Concentrations of human atrial natriuretic peptide-like immunoreactivity (hANP-LI) were measured by a highly sensitive and specific radioimmunoassay (Biochem Biophys Res Commun 1986;137:231–6) in normal subjects and in renal disease patients without accompanying congestive heart failure, hypertension, edema, diabetes, or pregnancy. We attempted to clarify whether the hANP-LI concentration in plasma was increased by loss of renal mass. We found no correlation between the hANP-LI concentration in plasma and creatinine clearance (Ccr, 4.6–122.3 mL/min) in patients with renal disease (n = 63, r = −0.196), nor between hANP-LI concentrations in plasma and urine (n = 97, r = −0.207). The fractional excretion of hANP (FEhANP) correlated significantly with Ccr (n = 63, r = 0.520, P < 0.01) and with FEhANP (n = 35, r = −0.503, P < 0.01). Increased FEhANP in patients with chronic renal failure may have resulted because of an increase in single-nephron glomerular filtration rate similar to the FEhANP increase in these patients. The present data indicate that decreased renal function itself does not increase the concentration of hANP-LI in plasma.

Additional Keyphrases: atrial natriuretic peptide-like immunoreactivity · chronic renal failure · fractional excretion of ANP · glomerular filtration rate

In 1981, DeBold et al. (1) first reported a rapid and potent natriuretic response to intravenous injections of atrial myocardial extract in rats. Many workers confirmed that a specific substance in this extract might have a role in homeostasis of electrolytes and fluid volume (2, 3). The complete amino acid sequence of this substance was determined by Kangawa and Matsuo (4) as α-human atrial natriuretic peptide (α-hANP); they also identified two other distinct types of hANP as β-hANP (antiparallel dimer of α-hANP) and γ-hANP (precursor of α-hANP) (5). Soon after their reports, synthesized α-hANP became available commercially, and several anti-hANP antibodies were made by independent researchers. Radioimmunoassay (RIA) is widely used to measure the concentrations of hANP-like immunoreactivity (hANP-LI). Concentrations of hANP-LI in plasma are reported to increase in patients with congestive heart failure (6, 7), hypertension (8), syndrome of inappropriate antidiuretic hormone secretion (9), and Bartter’s syndrome (10); in diazylized patients with chronic renal failure (CRF) (11, 12); and in normal pregnancy (13). In the present study, we measured the concentrations of hANP-LI in plasma and urine from normal subjects and from patients with kidney disease but without volume overloading and hypertension, to clarify whether renal function affects the hANP concentration in plasma.

Subjects and Methods

We studied 97 patients with renal disease: 56 males and 41 females, ages 14 to 79 years (mean 46.5, SD 14.7). Sixty-six patients had chronic glomerulonephritis, 16 had nephrosclerosis, and 15 had cystic kidney disease. Patients with serum creatinine concentrations >50–60 mg/L (0.44–0.53 mol/L) were advised by nutritionists to restrict their daily protein intake to between 0.8 and 1.0 g/kg body weight and salt to 5–7 g per day. We excluded from the present study, except as noted in the text, patients with systolic blood pressure >160 mmHg, congestive heart failure, or diabetes and patients who were edematous, receiving digitalis or diuretics, or pregnant. Ccr was measured in 63 patients, 42 men and 21 women, ages 19 to 79 years (mean 48.6, SD 13.9). Sixty-five healthy subjects, 36 men and 26 women, ages 16 to 76 years (mean 27.0, SD 12.0) were also studied as controls. Blood and urine samples were obtained from outpatients when they visited our clinic. We calculated the fractional excretion of hANP-LI (FEhANP) and of Na (FENa) for patients and healthy persons and evaluated the correlation between these values and Ccr.

We measured hANP-LI in plasma and urine without extraction, analyzing 0.1 mL of sample with our highly sensitive and specific RIA (14). The assay buffer used to dissolve all reagents contained 10 mmol of K2EDTA, 20 mmol of glycine, 10 mmol of e-aminoacapric acid, 1 mmol of sodium azide, and 1.1 g of heat-inactivated human serum albumin per liter. Briefly, we incubated 0.1 mL of sample or hANP standard (Peptide Institute, Inc., Osaka, Japan) 0.1 mL of assay buffer, and 0.1 mL of anti-hANP serum (final dilution 1:80 000) at 4 °C for 20 h, then added 50 μL of [125I]α-hANP (120 ng/L; specific activity of 74 × 105 Bq/moL; Amersham International plc, Bucks., U.K.) and further incubated it at 4 °C for 24 h. We separated the bound from free ligands by the double antibody/polyethylene glycol method (14). The minimum detectable quantity in the hANP-RIA was 0.3 pg/tube with 99% confidence; the 50% intercept was 3.6 pg/tube. The within- and between-assay variations were 7.8% and 6.1%, respectively. The dilution curves generated with unextracted human plasma and urine were parallel to that of standard α-hANP. The antibody cross-reacted 100% with β-hANP, γ-hANP [Met(O)12]-α-hANP, and α-hANP (7-28); 80% with α-hANP (7-27) and α-hANP (5-27); 60% with α-rat-ANP; 50% with α-hANP (7-23); 40% with α-hANP (5-25), parallel dimer of α-hANP, and α-rat-ANP (7-28); and 0.01% with α-rat-ANP (5-25) and α-hANP (17-28). RIA with the antisemur mainly recognized the carboxyl-terminal segment of α-hANP.

