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Effects of Sodium Intake, Furosemide, and Infusion of Atrial Natriuretic Peptide on the Urinary and Metabolic Clearances of Arginine Vasopressin in Normal Subjects*

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ABSTRACT. Arginine vasopressin (AVP) and atrial natriuretic peptide (ANP) have important influences on water and electrolyte metabolism, and studies on the interactions between these hormones may have important implications. We have investigated the effects of sodium intake, furosemide, and infusion of ANP on the urinary and metabolic (nonurinary) clearances of AVP in hydrated normal subjects. On a high sodium diet there was an increase in urine volume, sodium excretion, osmolal clearance, plasma ANP concentration, and urinary clearance and fractional excretion of AVP, with a decrease in PRA. The infusion of furosemide increased urine volume, sodium excretion, osmolal clearance, and PRA, but decreased circulating ANP levels and urinary clearance and fractional excretion of AVP. Since there was a positive correlation between

circulating ANP and urinary clearance of AVP in these experiments, we infused human α ANP in physiological amounts and found increases in the urinary and metabolic (nonurinary) clearances of AVP. The changes in urinary clearance of AVP in all three experiments occurred even in relation to creatinine clearance. These observations demonstrate that urinary clearance of AVP does not correlate with urine volume, sodium or solute excretion, or PRA. The observations support a physiological role for ANP in modulating the renal action of AVP, probably at the level of the renal tubules, and indicate a need for caution when using plasma or urinary AVP as an indicator of AVP release from the neurohypophysis. (*J Clin Endocrinol Metab* 70: 222, 1990)

THE INFUSION of arginine vasopressin (AVP) in amounts ranging from 10-350 μ U/kg·min into hydrated normal subjects on uncontrolled sodium intakes causes dose-related increases in urinary AVP concentration and excretion rate, plasma AVP concentration, and urinary and metabolic clearances of AVP. In contrast the fractional excretion of infused AVP was quite consistent (~6.5%) at each infusion rate of AVP (1). The results of these experiments in man have been very closely duplicated in conscious dogs (2).

The present studies were designed and conducted in hydrated normal subjects to clarify the roles of sodium excretion, solute excretion, urinary volume, plasma volume, atrial natriuretic peptide (ANP), and PRA on the metabolic (nonurinary) and urinary clearances of infused AVP in hydrated normal subjects. Our interest in doing this was stimulated by the reports that the urinary clearance of vasopressin is increased in sodium-retaining

states, such as occurs in patients with primary aldosteronism (3) and when desoxycorticosterone is administered to the pig on an unrestricted sodium intake (4). The reverse has been demonstrated (decreased urinary clearance of AVP) in the presence of dehydration (3).

Materials and Methods

The project was approved by the Committee for the Protection of Human Subjects, and informed consent was obtained from each subject. No subjects were taking medication, including oral contraceptives, and female subjects were studied during the first half of the menstrual cycle. The subjects were instructed not to consume alcohol for 48 h or to smoke tobacco for 12 h before each study. Intravenous cannulas were placed in both forearms for administration of infusions and withdrawal of blood samples. Subjects remained recumbent in bed, except for standing briefly to urinate. Specimens from several subjects were not assayed or included in the data because they were unable to consistently empty their bladders, as reflected by variable creatinine and osmolal clearances. Recordings of blood pressure and heart rate were made every 5 min using an automatic device with a self-inflating/deflating cuff and a digital printout.

Three groups of studies were conducted. The purpose of the first set of experiments was to investigate the effect of sodium intake on the metabolism of and response to infused AVP.

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Nine normal subjects (seven males and two females) between 23–37 yr of age (median, 27 yr) were admitted twice to the Clinical Research Center, University Hospital. Each subject was studied once on a diet containing 250 meq sodium/day for 4 days and another time, in random order, when they had consumed 10 meq/day sodium diets for 4 days. At approximately 0800 h after an overnight fast, each subject was given an oral water load of 20 mL/kg over 15–20 min. Urine was passed spontaneously every 15 min, and the volume voided was replaced orally with tap water. After three consecutive periods of constant diuresis, synthetic AVP (Pitressin, Parke-Davis, Morris Plains, NJ), diluted in normal saline, was infused over consecutive periods of 1 h at each of the following rates: 10, 35, and 100 μ U/kg·min. During the AVP infusion subjects voided every 30 min, and the voided volume was replaced with tap water minus the amount infused over the 30-min period. Blood for AVP, PRA, ANP, osmolality, and creatinine determinations was obtained at 30 and 60 min of each infusion rate. We had previously demonstrated under these conditions that plasma

levels of AVP at 30 and 60 min of each infusion rate of AVP were similar, and therefore, an equilibrium state existed from 30–60 min of each infusion rate (1). The urine data used in the calculations are from the second 30-min collection period of each infusion rate.

