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Increased renal ANP synthesis, but decreased or unchanged cardiac ANP synthesis in water-deprived and salt-restricted rats

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Increased renal ANP synthesis, but decreased or unchanged cardiac ANP synthesis in water-deprived and salt-restricted rats.

Background. Experiments were performed to examine the effect of water deprivation and salt restriction on ANP synthesis in the kidneys and hearts of normal rats.

Methods. A 4-day water deprivation (WD) and 7-day salt restriction (SR; 0.01% NaCl) were performed in 12 and 14 rats, respectively. Atrial natriuretic peptide (ANP) mRNA expression in the kidney was assessed with reverse transcription-polymerase chain reaction coupled with Southern blot hybridization, while the ANP mRNA in the hearts was measured by Northern blot hybridization. ANP and angiotensin II concentrations in the extracted plasma were measured by radioimmunoassay. The molecular form of renal ANP-like protein was characterized by reverse phase—high-performance liquid chromatography (RP-HPLC).

Results. Renal outer and inner medullary ANP mRNA showed a respective 11-fold and ninefold increase in WD rats, and an eightfold and fivefold increase in SR rats as compared to corresponding control groups. Inversely, cardiac atrial ANP mRNA and plasma ANP were decreased in WD rats, whereas they did not change in the SR group. Plasma angiotensin II concentration increased in conjunction with the decrease of urine sodium excretion in both groups. RP-HPLC analysis revealed a 45% extraction of ANP in the WD rat kidneys, whereas only 3% ANP in the control kidneys migrated in a molecular form similar to cardiac atrial proANP.

Conclusions. Our results demonstrate that water deprivation and salt restriction markedly enhance renal ANP mRNA, whereas water deprivation suppresses cardiac atrial ANP mRNA and plasma ANP concentrations. The current study indicates that renal ANP and cardiac atrial ANP appear to be two distinct systems regulated by different mechanisms and possibly exhibiting different intra-renal paracrine and systemic endocrine functions.

Atrial natriuretic peptide (ANP), secreted primarily by the cardiac atrium [1], has been known to possess potent diuretic, natriuretic, vasorelaxant, and aldosterone-inhibitory activities [2–4]. Increased intravascular blood volume has been found to enhance atrial ANP synthesis and plasma ANP concentration [5–7], whereas volume depletion causes opposite alterations [8, 9]. Using the immunocytochemical technique and cDNA probes, the presence of proANP-like immunoreactive peptide and ANP mRNA was found in extra-atrial organs [10–13], including the kidney [14–16]. Using reverse transcription-polymerase chain reaction (RT-PCR) followed by Southern blot analysis, we recently demonstrated that renal ANP synthesis was markedly enhanced in deoxycorticosterone acetate (DOCA) salt-treated [17] and diabetic [18] rats. This finding, namely that plasma renin activity was suppressed in DOCA salt-treated [17] and diabetic [18] rats, indicates that the increase of renal ANP synthesis may be responsive to volume expansion. In the above experiments [17, 18], daily urinary ANP excretion showed a significant correlation with daily urinary sodium excretion and daily urine volume. Thus, we suggested that ANP synthesized from the kidney may produce natriuretic and diuretic action in volume-expanded status. However, the exact pathophysiological significance and the regulation of ANP synthesized from the kidney remain to be clarified. To investigate the action of renal-derived ANP and its regulation, it is necessary to first elucidate the change of ANP gene message in the kidney and urinary ANP excretion in volume-depleted status. We hypothesized that both renal and cardiac ANP synthesis might decrease in volume-depleted disorders. Therefore, in the present study, ANP mRNA levels were measured in the kidney and the heart, and plasma and urine ANP levels of normal rats were measured after four days of water deprivation and seven days of salt restriction. Rather surprisingly, increased renal ANP mRNA and urine ANP excretion rates with decreased or unchanged cardiac ANP synthesis were found in water-deprived and salt-restricted rats.

Key words: atrial natriuretic peptide, diuresis, vasorelaxation, aldosterone inhibition, volume depletion disorders, hypertension.

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METHODS

Animal experiments

Experiments were performed on male Wistar rats weighing 262 to 308 g. These rats were individually housed in

metabolic cages for seven days before the start of the three experiments. In experiment 1, the influence of water deprivation on renal ANP synthesis was investigated. Twelve normal rats were completely water depleted (WD) from the first to the fourth days. Fourteen body weight- and age-matched rats were included as normal controls (NC1), and were provided normal rat chow diet (0.20% NaCl) and tap water *ad libitum*. In experiment 2, the influence of salt restriction on renal ANP synthesis was studied. Fourteen normal rats were maintained on NaCl-restricted diet (addition of 0.01% NaCl to catalog no. 960232; ICN Biochemicals, Irvine, CA, USA) and distilled water *ad libitum* (SR) from the first to the seventh days. Another group of 15 body wt- and age-matched normal rats were studied as controls (NC2) and given 0.26% NaCl food (catalog no. 905453; ICN Biochemicals) and tap water *ad libitum* during the study period. All rats were sacrificed by decapitation to collect blood for the measurement of plasma ANP, angiotensin II, arginine vasopressin (AVP) and sodium level on the end day of both experiments. Kidneys, cardiac atria and ventricles were removed, and renal tissues were immediately divided into cortex, outer and inner medulla for ANP mRNA analysis. Urine sodium and ANP immunoreactivity were also measured on days 4 and 7 in experiments 1 and 2, respectively. In experiment 3, the identity of this renal ANP-like protein was further characterized by its migratory properties by using reverse phase—high-performance liquid chromatography (RP-HPLC). Six normal control and water-deprived rats were killed by decapitation, and trunk blood was collected into ice-cooled tubes containing 500 KIU/ml aprotinin (Sigma, St. Louis, MO, USA) and 1 mg/ml ethylenediaminetetraacetic acid, and immediately centrifuged. The kidneys and cardiac atria were rapidly removed and immediately extracted.

RNA isolation and reverse transcription

Total RNA was extracted from the renal cortex, outer and inner medulla, cardiac atrium and ventricle using a modified guanidium isothiocyanate method [19]. Two micrograms of total RNA from renal cortex, outer and inner medulla were reverse transcribed by incubating with a 20 μl reverse transcription mixture containing: 20 pmole oligo $(dT)_{18}$ primer, 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 20 units of RNase inhibitor, 0.5 mM dNTPs, and 50 U of MMLV reverse transcriptase (Stratagene Laboratories Inc., Palo Alto, CA, USA) at 37°C for two hours. The reverse transcriptase was inactivated by heating for five minutes at 94°C.

