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Modesti, Pietro Amedeo, Ilaria Cecioni, Angela Migliorini, Alessandra Naldoni, Alessandro Costoli, Simone Vanni, and Gian Gastone Neri Serneri. Increased renal endothelin formation is associated with sodium retention and increased free water clearance. *Am. J. Physiol.* 275 (*Heart Circ. Physiol.* 44): H1070–H1077, 1998.—To investigate whether renal endothelin (ET)-1 participates in water and sodium handling, we investigated the influence of different sodium intakes on renal production of ET-1 in eight healthy subjects. The functional relationship with the renin-angiotensin system was also studied. Renal ET-1 formation is affected by sodium intake, because 1 wk of high sodium decreased urinary ET-1 excretion (−34%, $P < 0.05$), whereas a low-sodium diet increased ET-1 excretion (66%, $P < 0.05$) and mRNA expression for preproendothelin-1 in epithelial cells of medullary collecting ducts and endothelial cells of the peritubular capillary network. Increased ET-1 renal synthesis was associated with sodium retention and increased free water clearance. Urinary ET-1 excretion changes from normal to low-sodium diet were negatively related to contemporary changes in sodium excretion ($r = 0.97$, $P < 0.05$) and were positively correlated with free water clearance ($r = 0.97$, $P < 0.05$). These correlations were maintained during angiotensin-converting enzyme inhibition, which only partially reduced ET-1 renal excretion. These results indicate that renal ET-1 production is indeed modulated by varying sodium intakes and may exert a role in sodium and water handling.

sodium balance; renal function; urine; angiotensin II

ABUNDANT EXPRESSION of endothelin (ET)-1 peptide and mRNA for its precursor, preproendothelin-1 (prepro-ET-1), have been found in the vascular endothelium of the renal vascular bed, including glomerular capillaries, arterioles, and peritubular capillaries (15, 24). Subsequent studies have demonstrated that ET-1 is present in high concentrations in the inner medulla of the kidney (24), where ET-1 receptors are also located (5), and that ET-1 secretion is not limited to vascular endothelium, because primary epithelial cell cultures derived from the renal tubule secrete abundant amounts of ET-1 (10). ET-1 secretion in the renal medulla is highly compartmentalized in tubules with the following secretion hierarchy: inner medullary collecting ducts (IMCD) > medullary thick ascending limb > cortical collecting ducts > proximal tubule (11, 29).

The physiological activities of ET-1 on renal sodium and water handling still remain to be clarified (11). Convincing evidence exists that ET-1 may inhibit arginine vasopressin (AVP)-stimulated water reabsorption in the collecting ducts. Several studies have demonstrated that ET-1 binding to the ET_B receptor subtype reduces Na^+-K^+-ATPase activity in the IMCD (34) and inhibits vasopressin-stimulated cAMP accumulation and water transport on isolated cortical collecting ducts from rats (23, 30). These and other studies (11, 29) indicate that ET-1 might regulate water reabsorption independently of its effects on Na^+ reabsorption or renal hemodynamics.

Studies investigating the activity of ET-1 on sodium reabsorption have given conflicting or unconvincing results. ET-1 inhibited Na^+-K^+-ATPase activity in a suspension of rabbit IMCD (34), suggesting a possible natriuretic effect. Administration of anti-ET-1 antibodies to rats on a low-sodium diet increased urinary salt and water (32). Experiments performed by infusing ET-1 in isolated rat kidney (22) or in vivo gave conflicting results, because either a sodium-retentive (8) or a natriuretic (13, 31) effect was observed. However, the natriuresis observed after ET-1 infusion might result from release of the atrial natriuretic peptide (ANP) induced by ET-1 infusion (19). Moreover, the natriuretic effect might be secondary to hemodynamic changes such as the increase in arterial blood pressure (pressure natriuresis) (31). On the other hand, the sodium retention reported after ET-1 infusion both in rabbits (8) and in humans (25) might be caused by intrarenal blood flow redistribution even if the total renal blood flow was not decreased (25).

Thus the pharmacological approach to investigation of physiological function as performed by infusing ET-1 may be confounding because the active agents can provoke additional known or unknown effects. Studies performed in humans without ET-1 infusion but reproducing some physiological situations have shown that urinary ET-1 is inversely related to changes in blood volume. Acute volume expansion caused, in addition to the notable increase in urinary volume, a marked decrease in urinary ET-1 excretion (21). Conversely, a sustained enhancement of urinary ET-1 excretion with a considerable decrease in urinary volume and sodium excretion occurred during long-lasting standing posture (17). These findings suggest that renal ET-1 may be involved in the mechanisms of control of diuresis and natriuresis.

To investigate this hypothesis we studied whether different sodium intakes affect synthesis of renal ET-1. Because experimental studies (27) and investigations performed in humans (17) suggest that angiotensin-converting enzyme (ACE) inhibition may influence renal ET-1 excretion, we studied renal ET-1 excretion during a low-sodium diet in the presence of inhibition of ANG II formation.

METHODS

Subjects Investigated

Eight healthy subjects of ages 25–38 yr (5 males and 3 females), volunteers for the present investigation, were studied. Experimental procedures and the purposes of the study were explained to the subjects, and all of them gave their informed consent. No subject was a smoker or had taken any drug for at least 4 wk. Before the investigation serum electrolytes, fasting plasma glucose, creatinine clearance, and urinalysis were tested in all subjects. No pathological finding emerged, and creatinine clearance ranged within commonly accepted normal values (range of subjects investigated: 97–135 ml/min). All subjects had the same lifestyle during the sample-collecting days: breakfast at about 8:30 AM, lunch at about 1:00 PM, and an evening meal at 8:00 PM. They went to sleep at 11:00 PM and woke up at 7:00 AM.

