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Natriuretic Effect of Atrial Natriuretic Peptide in Diabetes Insipidus Rats (42650)

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Abstract. Washout of the solute concentration gradient in the renal medullary interstitium has been suggested to play a role in mediating the natriuretic response to atrial natriuretic peptide (ANP). The purpose of this study was to determine the effects of ANP 8-33 on sodium excretion in Brattleboro diabetes insipidus (DI) rats, in which medullary tonicity is known to be decreased as compared to Long–Evans (LE) control rats. Basal urine osmolality (Uosm) was significantly lower in DI rats as compared to LE rats (123 ± 6 vs 673 ± 38 mOsm/kg). Infusion of ANP 8-33 at a rate of 4 μg/kg/hr for 60 min resulted in a significantly greater increase in UnaV (Δ6.1 ± 1.2 vs Δ2.9 ± 0.7 μEq/min) and urine flow (Δ40 ± 12 vs Δ8 ± 7 μl/min) in the LE rats than in the DI rats. The greater natriuresis occurred in the LE rats despite no significant change in Uosm. Fractional lithium reabsorption (an indicator of proximal sodium reabsorption) decreased similarly in both groups. Infusion of ANP had no effect on mean arterial pressure in LE and DI groups. In summary, infusion of ANP in the DI rat resulted in a significant natriuresis, albeit less than in LE rats. The natriuresis in the LE rats occurred despite no significant change in Uosm. These data suggest that mechanisms other than medullary washout are responsible for the natriuretic effects of ANP.

Atrial natriuretic peptides (ANP), which are synthesized and stored in atrial myocytes, have been shown to have potent natriuretic and diuretic activity (1–3). However, the mechanism whereby this peptide alters sodium excretion has not yet been elucidated.

One proposed mechanism for the natriuretic action of ANP is that ANP reduces sodium reabsorption via washout of the medullary osmotic gradient (4–6). In support of this concept, ANP has been reported to increase medullary blood flow (7), as well as papillary plasma flow (8). In addition, preliminary studies have found that infusion of ANP reduces the renal interstitial solute and urea concentrations (9).

Although most components of the medullary washout mechanism have been demonstrated with ANP, the importance of this mechanism in mediating the natriuresis is unclear. In a previous study, Reineck and Parma examined the effect of salt loading in rats and showed that medullary tonicity influences renal sodium handling only when sodium delivery from the proximal tubules is increased (10). ANP is known to enhance delivery of sodium from the proximal tubule either by increasing glomerular filtration rate (5) or by inhibiting tubular reabsorption (6). Thus, enhanced delivery of sodium from the proximal tubules in combination with a reduction in the solute concentration gradient in the renal medullary interstitium during ANF infusion should enhance sodium excretion. The importance of this effect of ANF in contributing to the natriuresis of ANF infusion is not known.

The objective of this study was to determine the effect of ANP on sodium excretion in Brattleboro diabetes insipidus (DI) rats and Long–Evans (LE) controls.

Materials and Methods. Experiments were performed in male Brattleboro homozygous rats and Long–Evans rats (120–160 g body weight, 6–7 weeks old, Blue Spruce Farms, Inc.). The rats were fed normal rat chow (Ralston Purina Co., St. Louis, MO) containing 0.1 mEq sodium per gram. One to two days prior to the experiment, 0.02 m*M* lithium per gram was added to the rat chow. The rats were anesthetized with Inactin (100 mg/kg body weight, ip), and placed on a heated table to maintain body temperature of 36–38°C. Following a tracheotomy, catheters were inserted into a jugular vein for infusions, a carotid artery for blood sampling and blood pressure monitoring, and the dome of the bladder for urine collection. The

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rats were infused with a solution containing 5% inulin, 6.25% albumin, and 3 m*M* lithium chloride in isotonic saline and the rate of infusion was 1 ml/hr for the duration of the experiment. Simultaneously, 10 m*M* sodium chloride in 1.4% glucose for DI and isotonic saline for LE were infused to be adjusted to equal the urine flow at approximately 6 and 0.5 ml/hr, respectively. After urine flow became constant, two 20-min clearances were obtained with a 0.5-ml blood sample taken at midpoint of the two clearance periods. After the removal of the plasma, the packed red cells were resuspended in isotonic saline and returned to the rat. ANP (atrial natriuretic factor 8-33, rat, Peninsula Laboratories, Inc.) was then infused at a rate of 4 μg/kg/hr. This dose of ANP has been previously shown to have a maximal natriuretic effect without changes in renal perfusion pressure. Following a 20-min equilibration period, clearance measurements were repeated. Urinary losses were replaced with an equivalent volume of isotonic saline for LE and with 10 m*M* sodium chloride in 1.4% glucose for DI.

Inulin concentrations were determined by the anthrone method (11). Sodium and potassium concentrations were measured with Beckman E2A electrolyte analyzer. Lithium was measured by atomic absorption photometry.

To estimate sodium reabsorption by proximal tubules of the kidney as a whole, the lithium-clearance method was used. This method is based on evidence that lithium is reabsorbed almost exclusively in the proximal tubule, including the pars recta, in parallel with sodium and water (12–14).

All values are expressed as means ± SE. Control and ANP values are averages of two collection periods. Statistical comparisons were made using the Student paired or group *t* test as appropriate. Significance was designated as $P < 0.05$.

Results. Table I summarizes the data during control and infusion of synthetic ANP for LE and DI rats. There was no change in mean arterial pressure with ANP infusion in either group. Basal level of glomerular filtration rate (GFR) was significantly less in DI rats. With infusion of ANP, GFR tended to increase in both groups although it did not reach statistical significance.