The second group of studies concerned the effect of furosemide on the response to infused AVP. Nine normal subjects (eight males and one female) between 23–36 yr of age (median, 27 yr), including five who were investigated for the effect of sodium intake, were twice placed on 250-meq sodium diets for 4 days. The experiments proceeded as described above (but with two rates of AVP infusion), except that each subject was once infused with 40 mg furosemide iv over a 5-min period at the beginning of the first rate of infusion of AVP.

The third group of studies was undertaken to determine the effect of infused ANP on the response to AVP. Ten normal subjects (6 males and 4 females) between 22–40 yr of age (median, 26.5 yr), different from those investigated in the above-described studies, were placed on 50-meq sodium diets

FIG. 1. The effect of dietary sodium intake on the response to infused ANP in three different amounts for 1 h each. The studies were conducted in nine normal subjects who had consumed either 10 or 250 meq sodium/day. PRA (B) may be converted to nanograms per (L·s) by multiplying nanograms per mL/h by 0.2778. The *P* values refer to the effect of diet at all infusion rates of AVP, based on analysis of variance for repeated measures.

TABLE 1. Data obtained from infusion of AVP into nine hydrated normal subjects who consumed two different sodium diets for 4 days

Dietary Na (meq/day)	AVP infusion rate ($\mu\text{U/kg} \cdot \text{min}$)			P
	10	35	100	
Urine vol (mL/min)				
250	2.06 $\pm$ 0.39	1.26 $\pm$ 0.20	0.87 $\pm$ 0.13	<0.001
10	0.81 $\pm$ 0.10	0.60 $\pm$ 0.09	0.54 $\pm$ 0.11	
Urine osmolality (mmol/kg)				
250	612 $\pm$ 60	734 $\pm$ 32	815 $\pm$ 33	NS
10	606 $\pm$ 42	689 $\pm$ 39	748 $\pm$ 44	
Osmolal clearance (mL/min)				
250	3.94 $\pm$ 0.43	3.18 $\pm$ 0.38	2.49 $\pm$ 0.30	<0.001
10	1.70 $\pm$ 0.17	1.43 $\pm$ 0.18	1.41 $\pm$ 0.30	
Creatinine clearance (mL/min)				
250	126 $\pm$ 6	129 $\pm$ 8	124 $\pm$ 6	NS
10	118 $\pm$ 8	118 $\pm$ 13	126 $\pm$ 12	
Urinary K excretion ($\mu\text{eq/min}$)				
250	75 $\pm$ 7	58 $\pm$ 10	52 $\pm$ 10	NS
10	80 $\pm$ 11	68 $\pm$ 11	66 $\pm$ 10	
Plasma AVP ($\mu\text{U/mL}$)				
250	1.4 $\pm$ 0.2	2.7 $\pm$ 0.3	5.9 $\pm$ 0.7	NS
10	1.2 $\pm$ 0.2	2.6 $\pm$ 0.2	6.8 $\pm$ 0.5	
Urinary AVP ($\mu\text{U/min}$)				
250	76 $\pm$ 13	193 $\pm$ 23	469 $\pm$ 64	NS
10	45 $\pm$ 5	160 $\pm$ 25	385 $\pm$ 60	
Fraction of AVP excreted				
250	.102 $\pm$.018	.074 $\pm$.009	.062 $\pm$.008	<0.003
10	.061 $\pm$.007	.060 $\pm$.010	.051 $\pm$.008	
Metabolic clearance of AVP (mL/kg $\cdot$ min)				
250	8.30 $\pm$ 1.77	14.1 $\pm$ 2.01	17.9 $\pm$ 2.12	NS
10	8.55 $\pm$ 0.91	13.0 $\pm$ 0.85	14.5 $\pm$ 1.11	
Systolic blood pressure (mm Hg)				
250	125 $\pm$ 4	125 $\pm$ 3	124 $\pm$ 5	<0.005
10	117 $\pm$ 3	122 $\pm$ 7	116 $\pm$ 3	
Diastolic blood pressure (mm Hg)				
250	56 $\pm$ 5	56 $\pm$ 4	57 $\pm$ 4	NS
10	57 $\pm$ 3	61 $\pm$ 4	57 $\pm$ 2	

Values are the mean $\pm$ SEM. P values refer to the effect of diet at all infusion rates of AVP, based on analysis of variance for repeated measures.

for 4 days. They were given the same maintained 20 mL/kg water load as above and then infused with AVP at the rate of 35 $\mu\text{U/kg} \cdot \text{min}$ for 2.5 h. During the last hour they were also infused with 20 μg of the 28-amino acid peptide human αANP (lot FH ANP 28O285C, Bachem, Inc., Torrance, CA). Urine and blood samples were collected as described for the above experiments (except for PRA). The same experiment was subsequently conducted in 9 additional normal subjects (7 males and 2 females) between 23–42 yr of age (median, 25 yr), but with the infusion of 40 μg ANP. The use of ANP was approved by an IND from the FDA. Before use, the ANP was appropriately diluted, filtered, and checked for sterility and the absence of pyrogenicity.