Polymerase chain reaction amplification and Southern blot hybridization

Primers for the PCR were designed to flank at least one putative intron site for rat ANP and β-actin. For ANP, the sense primer (5′-ATGGGCTCCTTCTCCATCACC-3′) corresponded to base pair 1 to 21, and antisense primer (5′-TGTTATCTTCGGTACCG-3′) corresponded to bp 445 to 461 of rat ANP cDNA. For β-actin, the sense primer (5′-CGT AAAGACCTCTATGCCAA-3′) corresponded to bp 2750 to 2769, and the antisense primer (5′-AGCCAT GCCAAATGTCTCAT-3′) corresponded to bp 3203 to 3222 of rat β-actin gene. The size of expected PCR products was 461 bps for ANP and 349 bps for β-actin. For amplification, PCR was performed at a final concentration of 1 × PCR buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl; 1.5 mM $MgCl_2$), 0.2 mM dNTP, 0.4 μM sense and antisense oligos, 2.0 units of *Taq* DNA polymerase (Boehringer Mannheim, Indianapolis, IN, USA) to a total volume of 50 μl. The amplification cycles were 45 seconds at 94°C, 45 seconds at 60°C and 90 seconds at 72°C in a Perkin-Elmer Cetus 9600 thermocycler (Perkin-Elmer Cetus, Norwalk, CT, USA). The cycle for the amplification of ANP mRNA was repeated 30 times for renal cortex, outer medulla, and inner medulla as described in our previous studies [17, 18]. The amplified products were then electrophoresed on 1.5% agarose gels and transferred to nylon membranes (Schleicher & Schuell GmbH, Dassel, Germany). The blots were hybridized with [^{32}P] labeled, randomly-primed, 426 bp rat ANP cDNA prepared by PCR cloning of the rat atria extracts for 16 hours at 65°C, according to the standard technique [20]. After each hybridization, the blots were washed twice in a solution containing 0.1% sodium dodecyl sulfate (SDS) and 2×SSC (0.3 M NaCl, 30 mM sodium citrate) for 15 minutes at room temperature and then twice in 0.1% SDS and 0.1×SSC at 65°C. Blots were exposed to Kodak BIOMAX-MR (Eastman Kodak Company, Rochester, NY, USA) film at −70°C. A radioisotope-labeled probe for β-actin used as an internal control was also made using the primer extension method. After autoradiography, the x-ray film was scanned by a laser densitometer (Molecular Dynamics, Sunnyvale, CA, USA), and the data were analyzed by MD ImageQuant software release version 3.22. The value obtained for each ANP PCR product was normalized by its β-actin levels. To control the accuracy of RT-PCR followed by Southern hybridization, serially diluted total RNA of renal tissues were subjected to RT-PCR amplification. Moreover, the intra-assay variation of semiquantitative RT-PCR followed by Southern blot analysis from renal tissues was analyzed.

Northern blot hybridization

RNA samples (20 μg/lane) were denatured, electrophoresed through 1% agarose gel containing 1.1% formaldehyde, and transferred onto the nylon membrane. Hybridization was carried out at 42°C with a randomly primed, [^{32}P]-labeled rat ANP cDNA probe. The membranes were washed twice in 2 × SSC and 0.1% SDS at room temperature, and were then further washed in 0.2 × SSC and 0.1% SDS at 65°C. The membranes were then exposed to Kodak BIOMAX-MR film (Eastman-Kodak) at −70°C with the

aid of an intensifying screen. Membranes were subsequently rehybridized with a 700-bp *pstI* fragment of a β-actin probe to correct for differences in RNA loading. The signals on the autoradiograph were scanned and analyzed.

Extraction of plasma, renal and cardiac tissues

Kidneys and atria were minced and boiled for five minutes in 10 vol 0.1 M acetic acid. Once cooled to 4°C, the acid tissues were homogenized with a Polytron homogenizer (Janke & Kunkel IKA-Labortechnik, Germany) for 60 seconds. The homogenate was centrifuged at 4500 rpm for 40 minutes at 4°C. The acid supernatants were then extracted using Sep-Pak C_{18} cartridges (Waters Associates, Milford, MA, USA) that were premoisturized with 4 ml of 60% acetonitrile (ACN) in 0.1% trifluoacetic acid (TFA). The cartridges with the absorbed peptides were washed with 6 ml of 0.1% TFA and then eluted with 3 ml of 60% ACN in 0.1% TFA. Plasma samples were also extracted using Sep-Pak C_{18} cartridges as described for renal and atrial tissues. The eluates were lyophilized and reconstituted for RP-HPLC and RIA.

Characterization of plasma, renal and atrial ANP profiles by RP-HPLC

The dry eluates were reconstituted in HPLC buffer A (10% ACN in 0.1% TFA). The HPLC analysis was performed with a Beckman System Gold HPLC system, equipped with a reverse-phase column (Cosmosil $5C_{18}$-AR Column; Nacacai Tesque Inc., Kyoto, Japan). The column was equilibrated at room temperature with buffer A and eluted by increasing the ACN concentration (buffer B, 60% ACN-0.1% TFA). The starting buffer was 100% buffer B. A linear gradient elution system was employed using from 10 to 60% ACN by an increase in the ACN of 0.5% per minute. The flow rate was 1 ml/min, and fraction collection was 0.5 ml/tube. The collections were lyophilized and reconstituted for RIA.

Assay methods

ANP, AVP and angiotensin II immunoreactivities from samples of plasma and ANP from urine were determined by radioimmunoassay methods after extraction as previously reported [17, 18]. Briefly, 3 ml of plasma were passed through Sep-Pak C_{18} cartridges (Waters Corporation) and eluted with 5 ml 60% ACN in 0.1% TFA. The eluate was lyophilized and reconstituted for RIA. The concentrations of plasma and urinary sodium and plasma osmolality levels were determined in an automatic analyzer (Nova Biochemical, Newton, MA, USA).

Statistical analysis

The data are expressed as mean values ± SEM. To test the difference between the two groups, an unpaired Student's *t*-test was performed. A P value <0.05 was considered statistically significant.