TABLE I. THE EFFECTS OF ATRIAL NATRIURETIC PEPTIDE INFUSION ON RENAL FUNCTION

		LE (n = 7)	DI (n = 7)
MAP (mm Hg)	Basal	108 ± 4	115 ± 4
	ANP	106 ± 4	114 ± 4
GFR (ml/min)	Basal	1.8 ± .1	1.3 ± .1**
	ANP	1.9 ± .1	1.5 ± .2
UV (μl/min)	Basal	25 ± 3	108 ± 7**
	ANP	66 ± 11*	115 ± 11
$U_{Na}V$ (μEq/min)	Basal	3.0 ± 1.7	2.0 ± .5
	ANP	9.1 ± 1.8*	4.9 ± .7*
FE_{Na} (%)	Basal	1.1 ± .2	1.1 ± .3
	ANP	3.3 ± .6*	2.5 ± .4*
FR_{Li} (%)	Basal	80 ± 2	83 ± 3
	ANP	68 ± 4*	73 ± 3*
Uosm (mOsm/kg)	Basal	673 ± 38	123 ± 6**
	ANP	573 ± 92	178 ± 11
Posm (mOsm/kg)	Basal	287 ± 4	295 ± 5
	ANP	286 ± 2	299 ± 4
P_{Na} (mEq/liter)	Basal	142.4 ± .8	140.1 ± 2.6
	ANP	142.6 ± 1.0	142.8 ± 2.0

Note. Values are means ± SE. LE, Long-Evans control rats; DI, Brattleboro diabetes insipidus homozygous rats; MAP, mean arterial pressure; GFR, glomerular filtration rate; UV, urine flow; U_{Na} V, urinary sodium excretion; FR_{Li}, fractional reabsorption of lithium; Uosm, urine osmolality; Posm, plasma osmolality; P_{Na}, plasma sodium concentration.

* $P < 0.05$ Basal vs ANP.

** $P < 0.05$ DI vs LE in basal value.

Urine flow increased significantly in LE, whereas there was no change in DI (UV, Δ40 ± 12 vs Δ8 ± 7 μl/min, $P < 0.05$). Absolute and fractional excretion of sodium increased in both LE and DI. The increase in absolute excretion of sodium was significantly greater in LE compared to DI ($U_{Na}V$, Δ6.1 ± 1.2 vs Δ2.9 ± 0.7 mEq/min, $P < 0.05$).

Fractional excretion of sodium increased significantly in both the LE and the DI groups after ANP infusion. Fractional reabsorption of lithium decreased significantly in both groups. There was no difference in the decrease between LE and DI (Δ − 13 ± 3 vs Δ − 10 ± 3%).

Urinary osmolality in DI was significantly lower than LE controls. There was no significant change in this variable with ANP infusion in either group.

Discussion. Intravenous infusion of ANP increases inner medullary blood flow (7) and papillary flow (8). It also decreases papillary urea and salt concentration (9), suggesting that ANP induces a medullary washout. In dogs, continuous infusion of ANP leads to a

decrease in urine osmolality without a change in free water clearance (6). Therefore, an increase in inner medullary blood flow and medullary washout may be an important contributing factor to the ANP-induced natriuresis.

Previous studies by Reineck and Parma showed that volume expansion induced natriuresis is enhanced in the presence of a reduced solute gradient in the renal medullary interstitium (10). They suggested that enhanced delivery from the proximal tubule is necessary to demonstrate an effect of medullary washout on urinary sodium excretion (10). In Brattleboro rats, papillary plasma flow is high (15) and medullary tonicity is decreased (16) because of the absence of circulating vasopressin. In the present study, the fractional reabsorption of lithium, an indicator of proximal reabsorption of sodium (12–14), decreased to similar extent in Brattleboro rats and Long–Evans controls. However, despite similar reductions in proximal sodium reabsorption, the natriuresis was significantly lower in the DI group than in the LE group. In contrast to other studies, a significant natriuresis was observed in the LE group despite no change in urine osmolality (6, 7). These findings suggest that the medullary washout mechanism plays no major role in mediating the natriuresis of ANP. In support of this suggestion are recent studies by Kiberd *et al.* which dissociated ANP-induced natriuresis with increases in medullary blood flow (17). They reported that the increase in urine flow and sodium excretion occurred before an increase in vasa recta blood flow (17). Similar studies by Takewaza *et al.* also demonstrated a dissociation between papillary blood flow and sodium excretion after ANP infusion (18). In addition, Hildebrandt and Banks reported that a functional papilla was not required for the action of ANP in the rat (19). Thus, these findings and those of the present study would suggest that the increase in sodium excretion during ANP infusion is mediated by mechanisms other than medullary washout.

Glomerular filtration rate in Brattleboro rats is significantly less than Long–Evans rats since the single nephron glomerular filtration rate is abnormally low in deep nephrons of rats with diabetes insipidus (20, 21). Atrial natriuretic peptide tended to increase glomerular filtration rate in both groups, although it did not reach statistical significance. The lower baseline glomerular filtration rate in the DI rats may have contributed to the lower natriuretic response to ANP.

In summary, infusion of synthetic ANP resulted in a significant natriuresis in the DI rat, albeit less than LE rats. In addition, a significant natriuresis occurred in LE rats despite no change in urine osmolality. These data suggest that the natriuresis of ANP is mediated by mechanisms other than medullary washout.

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