Experimental Protocols

All subjects underwent three 7-day periods of different sodium intake (low, 20 meq/day; normal, 108 meq/day; high, 300 meq/day) in a randomized order. After the 7 days of low-sodium diet subjects maintained this regimen and were treated with an ACE inhibitor (ramipril, 5 mg/day) for another 7 days. The three diet periods were separated from one another by 1 wk of a normal-sodium diet.

In another separate set of experiments, after one wash-out week on a normal-sodium diet, the subjects were treated with ramipril (5 mg/day) for 7 days and then underwent a 7-day low-sodium diet period while they continued ramipril treatment.

Throughout the experimental periods the subjects had the same basic diet containing a constant amount of protein (1.3 g/kg), calories (126 kJ/kg), calcium (800 mg/24 h), and potassium (80 meq/day). Urinary and plasma samples for the determination of ET-1, sodium, lithium, osmolality, creatinine, and plasma renin activity (PRA) were taken daily during the last 3 days before the different diet regimens started and every day throughout the study periods.

Sampling Procedure and Radioimmunoassays

Blood and urine sample collection and ET-1 extraction were performed as previously described (17, 21). Briefly, plasma and urine samples were extracted with Sep-Pak C_{18} columns (Waters, Milford, MA) that had been previously activated by consecutive washing steps with methanol (for plasma) or acetonitrile-trifluoracetic acid (TFA) (for urine). After washing, the adsorbed ET-1 was eluted with methanol (for plasma) or acetonitrile-TFA (for urine). The ET-1 recovery rate, calculated by the addition of different concentrations of cold ET-1, was 70 ± 9% from plasma and 90 ± 5% from urine. Samples were then assayed by specific radioimmunoassay, using polyclonal rabbit anti-ET-1 serum (Peninsula, Belmont, CA), as previously described (17, 21). Briefly, the extracted samples were resuspended in phosphate buffer immediately before the assay was performed. The standard ET-1 (Peninsula) or plasma samples (100 µl) were mixed with 100 µl of rabbit anti-ET-1 serum, diluted with radioimmunoassay buffer to a final dilution of 1:72,000 (vol/vol) for plasma and 1:24,000 (vol/vol) for urine. Antibody cross-reactivities were 100% with ET-1 (porcine, human), 7% with ET-2 (human), 7% with ET-3 (rat, human), 35% with Big ET (porcine), 17% with Big ET (human), and 3% with sarafotoxin S6b. There was no cross-reactivity with α-human ANP, brain natriuretic peptide (BNP; porcine), ANG I, ANG II, ANG III, vasopressin, ACTH, or vasoactive intestinal peptide (VIP). The minimum detectable ET-1 concentration was 0.1 pg/ml. The coefficients of intra-assay and interassay variations were 4 (n = 11 samples) and 10 (n = 11)%, respectively. Results were expressed as picograms per milliliter (pg/ml) for plasma. Urinary ET-1 excretion was expressed as picograms per milliliter and as amount excreted per minute (pg/min).

Blood samples for PRA were collected in ice-cold tubes containing EDTA (0.084 ml tripotassic EDTA in 7 ml blood; final concentration 0.34 mol/l) and immediately centrifuged, then stored at −20°C. PRA measurement was performed by measuring the quantity of ANG I generated in vitro by radioimmunoassay with a commercial kit (Sorin Biomedica) and was expressed as nanograms of ANG I per milliliter of plasma per hour of incubation.

Analytical Methods and Calculations

Plasma and urine were assayed for electrolytes (sodium and lithium) with an ion-sensitive electrode (Instrumentation Beckmann Astra, Brea, CA) and for creatinine with the Jaffe reaction (Instrumentation Beckmann Astra). Glomerular filtration rate (GFR) was estimated by the clearance of endogenous creatinine. To better appreciate the effects of ACE inhibition on urinary volume and sodium excretion regardless of GFR variation, we also expressed urinary volume and 24-h sodium excretions as the rate of excretion per unit of creatinine clearance (C_{Cr}).

Free water clearance (C_{H_2O}) was calculated as

$$C_{H_2O} = (1 - U_{osm}/P_{osm}) \times V$$

where U_{osm} (mosmol/kg H_2O) and P_{osm} (mosmol/kg H_2O) are the simultaneous urinary and plasma osmolalities, respectively, and V (ml/min) is the simultaneous rate of urinary volume flow. Negative values of C_{H_2O} occur when the urine is hypertonic to the plasma.

Osmolar clearance, sodium clearance (C_{Na}), lithium clearance (C_{Li}), and C_{Cr} were calculated using standard formulas. C_{Li} is a measure of fluid delivery from the proximal tubule to the loop of Henle. The ratio of sodium to lithium clearances (C_{Na}/C_{Li}) is thus a measure of that fraction of the sodium delivered to the distal tubule which is finally excreted, and $C_{Li} - C_{Na}$ is a measure of the absolute rate of sodium reabsorption from the distal tubule (3). Finally, we calculated the cumulative sodium balance during the last 3 days of sodium diet or ACE treatment period.

In Situ Hybridization

The expression of mRNA for prepro-ET-1 was investigated in kidneys obtained in surgery from six patients affected with localized renal tumors (age range 47–66 yr). Before surgery patients underwent a 7-day period of normal (3 patients, 108 meq/day)- or low (3 patients, 20 meq/day)-sodium intake.

