Role of Atrial Natriuretic Peptide in Adaptation of Sodium Excretion with Reduced Renal Mass

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Abstract

The kidney maintains constancy of body fluid volume by regulating urinary sodium (Na) excretion. In chronic renal failure, the reduction in glomerular filtration rate (GFR) is accompanied by an increase in Na excretion per nephron if dietary Na intake is not changed. Reduction in Na intake in proportion to reduced GFR obviates this adaptive increase in tubule Na excretion. To examine the potential role of endogenous atrial natriuretic peptide (ANP) in modulating the enhanced Na excretion per nephron in chronic renal failure, we studied rats subjected to 5/6 nephrectomy or sham operation on low, normal, and high Na intakes. Urinary Na excretion increased with increasing dietary Na in all groups, and Na excretion per nephron was increased in 5/6 nephrectomized rats as compared with sham-operated rats on the higher Na intakes. Plasma ANP levels were unaffected by dietary Na manipulations in sham-operated rats, but rose progressively in 5/6 nephrectomized rats with increasing Na intake. Despite extensive nephron reduction, however, plasma ANP levels failed to rise in uremic rats on low Na diets and in this group Na excretion per nephron also failed to rise. We conclude that enhanced ANP secretion may play an important role in promoting the adaptive increase in Na excretion per nephron in chronic renal failure. Restriction of dietary Na in the setting of reduced GFR obviates the stimulation of ANP secretion as well as the adaptive increase in Na excretion rate per nephron.

Introduction

The normal kidney possesses a remarkable ability to maintain constancy of extracellular fluid volume (ECFV)[1] despite a wide range of dietary sodium (Na) intakes. As Na intake increases, proximal and distal tubule Na reabsorption rates fall, and the fractional excretion of sodium (FE_{Na}) increases, so that external Na balance is maintained. However, when the glomerular filtration rate (GFR) is reduced by surgical renal ablation or by intrinsic renal disease, FE_{Na} must increase to maintain Na balance in the face of unaltered Na intake. Studies in rats have demonstrated that this increased FE_{Na} occurs immediately, well before changes in whole kidney or single nephron GFR are demonstrable (1).

That this increase in FE_{Na} in chronic renal failure is dictated by the requirements for external Na balance has been elegantly demonstrated in studies by Bricker and colleagues (2, 3), who showed that the obligatory increase in FE_{Na} is abolished if Na intake is reduced in proportion to the reduction in GFR. Thus, as dietary Na intake was restricted in proportion to the decrease in GFR in dogs with chronic renal failure, Na excretion rate also decreased and thereby obviated the adaptive increase in FE_{Na}. Despite extensive investigation, however, the physiologic mechanisms responsible for the adaptation in Na excretion per nephron in chronic renal failure have not been established.

A potential mediator of this adaptive response is atrial natriuretic peptide (ANP), a hormone which is synthesized and released by cardiac atrial myocytes and which possesses potent natriuretic, diuretic, and vasorelaxant properties (4–6). Distention of the right atrium, whether induced by mechanical stretch or by ECFV expansion, results in a brisk natriuresis and diuresis (7). Recently, increased plasma levels of immunoreactive ANP (iANP) have been noted in patients with atrial tachyarrhythmias (8) and congestive heart failure (9), which suggests that elevation of atrial pressures may lead to hypersecretion of ANP. Circulating plasma iANP levels also increase in the rat when ECFV is expanded by administration of mineralocorticoid (10) or by chronic dietary Na excess (11). Thus, the diuretic and natriuretic responses to atrial distention, the demonstration that ANP is released from the heart when right atrial pressure increases, and the increase in circulating ANP levels with acute and chronic ECFV expansion, all suggest that ANP may play an important role in ECFV homeostasis.

The purpose of this study was to examine the potential participation of ANP in modulating the adaptive natriuresis per nephron typically observed in the setting of reduced renal mass. The experimental protocol was designed to characterize the plasma ANP response, both before and after the development of compensatory functional and structural adaptation of nephrons to reduced renal mass.