Table 1. Characteristics of normal control (NC1) and water-deprived normal (WD) rats on the fourth study day

	NC1	WD
Number	14	12
Body weight, *g*	311 ± 5.0	261 ± 9.18[a]
Kidney weight, *g*	1.59 ± 0.03	1.25 ± 0.03[a]
KW/BW, *g/g* ($\times 10^{-3}$)	3.69 ± 0.05	3.47 ± 0.09[c]
Water intake, *ml/24 hr*	26.5 ± 1.5	0[a]
UV, *ml/24 hr*	16.5 ± 0.9	2.3 ± 0.1[a]
Blood glucose, *mg/dl*	125 ± 4.3	107 ± 6.5[b]
Plasma sodium, *mmol/liter*	140 ± 1.8	149 ± 1.6[b]
Plasma osmolality, *mOsm/kg*	287 ± 2.2	299 ± 2.2[b]
Plasma ANP, *pg/ml*	42 ± 3.7	24 ± 2.2[a]
Plasma AVP, *pg/ml*	1.4 ± 0.03	2.1 ± 0.16[a]
Plasma angiotensin II, *pg/ml*	18 ± 1.1	197 ± 32.6[a]
$U_{Na}V$, *mmol/24 hr*	0.62 ± 0.04	0.15 ± 0.02[a]
$U_{ANP}V$, *pg/24 hr*	44 ± 6.6	673 ± 84[a]

Values are mean ± SEM.
[a] $p < 0.001$, [b] $p < 0.01$, [c] $p < 0.05$ vs. NC1

RESULTS

The characteristics of NC1 and WD groups on the fourth study day are shown in Table 1. The mean amount of daily water intake in NC1 group was 26.5 ± 1.5 ml, whereas the complete water deprivation was performed in the WD group. Body wt and kidney wt in WD rats were significantly decreased as compared to NC1 rats. The mean plasma AVP and angiotensin II levels were significantly increased along with the elevation of mean plasma sodium and osmolality levels, whereas plasma ANP concentrations were significantly decreased in the WD group after a four-day water deprivation. The mean daily urinary sodium excretion and urine volume were significantly decreased in WD rats in comparison to NC1 group. However, the urinary ANP excretion rates were increased on the fourth day in WD rats.

RT-PCR coupled with Southern blot analysis revealed a 461 bp product for ANP from rat kidney tissue RNA extracts. To determine the relative changes in tissue ANP mRNA expression, the yield of ANP PCR products was normalized to the amount of β-actin cDNA amplified from the same RT cDNA of tissue samples, a method that has been used in our previous reports [17, 18]. The accuracy of the semiquantitative RT-PCR with Southern hybridization in this study was also tested. Serial dilutions (0.4, 0.8, 1.2, 1.6, 2.0 and 2.4 μg) of total RNA were subjected to RT-PCR amplification f or ANP and β-actin, respectively. The correlation coefficients between RNA concentrations and corresponding densities from renal cortex, outer and inner medulla were: (*1*) for ANP mRNA, $r = 0.968$, $r = 0.992$ and $r = 0.966$; (*2*) for β-actin mRNA, $r = 0.972$, $r = 0.958$ and $r = 0.963$. An intra-assay test of RT-PCR followed by Southern blot analysis in 2.0 μg total RNA extracted from renal tissues was performed. The variation

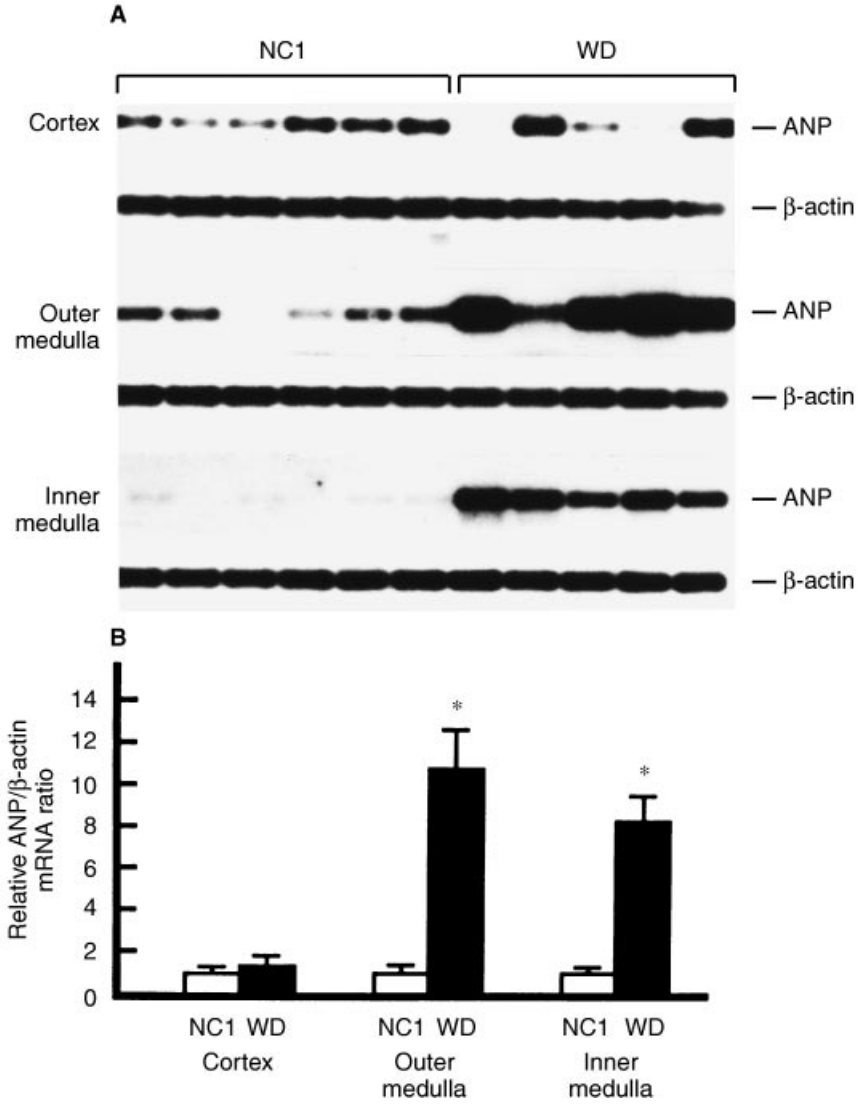

Fig. 1. (*A*) Autoradiographs of the amplification of ANP and β-actin mRNA by RT-PCR followed by Southern blot analysis in renal cortex, outer and inner medullas from 6 representative normal control (NC1) and 5 representative water-deprived normal (WD) rats on the fourth day after complete water deprivation in the WD group. Each lane represents an individual rat. (*B*) Relative ratios (mean ± SEM) of the densitometry readings for RT-PCR amplification of ANP and β-actin mRNA in the cortex, outer and inner medulla from 14 normal control (NC1) and 12 water-deprived (WD) rats after a 4-day water deprivation. $^{*}P < 0.001$ versus the NC1 group.

coefficients were 5.6%, 6.8% and 7.2% for ANP mRNA ($N = 8$), and 7.3%, 5.2% and 6.6% for β-actin mRNA ($N = 8$) in renal cortex, outer and inner medullas, respectively.