Kidney specimens were cut from the pole opposite the tumor. Kidney portions containing cortex and medulla from the six kidneys were fixed in 4% paraformaldehyde in 0.12 M phosphate buffer (pH 7.2). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and prepro-ET-1 probes were transformed from *Escherichia coli* with separate plasmid vectors harboring the cDNA for GAPDH (pHcGAP, ATCC no. 57090) and prepro-ET-1 (ET1c, ATCC no. 65698). Recombinant plasmids were purified from 5 ml each of *E. coli* growth using the FlexyPrep kit (Pharmacia). After digestion we separated the cDNA fragments from the plasmid by electrophoresis on a 1% agarose gel and then purified by cutting the gel slice of the corresponding molecular insert size (1.2 kb for both ET-1 and GAPDH) using the BandPrep Kit Sephaglas (Pharmacia).

Streptavidin-biotinylated horseradish peroxidase complex in buffered sodium chloride was used as the detection reagent (28). Specimens were stained with 3-amino-9-ethyl carbazole (Sigma) for 10 min at 37°C and counterstained with Mayer's hematoxylin. To ensure the specificity of the in situ hybridization signals, negative controls were performed by testing the sections with hybridization mixture *1*) without the probe and *2*) after incubation with RNAase A (1 Kunitz unit/l) for 1 h at 37°C. Positive controls were obtained for each sample using a cDNA probe for the constitutively expressed GAPDH to ensure that mRNA in kidney specimens was intact. In situ hybridization staining was performed at the same time for all specimens and at least twice on serial sections in each specimen. The presence of mRNA signals was assessed by light microscopy.

Statistical Analysis

Data are presented as means ± SD unless otherwise indicated. Comparisons of a single observation between groups were made with ANOVA and Student's *t*-test. Multiple comparisons among diet groups were made with Tukey's test. Repeated observations over the experimental days were analyzed with ANOVA for repeated measures, with a split-plot design for differences between days (multivariate analysis of variance). A value of $P < 0.05$ was considered significant. All statistical analyses were performed with BMDP statistical software (BMDP Statistical Software, Los Angeles, CA).

RESULTS

Effects of Different Sodium Intakes

The three sodium regimens deeply affected urinary ET-1 excretion, which increased during the low-sodium diet ($P < 0.05$) and decreased during high sodium intake ($P < 0.05$) (Fig. 1). ET-1 plasma concentration was not affected by the low-sodium diet, whereas it increased significantly after high sodium intake ($P < 0.05$) (Fig. 1).

The values of the different analytical parameters obtained at the end of the 7-day normal-sodium diet are reported in Table 1.

Urinary ET-1 excretion at the end of the high-sodium diet period had significantly decreased (−28%, $P < 0.05$), whereas ET-1 plasma concentration had on average increased by 103% ($P < 0.05$) (Table 1). The decrease in urinary ET-1 excretion became significant from *day 2* (−22 %, $P < 0.05$) and reached a nadir at *day 3* (−34%, $P < 0.05$), and then values plateaued (Fig. 1, Table 1). PRA decreased from 1.50 ± 0.21 to 0.28 ± 0.10 ng ANG I · ml^{-1} · h^{-1} ($P < 0.05$). GFR did not change, whereas urinary flow rate and C_{Na} increased and C_{H_2O} decreased (Table 1).

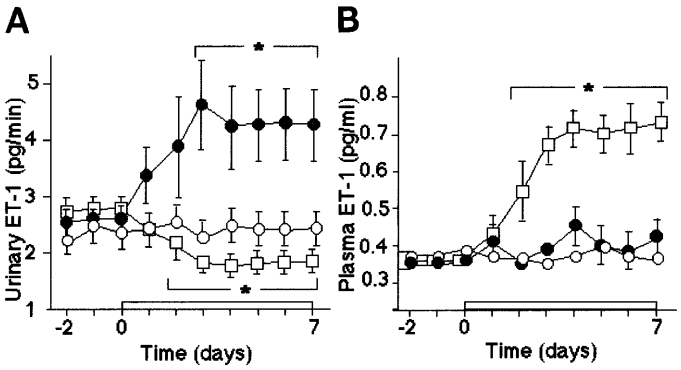

Fig. 1. Urinary endothelin (ET)-1 excretion (*A*) and plasma ET-1 concentration (*B*) in healthy subjects at baseline (*day −2* to *day 0*) and during three 7-day sodium diet regimens (*day 1* to *day 7*), indicated by bars. ●, Low-sodium diet, 20 meq/day; ○, normal-sodium diet, 108 meq/day; □, high-sodium diet, 300 meq/day. *$P <$ 0.05 vs. baseline (*day 0*).

The low-sodium diet was associated with an increased daily ET-1 urinary excretion (from 2.58 ± 0.62 to 4.28 ± 1.34 pg/min, +66%, $P < 0.05$). Plasma ET-1 concentration did not change significantly (Fig. 1, Table 1). PRA increased after 2 days of diet ($P < 0.05$), and it was 4.26 ± 0.37 ng ANG I · ml^{-1} · h^{-1} ($P < 0.05$) at the end of the low-sodium diet. During the low-sodium diet period, GFR did not significantly change, whereas the urinary flow rate decreased from 54 ± 16 to 46 ± 15 ml/h at the end of the diet period ($P < 0.05$). Similarly, there was a marked reduction in sodium excretion (from 117 ± 5 to 21 ± 3 meq/24 h) and C_{Na} (from 0.57 ± 0.02 to 0.10 ± 0.01 ml/min, $P < 0.05$).