Blood for AVP assay was collected into evacuated glass tubes containing EDTA, chilled immediately, and centrifuged, and the plasma was stored at -20°C for no longer than 2 months. When ready for assay, the plasma was thawed, and the pH adjusted by the addition of 0.1 mL 1 N HCl/mL. Urine for AVP assay was acidified to pH 3.5 with glacial acetic acid and stored at -20°C . Thawed specimens for assay were extracted on Sep-Pak C_{18} columns (Waters Associates, Milford, MA) using absolute methanol for the elution. The RIA was conducted using Ferring K3 antibody (Ferring Pharmaceuticals, New York, NY) and [^{125}I]AVP (DuPont/New England Nuclear Research Products, Boston, MA). The standard curves were constructed from the same batch of AVP that was infused. The maximum sensitivity of the RIA standard curve was 0.4 $\mu\text{U/mL}$ (0.15 μU /assay tube). Intra- and interassay coefficients of variation were 4.6% and 6.8%, respectively.

Extracted recovery of AVP ranged from 83.0–91.5%. The concentration of urine before extraction had little or no effect on the amount of AVP extracted. The AVP obtained from the infusion tubing was assayed and found to have the calculated potency, indicating that no AVP adhered to the plastic bag or tubing.

For measurement of plasma ANP 10 mL blood were collected in EDTA tubes on ice and spun within 1 h in a refrigerated centrifuge. The plasma was stored at -20°C . Within the next 2 weeks the plasma was thawed and spun at 4°C . The supernatant was decanted and pipetted into plastic tubes containing 0.1 N HCl in the ratio of 0.1 mL/mL plasma, centrifuged, and decanted onto Sep-Pak C_{18} cartridges, prepared by sequential washing with 5 mL 100% methanol and 10 mL distilled water. A distilled water wash of 10 mL was followed by a second wash of 5 mL 4% trifluoroacetic acid. The ANP was then eluted with 5 mL 90% ethanol containing 4% trifluoroacetic acid. The eluate was dried with air at room temperature and stored at -20°C until assayed.

For RIA of human αANP , we used antibody and [^{125}I]ANP from Peninsula Laboratories (Belmont, CA; catalog no. RAS 8798 and Y8798, respectively). The antiserum was incubated first in the presence of standard or sample for 24 h and then with labeled ANP for an additional 16 h, both at 4°C . Free and bound αANP were separated by incubating with goat antirabbit immunoglobulin G serum and normal rabbit serum for 2 h at room temperature and then centrifuging. The maximum sensitivity of the RIA was 2.5 pg/mL (2 pg/assay tube). Total binding at the antibody dilution used was 30–40%. Intra- and interassay coefficients of variation were 7.5% and 9.5%, respectively. Recovery of ANP through extraction of ANP from blood ranged from 81.8–88.3%.

Plasma concentrations of PRA were determined by using the angiotensin-1-Biotecx Radioimmunoassay Kit (Biotecx Laboratories, Inc., Friendswood, TX). Osmolality was determined by freezing point depression using an Advanced Instruments Digimatic Osmometer (Advanced Instruments, Needham, MA).

Creatinine concentrations were determined using a Technicon AutoAnalyzer. Sodium and potassium concentrations were determined by flame photometry.

The MCRs of AVP were determined by dividing the infusion rates of AVP in microunits per kg/min by the mean plasma

FIG. 2. The effect of furosemide on the response to infused AVP in different amounts for 1 h each. The studies were conducted in nine normal subjects who had been eating 250-meq/day sodium diets. The conversion factor for PRA is explained in Fig. 1. The P values refer to the effect of the diuretic at both infusion rates of AVP, based on analysis of variance for repeated measures.

AVP (in microunits per mL) at 30 and 60 min of each infusion rate. This calculation is valid because endogenous secretion of AVP is minimal in the water-loaded state. To obtain the true (nonurinary) MCR of AVP (Tables 1, 2, and 3), urinary clearance of AVP was subtracted. Plasma PRA and ANP values (Figs. 1, B and C; 2, B and C; and 3B) are means of specimens collected at 30 and 60 min of each infusion rate of AVP.

