volume of warmed Ringer's solution. After completion of the baseline period group II rats had ANP (28 amino acid α human-ANP, Peninsula Laboratories, Belmont, CA) added to their intravenous infusions at a rate of 15 μg/kg/hr, a dose shown to cause maximum diuresis and natriuresis in rats (15). Group I received Ringer's without ANP. The infusions were continued for 240 min. During this time urine was collected at 30-min intervals; heart rate and blood pressure were recorded every 15 min and averaged for the 30-min interval. At the conclusion of the experiment another blood sample was obtained from the arterial catheter for hematocrit and plasma levels of inulin, PAH, and ANP. The rats were sacrificed and weighed and their kidneys were removed and weighed.

Urine volumes were determined gravimetrically. Urinary sodium and potassium were analyzed by flame photometry (Instrumentation Laboratory Model 443, Lexington, MA). Plasma and urine inulin were determined by the method of Walser *et al.* (16) and PAH by the method of Smith *et al.* (17). The values for plasma inulin and PAH used in calculating GFR and ERPF for each rat were obtained by averaging the values measured from the two plasma samples. Urinary cGMP was determined by radioimmunoassay on previously collected samples that had been stored at −70°C (New England Nuclear, Boston, MA). Whole blood samples for plasma ANP determinations were collected in chilled glass tubes that contained heparin and aprotinin. After centrifugation plasma was stored at −70°C until assay. Plasma ANP was determined by radioimmunoassay (Peninsula Laboratories, Belmont, CA) following extraction on C18 Bond Elut columns (Analytichem, Harbor City, CA). The intraassay and interassay variabilities were 11.5 and 12.9%, respectively. Data variables not measured directly were calculated from standard formulae.

Rats were excluded from analysis if, during the baseline period, MAP was less than 100 mm Hg (one rat), inulin clearance was less than 0.65 ml/min (one rat), or urinary flow rate was less than 10 μl/min (three rats); rats were also excluded if the kidneys had gross abnormalities by visual examination after sacrifice (one rat). All data are presented as means ± SE. Two-way analysis of variance for repeated measures followed by Dunnett's comparison was used to test for differences between baseline and experimental periods within groups. Two-way analysis of variance for repeated measures followed by Student's t test for unpaired data was used to test for differences between corresponding intervals of the two groups, as appropriate. Differences were considered significant at $P < 0.05$.

Results. MAP and heart rate results are shown in Figs. 1A and B. There were no significant differences in MAP or heart rate between the control rats (group I) and the ANP-infused rats (group II) during the baseline period. In group I neither heart rate nor MAP varied significantly from baseline dur-