At the end of the low-sodium diet, C_{Li} was reduced by 51% ($P < 0.05$). At the same time, the fraction of sodium delivered to the distal tubule that is finally excreted, expressed by the ratio C_{Na}/C_{Li}, decreased by 62% ($P <$ 0.05) (Table 1). C_{H_2O} significantly increased on the second day (from −0.88 ± 0.30 to −0.38 ± 0.40 ml/min, $P < 0.05$) and rose further up to the seventh day (−0.17 ± 0.13 ml/min, $P < 0.05$) (Table 1).

Urinary ET-1 excretion changes from a normal- to a low-sodium diet were negatively related to contemporary changes in sodium excretion ($r = 0.97$, $P < 0.05$) and were positively correlated to C_{H_2O} ($r = 0.97$, $P <$ 0.05) (Fig. 2).

In situ hybridization. In situ hybridization studies performed in kidneys from patients on a normal-sodium diet showed that the prepro-ET-1 mRNA signal was present in the smooth muscle cells and endothelial cells of arcuate and interlobular arteries, whereas almost no signal was observed within the glomeruli (Fig. 3). The hybridization signal was observed in the peritubular capillary network in the cortical region (Fig. 3), in vasa recta, and in the loose connective tissue of the inner medulla. In the cortical region the epithelial cells did not show the hybridization signal in either the proximal or the distal tubule (Fig. 3). A weak, spotted hybridization signal was observed in the epithelial cells of the medullary collecting tubule (Fig. 3).

In kidneys from patients on the low-sodium diet the expression of prepro-ET-1 mRNA increased at both the vascular capillaries and epithelial cells of the collecting tubule. The hybridization signal was present in the smooth muscle cells of large vessels located in the renal columns and in the arcuate and interlobular arteries, as well as in the radial arteries. In comparison to the normal-sodium diet, prepro-ET-1 mRNA signal increased in capillary vessels originating from the efferent arteriola and circumventing the proximal and distal tubules (Fig. 4). No signal was present in the epithelial cells of the proximal or distal tubules (Fig. 4). The hybridization signal of mRNA expression had

Table 1. *Effects of 3 different 7-day sodium diet regimens: angiotensin-converting enzyme inhibition during low-sodium diet*

			20 meq Na			20 meq Na + Ramipril		
	300 meq Na	108 meq Na	*Day 1*	*Day 7*	% vs. baseline	*Day 8*	*Day 14*	% vs. baseline
Plasma ET-1, pg/ml	0.75 ± 0.09*	0.37 ± 0.03	0.41 ± 0.08	0.44 ± 0.08	+19	0.40 ± 0.09	0.35 ± 0.08	−5
Urinary ET-1, pg/ml	1.75 ± 0.45	4.17 ± 1.82	3.93 ± 1.52	5.52 ± 2.42*†	+32	2.31 ± 0.89*†‡	4.88 ± 2.30‡§	+17
Urinary ET-1, pg/min	1.85 ± 0.44*	2.58 ± 0.62	3.42 ± 1.01	4.28 ± 1.34*	+66	1.86 ± 0.56*†‡	3.38 ± 1.22*‡§	+31
Urinary flow rate, ml/h	65 ± 13*	54 ± 16	51 ± 16	46 ± 15*	−15	52 ± 25	51 ± 21	−5
C_{Cr}, ml/min	118 ± 21	112 ± 20	115 ± 18	114 ± 33	+2	100 ± 25	115 ± 45	+3
C_{Na}, ml/min	1.65 ± 0.37	0.57 ± 0.02	0.45 ± 0.04*	0.10 ± 0.01*†	−82	0.23 ± 0.04*†‡	0.13 ± 0.02*†‡§	−77
C_{Li}, ml/min	29.3 ± 6.3	25.1 ± 5.9	24.4 ± 5.6	12.3 ± 3.9*†	−51	17.2 ± 3.4*†‡	14.5 ± 3.0*†§	−42
C_{Na}/C_{Li}, %	5.8 ± 1.6	2.4 ± 0.5	1.9 ± 0.4*	0.9 ± 0.3*†	−62	1.4 ± 0.3*†‡	0.9 ± 0.3*†§	−62
C_{H_2O}, ml/min	−2.87 ± 0.56*	−0.88 ± 0.30	−0.78 ± 0.16	−0.17 ± 0.13*†	+81	−0.52 ± 0.11*†‡	−0.29 ± 0.16*†‡§	+67
$C_{Li} - C_{Na}$, ml/min	27.7 ± 6.2*	24.5 ± 5.9	23.9 ± 5.6	12.2 ± 3.9*†	−50	17.0 ± 3.4*†‡	14.4 ± 3.0*†§	−41

Values are means ± SD. ET-1, endothelin-1; C_{Cr}, creatinine clearance; C_{Na}, sodium clearance; C_{Li}, lithium clearance; C_{H_2O}, free water clearance. *$P < 0.05$ vs. baseline; †$P < 0.05$ vs. *day 1;* ‡$P < 0.05$ vs. *day 7;* §$P < 0.05$ vs. *day 8*.

markedly increased at the epithelial cells of the collecting tubules and ducts (Fig. 4) in comparison to the normal-sodium diet (Fig. 3).

In conclusion, on a low-sodium diet prepro-ET-1 mRNA expression increased not only in the capillary network but also in the epithelial cells of the collecting tubule.