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1. *Abbreviations used in this paper:* ANP, atrial Na natriuretic peptide; ECFV, extracellular fluid volume; FE_{Na}, fractional excretion rate; GFR, glomerular filtration rate; iANP, immunoreactive ANP; PUN, plasma urea nitrogen; $U_{Na}V$, sodium excretion rate.

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Methods

Adult male Munich-Wistar rats (200–250 g) were subjected to sham operation or to 5/6 nephrectomy by removal of the right kidney and infarction of ~⅔ of the left kidney. In the first protocol (the acute studies), animals were studied 48 h after surgery, at a time before full functional and structural hypertrophic adaptive responses occur (1). Rats in each group received a total of 0, 2, or 4 mEq of Na per 24 h administered in two daily intraperitoneal injections of 5% dextrose, 1% NaCl, or 2% NaCl solutions, respectively. This route was chosen to obviate irregularities in food intake in the acute postoperative period. Animals were housed in individual metabolic cages and given ad libitum access to deionized water and to Na-deficient chow (ICN K & K Laboratories, Inc., Plainview, NY). Urine was collected at 24-h intervals for determination of volume, and of Na concentration using flame photometry. At 48 h after surgery, animals were sacrificed and trunk blood was obtained for determination of plasma urea nitrogen (PUN) and iANP levels. In the second protocol (the chronic studies), rats were subjected to 5/6 nephrectomy and maintained for 14 d on daily diets providing either <1 mEq Na (Na-deficient chow and deionized water) or 4–5 mEq Na (Na-deficient chow and 1% NaCl as drinking water). The 14-d interval was chosen to allow compensatory single nephron hyperfiltration and hypertrophy (1). Rats were housed in individual metabolic cages and three 24-h urine collections were made for determination of daily Na excretion over the last 72 h of the study. Rats were then sacrificed for determination of PUN and iANP levels as per the first protocol.

For determination of iANP levels, rats were killed by rapid decapitation and trunk blood was collected in iced tubes containing 10 mg EDTA, 1,500 KIU aprotinin, and 10 Nα-benzoyl-L-arginine ethyl ester units soybean trypsin inhibitor (Sigma Chemical Co., St. Louis, MO). Plasma ANP was extracted using the method of Lang et al. (12). Briefly, 2 ml of plasma with 6 ml of 4% acetic acid were passed over an ODS Sep-Pak cartridge (Waters Associates, Millipore Corp., Milford, MA), washed twice with 5 ml of distilled water, eluted with 90% ethanol and 0.4% acetic acid, and evaporated to dryness. The dried eluate was reconstituted in 2 ml of ANP-radioimmunoassay buffer (Peninsula Laboratories, Inc., Belmont, CA) and all eluates were stored at −20°C for equivalent periods of time. 100 μL of serial dilutions of each plasma extract were assayed in duplicate for iANP using a commercially available radioimmunoassay kit (Peninsula Laboratories, Inc.). iANP was precipitated using the double antibody technique and pellet radioactivity was quantitated by gamma counter. Human ANP (1–28), which was shown to be 100% cross-reactive with rat ANP (1–28), was used to construct the standard curve over the range of 2–128 pg/ml. Recovery of ANP averaged 72% as determined by addition of radiolabeled ANP to rat plasma ($n = 4$). Plasma iANP concentration determined from two different dilutions of the plasma extracts yielded essentially identical values ($n = 8$).

Values are given as means±standard error of the mean. Significance was assessed using analysis of variance, and statistical significance was defined as $P < 0.05$.

Results

As demonstrated in Table I, all animals were in external Na balance at the time of sacrifice, since Na excretion rate ($U_{Na}V$) increased with increasing dietary Na intake in each group. Azotemia developed promptly in the 5/6 nephrectomized groups. Of note, PUN levels tended to be numerically lower in 5/6 nephrectomized rats on the high Na intakes in both the acute and chronic studies, presumably secondary to mild ECFV expansion. On the other hand, the lower PUN values in the 14-d rats as compared with values measured in 48-h rats presumably also reflect the added influence of compensatory single nephron hyperfiltration.

