*Kidney International, Vol. 52 (1997), pp. 1028–1034*



# Urine and plasma levels of uroguanylin and its molecular forms in renal diseases

**Hiroshi Kinoshita, Shouichi Fujimoto, Masamitsu Nakazato, Naoto Yokota, Yukari Date, Hideki Yamaguchi, Shuichi Hisanaga, and Tanenao Eto**

*First and Third Departments of Internal Medicine, Miyazaki Medical College, Miyazaki, Japan*

**Urine and plasma levels of uroguanylin and its molecular forms in renal diseases.** Uroguanylin activates the intestinal and possibly the renal guanylate cyclase C receptor, and stimulates $Cl^-$ secretion. We developed a sensitive radioimmunoassay (RIA) for human uroguanylin and measured its concentration in the urine and plasma. Twenty-four-hour urinary excretion of immunoreactive (ir-) uroguanylin for persons with a high-salt diet (10 g/day) was 137.8 ± 14.4 pmol/day, significantly higher than that for persons with a low-salt diet (7 g/day, 95.1 ± 16.3 pmol/day, $P < 0.05$). There were significantly positive correlations between the urinary excretion of ir-uroguanylin and $Na^+$, $Cl^-$, $K^+$ or cyclic GMP (cGMP). We demonstrated the presence of messenger RNA of guanylate cyclase C in the medulla of human kidney. The concentration of plasma ir-uroguanylin significantly correlated with that of serum creatinine ($r = 0.71$, $P < 0.001$). Biologically active uroguanylin-16 accounted for 99% of the endogenous uroguanylin molecules in normal urine and 60% in plasma, the remainder being the 10 kDa precursor. The precursor content increased in the urine and plasma as the severity of renal impairment increased. These findings suggest that bioactive uroguanylin-16 is involved in the regulation of electrolyte homeostasis and that the kidney participates in the metabolism and excretion of uroguanylin.

Uroguanylin, a 16-amino acid peptide recently isolated from human and opossum urine, shares 50% amino acid sequence homology with guanylin [1, 2]. These two peptides enhance cyclic GMP (cGMP) production by activating the transmembrane guanylate cyclase C receptor (GC-C), thereby stimulating $Cl^-$ secretion by means of the cystic fibrosis transmembrane conductance regulator protein (CFTR) [3, 4]. Heat-stable enterotoxins (STs), which comprise 15 to 30 amino acid peptides, have high degrees of structural homology with uroguanylin and guanylin and act on intestinal GC-C by molecular mimicry, causing secretory diarrhea. The proximal tubules of opossum kidney have apical membrane receptors that bind $^{125}$I-labeled STs [5]. STs increase the cGMP contents in opossum renal slices and cultured opossum kidney cells [6]. STs administered intravenously or intramuscularly elicit large increases in the cGMP concentration of opossum urine [5, 6]. Administration of STs to perfused rat kidney decreases fractional sodium transport in the proximal tubules [7]. CFTR also is abundant in the apical membrane of the tubular cells [8, 9]. These results suggest the existence of a GC-C ligand in the kidney. Recently, Forte et al hypothesized that uroguanylin acts as an intestinal natriuretic hormone influencing salt and water homeostasis through an endocrine axis between the intestine and the kidney [10], because it is abundant in urine [1, 2]. However, Northern analysis showed that uroguanylin messenger RNA (mRNA) is expressed in the intestine, but not in the kidney [11, 12]. Using a sensitive radioimmunoassay (RIA) for human bioactive uroguanylin [13], we examined the effects of changes in dietary salt intake on the urine excretion of immunoreactive (ir-) uroguanylin and studied the relationships between the urine excretion of uroguanylin and $Na^+$, $K^+$, $Cl^-$ and cGMP. We also determined the plasma concentration of ir-uroguanylin in normal individuals and in patients with various degrees of renal impairment, and identified the endogenous molecular forms of uroguanylin in their plasma and urine. Finally, we have shown for the first time the existence of mRNA for GC-C in the medulla of human kidney by the reverse transcription-polymerase chain reaction (RT-PCR), thus confirming the ligand/receptor system.

**Key words:** uroguanylin, natriuretic hormone, electrolyte hemostasis, peptides, ligand/receptor system.

Received for publication August 15, 1996
and in revised form May 30, 1997
Accepted for publication June 2, 1997



## METHODS

### Peptides

Human bioactive uroguanylin and Tyr$^0$-uroguanylin were synthesized by the solid phase method with an ABI 430A or 433A peptide synthesizer. Human bioactive uroguanylin and generated Tyr$^0$-uroguanylin were isolated by reversed-phased high performance liquid chromatography (RP-HPLC) [14]. Synthetic Tyr$^0$-uroguanylin was radioiodinated by the lactoperoxidase method.

### Radioimmunoassay procedure

Antiserum was raised in rabbits against synthetic bioactive human uroguanylin conjugated with bovine thyroglobulin as previously reported [13]. The bioactivity of the uroguanylin and the guanylin molecules was confirmed by their production of cGMP in T84 human colon cancer cells that express GC-C. The RIA incubation buffer was 0.05 M sodium phosphate (pH 7.4) that contained 0.25% bovine serum albumin (BSA) treated with N-ethylmaleimide, 0.08 M NaCl, 0.025 M EDTA · 2Na, 0.05% $NaN_3$, and 0.1% Triton X-100. The diluted sample or a standard peptide solution (100 μl) was incubated with 100 μl of the antiserum diluent (final dilution 1/20,000) for 24 hours. One

hundred microliters of $^{125}$I-labeled ligand (16,000 cpm) then were added, and the mixture was incubated for 24 hours. The bound and free ligands were separated with a polyethylene glycol solution. All procedures were conducted at 4°C. Samples were assayed in duplicate.