Effects of ACE Inhibition

Ramipril administration started on the eighth day of the low-sodium diet caused a further increase in PRA values in the first 24 h (from 4.26 ± 0.37 to 12.45 ± 0.98 ng ANG I·ml^{-1}·h^{-1}, $P < 0.05$), without any further significant variation during the following 6 days. Mean arterial blood pressure had mildly decreased 3 h after ramipril administration (from 85 ± 6 to 81 ± 7 mmHg, $P < 0.05$) but returned to baseline levels at the 6-h measurement (data not shown). ACE inhibition did not affect ET-1 plasma concentration, although it caused a sharp and brief decrease in ET-1 urinary excretion in the first 24 h (from 4.28 ± 1.34 to 1.86 ± 0.56 pg/min, −56%, $P < 0.05$); this, however, partially recovered in the following days (Table 1, Fig. 5*A*). At the end of the 7-day ramipril treatment ET-1 urinary excretion was lower than pretreatment (−20%, $P < 0.05$) but significantly higher than baseline (+31%, $P < 0.05$) (Table 1).

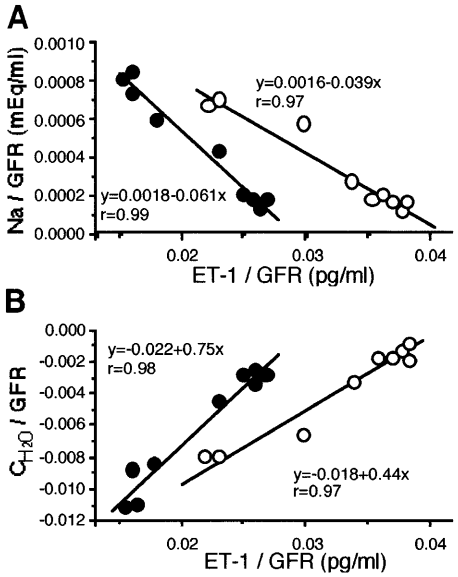

Fig. 2. Relationships of urinary ET-1 with sodium excretion (*A*) and free water clearance (C_{H_2O}; *B*) during low-sodium diet without (○) and with (●) angiotensin-converting enzyme (ACE) inhibition. Values are expressed per unit of glomerular filtration rate (GFR).

Sodium excretion showed consensual modifications to those of ET-1 excretion (Fig. 5*B*). On the first day of ramipril administration there was a marked and transient increase in C_{Na} (from 0.10 ± 0.01 to 0.23 ± 0.04 ml/min, $P < 0.05$), which, however, showed a tendency to return to the pretreatment values (0.13 ± 0.02 ml/min, $P < 0.05$ vs. both pretreatment values and vs. the first day of ramipril administration). The cumulative sodium balance of the last 3 days of ramipril treatment, when sodium and ET-1 excretion remained steady, was −16 ± 12 meq in comparison to −2 ± 5 meq ($P < 0.05$), observed during the low-sodium diet before ramipril treatment (Fig. 5*C*).

C_{Li} and C_{Na}/C_{Li} increased during the first 24 h of ramipril administration (Table 1), whereas C_{H_2O} decreased. After the first day of ACE inhibition all these functional indexes returned to the pretreatment values, whereas C_{H_2O} remained significantly reduced in comparison to pretreatment values (Table 1; Fig. 5*D*). Thus the partial recovery of ET-1 excretion despite ACE inhibition was associated with consensual changes in C_{H_2O} (from −0.52 ± 0.11 to −0.29 ± 0.16 ml/min, $P < 0.05$; Fig. 5*D*).

When ACE inhibition was started before the low-sodium diet no significant differences in ET-1 excretion, urinary volume, sodium excretion, or C_{H_2O} were found in comparison to ACE inhibition started during the low-sodium diet (data not shown). The very good correlations found between ET-1 excretion and both sodium