Calculated values for FE_{Na} also increased with increasing sodium intake, particularly in rats with reduced renal mass. Since direct measurements of GFR could not be made without introducing acute perturbations in ECFV and thereby altering plasma iANP levels, values for FE_{Na} were calculated using measured $U_{Na}V$ values and assuming reasonable total GFR values of 2.4 ml/min in sham-operated rats (13), 0.4 ml/min in acutely nephrectomized rats (reduction to ⅙ of the normal nephron number without compensatory hyperfiltration), and 0.8 ml/min in 5/6 nephrectomized rats studied after 14 d (when single nephron GFR would have been expected to double [1]). Conclusions reached in this study regarding FE_{Na} values among groups would not differ even with GFR values 50% above or below those assumed herein. These calculated values agree with previous findings that FE_{Na} rises both in response to increases in Na intake and to reduction in GFR, and that restriction of Na intake in proportion to GFR reduction obviates the need for increased natriuresis per nephron and hence increased FE_{Na} (3).

Table I. Effect of Alteration of Sodium Intake and Renal Mass on Sodium Handling and Plasma iANP Levels

	Sodium intake		
Group	0–1.0	1.1–3.0	3.1–5.0
	mEq/d	*mEq/d*	*mEq/d*
Sham, 48 h	($n = 4$)	($n = 4$)	($n = 4$)
PUN (*mg/dl*)	15±2	16±1	12±1
$U_{Na}V$ (*mEq/d*)	0.39±0.05	1.70±0.29*	3.29±0.45*
FE_{Na} (*percent*)	0.1	0.4	0.8
iANP (*pg/ml*)	109.5±28.9	171.2±14.8	163.8±40.4
5/6 Nephrectomy, 48 h	($n = 4$)	($n = 4$)	($n = 4$)
PUN (*mg/dl*)	58±7‡	50±7‡	45±6‡
$U_{Na}V$ (*mEq/d*)	0.68±0.14	2.43±0.19*	4.30±0.10*
FE_{Na} (*percent*)	0.6	2.5	4.9
iANP (*pg/ml*)	60.8±10.0	183.8±17.8*	520.0±41.8*‡
5/6 Nephrectomy, 14 d	($n = 9$)		($n = 10$)
PUN (*mg/dl*)	38±4		29±3
$U_{Na}V$ (*mEq/d*)	0.53±0.11		4.93±1.0*
FE_{Na} (*percent*)	0.3		2.5
iANP (*pg/ml*)	173.3±23.5		432.0±46.0*

* $P < 0.05$ vs. 0–1.0 mEq Na intake/d. ‡ $P < 0.05$ vs. sham on same Na intake.

Despite comparable Na intakes, plasma iANP levels responded very differently in 5/6 nephrectomized rats than in sham-operated rats. As summarized in Table I and depicted graphically in Fig. 1, plasma iANP levels in sham-operated rats did not differ significantly across the range of Na intakes studied, which suggests that the intact kidney is able to maintain external Na balance without ECFV expansion or stimulation of ANP across this range. In contrast, iANP levels rose progressively in the acute and chronic 5/6 nephrectomized rats on the high Na intake, which indicates that this hormonal system is activated when Na intake exceeds the excretory capacity of a reduced number of functioning nephrons. However, even in the setting of reduced GFR, restriction of dietary Na mitigated the rise in plasma iANP levels and also prevented increases in $U_{Na}V$ and FE_{Na}. Presumably, dietary Na restriction prevented ECFV expansion, and thus eliminated the stimulus for enhanced atrial ANP secretion.