Laboratory determinations from paired studies were analyzed by analysis of variance for repeated measures to determine the effect of treatment at all infusion rates of AVP. The effects of AVP plus ANP *vs.* AVP alone were analyzed by paired one-tailed t tests based on the hypothesis that ANP would increase AVP clearance and the associated phenomena.

Results

When subjects consumed the high sodium diet (250 meq/day), urine volume, sodium excretion, and solute clearance were significantly greater during the AVP infusion than when they consumed the low sodium (10 meq/day) diet (Fig. 1A). The high sodium diet caused a decrease in PRA and an increase in circulating ANP

levels (Fig. 1, B and C). When on the high sodium diet, urinary clearance of infused AVP was greater (Fig. 1D), even factored for creatinine clearance (Fig. 1E). The high sodium diet caused the fractional excretion of infused AVP and the systolic blood pressure to be increased (Table 1). Heart rate did not change with a change in either diet or AVP infusion rate.

Administration of furosemide to subjects receiving the 250 meq/day sodium diet markedly increased urine volume, sodium, potassium, and osmolal clearance during the AVP infusion studies (Fig. 2A and Table 2). Conversely, the furosemide decreased urine osmolality (Table 2). Furosemide increased the PRA and decreased circulating ANP levels (Fig. 2, B and C). Furosemide decreased the urinary clearance of infused AVP (Fig. 2D), even in relation to creatinine clearance (Fig. 2E). Furosemide caused the fractional excretion of AVP to be decreased, and it increased diastolic blood pressure (Table 2). There were no changes in heart rate when furosemide was given or when the AVP infusion rate was altered.

TABLE 2. Effect of furosemide on the action of AVP in nine hydrated normal subjects

Furosemide	AVP infusion rate ($\mu\text{U/kg} \cdot \text{min}$)		P
	10	35	
Urine vol (mL/min)			
0	1.72 $\pm$ 0.35	1.14 $\pm$ 0.16	<0.001
+	22.91 $\pm$ 1.11	5.78 $\pm$ 0.64	
Urine osmolality (mmol/kg)			
0	652 $\pm$ 55	731 $\pm$ 29	<0.001
+	272 $\pm$ 3	358 $\pm$ 11	
Osmolal clearance (mL/min)			
0	3.60 $\pm$ 0.41	2.98 $\pm$ 0.39	<0.001
+	22.35 $\pm$ 0.95	7.45 $\pm$ 0.69	
Creatinine clearance (mL/min)			
0	131 $\pm$ 8	132 $\pm$ 10	NS
+	129 $\pm$ 14	127 $\pm$ 27	
Urinary K excretion ($\mu\text{eq/min}$)			
0	74 $\pm$ 8	58 $\pm$ 8	<0.001
+	248 $\pm$ 23	137 $\pm$ 12	
Plasma AVP ($\mu\text{U/mL}$)			
0	1.1 $\pm$ 0.2	2.4 $\pm$ 0.2	NS
+	1.2 $\pm$ 0.2	2.7 $\pm$ 0.3	
Urinary AVP ($\mu\text{U/min}$)			
0	75 $\pm$ 11	213 $\pm$ 35	NS
+	60 $\pm$ 8	161 $\pm$ 22	
Fraction of AVP ex- creted			
0	.099 $\pm$.015	.079 $\pm$.012	<0.05
+	.078 $\pm$.010	.060 $\pm$.007	
Metabolic clearance of AVP (mL/kg $\cdot$ min)			
0	11.0 $\pm$ 1.78	15.1 $\pm$ 1.79	NS
+	9.64 $\pm$ 1.70	13.2 $\pm$ 1.07	
Systolic blood pres- sure (mm Hg)			
0	122 $\pm$ 4	122 $\pm$ 4	NS
+	121 $\pm$ 4	122 $\pm$ 3	
Diastolic blood pres- sure (mm Hg)			
0	70 $\pm$ 2	67 $\pm$ 2	<0.01
+	75 $\pm$ 4	75 $\pm$ 4	

Values are the mean $\pm$ SEM. P values refer to the effect of furosemide at all infusion rates of AVP, based on analysis of variance for repeated measures.

Both rates of infusion of ANP (20 and 40 $\mu\text{g/h}$) into subjects being infused with AVP caused increases in urine volume, osmolal clearance, urine sodium excretion, urine AVP excretion per min, urinary clearance of AVP (in milliliters per min and in relation to creatinine clearance), fractional excretion of infused AVP, and plasma ANP concentration (Fig. 3, A-D, and Table 3). Both rates of ANP infusion decreased urine potassium excre-

tion (Table 3). In contrast, only the 40- μg infusion of ANP decreased plasma AVP and increased urine osmolality and the MCR (nonurinary) of AVP (Table 3). There was no change in heart rate when 20 μg ANP were infused. However, heart rate increased 5 beats/min during infusion of 40 μg ANP ($P < 0.005$).