### Quantification of immunoreactive uroguanylin

*Urine*. Twenty-four-hour urine samples were obtained from the following groups: 19 controls who had no signs of heart failure, renal diseases or gastrointestinal diseases (8 men and 11 women; mean age ± SEM, 59.5 ± 3.7 years); 13 patients with chronic glomerulonephritis (CGN patients; 3 men and 10 women; mean age, 47.8 ± 5.8 years) whose serum creatinine levels were ≤ 1.0 mg/dl; 13 patients with chronic renal failure (CRF patients; 10 men and 3 women; mean age, 51.6 ± 5.8 years) whose serum creatinine levels were ≥ 1.5 mg/dl (3.0 ± 0.8 mg/dl); and 12 patients on maintenance hemodialysis (HD patients; 9 men and 3 women; mean age, 58.9 ± 5.7 years). Renal biopsies of the CGN patients showed that 6 had membranous glomerulopathy, 3 minor glomerular abnormalities, 2 IgA nephropathy, and 2 lupus nephritis. The underlying diseases of the CRF patients were chronic glomerulonephritis in 7, hypertension in 3, collagen disease in 2 and amyloidosis in 1. Those of the HD patients were hypertension in 5, chronic glomerulonephritis in 4, diabetes mellitus in 2 and medullary cystic disease in 1. Twenty-eight subjects (19 controls and 9 CGN patients without nephrotic syndrome) were divided into groups with a high-salt diet (10 g/day, 2 men and 10 women; mean age, 53.5 ± 4.2 years) and a low-salt diet (7 g/day, 6 men and 10 women; mean age, 53.9 ± 5.8 years). The subjects in both groups had been on "low" or "high" salt diets for more than three days. The urine specimens were centrifuged at 3,000 rpm for 15 minutes at 4°C. The supernatant was diluted by one half with 0.9% saline and applied to a Sep-Pak C-18 cartridge (Waters Associates, Milford, MA, USA) that had been equilibrated with 0.9% saline, after which the cartridge was washed with saline and 10% acetonitrile ($CH_3CN$) solution containing 0.1% trifluoroacetic acid (TFA). Peptides were eluted with 60% $CH_3CN$ solution containing 0.1% TFA. The eluate was used for the RIA. The urine $Na^+$, $K^+$, $Cl^-$, creatinine and cGMP concentrations were also measured in 18 different patients, and plasma atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) values were measured in 16 of the 18 patients whose serum creatinine levels were ≤1.0 mg/dl; patients with nephrotic syndrome, gastrointestinal diseases and congestive heart failure were excluded. These patients were allowed free diet selection. The plasma ANP and BNP concentrations were measured with specific immunoradiometric assays for human ANP and BNP (ShionoRIA kits, Shionogi Co., Osaka, Japan).

*Plasma*. The subjects comprised four groups: 13 normal individuals (7 men and 6 women, mean age 27.7 ± 0.95 years); 12 CGN patients whose serum creatinine levels were ≤ 1.0 mg/dl and creatinine clearance levels ≥ 80 ml/min (3 men and 9 women, mean age 32.8 ± 5.1 year, serum creatinine 0.7 ± 0.1 mg/dl); 15 CRF patients whose serum creatinine levels were ≥ 1.5 mg/dl (7 men and 8 women, mean age 56.9 ± 3.8 years, serum creatinine 5.2 ± 0.8 mg/dl); and 10 hemodialysis (HD) patients (2 men and 8 women, mean age 52.6 ± 5.3 years). Blood was taken from the CGN and CRF patients after an overnight fast and from the HD patients just before the beginning of HD. Blood was drawn into chilled polypropylene tubes containing EDTA · 2Na (1 mg/ml of blood) and aprotinin (500 units/ml of blood) and immediately centrifuged at 3,000 rpm for 15 minutes at 4°C. The supernatant was diluted to one half with 0.9% saline and applied to a Sep-Pak C-18 cartridge as described above. The eluate was used for the RIA.

### Characterization of immunoreactive uroguanylin in urine and plasma

Urine was obtained from 3 normal controls, 3 CGN patients (serum creatinine < 1 mg/dl), 3 moderate CRF patients (serum creatinine 2 to 4 mg/dl), and 3 HD patients. Plasma was obtained from these same individuals. The urine and plasma samples were applied to individual Sep-Pak C-18 cartridges as described above. The eluates from the cartridges were subjected to RP-HPLC on a TSK ODS SIL 120 A column (4.6 × 150 mm). A 10% to 60% linear gradient of $CH_3CN$ in 0.1% TFA was run for 40 minutes at a flow rate of 1.0 ml/minute. RIA was used to quantify the uroguanylin content of each fraction. Synthetic human bioactive uroguanylin was chromatographed under the same conditions. The ir-fractions, #41 to 43 in Figure 6C, were analyzed by gel permeation chromatography on a TSK gel G2000 SW column under the conditions described in the legend to Figure 6D. Human uroguanylin and rat 10-kDa proguanylin purified from rat intestine [15] also were chromatographed under the same conditions.

### RT-PCR

Total RNA was extracted from human renal medulla and cortex, and colon by the acid guanidinium thiocyanate-phenol-chloroform method [16]. To digest the genomic DNA, 3 units of RNase-free DNase I (Pharmacia, Uppsala, Sweden), 110 U of RNase inhibitor, 40 mM Tris-HCl (pH 7.6) and 6 mM $MgCl_2$ were added to the 2.5 μg RNA samples. The samples then were incubated for 30 minutes at 37°C, after which they were heated to 90°C for five minutes to inactivate the DNase. The sequences and names of primers used in the RT-PCR assay are given in Table 2. The first strand of cDNA was synthesized with 0.4 μg of an RNA sample that had been treated with DNase, 2.5 μM GCC-AS1 primer, a dNTP mixture (1 mM each), 110 U of RNase inhibitor and 200 U of reverse transcriptase (GIBCO BRL, Gaithersburg, MD, USA). Reverse transcription was conducted for 30 minutes at 42°C followed by incubation for three minutes at 94°C to inactivate the reverse transcriptase. The resulting cDNA was subjected to PCR amplification with 2 μM GCC-S and GCC-AS1 primers, and 1.25 U of *Taq* DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT, USA). The reaction volume was 25 μl, and the PCR conditions were 35 cycles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 51°C and extension for 60 seconds at 72°C. Nested PCR was conducted with 1 μl of the first-step RT-PCR product and 2 μM GCC-S and GCC-AS2 primers under the PCR conditions described above. The PCR products were electrophoresed on a 2% agarose gel (FMC BioProducts, Rockland, ME, USA). Human β-actin was used as a positive control for RT-PCR as described elsewhere [16].