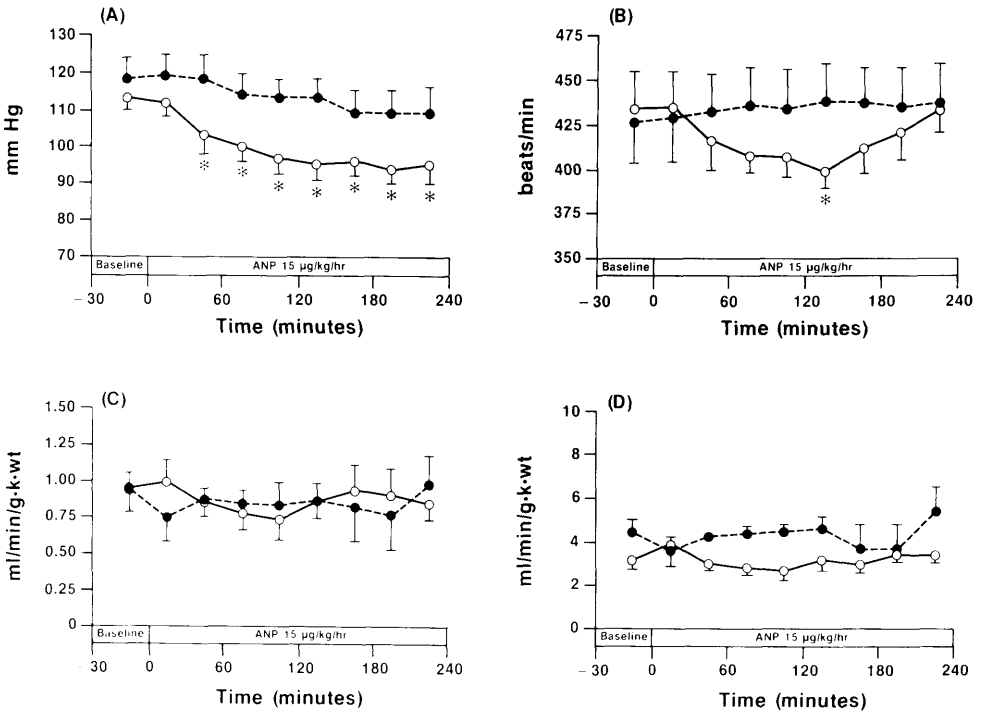

FIG. 1. Time course of values for mean arterial blood pressure (A), heart rate (B), glomerular filtration rate (C), and effective renal plasma flow (D). Closed circles, group I; open circles, group II. ANP was begun in the group II animals at time 0. *, different from corresponding baseline value ($P < 0.05$).

ing the 4-hr infusion protocol. In group II MAP fell significantly 60 min following the start of ANP and was lower than baseline for the remainder of the infusion; MAP values were also significantly lower than those of the corresponding periods of group I. Heart rate did not increase in group II despite the decline in blood pressure.

The effects of ANP infusion on GFR and ERPF are shown in Figs. 1C and D. There appeared to be a small increase in GFR and ERPF following the initiation of ANP in group II; however, significance was not achieved between baseline and experimental periods for either group. Similarly, there were no significant differences seen in GFR and ERPF between groups during any of the experimental intervals.

The effects of the ANP infusion on urinary flow rate and excretion rates of sodium and potassium are shown in Fig. 2. Urinary flow rate (panel A) was comparable between the two groups during the baseline period. In group II urinary flow rate increased immediately following the initiation of the ANP infusion and peaked in the second collection period (30–60 min after ANP was begun). By 120 min after beginning ANP, however, urinary flow rate had returned to a level comparable to baseline and remained at that level for the remainder of the infusion. Urinary sodium excretion rates (panel B) were not significantly different during the baseline period for the two groups. For group I the sodium excretion rate did not vary significantly from baseline during the experiment. For group II, however, the urinary sodium excretion rate increased immediately after beginning the ANP infusion. As with urinary flow rate, the peak sodium excretion rate was seen during the period of 30–60 min but by 120 min had returned to a value comparable to baseline and remained at this level for the remainder of the experiment. The rate of urinary potassium excretion (panel C) declined during the experiment for the rats in group I; by 150 min the urinary potassium excretion rate was significantly lower than baseline.