Figure 1 shows the autoradiographs of RT-PCR amplification of ANP and β-actin mRNA in the renal cortex, outer and inner medullas from six representative NC1 and five representative WD rats on day 4. The relative ratios of the densitometry measures of the PCR products for ANP and β-actin mRNA from 14 NC1 and 12 WD rats are shown in Figure 1B. The relative ratio of PCR products for ANP and β-actin mRNA from renal outer and inner medulla in the WD group increased 11 and 9 times as compared with those in NC1 group; no significant difference for ANP mRNA expression in the renal cortex was found between NC1 and WD rats.

Table 2 shows the characteristics of NC2 and SR groups on the seventh day. The mean body wt value was significantly decreased while mean kidney wt value was not changed in the SR group as compared to the NC2 group. The mean daily water intake and urine amount, as well as plasma sodium and osmolality levels in SR rats were not

Table 2. Characteristics of normal control (NC2) and salt-restricted normal (SR) rats on the seventh study day

	NC2	SR
Number	15	14
Body weight, *g*	314 ± 3.0	280 ± 3.4[a]
Kidney weight, *g*	1.48 ± 0.02	1.46 ± 0.04
Water intake, *ml/24 hr*	28.1 ± 1.6	23.5 ± 1.9
UV, *ml/24 hr*	18.2 ± 1.4	13.6 ± 2.2
Plasma sodium, *mmol/liter*	142 ± 1.0	144 ± 1.5
Plasma osmolality, *mOsm/kg*	288 ± 2.3	288 ± 4.1
Plasma ANP, *pg/ml*	37.2 ± 4.2	28.5 ± 3.7
Plasma AVP, *pg/ml*	1.5 ± 0.08	1.8 ± 0.17
Plasma angiotensin II, *pg/ml*	22 ± 2.3	64 ± 5.0[a]
$U_{Na}V$, *mmol/24 hr*	0.65 ± 0.05	0.04 ± 0.01[a]
$U_{ANP}V$, *pg/24 hr*	68 ± 7.1	235 ± 70.2[b]

Values are mean ± SEM.
[a] $P < 0.01$, [b] $P < 0.05$ vs. NC2

different from those of NC2 group. The mean plasma angiotensin II value was significantly increased, while there was no significant difference in the plasma AVP and ANP concentrations between the SR rats and NC2 group. The mean daily urinary sodium excretion rate was significantly decreased, but the mean urinary ANP excretion rate was increased on the seventh salt-restricted day in the SR group when compared to the NC2 group.

Autoradiographs of the RT-PCR amplification of ANP and β-actin mRNA in the renal cortex, outer and inner medullas from three representative NC2 and SR rats on the seventh study day are shown in Figure 2. The relative ratios of the densitometry measures of the PCR products for ANP and β-actin mRNA from the two groups are shown in Figure 2B. The relative ratios of PCR products for ANP and β-actin mRNA from renal outer and inner medullas of 14 SR rats increased 8 and 5 times when compared with the ratios of 15 rats in the NC2 group. No significant difference for ANP mRNA expression in the renal cortex was found between the NC2 and SR groups.

The RP-HPLC analysis of the ANP molecular form is shown in Figure 3. In normal rat kidneys (Fig. 3A), the majority (92%) of irANP comigrated with synthetic rat α-ANP-(1-28), while a small amount (3%) of total irANP migrated with an molecular form of proANP as compared with the HPLC profile of atrial extract from the NC and WD rats. The ANP molecular profile from kidneys extracted from these rats with a four-day water deprivation were markedly different when compared with normal kidneys. Approximately 45% of the extracted irANP in the WD rat kidneys (Fig. 3B) migrated at an apparent molecular form similar to heart proANP, whereas 46% of irANP comigrated with synthetic rat α-ANP-(1-28). To determine whether the existence of ANP prohormone in the WD rat kidney was the result of ANP extraction from the plasma, we analyzed the molecular form of plasma ANP in control (Fig. 3C) and water-deprived (Fig. 3D) rat plasma by RP-HPLC. The results revealed only ANP-(1-28), but not pro-ANP, in the plasma of both groups.

Figure 4A is an autoradiograph of a Northern blot of right atrial and left ventricular ANP and β-actin RNA extracted from two representative NC1 and two WD rats that were sacrificed on the fourth study day. Figure 4B shows the relative ANP mRNA and β-actin mRNA levels from 14 NC1 and 12 WD rats. The relative ratio of ANP and β-actin mRNA levels of right atrium from WD rats was significantly decreased as compared to NC1 rats, while no difference in the left ventricular ANP mRNA level was observed between the NC1 and WD groups. Figure 5 is Northern blot analysis of mRNA for ANP and β-actin in the tissues of right atria and ventricles from two representative NC2 and two SR rats; Fig. 5B demonstrates that there was no significant difference to be found for the relative ratio of ANP and β-actin mRNA from cardiac atrium or ventricles between 15 NC2 and 14 SR rats.