### Statistical analysis

Data are expressed as means ± SE. Simple linear regression analysis was used for the correlations. The unpaired Student's *t*-test was used for the comparisons of the two randomized groups.

**Table 1.** Plasma and urine concentrations of immunoreactive uroguanylin

| | Control | CGN | CRF | HD |
|---|---|---|---|---|
| Urine | 103.1 ± 15.9 | 137.6 ± 34.0 | 196.2 ± 34.3[a] | 632.8 ± 273.1[a] |
| *pmol/day* | *N* = 19 | *N* = 13 | *N* = 13 | *N* = 12 |
| Plasma | 5.0 ± 0.3 | 7.5 ± 0.8[b] | 44.3 ± 12.1[b] | 216.9 ± 66.4[c] |
| *fmol/ml* | *N* = 13 | *N* = 12 | *N* = 15 | *N* = 10 |

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ compared with control

**Table 2.** Primers for RT-PCR of GC-C

| Primer name | Oligonucleotide sequence | Nucleotide number in [17] |
|---|---|---|
| GCC-S | 5′-TTCAAATGCACAACGGATGG-3′ | 375-394 |
| GCC-AS1 | 5′-TCCTCCAAGTACTGGTCATT-3′ | 877-896 |
| GCC-AS2 | 5′-CGGTCACCCTTCAGCTTGTA-3′ | 814-833 |

GCC-S is a sense primer and GCC-AS1 and -AS2 are antisense primers.

Differences were considered significant if the $P$ value was less than 0.05.

## RESULTS

When the antiserum was used at the final dilution of 1/20,000, bioactive uroguanylin was measurable at a minimal concentration of 0.4 fmol/tube (10% replacement), and the half-maximum inhibition on the standard RIA curve was 4 fmol/tube (Fig. 1). The antiserum showed no cross-reactivity with human guanylin. Twenty-four-hour urinary excretions of ir-uroguanylin of the CRF and HD patients were significantly higher than the control value (Tables 1 and 2). The daily excretion of ir-uroguanylin was significantly higher in the high-salt group than in the low-salt group (137.8 ± 14.4 pmol/day vs. 95.1 ± 16.3 pmol/day, respectively, $P < 0.05$; Fig. 2). The daily urine excretions of $Na^+$, $K^+$ and $Cl^-$ in the high-salt group were 143.1 ± 29.7 mEq/day, 36.3 ± 6.2 mEq/day and 142.8 ± 25.5 mEq/day, and those in the low-salt group were 93.3 ± 14.7 mEq/day, 32.2 ± 6.7 mEq/day and 99.8 ± 19.5 mEq/day, respectively. The urinary excretions of $Na^+$, $K^+$ and $Cl^-$ significantly correlated with that of ir-uroguanylin (Fig. 3). The urinary excretion of cGMP also significantly correlated with that of ir-uroguanylin ($r = 0.78$, $P < 0.01$). The plasma concentrations of ANP and BNP were 22.5 ± 5.4 pg/ml and 13.5 ± 5.1 pg/ml, and did not significantly correlate with the urinary excretion of $Na^+$, $K^+$ and $Cl^-$, but did correlate significantly with the urinary excretion of cGMP (ANP, $r = 0.71$, $P < 0.05$; BNP, $r = 0.50$, $P < 0.05$).

The plasma ir-uroguanylin concentrations of the CGN, CRF and HD patients were significantly higher than the control value (Table 1). The plasma ir-uroguanylin concentrations of the CGN and CRF patients ranged from 3.5 to 168.2 fmol/ml and correlated with their serum creatinine concentrations ($r = 0.71$, $P < 0.001$; Fig. 4).

Uroguanylin molecules in human urine and plasma were analyzed by RP-HPLC coupled with RIA. Representative RP-HPLC profiles of the molecules in the urine are shown in Figure 5. Uroguanylin immunoreactivity in samples from controls and CGN patients (Fig. 5A) was detected only at the elution position of uroguanylin-16 (fractions #24 and #25). Two major immunoreactive peaks were found in patients with moderate CRF, one at the elution position of uroguanylin-16 and the other in fractions #41 to 43 (Fig. 5B). The HD patients showed a major peak at fractions #41 to 43 and a very minor peak in fractions #24 and #25 (Fig. 5C).

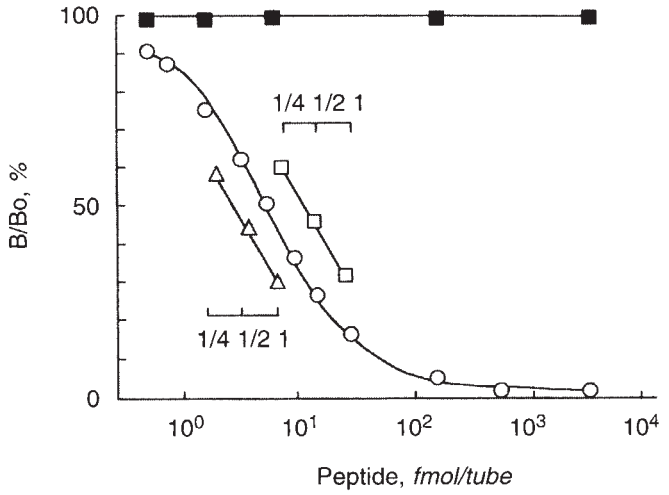


**Fig. 1. Standard RIA curve for human bioactive uroguanylin.** Inhibition of $^{125}$I-Tyr$^0$-human bioactive uroguanylin binding to antiserum by serial dilution of human uroguanylin (○), human guanylin (■), plasma extract from a CRF patient (△), and urine extract from a control subject (□). The unity scale under the plasma dilution curve represents 20 μl and that above the curve for urine indicates 4 μl.