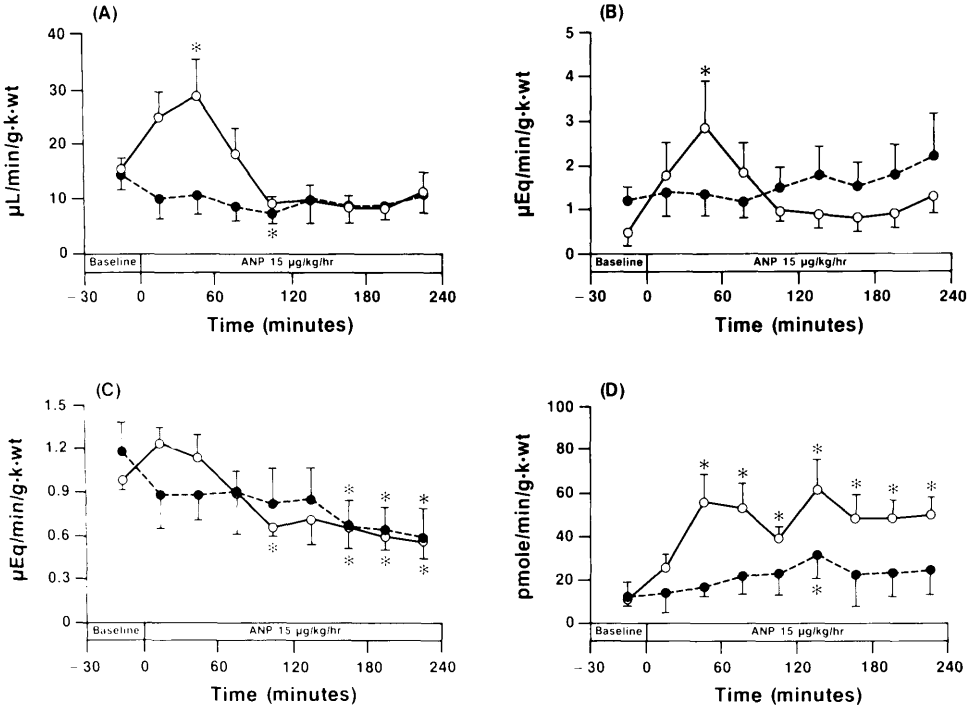

FIG. 2. Time course of values for urinary flow rate (A), urinary sodium excretion rate (B), urinary potassium excretion rate (C), and urinary cGMP excretion rate (D). Scale and symbols as in Fig. 1.

There was a small but statistically insignificant increase in the urinary potassium excretion rate following the initiation of ANP in group II. As with the group I rats the excretion rate of potassium fell throughout the course of the experiment and was significantly below baseline for three of the last four periods. There were no significant differences between groups for corresponding time intervals.

The cGMP excretion rates from groups I and II (Fig. 2D) were not significantly different during the baseline period. For group I the cGMP excretion rate appeared to increase modestly during the course of the experiment but was significantly different from baseline during only one of the experimental periods. The cGMP excretion rate for group II increased immediately following ANP infusion, achieving statistical significance 60 min after ANP was begun. In contrast to the effects of ANP on rates of urinary flow and sodium excretion, the rate of urinary cGMP excretion was significantly higher than baseline throughout the 4-hr infusion period. The mean cGMP excretion rates of the rats in group II were significantly higher than those of the rats in group I for every period during the ANP infusion.

The hematocrits and weights of the rats in the two groups are shown in Table I. The hematocrit fell in both groups, though statistical significance was reached only in group I. Group I had a small but statistically insignificant increase in weight during the experiments; group II had a similarly small but significant decline in weight during the experiments. Immunoreactive ANP at the end of the infusion was 249 ± 89 pg/ml (81 ± 29 p*M*) for group I and 1936 ± 35 pg/ml (628 ± 41 p*M*) for group II ($P < 0.05$).

Discussion. ANP has potent diuretic and natriuretic activity when given acutely in a number of species (8, 9) including man (10, 19, 20). Immunoreactive levels of ANP increase in response to acute volume loads (2, 3), following chronic ingestion of a high salt diet (21, 22), in mineralocorticoid escape (23, 24), and in some pathophysiologic states associated with edema formation, such as

TABLE I. TOTAL BODY WEIGHTS, HEMATOCRITS, AND PLASMA iANP LEVELS BEFORE AND AFTER THE 4-hr INFUSION IN CONTROL AND ANP-INFUSED RATS