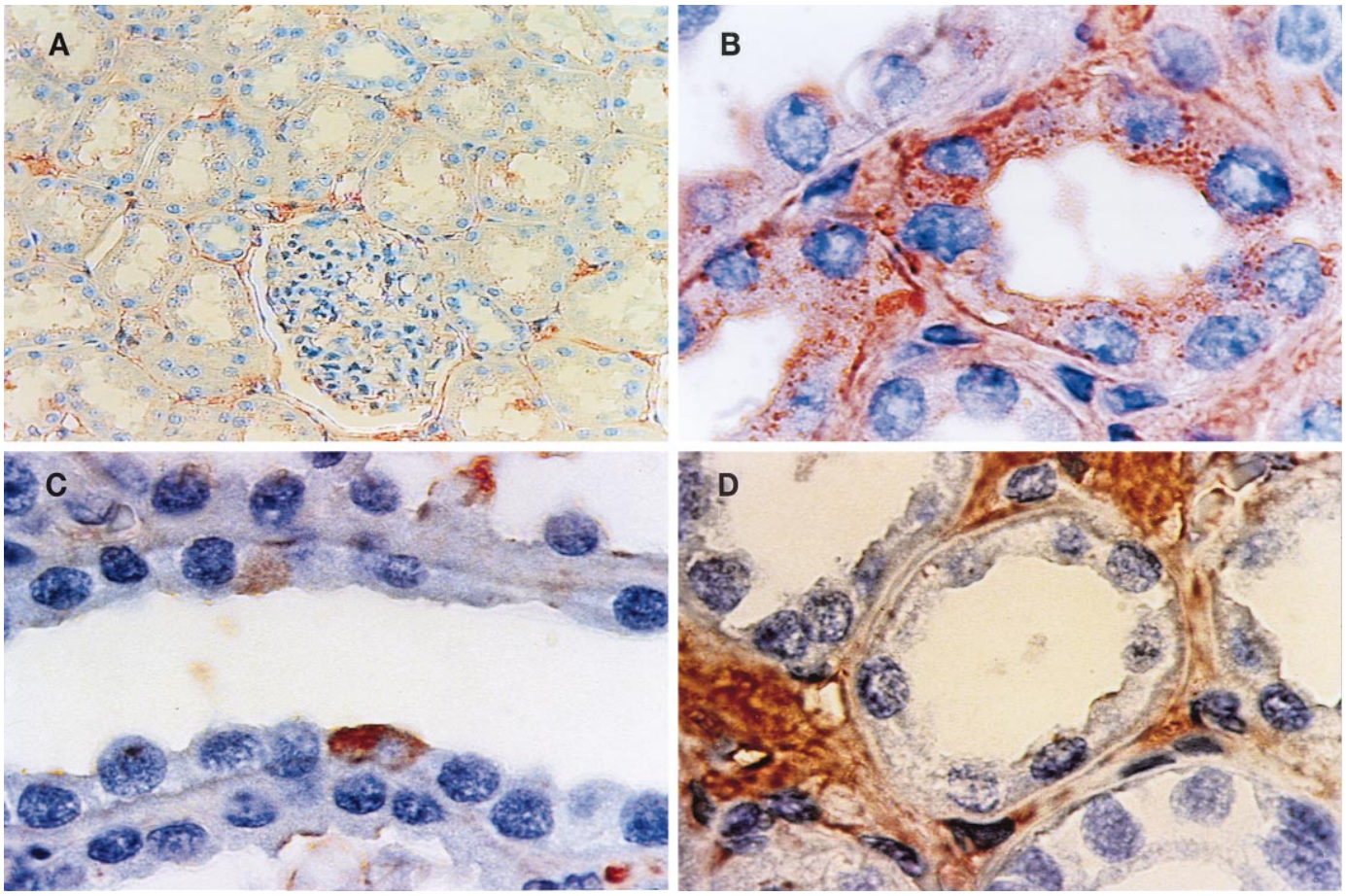

Fig. 3. In situ hybridization in kidney specimens from patients on normal-sodium diet. *A*: no signal for preproendothelin (prepro-ET-1) mRNA was observed within glomeruli and in epithelial cells; positive prepro-ET-1 mRNA hybridization signal in peritubular capillary network (×200). *B*: glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression in epithelial cells of inner medulla (×1,000). *C* and *D*: weak, spotted prepro-ET-1 mRNA hybridization signal was observed in epithelial cells of medullary collecting tubule in longitudinal (*C*) and transverse (*D*) sections (×1,000).

excretion and C_{H_2O} during the low-sodium diet were also maintained when the low-sodium diet was started under ACE inhibition ($r = 0.99$, $P < 0.05$ and $r = 0.98$, $P < 0.05$, respectively), although they had slightly shifted to the left (Fig. 2).

DISCUSSION

The present findings indicate that a low sodium intake is associated with an increase in prepro-ET-1 mRNA expression in endothelial cells of the peritubular capillary network and, especially, in epithelial cells of the medullary collecting tubules. These changes of mRNA expression for prepro-ET-1 were associated with increased urinary ET-1 excretion. Conversely, the high-sodium diet was associated with a decrease in urinary ET-1 excretion.

Our results demonstrate for the first time that renal ET-1 synthesis in humans is affected by changes in sodium intake. The modifications in renal prepro-ET-1 mRNA expression were associated with consensual variations in urinary ET-1 excretion, thus indicating that urinary ET-1 excretion is the main expression of renal ET-1 formation. Previous studies showed that infusion of 30 ml/kg of a 5% glucose solution over 1 h caused a marked increase in plasma ET-1 concentration associated with an almost complete disappearance of urinary ET-1 (21). In the present study the increase in urinary ET-1 excretion without any changes in plasma ET-1 during low sodium intake, and, conversely, the increased plasma ET-1 concentration with a contemporary decrease in ET-1 excretion after a high-sodium diet, suggest that plasma ET-1 and renal ET-1 are two independent compartments.

The expression of mRNA for prepro-ET-1 during the low-sodium diet increased in the endothelial cells of the peritubular capillary network and in the epithelial cells of the medullary collecting tubules. Although the contribution of this ET-1 arising from endothelial cells to the total amount of urinary ET-1 cannot be ruled out, it is probably negligible, because studies performed in rats showed that only trace amounts of injected ^{125}I-labeled ET-1 passed in the urine (from 0.27 to 0.30% of the total infused ^{125}I-labeled ET-1) (1); as a consequence, the urinary ET-1 almost entirely derives from the renal tubule.