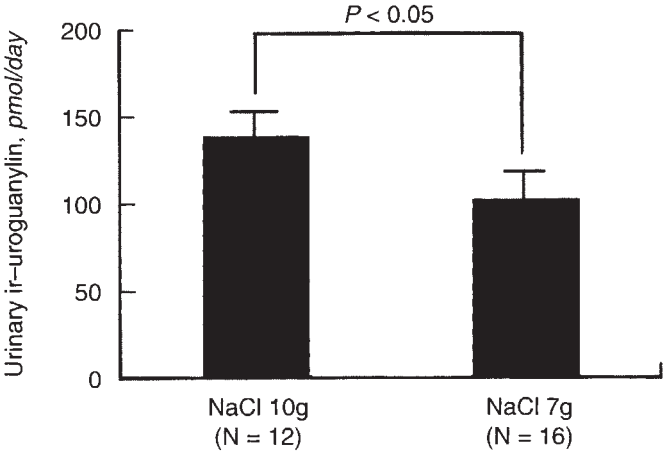


**Fig. 2. Daily excretion of immunoreactive (ir-) uroguanylin for the groups with high-salt (10 g/day) and low-salt (7 g/day) diets.** Values are expressed as mean ± SE.

About 60% of the uroguanylin immunoreactivity in normal plasma was detected at the elution position of uroguanylin-16 (#24 and #25) and the rest in fractions #41 to 43 (Fig. 6A). The major peak of immunoreactivity in the plasma from patients with CGN or HD was in fractions #41 to 43, and there was a very minor peak, which corresponded to uroguanylin-16, in fractions #24 and #25 (Fig. 6B and C). To further characterize the uroguanylin-immunoreactive peptide from fractions #41 to 43 (Fig. 6C), we estimated its molecular weight by gel permeation chromatography. The peptide was eluted at a position identical to

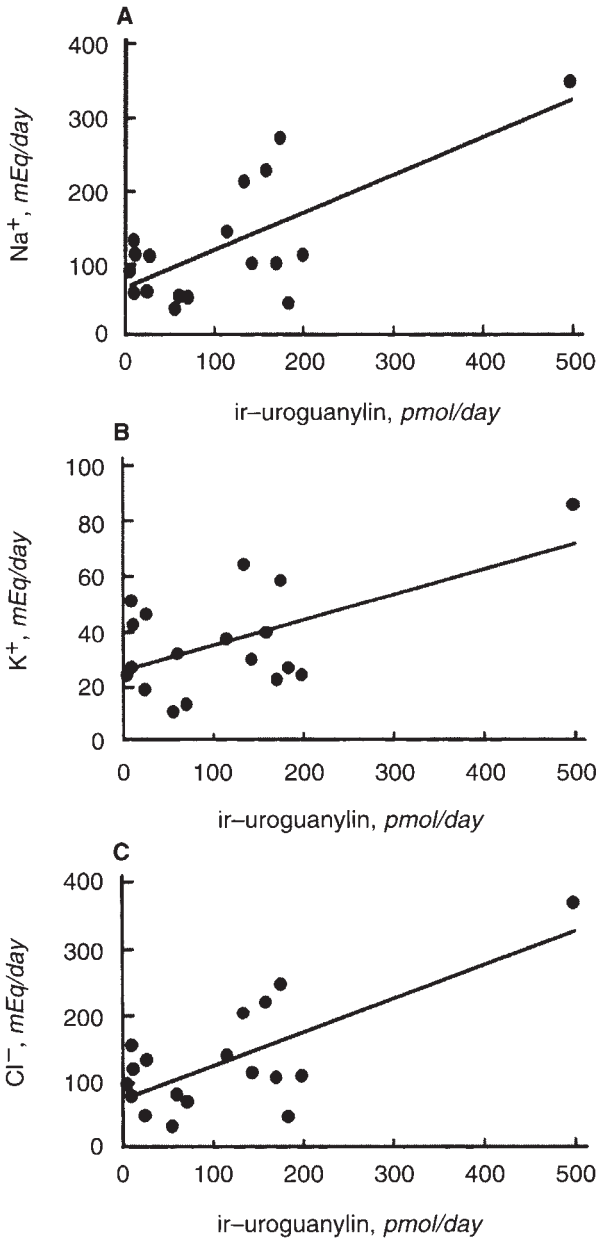


**Fig. 3. Correlation between the urine excretion of ir-uroguanylin and $Na^+$ (*A*; r = 0.71; P < 0.01), $K^+$ (*B*; $r = 0.56$; $P < 0.05$), and $Cl^-$ (*C*; $r = 0.71$; $P < 0.001$) in 18 patients with normal renal function.** Patients with nephrotic syndrome, gastrointestinal diseases and heart failure were excluded.

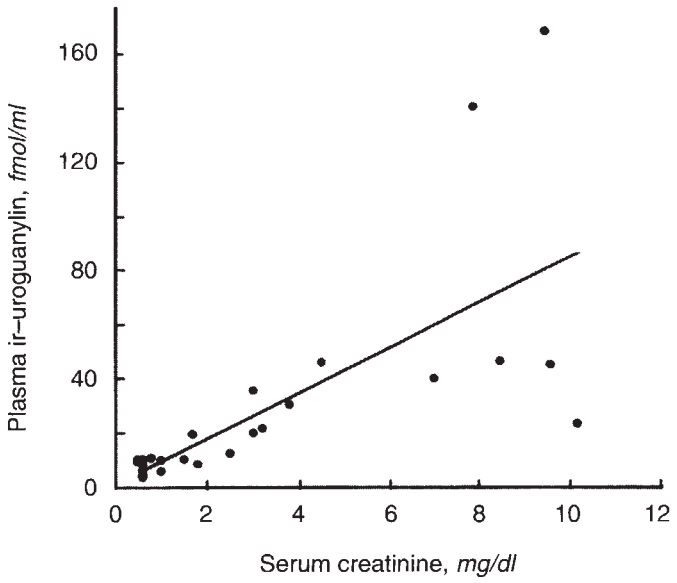


**Fig. 4. Correlation between plasma immunoreactive (ir-) uroguanylin and serum creatinine concentrations in 27 patients with proteinuria of more than 300 mg/day (CGN and CRF patients; $r = 0.71$; $P < 0.001$).**

that of rat 10-kDa proguanylin (Mr = 10.4 k; Fig. 6D). The plasma concentrations of bioactive uroguanylin-16 were calculated from the RP-HPLC findings. The mean concentrations were 3.1 fmol/ml in the controls, 1.9 fmol/ml in the CGN patients, 5.9 fmol/ml in the CRF patients, and 29.1 fmol/ml in the HD patients.