	Control rats (n = 5)		ANP-infused rats (n = 6)	
	Pre	Post	Pre	Post
Body weight (g)	230.9 ± 7.5	234.0 ± 6.7	223.7 ± 5.2	221.0 ± 5.6
	N.S.		$P < 0.05$	
Hematocrit (%)	32.6 ± 1.3	27.2 ± 1.3	32.0 ± 3.8	28.2 ± 5.0
	$P < 0.05$		N.S.	
Plasma iANP (pg/ml)	—	249 ± 89	—	1936 ± 435
		$P < 0.05$		

congestive heart failure (12, 13). The role of these peptides in regulating day to day salt and water balance, however, is unclear (1). During chronic salt loading or during the escape phase of hypermineralocorticoid disorders increased release and elevated plasma levels of ANP may be important in mediating the natriuresis and diuresis that result in the maintenance of salt and water balance (21–24). In contrast, in pathophysiologic states associated with edema formation increased release and elevated plasma levels of ANP do not result in diuresis and natriuresis sufficient to restore normal salt and water balance (12, 13). Thus resistance to the diuretic and natriuretic activity of ANP may develop in some settings but not in others. An understanding of the mechanisms that result in resistance to ANP's diuretic and natriuretic activity may be important in understanding better the long-term regulation of salt and water balance in both normal and pathophysiologic states.

Several groups have shown that the diuretic and natriuretic effects of ANP are transient when given as continuous infusions (9, 11) or as repeated bolus injections (8, 10). In our studies resistance to the diuretic and natriuretic effects of ANP developed after 120 min of ANP infusion. The effects of ANP on blood pressure persisted throughout the study. We did not see a significant effect of ANP on ERPF or GFR even during periods when diuresis and natriuresis were greatest though the number of rats in this study may not have been sufficient to detect a difference. Of most interest was the finding that the rate of urinary cGMP excretion remained elevated after resistance to the natriuretic and diuretic effects of ANP had occurred. These data suggest that resistance to the diuretic and natriuretic effects of ANP does not occur through a cGMP-related mechanism.

We used the urinary cGMP excretion rate as an index of ANP-stimulated renal cGMP production. Huang *et al.* (4) found that the intravenous administration of ANP in rats resulted in a 10- to 12-fold increase in the urinary excretion rate of cGMP, a result similar to ours. These investigators demonstrated that the increment in urinary cGMP excretion resulted from increased renal production of cGMP rather than from changes in the filtered load of cGMP or its renal clearance. Based on these findings we believe it reasonable to use the cGMP excretion rate as an index of renal cGMP production.

Our data suggest that resistance to ANP is not a result of mechanisms that involve cGMP metabolism (such as reduction in number of renal ANP receptors, loss of receptor sensitivity to ANP, or increased rates of renal cGMP degradation). In our ANP-infused rats the rate of cGMP excretion (and presumably production) was increased long after resistance to ANP's effects on sodium and water excretion had occurred. There are three possibilities to explain these results. First, cGMP may not be the second messenger for the diuretic and natriuretic effects of ANP. Against this explanation is a substantial body of data (2, 4–6) that demonstrates ANP-induced stimulation of renal cGMP synthesis, and data from *in vivo* studies that demonstrate cGMP-induced diuresis and natriuresis that mimics ANP (7). Second, cGMP may be the second messenger for ANP's diuretic and natriuretic effects, but two or more sites of binding and action for

ANP exist (glomerular and tubular), with receptor down-regulation and loss of cGMP production occurring at one site but not the other. Nonoguchi *et al.* (6) showed two distinct nephron binding sites for ANP (glomerulus and inner medullary collecting duct) that result in cGMP production. cGMP production was noted from glomeruli at ANP concentrations of 100 n*M* or greater, whereas cGMP production from inner medullary collecting ducts occurred at ANP concentrations as low as 1 n*M*. In our studies ANP-infused rats had plasma iANP levels of 0.63 ± 0.14 n*M*, presumably high enough to stimulate cGMP production in collecting ducts but not glomeruli. Though we cannot conclusively rule out the possibility that the urinary cGMP we measured was from two different nephron sites, only one of which was involved in the development of resistance, we feel this is unlikely. A third explanation for our results is that resistance to ANP develops because of mechanisms superimposed on the ANP-exposed kidney which override the ANP/cGMP stimulus for diuresis and natriuresis. We believe this is the most likely explanation for our observations and for the phenomenon of ANP resistance seen in some of the edema-forming pathophysiologic states as discussed below.