Plasma levels of ANP were compared at 30 and 60 min of each infusion rate of ANP (20 and 40 $\mu\text{g/h}$), and there were no differences at the two time intervals (change, $+2.6 \pm 7.6$ pg/mL; $P = \text{NS}$). Therefore, an equilibrium state existed from 30–60 min during the ANP infusions.

Discussion

These studies, which were conducted in hydrated normal subjects under steady state conditions of AVP infusion, demonstrate that the increased urinary sodium excretion, osmolal clearance, and urine volume caused by a high sodium intake (250 vs. 10 meq/day) are associated with increased urinary clearance of AVP. In contrast, a much greater natriuresis, solute excretion, and urine volume caused by furosemide is associated with a decreased urinary clearance of AVP. The fractional excretion of AVP in each experiment was changed significantly in the same direction as the urinary clearance of AVP. It is possible that furosemide *per se* decreased urinary clearance of AVP. Nonetheless, these observations do not support the suggestion that the urinary excretion and clearance of AVP are closely correlated with solute clearance (5).

Plasma volume is expected to be expanded when subjects consume high sodium diets vs. low sodium diets. This is confirmed by the decreased circulating PRA values and increased circulating ANP (6–8). Conversely plasma volume is expected to be contracted during furosemide-induced diuresis. This is confirmed by the finding that circulating PRA was increased and ANP was decreased (8–10). The urinary clearance of AVP in these experiments correlates with the plasma volume changes and circulating ANP levels. Changes in MCRs (nonurinary) of AVP induced by the high sodium diet and by furosemide are in the same direction as changes in urinary clearances, but are not statistically significant. The increased urinary clearance of AVP caused by plasma volume expansion in our studies may explain the increased urinary clearance of vasopressin in deoxycorticosterone-treated pigs (4) and in patients with primary aldosteronism (3) noted in the *Introduction*.

Since the urinary clearance of AVP correlated with endogenously attained ANP levels, we infused ANP in amounts that we estimated would attain plasma levels that would produce physiological effects in normal subjects (11, 12). The levels of ANP attained increased urine volume, sodium excretion, and osmolal clearance and

FIG. 3. The effect of infusing 20 μ g ANP over 1 h on the response to AVP infused at the rate of 35 μ U/kg·min. The studies were conducted in 10 normal subjects who had been eating 50-meq/day sodium diets. The mean plasma ANP level attained was 54.9 pg/mL.

also increased AVP excretion, fractional excretion, and urinary clearance of AVP. The infusion of 40 μ g ANP lowered plasma AVP levels and increased the MCR of AVP. The changes in urinary clearance of AVP that were caused by alterations in plasma volume (diet or diuretic) or by infusion of ANP were significant even when factored for creatinine clearance. Therefore, the effect of altered plasma volume and/or ANP may be exerted at the level of the renal tubules. Binding sites for ANP are present in proximal tubules, outer medulla, and inner medullary collecting ducts (13). Chai *et al.* (14) have described receptors in the inner and outer renal medulla of the rat. Alterations in urinary clearance of AVP by tubular actions are possible because there is considerable evidence that after vasopressin is filtered through the glomerulus it is reabsorbed or degraded proximally and secreted distally (15, 16). The proximal tubular actions apparently involve two mechanisms, one involving hydrolysis by luminal peptidases, and another involving endocytosis with transfer of the hormone to lysosomes and rapid degradation therein (17). Since our calculations of the MCR of AVP exclude urinary clearance, the increase in the MCR of AVP that resulted from the infusion of 40 μ g ANP would apparently result from increased visceral metabolism of AVP. In dog experiments, liver and prehepatic viscera, presumably intestine, account for most of the metabolism of AVP, although there may be substantial clearance of AVP by other organs (18).

Despite the known vasodilatory action of ANP, the infusion of ANP caused no change in blood pressure, which was also noted by Cody *et al.* (19) in supine normal subjects infused with much larger amounts of ANP. Twenty micrograms of ANP did not alter the pulse rate.

However, the infusion of 40 μ g ANP increased the pulse rate by 5 beats/min, which could be a subtle consequence of vasodilatation. Both rates of infusion of ANP decreased the rate of potassium excretion. This effect of AVP has previously been observed in man (20) and could be due to increased distal tubular sodium potassium exchange.