GC-C gene expression in human kidney and colon was examined by RT-PCR using nested PCR. A GC-C transcript product corresponding to the predicted size was detected at 459 bp in the renal medulla and colon, but not in the renal cortex (Fig. 7).

## DISCUSSION

An intestinal sensory mechanism for sodium that controls renal sodium excretion may exist because oral salt loads stimulate urinary sodium excretion more rapidly than intravenous salt load [18, 19]. When dietary sodium chloride is low, the regulation of salt homeostasis is maintained by mineralocorticosteroids under the influence of the renin-angiotensin pathway. The maintenance of homeostasis during the intake of high sodium chloride is not likely to be accomplished simply by turning off the aldosterone stimulus. ANP is known to be an important hormone in the control of sodium balance. Ingestion of a high-salt meal elicited a rapid increase in urinary sodium excretion, but plasma ANP levels did not increase for seven hours [20], whereas continuous ingestion of high-salt meals for 4 or five days elicited a significant increase in the plasma ANP level [21, 22]. It was suggested that ANP contributed to the increase in renal sodium excretion when the high sodium diet was continued for several days, but the postprandial increase in urinary excretion did not seem to be mediated by ANP [20]. Uroguanylin activates the receptor-guanylate cyclase effector molecules found on the apical membranes of human T84 intestinal cells and was originally isolated from opossum and human urine [1, 2]. Two research groups recently reported that human uroguanylin mRNA is expressed in the stomach and intestine, but not in the kidney [11, 12]. Human uroguanylin therefore is presumably produced in the gastrointestinal tract and then enters the circulatory system, reaching its receptors on renal tubular cells via glomerular filtration. We showed that urinary excretion of ir-uroguanylin was quite large and that bioactive uroguanylin-16 is abundant in control urine, suggesting that uroguanylin functions as a bioactive peptide in the kidney. We also demonstrated an increased excretion of urinary ir-uroguanylin in the group with a high-salt diet compared with a low-salt group and found a significant correlation between the urine excretion of ir-uroguanylin and $Na^+$, $K^+$, $Cl^-$ and cGMP. Plasma levels of ir-ANP and ir-BNP significantly correlated with

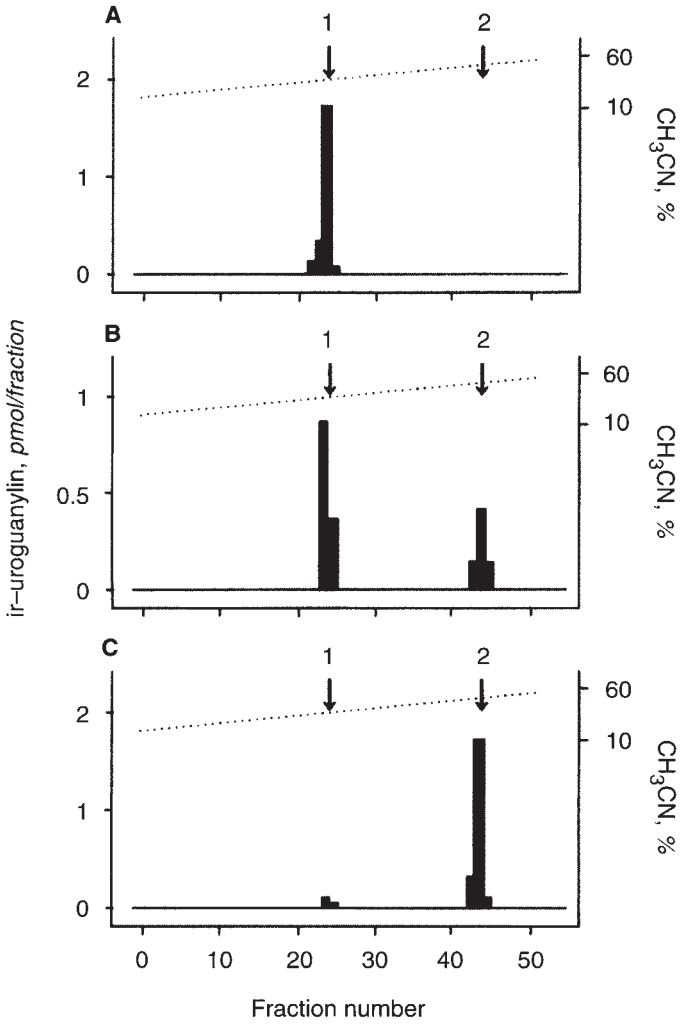


**Fig. 5. Representative RP-HPLC profiles of uroguanylin immunoreactivity in urine.** (*A*) Sample: 1 ml of urine from a CGN patient (serum creatinine 0.8 mg/dl and creatinine clearance 91 ml/minute). (*B*) Sample: 1 ml of urine from a CRF patient (serum creatinine 3.2 mg/dl and creatinine clearance 26 ml/minute). (*C*) Sample: 1 ml of urine from an HD patient (serum creatinine 10.1 mg/dl before HD). HPLC was performed with a TSK ODS SIL 120 A column (4.6 × 150 mm). A linear gradient of $CH_3CN$ from 10% to 60% in 0.1% TFA was run for 40 minutes at a flow rate of 1.0 ml/min. Each fraction volume was 0.5 ml. Black bars show uroguanylin immunoreactivity. Arrows indicate the elution positions of human uroguanylin-16 (1) and 10 kDa uroguanylin (2).

the urinary excretion of cGMP, but not with the excretion of $Na^+$, $K^+$ and $Cl^-$. When a high-salt diet is present, ANP seems to be partly responsible for the natriuresis [21, 22], but one or more natriuretic factors that relate to an intestinal sensory mechanism for salt are required [18–20]. Uroguanylin may be a candidate for an intestinal natriuretic factor because it is primarily synthesized in the intestine.