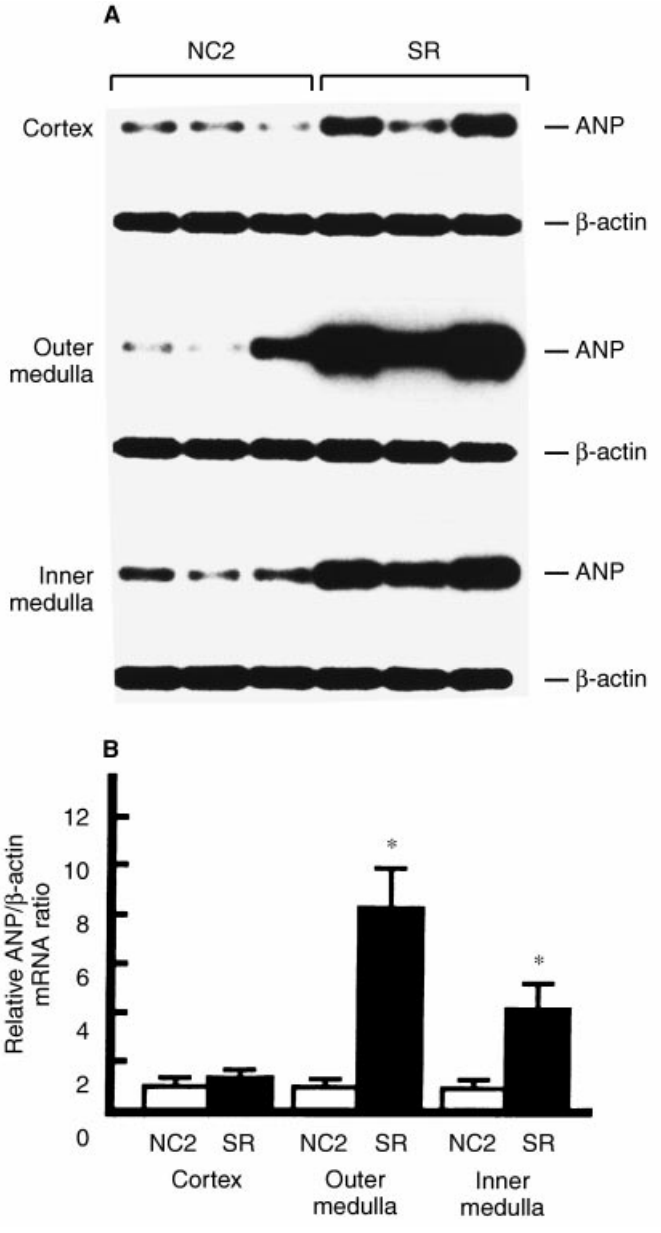

Fig. 2. (*A*) Autoradiographs of the amplification of ANP and β-actin mRNA by RT-PCR followed by Southern blot analysis in the renal cortex, outer and inner medullas from 3 representative normal control (NC2) and 3 representative salt-restricted normal (SR) rats after a 7-day salt restriction in the SR group. Each lane represents an individual rat. (*B*) Relative ratios (mean ± SEM) of the densitometry readings for RT-PCR amplification of ANP and β-actin mRNA in the cortex, outer and inner medulla from 15 normal control (NC2) and 14 salt-restricted normal (SR) rats after a 7-day salt restriction. $^{*}P < 0.001$ versus the NC2 group.

DISCUSSION

In the present study, we have demonstrated the influence of water deprivation and salt restriction on renal and cardiac ANP synthesis in normal rats. These results are: (*1*) renal medullary ANP mRNA expression was markedly enhanced after a four-day water deprivation and seven-day

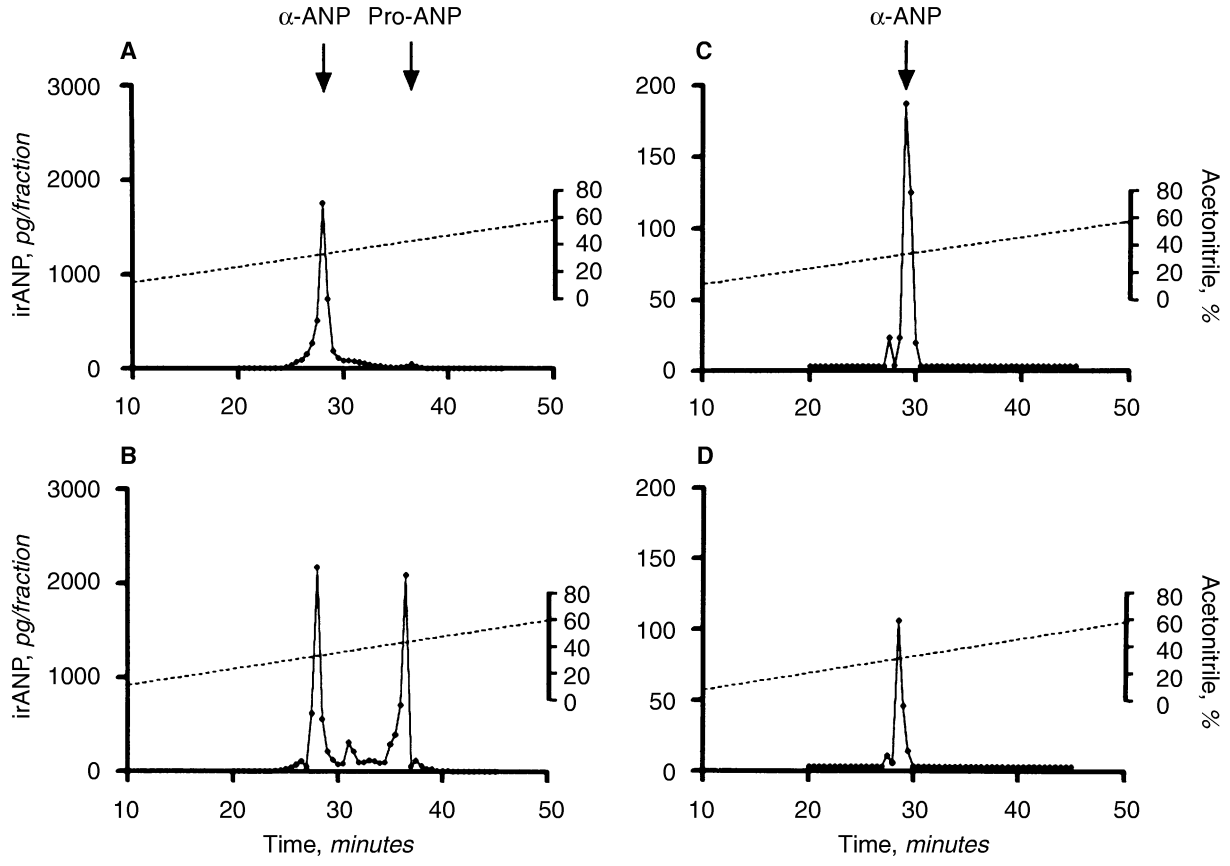

Fig. 3. Reverse phase—high-performance liquid chromatography (HPLC) profiles. (*A*) Kidney (5.0 g) from 3 normal control rats, (*B*) kidney (5.0 g) from 3 water-deprived rats, (C) pooled plasma (2.5 ml) from 3 control rats, (D) pooled plasma (2.5 ml) from 3 water-deprived rats. Extracted samples were applied to HPLC system equipped with a reverse-phase column (Cosmosil 5 C_{18}-AR column). The column was eluted by increasing the ACN concentration in Buffer B (60% ACN in 0.1% TFA). A linear gradient elution system was employed from 10% to 60% ACN by an increase in the ACN concentration of 0.5% per minute. The flow rate was 1 ml/min, and fraction collection was 0.5 ml/tube. Elution positions for standard rat α-ANP-(1-28) and rat atrial proANP are indicated by the arrows.

salt restriction; (*2*) while urinary ANP excretion was significantly increased, it was accompanied by decreased urine sodium excretion in water deprived and salt-restricted rats; (*3*) RP-HPLC analysis revealed that 45% of the extracted ANP in the water-deprived kidneys and only 3% in the normal kidneys migrate in the molecular form of atrial proANP; (*4*) atrial ANP mRNA expression and plasma ANP concentration was significantly decreased after water restriction; and (*5*) the seven-day salt restriction did not reduce cardiac ANP mRNA expression or plasma ANP concentration.