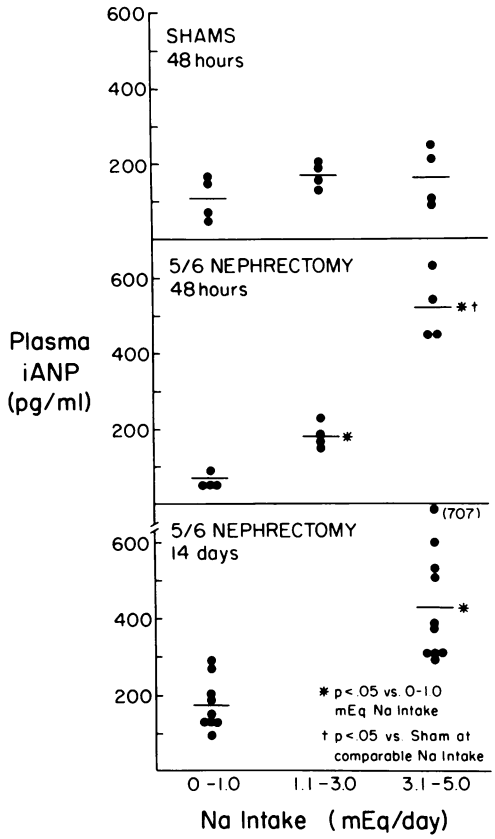

Figure 1. Plasma iANP levels in sham-operated and 5/6 nephrectomized rats studied 48 h or 14 d after operation. Plasma iANP levels were unaffected by changes in dietary Na intake in sham-operated rats, but rose progressively in 5/6 nephrectomized rats studied at 48 h and 14 d after renal ablation. *$P < 0.05$ vs. 0–1.0 mEq Na intake/d; †$P < 0.05$ vs. sham on same Na intake.

Discussion

These findings are consistent with the hypothesis that ANP plays an important role in the maintenance of external Na balance in the setting of a reduction in GFR. Reduction in nephron number without a proportional reduction in dietary Na intake leads to ECFV expansion. In response to right atrial distention induced by the increased plasma volume, ANP is released and plasma ANP levels increase. The resultant natriuresis per nephron acts to restore plasma volume toward normal levels, but with continuous acquisition of dietary Na in amounts that exceed the normal Na excretory rate per nephron, the need for high plasma ANP levels persists. In the present study, plasma ANP levels in remnant kidney rats with high Na intake and high FE_{Na} were comparable to those observed to produce natriuresis in rats and humans receiving acute saline infusions (14, 15), and in humans during the natriuretic and diuretic phase of supraventricular tachyarrhythmias (8).

While the mechanism whereby ANP induces natriuresis remains incompletely understood, recent evidence tends to exclude increased GFR (4) or decreased proximal tubule Na reabsorption (16, 17) as primary mechanisms. Evidence for net addition of Na along the papillary collecting duct (18), and preliminary observations that vasa recta Starling forces become less favorable for papillary fluid uptake after systemic ANP infusion (19), point to a papillary site of action. Accordingly, the suggested distal site of ANP natriuretic activity, and the probable distal mechanism of the natriuresis observed in chronic renal failure (1), are consonant with the hypothesis that ANP may modulate the natriuresis per nephron in chronic renal failure. In further support of this possibility, Cole et al. (20) recently reported enhanced Na excretion per nephron in response to exogenous ANP infusion in rats with reduced renal mass, relative to non-nephrectomized control rats.

The metabolism and disposition of endogenous or exogenous ANP have not yet been defined. However, the current findings of low plasma iANP levels in the Na-restricted nephrectomized rats exclude the possibility of a nonspecific accumulation of ANP due to decreased renal excretion or degradation of the peptide, and suggest that the elevated levels of iANP reported herein reflect true increases in cardiac ANP secretion.

In summary, plasma iANP levels increase progressively in rats with reduced renal mass when obligatory high Na excretion rates per nephron are required for maintenance of external Na balance. Dietary Na restriction obviates the necessity of large increases in FE_{Na} or plasma iANP levels. These findings suggest that ANP plays an important role in modulating the adaptive natriuresis per nephron regularly observed in animals and patients with chronic renal failure.

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