A number of factors such as prior water deprivation (25), changes in renal sympathetic tone (14, 26), and reduced renal perfusion pressure (27, 28) have been shown to blunt or abolish the diuresis and natriuresis seen following acute ANP infusion. In our studies resistance to the diuretic and natriuretic effects of ANP developed concurrently with reduced blood pressure and volume depletion. Although the reduction in systemic blood pressure persisted during the entire perfusion protocol, the volume lost during the initial ANP-induced diuresis was almost completely replaced by the end of the 240-min infusion (as judged by the small change in body weight), making it unlikely that a reduction in total body fluid volume was important in the development of resistance. ANP may act by altering the slope of the pressure/natriuresis relationship of the kidney (9, 29), such that small increases in renal perfusion pressure produce greater degrees of natriuresis in the presence of ANP than in its absence. Such a mechanism for ANP's action is consistent with our results if it is assumed that a certain threshold renal perfusion pressure is necessary for the expression of ANP's diuretic and natriuretic action on the kidney, and that during a prolonged ANP infusion the systemic vasodilatory actions of ANP result in a fall of renal perfusion pressure below this threshold. Renal sympathetic tone may also play an important role in modulating the action of ANP and perhaps in the development of resistance (26). Koepke and DiBona (14) showed that the renal response to ANP was blunted in nephrotic rats; sectioning the renal nerves restored the response to that seen with non-nephrotic rats. Further studies are necessary to examine these possible explanations for the development of resistance during prolonged ANP infusion.

Our data do not provide support for the hypothesis that a change in GFR is the mediator of the diuretic and natriuretic response to ANP. As others have shown (18, 20, 30, 31) the increase in urinary volume and sodium excretion occurred without a significant increase in GFR; similarly, resistance to ANP occurred without a significant decline in GFR. GFR and ERPF remained constant during ANP infusion in our rats despite a significant reduction of blood pressure, illustrating the integrity in renal autoregulation of GFR and blood flow despite ANP (15). Caution should be used in interpreting the GFR and ERPF data, however, because of two methodologic limitations. First, the number of rats studied may have been too few to detect small but important changes in these parameters. Second, the values for plasma inulin and PAH used in calculating GFR and ERPF were averaged from measurements made before and after the ANP infusion; transient changes from steady-state plasma levels would not have been detected. Because of these methodologic limitations, definite conclusions about the role of GFR and ERPF in the development of tolerance are not possible from this study.

Finally, we do not believe that increased degradation of ANP explains the resistance that develops during prolonged ANP infusion (11, 32). Plasma iANP levels at the end of the experiment were higher in the infused

rats than in the controls, and the effects of ANP on cGMP excretion rates and blood pressure persisted despite the development of resistance to the natriuretic and diuretic effects. It is possible, however, that our infusion rate of 15 μg/kg/hr exceeded the maximum endogenous production and degradation rates so that we missed a physiologically important mechanism in regulating ANP action. Further studies at lower infusion rates will be needed to test for this possibility.

In summary, we found that prolonged infusion of ANP in the rat results in resistance to the diuretic and natriuretic actions of this peptide at 90 min after initiation of the infusion. Resistance was not associated with a concomitant decline in the excretion rate of cGMP, the presumed second messenger for ANP action in the kidney. Resistance did not develop to the hypotensive effect of ANP at the doses infused. We conclude that resistance to the diuretic and natriuretic actions of ANP is not a result of changes in ANP binding and subsequent cGMP production in the kidney but a result of superimposed mechanisms that blunt or abolish renal ANP responsiveness.

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