ANP is originally isolated and identified from rat and human cardiac atrium. However, the presence of proANP-like immunoreactive peptide and ANP mRNA has been discovered in several extra-atrial tissues [10–13], including the kidney [14–16]. In a search of the renal ANP gene message, we [17, 18] and other investigators [21, 22] found that the renal ANP mRNA was too low to be measured by Northern blot hybridization. By using RT-PCR coupled with Southern blot hybridization, we [17, 18] and others [21, 22] previously demonstrated that a low abundance of the ANP RNA message could be detected in the rat kidney tissue. Moreover, we compared the relative amount of ANP mRNA expression in the kidney as well as in cardiac tissues by the combination of RT-PCR and Southern blot hybridization. Our results have shown that renal ANP mRNA level were enhanced in diabetic [18] and DOCA salt-treated [17] rats. In the present study, to our surprise, we show that water deprivation and salt restriction markedly increase renal ANP mRNA levels and urinary ANP excretion accompanied with the decreases in cardiac atrial ANP mRNA levels and plasma ANP concentrations in water-deprived rats. Furthermore, in the water-deprived rat kidneys, an apparent molecular form of ANP appeared to be cardiac atrial proANP. This peptide could not to be of blood origin because no ANP prohormone was detected in the plasma of these rats. These results suggest a pathophysiological role for renal-derived ANP as a paracrine or autocrine function in the kidney.

ANP synthesized from the atrium is intimately linked to changes in intravascular volume. Elevations of circulating ANP can decrease cardiac output and arterial blood pressure

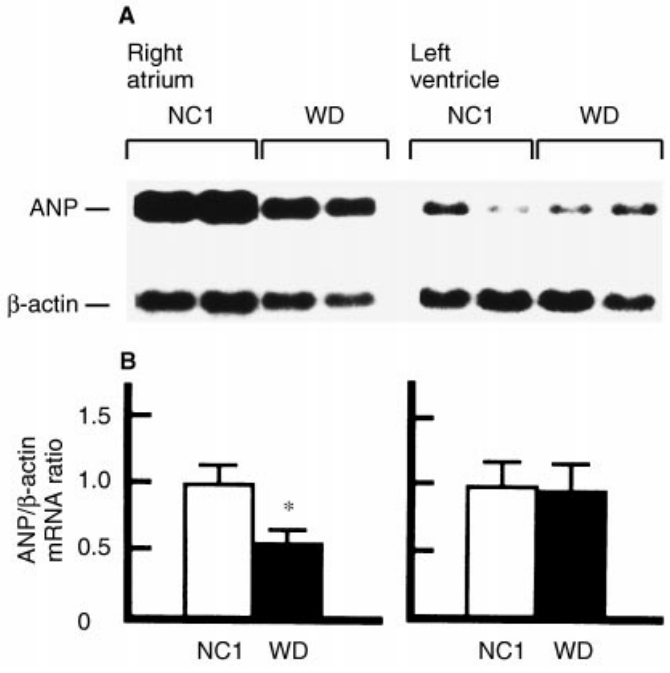

Fig. 4. ANP mRNA expression from right atrium and left ventricles in normal control (NC1) and water-deprived normal (WD) rats after a 4-day water deprivation in the WD group. (*A*) Each of two representative autoradiograms from NC1 and WD groups. (*B*) The relative ANP mRNA levels of 14 NC1 and 12 WD rats. The density of each RNA sample hybridized to the ANP probe was normalized to the β-actin probe. The mean corrected density values of the SR group were further divided by those of the NC1 group. $^{*}P < 0.05$ versus the NC1 group.

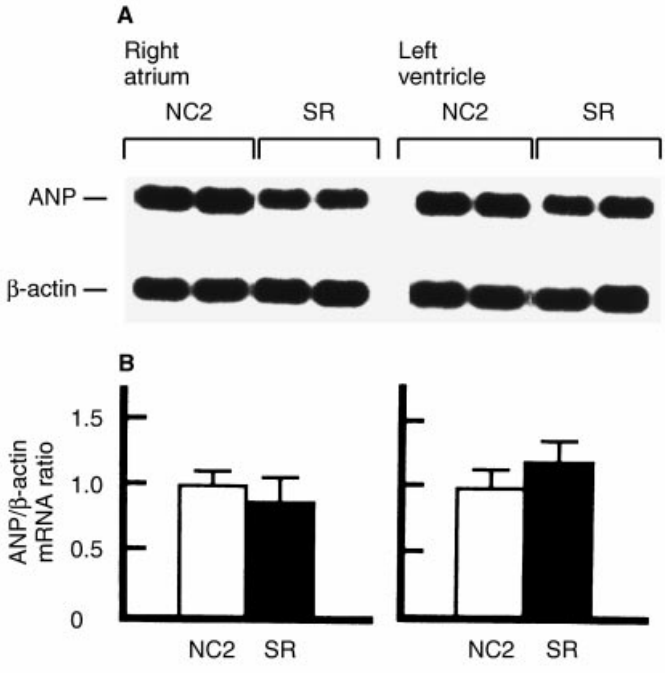

Fig. 5. ANP mRNA expression from the right atrium and left ventricles in normal control (NC2) and salt-restricted normal (SR) rats after a 7-day salt-restriction in the SR group. (*A*) Two representative autoradiograms from the NC2 and SR groups. (*B*) The relative ANP mRNA levels of 15 NC2 and 14 SR rats. The density of each RNA sample hybridized to the ANP probe was normalized to the β-actin probe. The mean corrected density values of the SR group were further divided by those of the NC2 group. No statistical change was found between the SR group and NC2 group.

[23] and alter the distribution of fluid across the capillary beds [24]. However, many studies have shown that circulating ANP is not a direct regulator of renal sodium excretion [25]. This controversial assertion is made based on the fact that plasma ANP levels do not correlate well with the degree of natriuresis in common physiological and pathophysiological conditions [25–31]. For instance, Goetz et al demonstrated that left atrial distension in dogs after cardiac denervation results in an elevation of plasma ANP concentrations without a concomitant natriuresis. In contrast, the excretion of urine urodilatin (ANP-95-126), a natriuretic peptide of renal origin, closely parallels renal sodium excretion under various conditions that influence body fluid regulation [28].