ANP inhibits the release of AVP *in vitro* (21), and ANP also has been claimed to inhibit AVP release in man under basal conditions and in response to osmotic and postural stimuli (22–24). The evidence for this concept is the presence of lower plasma AVP levels when ANP is infused. Our present observations should cause a reassessment of the postulated inhibition of AVP release by ANP, since the ANP might have lowered circulating AVP levels by increasing its clearance. In addition, there is increasing evidence that ANP may exert direct renal actions as part of the mechanism by which this hormone increases the excretion of water (in addition to sodium) (14). ANP inhibits AVP-stimulated water transport in rabbit collecting tubules perfused *in vitro* (25). A similar observation has been reported using the rat inner medullary collecting duct (26). Although our studies did not reveal an antagonism between ANP and the antidiuretic action of AVP, other studies in man, using 5–10 times as much ANP, have demonstrated such an action of ANP (27, 28). These observations suggest that in addition to its role in sodium balance, ANP may act as a hormone of water homeostasis by antagonizing the antidiuretic action of AVP. Our results suggest that ANP may, as part of its effect on water homeostasis, increase the urinary clearance and perhaps the metabolic clearance of AVP. This mechanism may be responsible for the renal resistance to AVP that occurs in the deoxycor-

TABLE 3. Effect of infusing 20 and 40 μg ANP during 1 h on the action of 35 $\mu\text{U}/\text{kg} \cdot \text{min}$ AVP in 9 and 10 hydrated normal subjects, respectively

20 μg ANP		P	40 μg ANP		P
Urine vol (mL/min)					
0	0.74 $\pm$ 0.11	<0.01	0	0.69 $\pm$ 0.11	<0.05
+	1.19 $\pm$ 0.19		+	1.05 $\pm$ 0.21	
Urine osmolality (mmol/kg)					
0	748 $\pm$ 41	NS	0	718 $\pm$ 24	<0.05
+	730 $\pm$ 26		+	781 $\pm$ 34	
Osmolal clearance (mL/min)					
0	1.88 $\pm$ 0.23	<0.005	0	1.72 $\pm$ 0.26	<0.005
+	2.98 $\pm$ 0.43		+	2.70 $\pm$ 0.40	
Creatinine clearance (mL/min)					
0	113 $\pm$ 17	NS	0	101 $\pm$ 12	NS
+	111 $\pm$ 10		+	124 $\pm$ 16	
Urinary Na excretion ($\mu\text{eq}/\text{min}$)					
0	103 $\pm$ 16	<0.01	0	93 $\pm$ 23	<0.005
+	193 $\pm$ 26		+	245 $\pm$ 51	
Urinary K excretion ($\mu\text{eq}/\text{min}$)					
0	72 $\pm$ 12	<0.05	0	82 $\pm$ 13	<0.01
+	51 $\pm$ 7		+	52 $\pm$ 9	
Plasma AVP ($\mu\text{U}/\text{mL}$)					
0	3.0 $\pm$ 0.2	NS	0	2.9 $\pm$ 0.3	<0.005
+	2.9 $\pm$ 0.2		+	2.5 $\pm$ 0.3	
Urinary AVP ($\mu\text{U}/\text{min}$)					
0	218 $\pm$ 42	<0.0005	0	191 $\pm$ 35	<0.05
+	274 $\pm$ 48		+	227 $\pm$ 24	
Urinary clearance of AVP (mL/min)					
0	70.9 $\pm$ 9.0	<0.0005	0	68.2 $\pm$ 9.4	<0.01
+	92.7 $\pm$ 10.0		+	100.7 $\pm$ 9.5	
Urinary clearance of AVP/creatinine clearance					
0	0.68 $\pm$ 0.08	<0.05	0	0.72 $\pm$ 0.12	<0.01
+	0.89 $\pm$ 0.10		+	0.92 $\pm$ 0.15	
Fraction of AVP excreted					
0	0.086 $\pm$ 0.012	<0.0005	0	0.078 $\pm$.012	<0.01
+	0.109 $\pm$ 0.014		+	0.095 $\pm$.009	
Metabolic clearance of AVP (mL/kg $\cdot$ min)					
0	11.2 $\pm$ 0.73	NS	0	12.6 $\pm$ 1.46	<0.05
+	11.2 $\pm$ 0.71		+	15.0 $\pm$ 2.07	
Plasma ANP (pg/mL)					
0	16.1 $\pm$ 2.3	<0.0005	0	12.7 $\pm$ 1.2	<0.0005
+	54.9 $\pm$ 3.3		+	71.5 $\pm$ 3.1	
Systolic blood pressure (mm Hg)					
0	114 $\pm$ 4	NS	0	115 $\pm$ 3	NS
+	115 $\pm$ 4		+	117 $\pm$ 2	
Diastolic blood pressure (mm Hg)					
0	70 $\pm$ 3	NS	0	67 $\pm$ 3	NS
+	68 $\pm$ 2		+	68 $\pm$ 2	

Values are the mean $\pm$ SEM. P values refer to the effect of either 20 or 40 μg ANP on the various measurements (by paired one-tailed t test).

ticosterone-treated dog (29).