Activation of the tubular receptors by *E. coli* ST caused a marked increase in the urinary excretion of both $Na^+$ and $K^+$ [7]. $^{125}I$-labeled STs were bound in the apical membrane receptors of the proximal tubules of opossum kidney [5]. These studies strongly indicate the existence of GC-C in the kidney. However, GC-C has not been rigorously identified in the kidney [23–25] and a different mechanism of action for uroguanylin, independent of GC-C, could be considered. There are reports that cGMP analogs had little effect on $Cl^-$ uptake by T84 cells and failed to activate CFTR $Cl^-$ channels [26, 27] and STs could stimulate phospholipase C activity in the intestine [28]. We now have found the mRNA of GC-C in the renal medulla. The medulla is composed of various portions of the nephron and each segment has a unique transport function. Henle's loop contributes greatly to the urinary concentration system by the passive transport of NaCl and water. Outer and inner medullary collecting ducts are important for the final regulation of urinary NaCl and water. Taken together with the significant correlation between the excretion of uroguanylin and cGMP in the urine, the presence of GC-C transcripts in the kidney will further support the notion that uroguanylin acts in the kidney via cGMP mediation. We did not find mRNA of GC-C in the cortex. This uneven distribution of GC-C may be one of the reasons why GC-C has not as yet been identified in the kidney. More detailed investigations, including determining the distribution of GC-C in the kidney and administration of uroguanylin to the renal artery, are needed to confirm the activity and mechanism of uroguanylin as a local regulator of electrolytes in the kidney.

We have shown significant increases in 24-hour urinary excretion of ir-uroguanylin in patients with CRF and HD. However, the urine concentration of bioactive uroguanylin-16, determined by RP-HPLC, decreased as residual renal function decreased. A cDNA encoding a precursor for human uroguanylin has been cloned and sequenced from the human colon cDNA library [11, 12]. On the basis of the human uroguanylin cDNA sequence, it is predicted that a 10-kDa prouroguanylin will be secreted after cleavage of a signal peptide in the 112-amino acid preprouroguanylin. The major immunoreactive uroguanylin molecule in the plasma of CGN and CRF patients was the 10-kDa uroguanylin precursor. Recently, chymotrypsin-like protease within renal tubules has been proposed to activate prouroguanylin through cleavage of the peptide bond [29]. These findings suggest that the kidney is the target organ where the uroguanylin precursor is processed into its active form and that this processing is impaired in CRF patients.

We found a close correlation between the plasma levels of ir-uroguanylin and serum creatinine in patients with various degrees of renal dysfunction. The kidney is important in the elimination and metabolism of circulating proteins that are smaller than albumin. These low molecular weight proteins are extensively filtered in the glomerulus, absorbed by the tubular epithelium, and eventually hydrolyzed to their constituent amino acids within the renal cells [30]. We reported that most of the ir-guanylin in human plasma is the 10 kDa precursor [31] and that there is an excellent correlation between the plasma levels of ir-guanylin and serum creatinine [32]. The 10-kDa uroguanylin precursor found in this study must be filtered in the glomerulus and absorbed by the tubules of the normal kidney. Obviously, the decreased filtration of this precursor in CRF patients contributes greatly to the increased plasma peptide levels. We cannot exclude the possibility that uroguanylin production is enhanced in order to increase the urinary excretion of electrolytes, because the plasma concentration of ir-uroguanylin in CGN patients with normal renal function was significantly higher than that in the controls.

We demonstrated a greater uroguanylin excretion in patients with a high-sodium diet than in those with a low-sodium diet as well as a significant correlation of the excretion of $Na^+$, $K^+$, $Cl^-$