The enhancement of renal ANP synthesis found in our [17, 18] and other [14] studies suggests that ANP synthesized in the kidney is one of the factors responsible for fluid and electrolyte homeostasis. This is to say that the kidney itself may sense and regulate fluid-electrolyte imbalance by the synthesis of ANP in the kidney to maintain fluid homeostasis. The distribution of ANP receptors in the nephron [32] suggests that renal synthesized natriuretic peptide may act in a paracrine manner to modulate intrarenal sodium and water homeostasis under some pathophysiological conditions. In the present study, the finding that markedly enhanced renal ANP synthesis with increased urine ANP excretion rate in association with decreased atrial ANP mRNA and plasma ANP level in water-deprived rats provides the evidence to confirm the hypothesis that local renal ANP may affect renal function that is independent of cardiac ANP. In other words, the present findings demonstrate that renal ANP and heart ANP represent two distinct systems regulated by different control mechanisms. Furthermore, levels of ANP mRNA in renal outer and inner medullas were approximately eightfold and fivefold higher in salt-restricted rats than in normal control rats, whereas ANP mRNA abundance in the heart was not significantly affected by dietary salt restriction. Similarly, in these salt-restricted animals, there was no significant change in plasma ANP concentration while urinary ANP excretion was increased. These findings clearly indicate that the renal ANP system may first sense and regulate the electrolyte imbalance prior to the response of the cardiac ANP.

In the kidney, exogenous ANP exerts renal hemodynamic and tubular actions that lead to increases in urinary excretion of sodium, fluid and other electrolytes [2, 3]. In our previous studies [17, 18] in which we considered plasma volume expansion in DOCA-salt-treated rats and diabetic

rats, significantly increased urinary ANP excretion and enhanced renal ANP mRNA expression, as well as a significant correlation between urinary ANP excretion and daily sodium excretion were found. These findings indicate that ANP synthesized in the kidney is responsible for diuresis and natriuresis in these volume-expanded diseases. In fact, the influence of hydration status on the actions of ANP on diuresis and natriuresis has been studied. In several animal studies, elevation of urine flow and sodium excretion occurred only in animals that were volume expanded [33-35]. Conversely, water deprivation and salt restriction in this study markedly increased renal ANP gene expression and daily urine ANP excretion, but were also associated with a profound reduction in the daily sodium excretion and urine flow. These results strongly indicate that it is unlikely that natriuresis and diuresis are the main actions of endogenously-synthesized ANP from the kidney in volume-depleted status.

In volume-depleted humans or experimental animals, a profound increase of systemic vascular resistance, presumably induced by the increased activity of renin-angiotensin system [36], vasopressin release [37] and sympathetic nervous system [38], has been noted. However, it has been shown that renal blood flow fell only slightly or did not change despite the strong elevation in the total peripheral vascular resistance in this condition [36–38]. The mechanisms responsible for the absence of an increase in renal vascular resistance during volume depletion in the face of the activated vasoconstrictive substances have been investigated [36–38]. In fact, many studies have confirmed that vasodilators, such as prostaglandins, are produced in response to the influence of volume depletion-induced elevation of circulating or even intrarenal vasoconstrictive substances, and therefore locally spare the renal circulation from the vasoconstrictor effects without counteracting the systemic vasoconstriction that may be necessary to preserve blood pressure [36–38]. In addition to increased prostaglandin production, a marked increase of neural form of the constitutive nitric oxide synthase mRNA levels with parallel changes in the expression of renin and angiotensinogen mRNA in renal cortical tissues of rats on a low-salt diet have been found recently [39]. The present study has yielded similar results in which profoundly enhanced renal ANP synthesis in normal rats is accompanied by the augmented plasma angiotensin II and vasopressin concentrations after a four-day water deprivation or seven-day salt restriction. In view of the ability of exogenous administration of ANP to raise renal blood flow in descending and ascending vasa recta [40] and its antagonizing action of vasoconstrictors [41], it is reasonable to propose that ANP derived from the kidney may locally preserve renal blood flow through counteracting the intrarenal vasoconstrictve effect from circulating vasoconstrictive substances to help the autoregulation of the kidney.

In summary, the present study has shown that renal ANP mRNA levels and urinary ANP excretion are markedly enhanced, but urine sodium excretion is significantly decreased, in water-deprived and salt-restricted normal rats. Water deprivation also decreases cardiac atrial ANP mRNA expression, plasma ANP concentration and daily urine volume. These results demonstrate that renal ANP and cardiac ANP are two distinctly different systems that may be regulated by different control mechanisms and may have different functions in the kidney.

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REFERENCES

1. De Bold AJ: Heart atrial granularity effects of changes in water-electrolyte balance. *Proc Soc Exp Biol Med* 161:508–511, 1979
2. De Bold AJ, Borenstein HB, Veress AT, Sonnenberg H: A rapid and potent natriuretic response to intravenous injection of atrial myocardial extracts in rats. *Life Sci* 28:89–94, 1981
3. Ballermann BJ, Brenner BM: Role of atrial peptides in body fluid homeostasis. *Circ Res* 58:619–630, 1986
4. Zeidel ML, Brenner BM: Actions of atrial natriuretic peptides on the kidney. *Semin Nephrol* 7:91–97, 1987
5. Kato J, Kida O, Nakamura S, Sasaki A, Takiguchi K, Tanaka K: Dissociation between plasma and atrial content of atrial natriuretic polypeptide (ANP) following sodium load in rats. *Life Sci* 39:2623–2627, 1986
6. Luft FC, Sterzel RB, Lang RE, Trabold EM, Veelken R, Ruskoaho H, Gao Y, Ganten D, Unger T: Atrial natriuretic factor determinations and chronic sodium homeostasis. *Kidney Int* 29:1004–1010, 1986
7. Lattion AL, Aubert JF, Fluckiger JP, Nussberger J, Waeber B, Brunner HR: Effect of sodium intake on gene expression and plasma levels of ANF in rats. *Am J Physiol* 255:H245–H249, 1988
8. Nakayama K, Ohkubo H, Hirose T, Inayama S, Nakanishi S: mRNA sequence for human cardiodilatin—atrial natriuretic factor precursor and regulation of precursor mRNA in rat atria. *Nature* 310:699–701, 1984
9. Takayanagi R, Tanaka I, Maki M, Inagami T: Effects of changes in water-sodium balance on levels of atrial natriuretic factor messenger RNA and peptide in rats. *Life Sci* 36:1843–1848, 1985
10. Nemer M, Lavigne JP, Drouin J, Thibault G, Gannon M, Antakly T: Expression of atrial natriuretic factor gene in heart ventricular tissue. *Peptides* 7:1147–1152, 1986
11. Tanaka I, Inagami T: Release of immunoreactive atrial natriuretic factor from rat hypothalamus in vitro. *Eur J Pharmacol* 122:353–355, 1986
12. Lee YJ, Lin SR, Shin SJ, Tsai JH: Increased adrenal medullary atrial natriuretic polypeptide synthesis in patients with primary aldosteronism. *J Clin Endocrinol Metab* 76:1357–1362, 1993
13. Gardner DG, Deschepper CF, Baxter JD: The gene for the atrial natriuretic factor in the aortic arch. *Hypertension* 9:103–106, 1987
14. Greenwald JE, Needleman P, Wilkins MR, Schreiner GF: Renal synthesis of atriopeptin-like protein in physiology and pathophysiology. *Am J Physiol* 260:F602–F607, 1991
15. Ritter D, Chao J, Needleman P, Tetens E, Greenwald JE: Localization, synthetic regulation, and biology of renal atriopeptin-like prohormone. *Am J Physiol* 263:F503–F509, 1992
16. Ramirez G, Saba SR, Dietz JR, Vesely DL: Immunocytochemical localization of proANF 1–30, proANF 31–67 and atrial natriuretic factor in the kidney. *Kidney Int* 41:334–341, 1992