The increased renal ET-1 formation was associated

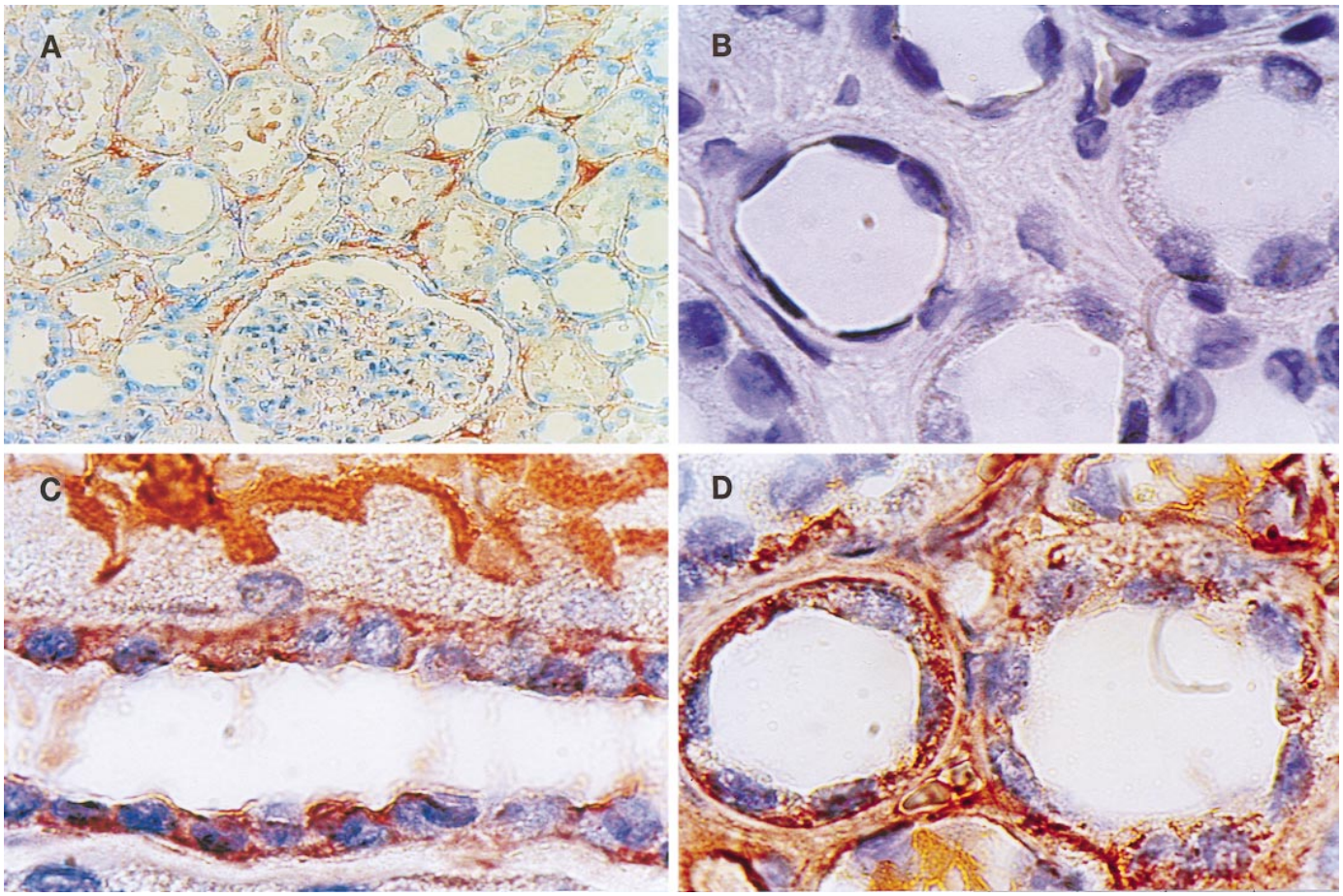

Fig. 4. In situ hybridization for prepro-ET-1 mRNA in kidney specimens from patients on low-sodium diet. *A*: increased hybridization signal in peritubular capillary network; no signal is detectable in epithelial cells of the proximal as well as of the distal tubule (×200). *B*: in situ hybridization of RNAase-treated specimen (×1,000). *C* and *D*: hybridization signal of prepro-ET-1 mRNA expression in epithelial cells of collecting tubules in longitudinal (*C*) and transverse (*D*) sections (×1,000).

with an enhancement in sodium retention. The increased prepro-ET-1 mRNA expression in endothelial cells of the postglomerular vascular capillary network circumventing the proximal and distal tubules probably contributes to the mild decrease in renal blood flow that occurs during low sodium intake (9). The decrease in renal blood flow mainly occurs in the renal medulla, where vasoconstrictor ET_A receptors are highly ex-

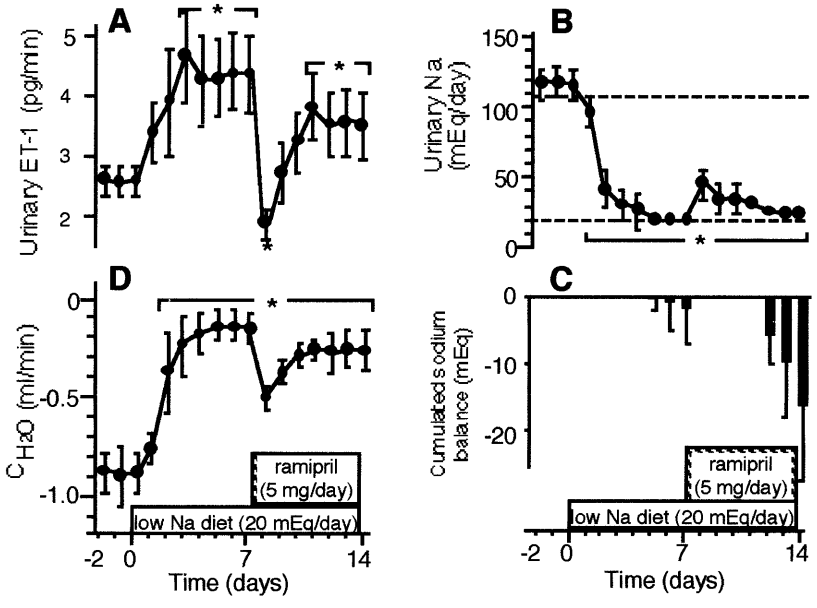

Fig. 5. Effects of ACE inhibition (*day 8* to *day 14*) in patients on low-sodium diet (*day 1* to *day 14*) on rate of ET-1 urinary excretion (*A*), urinary sodium excretion (*B*), and cumulative sodium balance of last 3 days of each period (*C*), and C_{H_2O} (*D*). $*P < 0.05$ vs. baseline (*day 0*).