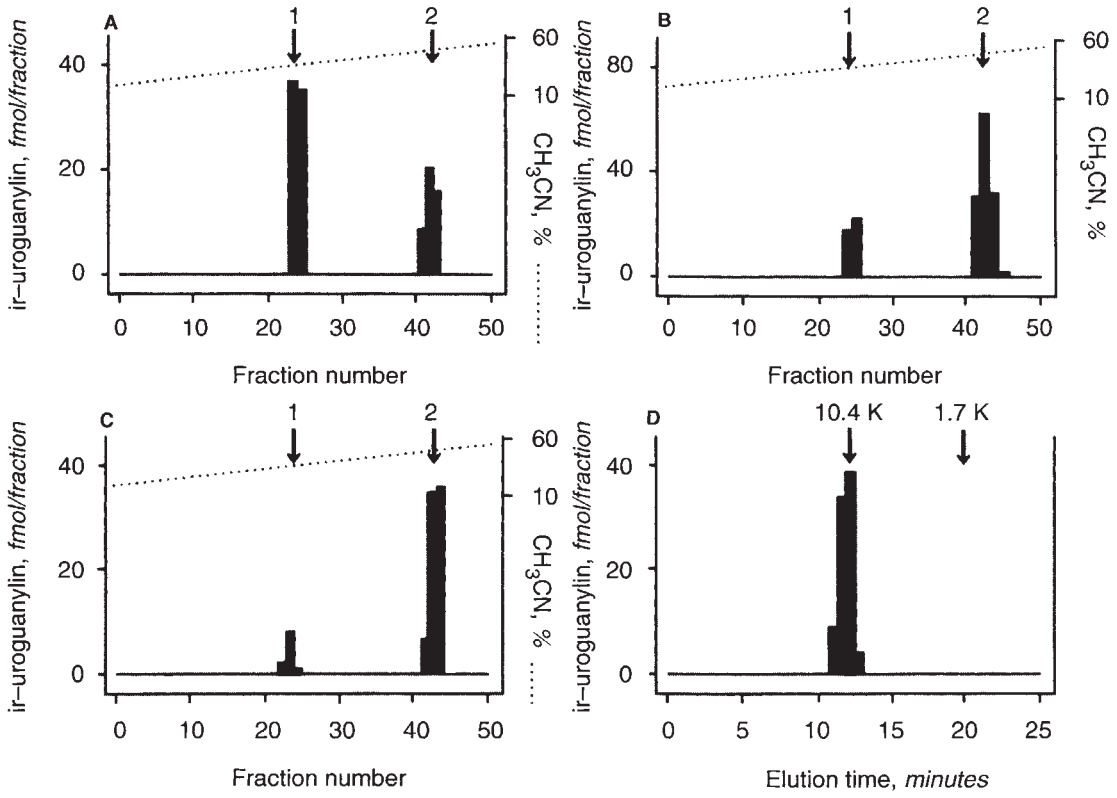


**Fig. 6. Representative RP-HPLC (A, B, C) and gel permeation chromatography (D) profiles of uroguanylin immunoreactivity in plasma.** (*A*) Sample: 27 ml of plasma collected from 3 normal controls. (*B*) Sample: 24 ml of plasma collected from 3 CGN patients with normal renal function. (*C*) Sample: 1 ml of plasma from an HD patient. HPLC was performed with a TSK ODS SIL 120 A column (4.6 × 150 mm). A linear gradient of 10% to 60% $CH_3CN$ in 0.1% TFA was run for 40 minutes at a flow rate of 1.0 ml/min. Each fraction volume was 0.5 ml. Black bars show uroguanylin immunoreactivity. Arrows indicate the elution positions of human uroguanylin-16 (1) and rat 10 kDa proguanylin (2). (*D*) Sample: fractions #41 to 43 in Figure 6C. Gel permeation chromatography was performed with a TSK gel G2000 SW column (7.5 × 600 mm). Each fraction volume was 0.5 ml. Peptides were eluted with 50% $CH_3CN$ in 0.1% TFA at a flow rate of 0.5 ml/min. Black bars show uroguanylin immunoreactivity. Arrows indicate the elution positions of human uroguanylin-16 (Mr = 1.7 kDa) and rat 10 kDa proguanylin (Mr = 10.4 kDa).

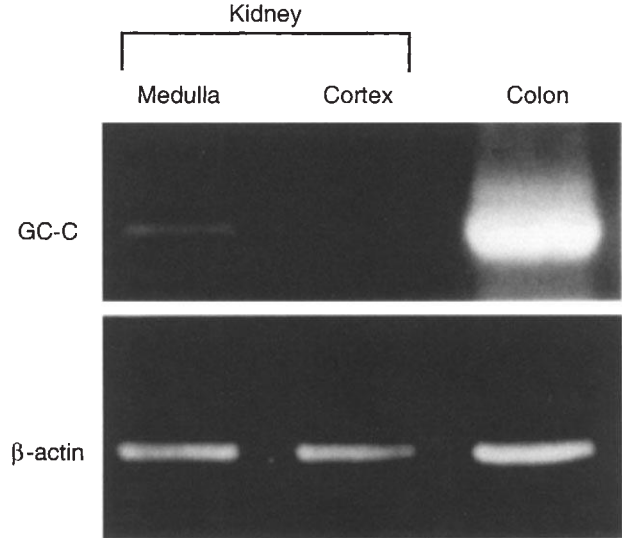


**Fig. 7. Electrophoretic analysis of RT-PCR products of human GC-C and β-actin transcripts in the renal medulla and cortex and the colon.**

and cGMP with ir-uroguanylin, thus suggesting that uroguanylin acts as a regulator of the metabolism of these electrolytes in the kidney. We also demonstrated the presence of mRNA of GC-C in the kidney, confirming the renal action of uroguanylin. We found that uroguanylin and its precursor circulate in the bloodstream and that the uroguanylin precursor is increased in CGN and CRF patients. Uroguanylin-16 was the main molecular form of urinary ir-uroguanylin in the controls and in CGN patients, but it decreased as renal failure progressed. These findings lead us to speculate that the kidney has an important function in the cleavage and elimination of uroguanylin. More detailed investigations of uroguanylin should furnish new insights into the regulation of electrolyte homeostasis.

*Reprint requests to Tanenao Eto, M.D., First Department of Internal Medicine, Miyazaki Medical College, 5200 Kihara, Kiyotake, Miyazaki 889-16, Japan.*

## APPENDIX

Abbreviations used in this paper are: ir-uroguanylin, immunoreactive uroguyanylin; GC-C, guanylate cyclase C; CFTR, cystic fibrosis transmembrane conductance regulator protein; mRNA, messenger RNA; STs, heat stable enterotoxins; RIA, radioimmunoassay; RP-HPLC, reversed-phased high performance liquid chromatography; CGN, chronic glomerulonephritis; PCR, reverse-transcription-polymerase chain reaction; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide.