17. Lee YJ, Shin SJ, Tan MS, Hsieh TJ, Tsai JH: Increased renal atrial natriuretic peptide synthesis in rats with deoxycorticosterone acetate-salt treatment. *Am J Physiol* 271:F779–F789, 1996
18. Shin SJ, Lee YJ, Tan MS, Hsieh TJ, Tsai JH: Increased atrial natriuretic peptide mRNA expression in the kidney of diabetic rats. *Kidney Int* 51:1100–1105, 1997
19. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156–159, 1987
20. Sambrook J, Fritsch EF, Maniatis T: *Molecular Cloning: A Laboratory Manual* (2nd ed). Cold Spring Harbor, Cold Spring Harbor Laboratory Press, 1989
21. Greenwald JE, Ritter D, Tetens E, Rotwein PS: Renal expression of the gene for atrial natriuretic factor. *Am J Physiol* 263:F974–F978, 1992
22. Golomb E, Friedman E, Abassi ZA, Trachewsky D, Keiser HR: Rare renal transcription of the atrial natriuretic factor gene in rats. Demonstration by polymerase chain reaction. *Am J Hypertens* 6:867–872, 1993
23. Currie MG, Geller DM, Cole BR, Boylan JG, Yu Sheng W, Holmberg SW, Needleman P: Bioactive cardiac substances: Potent vasorelaxant activity in mammalian atria. *Science Wash DC* 221:71–73, 1983
24. Trippodo NC, Cole FE, Frohlich ED, MacPhee AA: Atrial natriuretic peptide decrease circulatory capacitance in areflexic rats. *Circ Res* 59:291–296, 1986
25. Goetz K L: Evidence that atriopeptin is not a physiological regulator of sodium excretion. *Hypertension Dallas* 15:9–19, 1990
26. Drummer C, Fiedler F, Konig A, Gerzer R: Urodilatin, a kidney-derived natriuretic factor, is excreted with a circadian rhythm and is stimulated by saline infusion in man. *J Am Soc Nephrol* 1:1109–1113, 1991
27. Drummer C, Gerzer R, Heer M, Molz B, Bie P, Schlossbeeger M, Stadaeger C, Rocker L, Strollo F, Heyduck B, Bauer K, Warberg J, Baisch F, Christensen NJ, Konig A, Norsk P: Effects of an acute saline infusion on fluid and electrolyte metabolism in humans. *Am J Physiol* 262:F744–F754, 1992
28. Goetz K, Drummer C, Zhu JL, Leadley R, Fiedler F, Gerzer R: Evidence that urodilatin, rather than ANP, regulates renal sodium excretion. *J Am Soc Nephrol* 1:867–874, 1990
29. Heer M, Drummer C, Baisch F, Gerzer R: Long-term elevations of dietary sodium produce parallel increases in the renal excretion of urodilatin and sodium. *Pflügers Arch* 425:390–994, 1993
30. Saville MA, Geer PG, Wang BC Jr, Leadley RJ, Goetz KL: A high-salt meal produces natriuresis in humans without elevating plasma atriopeptin. *Proc Soc Exp Biol Med* 188:387–393, 1988
31. Singer DRJ, Shore AC, Markandu ND, Buckley MG, Sagnella GA, MacGregor GA: Dissociation between plasma atrial natriuretic peptide levels and urinary sodium excretion after intravenous saline infusion in normal man. *Clin Sci Lond* 73:285–289, 1987
32. Terada Y, Moriyama T, Martin BM, Knepper MA, Garcia-Perez A: RT-PCR microlocalization of mRNA for guanylyl cyclase-coupled ANP receptor in rat kidney. *Am J Physiol* 261:R1080–R1087, 1991
33. Shepperson NB, Barclay PL, Bennett JA, Samuels GMR: Inhibition of neutral endopeptidase (EC 3.4.24.11) leads to an atrial natriuretic factor-mediated natriuretic, diuretic and hypotensive response in rodents. *Clin Sci* 80:265–269, 1991
34. Bralet J, Mossiat C, Lecomte J-M, Charpentier S, Gros C, Schwartz JC: Diuretic and natriuretic responses in rats treated with enkephalinase inhibitors. *Eur J Pharmacol* 179:57–64, 1990
35. Field LJ, Veress AT, Steinhelper ME, Cochrane K, Sonnenberg H: Kidney function in ANF-transgenic mice: Effect of blood volume expansion. *Am J Physiol* 206:R1–R5, 1991
36. Watkins L Jr, Burton JA, Haber E, Cart JR, Smith FW, Barger AC: The renin-angiotensin-aldosterone system in congestive failure in conscious dogs. *J Clin Invest* 57:1606–1617, 1976
37. Yared A, Kon V, Ichikawa I: Mechanism of preservation of glomerular perfusion and filtration during acute extracellular fluid volume depletion: Importance of intrarenal vasopressin-prostaglandin interaction for protecting kidneys from constrictor action of vasopressin. *J Clin Invest* 75:1477–1487, 1985
38. Henrich WL, Anderson RJ, Berns AS, McDonald KM, Paulsen PJ, Berl T, Schrier RW: The role of renal nerves and prostaglandins in control of renal hemodynamics and plasma renin activity during hypotensive hemorrhage in the dog. *J Clin Invest* 61:744–750, 1978
39. Singh I, Grams M, Wang WH, Yang T, Killen P, Smart A, Schnermann J, Briggs J: Coordinate regulation of renal expression of nitric oxide synthase, renin, and angiotensinogen mRNA by dietary salt. *Am J Physiol* 270:F1027–F1037, 1996
40. Kiberd BA, Larson TS, Robertson CR, Jamison RL: Effect of atrial natriuretic peptide on vasa recta blood flow in the rat. *Am J Physiol* 252:F1112–F1117, 1987
41. Brenner BM, Ballermann BJ, Gunning ME, Zeidel ML: Diverse biological actions o f atrial natriuretic peptide. *Physiol Rev* 70:665–699, 1990