pressed on interstitial cells and pericytes of the vasa recta (6). As a consequence of the decreased blood flow, the medullary osmolarity increases; this in turn produces an augmented sodium reabsorption (9). This sequence of events is suggested by the decreased fraction of sodium delivered to the distal tubule that is finally excreted (C_{Na}/C_{Li}). This distal sodium reabsorption was largely independent of ANG II activity, because ramipril administration only mildly reduced distal sodium reabsorption (Table 1). Thus these findings suggest a sodium-retentive activity of ET-1 in vivo. Several experimental findings obtained in vitro seem to speak against a sodium-retentive activity of ET-1. In a suspension of isolated IMCD cells, ET-1 has been demonstrated to inhibit Na^+-K^+-ATPase activity (34) and to inhibit Na^+ channels in the luminal membrane by a Ca^+-dependent process in the cortical collecting duct cells (14), thus resulting in a decrease of sodium entry from the lumen of epithelial cells. However, the effects of ET-1 in vivo appear to be more complex. Clavell et al. (6), using selective ET_A and ET_B antagonists, elegantly showed in dogs that the natriuretic effect of ET-1 at the level of epithelial cells becomes evident only when ET_A vasoconstrictor receptors are selectively blocked. When ET-1 was infused in humans at low doses (1 $ng \cdot kg^{-1} \cdot min^{-1}$), it caused sodium retention accompanied by a fall in the fractional excretion of lithium (25). Thus, in vivo, the sodium-retentive activity of ET-1 seems to prevail over the natriuretic effect found in in vitro studies. The increased sodium retention resulting from the increased medullary osmolality found in the present investigation may be considered as an indirect effect of ET-1, but the net result is a reduced sodium excretion.

An increased C_{H_2O} was the other important functional modification associated with the increase in ET-1 urinary excretion. In addition to the peritubular capillaries, a marked increase in mRNA expression for ET-1 was also observed at the epithelial cells of inner medullary collecting tubules and ducts during low sodium intake. AVP secretion is the only condition affecting C_{H_2O}, and thus a decrease in this secretion might be responsible for the increase in C_{H_2O} observed in subjects during the low-sodium diet. We did not measure AVP plasma concentration, but it does not increase in low sodium intake (4). Several studies have shown that ET-1 negatively modulates the effects of AVP on the distal tubule by inhibiting both AVP-stimulated cAMP accumulation (12) and water permeability in the rat terminal IMCD (20, 23, 29). Therefore, the increased C_{H_2O} seems to be attributable to the increased ET-1 formation by the epithelial cells of the collecting tubules and ducts.

The factors responsible for the increased ET-1 renal formation remain to be clarified. Several studies have shown that ANG II increases mRNA expression for prepro-ET-1 in isolated cells (27). Therefore, the increased mRNA expression for prepro-ET-1 in the endothelial cells of the peritubular capillary network found during low sodium intake might be caused by the increased ANG II formation. In the present investigation, changes in ET-1 urinary excretion paralleled renin-angiotensin system activity. The further PRA increase after ramipril administration in patients on a low-sodium diet is an index of the inhibition of ACE and then of a reduced ANG II formation. However, ET-1 excretion was only partially reduced by ACE inhibition, thus indicating that renal ET-1-forming activity was largely independent of the renin-angiotensin system. Moreover, the increased kinin activity that occurs during ACE inhibition (2) might directly inhibit ET-1 renal production, because previous studies showed that bradykinin inhibited ET-1 production from cultured endothelial cells (18).

An increase in renal medulla osmolality was found to stimulate mRNA expression for prepro-ET-1 in rat and rabbit epithelial tubular cells (16, 33). Moreover, reduced renal perfusion in rats and rabbits considerably enhanced ET-1 synthesis by the epithelial cells of the medullary tubules (7). During low sodium intake, blood flow decreases and medullary osmolality increases (9). Thus the increased medullary osmolality rather than PRA appears to be the main factor causing the increased mRNA expression for prepro-ET-1.

Plasma ET-1 levels increased during high sodium intake in contrast to the decrease in urinary ET-1 excretion. The augmented plasma ET-1 levels are probably caused by the volume expansion that occurs in this condition (26), because experimental acute volume expansion (21) or spontaneous chronic volume expansion as observed in congestive heart failure (27) were both found to be associated with increased plasma ET-1 levels.

In conclusion, the present results indicate that renal ET-1 is influenced by sodium intake. More particularly, a low-sodium diet is associated with increased mRNA expression for prepro-ET-1 both in the endothelial cells of the peritubular capillaries and in the epithelial cells of the medullary collecting tubules. The increase in ET-1 synthesis is associated with an increase in both sodium retention and C_{H_2O}. The enhanced mRNA expression for prepro-ET-1 in the endothelial cells of the peritubular capillaries is indirectly responsible for the increased sodium reabsorption, which is mediated by the enhanced medullary osmolality, whereas the increased synthesis of ET-1 by the epithelial cells of the medullary collecting ducts may explain the increase in C_{H_2O} observed during a low-sodium diet.

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