## REFERENCES


1. Hamra FK, Forte LR, Eber SL, Pidhorodeckyj NV, Krause WJ, Freeman RH, Chin DT, Tompkins JA, Fok KF, Smith CE, Duffin KL, Siegel NR, Currie MG: Uroguanylin: Structure and activity of a second endogenous peptide that stimulates intestinal guanylate cyclase. *Proc Natl Acad Sci USA* 90:10464–10468, 1993
2. Kita T, Smith CE, Fok KF, Duffin KL, Moore WM, Karabatsos PJ, Kachur JF, Hamra FK, Pidhorodeckyj NV, Forte LR, Currie MG: Characterization of human uroguanylin: A member of the guanylin peptide family. *Am J Physiol* 266:F342–F346, 1994
3. Chao AC, de Sauvage FJ, Dong YJ, Wagner JA, Goeddel DV, Gardner P: Activation of intestinal CFTR $Cl^-$ channel by heat-stable enterotoxin and guanylin via cAMP-dependent protein kinase. *EMBO J* 13:1065–1072, 1994
4. Goldstein JL, Sahi J, Bhuva M, Layden TJ, Rao MC: *Escherichia coli* heat stable enterotoxin-mediated colonic $Cl^-$ secretion is absent in cystic fibrosis. *Gastroenterology* 107:950–956, 1994
5. Forte LR, Krause WJ, Freeman RH: *Escherichia coli* enterotoxin receptors: Localization in opossum kidney, intestine and testis. *Am J Physiol* 257:F874–F881, 1989
6. Forte LR, Krause WJ, Freeman RH: Receptors and cGMP signalling mechanism for *E coli* enterotoxin in opossum kidney. *Am J Physiol* 225:F1040–F1046, 1988
7. Lima AA, Monteiro HS, Fonteles MC: The effects of *Escherichia coli* heat-stable enterotoxin in renal tubular transport. *Pharmacol Toxicol* 70:163–167, 1992
8. Crawford I, Maloney PC, Zeitlin PL, Guggino WB, Hyde SC, Turley H, Gatter KC, Harris A, Higgins CH: Immunohistochemical localization of the cystic fibrosis gene product CFTR. *Proc Natl Acad Sci USA* 88:9262–9266, 1991
9. Riordan JR: The cystic fibrosis transmembrane conductance regulator. *Annu Rev Physiol* 55:609–630, 1993
10. Forte LR, Fan X, Hamra FK: Salt and water homeostasis: Uroguanylin is a circulating peptide hormone with natriuretic activity. *Am J Kid Dis* 28:296–304, 1996
11. Miyazato M, Nakazato M, Yamaguchi H, Date Y, Kojima M, Kangawa K, Matsuo H, Matsukura S: Cloning and characterization of a cDNA encoding a precursor for human uroguanylin. *Biochem Biophys Res Commun* 219:644–648, 1996
12. Hill O, Cetin Y, Cieslak A, Mägert HJ, Forssmann WG: A new human guanylate cyclase-activating peptide (GCAP-, uroguanylin): Precursor cDNA and colonic expression. *Biochim Biophys Acta* 1253: 146–149, 1995
13. Nakazato M, Yamaguchi H, Kinoshita H, Kangawa K, Matsuo H, Chino N, Matsukura S: Identification of biologically active and inactive human uroguanylins in plasma and urine and their increases in renal insufficiency. *Biochem Biophys Res Commun* 220:586–593, 1996
14. Chino N, Kubo S, Miyazato M, Nakazato M, Kangawa K, Sakakibara S: Generation of two isomers with the same disulfide connectivity during disulfide bond formation of human uroguanylin. *Lett Pept Sci* 3:45–52, 1996
15. Yamaguchi H, Nakazato M, Miyazato M, Kangawa K, Matsuo H, Matsukura S: Two novel rat guanylin molecules. guanylin-94 and guanylin-16 do not increase cyclic GMP production in T84 cells. *Biochem Biophys Res Commun* 214:1204–1210, 1995
16. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156–159, 1987
17. Sauvage FJ, Camerato TR, Goeddel DV: Primary structure and functional expression of the human receptor for *Escherichia coli* heat stable enterotoxin. *J Biol Chem* 266:17912–17918, 1991
18. Lennane RJ, Peart WS, Carey RM, Shaw J: A comparison of natriuresis after oral and intravenous sodium loading in sodium-depleted rabbits: Evidence for a gastrointestinal or portal monitor of sodium intake. *Clin Sci Mol Med* 49:433–436, 1975
19. Mu JY, Hansson GC, Bergström G, Lundgren O: Renal sodium excretion after oral or intravenous sodium loading in sodium-deprived normotensive and spontaneously hypertensive rats. *Acta Physiol Scand* 153:169–177, 1994
20. Saville MA, Geer PG, Wang BC, Leadly RJ Jr, Goetz KL: A high salt meal produces natriuresis in human without elevating plasma atriopeptin. *Proc Soc Exp Biol Med* 188:389–393, 1988
21. Gutkowska J, Schiffrin EL, Cantin M, Genest J: Effect of dietary sodium on plasma concentration of immunoreactive atrial natriuretic factor in normal humans. *Clin Invest Med* 9:222–224, 1986
22. Sagnella GA, Shore AC, Markandu ND, MacGregor GA: Effects of changes in dietary sodium intake and saline infusion on immunoreactive atrial natriuretic peptide in human plasma. *Lancet* 2:1208–1211, 1985
23. Schulz S, Chrisman TD, Garbers DL: Cloning and expression of guanylin. *J Biol Chem* 267:16019–16021, 1992
24. Laney DW Jr, Mann EA, Dellon SC, Perkins DR, Gianella RA, Cohen MB: Novel sites for expression of an *Escherichia coli* heat-stable enterotoxin receptor in the developing rat. *Am J Physiol* 263:G816–G821, 1992
25. Carrithers SL, Barber MT, Biswas S, Parkinson SJ, Park PK, Goldstein SD: Guanylyl cyclase C is a selective marker for metastatic colorectal tumors in human extraintestinal tissues. *Proc Natl Acad Sci USA* 93:14827–14832, 1996
26. Forte LR, Thorne PK, Eber SL, Krause WJ, Freeman RH, Francis SH, Corbin JD: Stimulation of intestinal $Cl^-$ transport by heat-stable enterotoxin: Activation of cAMP-dependent protein kinase by cGMP. *Am J Physiol* 263:C607–C615, 1992
27. Banik N, Ganguly U: Stimulation of phosphoinositides breakdown by the heat stable *E coli* enterotoxin in rat intestinal epithelial cells. *FEBS Lett* 236:489–492, 1988
28. Berger HA, Travis SM, Welsh MJ: Regulation of the cystic fibrosis transmembrane conductance regulator $Cl^-$ channel by specific protein kinases and protein phosphatases. *J Biol Chem* 268:2037–2047, 1993
29. Hamra FK, Fan X, Krause WJ, Freeman RH, Chin DT, Smith CE, Currie MG, Forte LR: Prouroguanylin and proguanylin: Purification from colon, structure, and modulation of bioactivity by proteases. *Endocrinology* 137:257–265, 1996
30. Maack T, Park CH, Camargo MJF: Renal filtration, transport, and metabolism of proteins, in *The Kidney: Physiology and Pathophysiology* (vol 3), edited by Seldin DW, Giebisch G, New York, Raven Press, 1992, pp 3005–3038
31. Nakazato M, Yamaguchi H, Shiomi K, Date Y, Fujimoto S, Kangawa K, Matsuo H, Matsukura S: Identification of 10-kDa proguanylin as a major guanylin molecule in human intestine and plasma and its increase in renal insufficiency. *Biochem Biophys Res Commun* 205:1966–1975, 1994
32. Kinoshita H, Nakazato M, Yamaguchi H, Matsukura S, Fujimoto S, Eto T: Increased plasma guanylin levels in patients with impaired renal function. *Clin Nephrol* 47:28–32, 1997