The circulating levels of ANP, on either a high or low sodium intake, are similar at each rate of infusion of AVP and to that measured before AVP was infused (7.0 $\pm$ 1.3 pg/mL on low sodium intake and 16.9 $\pm$ 3.2 pg/mL on high sodium intake). Since the plasma AVP levels

attained in these experiments cover the usual physiological range (in the absence of hypovolemia or hypotension), our data do not support the concept that in man AVP exerts a physiological role in stimulating the release of ANP, as has been observed *in vitro* (30) and in intact rats (31) and dogs (32). In contrast, when the PRA was stimulated by the low sodium diet to 1.58 $\pm$ 0.36 ng/(L $\cdot$ s) before the infusion of AVP, there was a progressive decline in PRA with increasing infusion rates of AVP (P < 0.005). This is consistent with the observations that AVP inhibits renin release in rats (33), dogs (34), and man (35).

In summary, these experiments demonstrate that in hydrated normal subjects infused with AVP, the administration of a high sodium diet and furosemide results in a positive correlation between urinary clearance of AVP and plasma ANP concentration, but not with urine volume, sodium or solute excretion, or PRA. Since the infusion of physiological amounts of human α ANP increases urinary clearance and perhaps metabolic (nonurinary) clearance of AVP, we believe that ANP may play a role in modulating the renal action of AVP. Our observations also indicate that endogenous ANP levels should be considered when using either plasma or urinary AVP as an indicator of AVP release from the neurohypophysis (36–38).

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References

1. Moses AM, Steciak E. Urinary and metabolic clearances of arginine vasopressin in normal subjects. *Am J Physiol*. 1986;251:R365–70.
2. Sondeen JL, Claybaugh JR. Clearance and urinary excretion of vasopressin in conscious dogs. *Am J Physiol*. 1989;256:R291–8.
3. Horky K, Sramkova J, Widimsky Jr J, Dvorakova J. Plasma concentration and urinary excretion of arginine-vasopressin in primary aldosteronism during the fluid deprivation tests. *Exp Clin Endocrinol*. 1983;82:347–55.
4. Brooks DP, Crofton JT, Share L, et al. Increased urinary vasopressin excretion in the DOCA-hypertensive pig. *Clin Exp Hypertension Theory Pract*. 1983(Suppl 10):1717–34.
5. Robertson GL. The regulation of vasopressin function in health and disease. *Recent Prog Horm Res*. 1977;33:333–74.
6. Sagnella GA, Markandu ND, Shore AC, MacGregor GA. Effects of changes in dietary sodium intake and saline infusion on immunoreactive atrial natriuretic peptide in human plasma. *Lancet*. 1985;2:1208–10.
7. Brown JJ, Davies DL, Lever AF, Robertson JIS. Variations in plasma renin concentration in several physiological and pathological states. *Can Med Assoc J*. 1964;90:201–6.
8. Williams GH, Cain JP, Dluhy RG, Underwood RH. Studies of the control of plasma aldosterone concentration in normal man. I. Response to posture, acute and chronic volume depletion, and sodium loading. *J Clin Invest*. 1972;51:1731–41.
9. Kimura T, Abe K, Ota K, et al. Effects of acute water load, hypertonic saline infusion, and furosemide administration on atrial natriuretic peptide and vasopressin release in humans. *J Clin*

- Endocrinol Metab. 1986;62:1003-10.
10. Anderson Jr GH, Miller M, Blakeman N, Streeten DHP. Effect of volume depletion and repletion on plasma concentrations of atrial natriuretic hormone in essential hypertension. In: Brenner B, Laragh J, eds. Advances in atrial peptide research. Am Soc Hypertension Symp Ser. New York: Raven Press; 1988;2:547-52.
 11. Richards AM, Tonolo G, Montorsi P, et al. Low dose infusions of 26- and 28-amino acid human atrial natriuretic peptides in normal man. J Clin Endocrinol Metab. 1988;66:465-72.
 12. Richards AM, McDonald D, Fitzpatrick MA, et al. Atrial natriuretic hormone has biological effects in man at physiological plasma concentrations. J Clin Endocrinol Metab. 1988;67:1134-9.
 13. Kramer HJ. Atrial natriuretic hormones. Gen Pharmacol. 1988;19:747-53.
 14. Chai SY, Sexton PM, Allen AM, et al. *In vitro* autoradiographic localization of ANP receptors in rat kidney and adrenal gland. Am J Physiol. 1986;250:F753-7.
 15. Walter R, Bowman RH. Mechanism of inactivation of vasopressin and oxytocin by the isolated perfused rat kidney. Endocrinology. 1973;92:189-93.
 16. Kimura T, Share L. Characterization of the renal handling of vasopressin in the dog by stop-flow analysis. Endocrinology. 1981;109:2089-94.
 17. Carone FA, Christensen EI, Flouret G. Degradation and transport of AVP by proximal tubule. Am J Physiol. 1987;253:F1120-8.
 18. Share L, Kimura T, Matsui K, et al. Metabolism of vasopressin. Fed Proc. 1985;44:59-61.
 19. Cody RJ, Atlas SA, Laragh JH, et al. Atrial natriuretic factor in normal subjects and heart failure patients. J Clin Invest. 1986;78:1362-74.
 20. Groban L, Ebert TJ, Kreis DU, et al. Hemodynamic, renal, and hormonal responses to incremental ANF infusions in humans. Am J Physiol. 1989;256:F780-6.
 21. Crandall ME, Gregg CM. *In vitro* evidence for an inhibitory effect of atrial natriuretic peptide on vasopressin release. Neuroendocrinology. 1986;44:439-45.
 22. Fujio N, Ohashi M, Nawata H, et al. Alpha-human atrial natriuretic polypeptide reduces the plasma arginine vasopressin concentration in human subjects. Clin Endocrinol (Oxf). 1986;25:181-7.
 23. Allen MJ, Ang VTY, Bennett ED, Jenkins JS. Atrial natriuretic peptide inhibits osmolality-induced arginine vasopressin release in man. Clin Sci. 1988;75:35-9.
 24. Williams TDM, Walsh KP, Lightman SL, Sutton R. Atrial natriuretic peptide inhibits postural release of renin and vasopressin in humans. Am J Physiol. 1988;255:R368-72.
 25. Dillingham MA, Anderson RJ. Inhibition of vasopressin action by atrial natriuretic factor. Science 1986;231:1572-3.
 26. Nonoguchi H, Sands JM, Knepper MA. Atrial natriuretic factor inhibits vasopressin-stimulated osmotic water permeability in rat inner medullary collecting duct. J Clin Invest. 1988;82:1383-90.
 27. Brown J, Forsling ML, Valdes G, et al. Antagonism of V₂-receptor effect of antidiuretic hormone by atrial natriuretic peptide in man. Experientia. 1988;44:513-6.
 28. Vierhapper H, Nowotny P, Waldhauser W. Effect of human atrial natriuretic peptide on 1-deamino-D-Arg⁸-vasopressin-induced antidiuresis in man. J Clin Endocrinol Metab. 1988;66:124-7.
 29. Gullner HG, West D, Gill Jr JR, Robertson GL. Diabetes insipidus with renal resistance to vasopressin in the desoxycorticosterone-treated dog: a possible role for prostaglandins. Renal Physiol (Basel). 1987;10:40-6.
 30. Sonnenberg H, Veress AT. Cellular mechanism of release of atrial natriuretic factor. Biochem Biophys Res Commun. 1984;124:443-9.
 31. Itoh H, Nakao K, Yamada T, et al. Modulatory role of vasopressin in secretion of atrial natriuretic polypeptide in conscious rats. Endocrinology. 1987;120:2186-8.
 32. Inoue M, Kimura T, Ota K, et al. Effect of vasopressin on atrial natriuretic peptide release and renal function in dogs. Am J Physiol. 1988;255:E449-55.
 33. Henderson IW, Balment RJ, Oliver JA. Vasopressin effects on plasma renin activity in male and female rats. Clin Sci Mol Med. 1978;55:301-7.
 34. Johnson MD, Kinter LB, Beeuwkes R. Effects of AVP and DDAVP on plasma renin activity and electrolyte excretion in conscious dogs. Am J Physiol. 1979;236:F66-70.
 35. Khokhar AM, Slater JDH, Forsling ML, Payne NN. Effects of vasopressin on plasma volume and renin release in man. Clin Sci Mol Med. 1976;50:415-24.
 36. Arisaka O, Shimura N, Hosaka A, et al. Water intoxication in asthma assessed by urinary arginine vasopressin. Eur J Pediatr. 1988;148:167-9.
 37. Miller M, Moses AM. Urinary antidiuretic hormone in polyuric disorders and in inappropriate ADH syndrome. Ann Intern Med. 1972;77:715-21.
 38. Moses AM. Clinical and laboratory observations in the adult with diabetes insipidus and related syndromes. Front Horm Res. 1985;13:156-75.