For chromatographic analysis of hANP in plasma, we performed reversed-phase HPLC, using an octadecyl silica column (0.45 × 25 cm, 5-μm-diameter particles, Nucleosil Macherey-Nagel, Düren, F.R.G.) and eluting with a linear gradient of acetonitrile from 150 to 600 mL/L in 90 mmol/L trifluoroacetic acid reagent for 1 h. The flow rate was 0.1 mL/min.
mL/min, and 0.7-mL fractions were collected. To prepare the sample for injection, we acidified 0.3 mL of plasma with HCl, applied this to a Sep-Pak C18 cartridge, and eluted the absorbed peptides with methanol and 0.1 mol/L acetic acid (30/20 by vol). The eluate was evaporated under a stream of nitrogen gas, reconstituted with 90 mmol/L trifluoroacetic acid, and injected (100 µL) into the reversed-phase HPLC. The elution positions of peptides were monitored by ultraviolet absorbance at 210 nm or by hANP-RIA.

We also analyzed plasma samples by gel-permeation chromatography, using a 2 × 45 cm Sephadex G-75 column and eluting with 0.5 mol/L acetic acid, as previously reported (16). The flow rate was 5 mL/h at 4 °C and 2-mL fractions were collected. We could apply 0.2 mL of the plasma acidified with HCl directly onto the column. The elution positions were calculated by using a peptide molecular mass calibration kit and authentic α-, β-, and γ-hANP, monitoring their absorbance at 280 nm, or using the hANP-RIA.

FE_hANP and FE_Na were calculated by conventional formulas. Renin activity and aldosterone concentration in plasma were measured by RIA. Values obtained were expressed as mean (SD) or as otherwise indicated in the text. All clinical data were represented on bilogarithmic graphs. A statistical study was made by using Wilcoxon's rank sum test.

Results

For the controls, hANP-LI concentrations in plasma and urine are 41.7 (16.9) and 36.5 (20.2) ng/L, respectively, with no evident correlation (n = 65, r = -0.193). FE_hANP and FE_Na are 0.70% (0.37%) and 0.90% (0.43%), respectively.

In the patients with kidney disease under study, plasma renin activity and plasma aldosterone concentration are normal, which indicates that plasma volume status is within normal range. Ccr ranges from 4.6 to 123 mL/min. hANP-LI concentrations in plasma and urine show no significant correlation (n = 97, r = 0.207) (Figure 1). The correlation between hANP-LI concentration in plasma and Ccr is not statistically significant (n = 63, r = -0.0796) (Figure 2). However, FE_hANP and Ccr correlate significantly (n = 63, r = -0.728, P < 0.01) (Figure 3). FE_Na also correlates with Ccr (n = 35, r = -0.503, P < 0.01) and with FE_hANP (n = 63, r = 0.520, P < 0.01).

The elution profile of hANP-LI in extracted plasma on reversed-phase HPLC and that in unextracted plasma on gel-permeation chromatography are shown in Figure 4. In both chromatograms, two components of hANP-LI in the plasma of a patient with end-stage renal disease are eluted in the positions corresponding to standard α-hANP and γ-hANP, with no peak for degraded hANP fragment.

Discussion

Because the kidney is a main target organ for hANP-induced natriuresis and diuresis, the cyclic structure, α-hANP (7-23), and the carboxyl terminal residues of α-hANP are important for expressing spasmylytic and natriuretic activity (15).

There are two conflicting results regarding the concentrations of plasma hANP-LI in nondialyzed CRF patients. Several investigators have reported that the plasma
hANP-LI concentration is increased not only in dialyzed but also in nondialyzed patients with CRF, disregarding their general medical conditions (16-18). In CRF patients, the increased hANP-LI concentrations in plasma should be considered in hypertension or volume-overload conditions. Furthermore, the antibody used by Hasegawa et al. (17) recognized the amino-terminal segment of α-hANP, and the antibody used by Yoshinaga et al. (16, 19) recognized not only the carboxyl- but also the amino-terminal site of α-hANP. Other antibodies may recognize α-hANP sites that are not biologically active (11, 12, 16-18). Yandle et al. (20) identified the amino-terminal-deleted peptide, α-hANP (7-28), in plasma. Their report suggests that the amino-terminal fragment of α-hANP exists in plasma, because α-hANP is processed from γ-hANP by a membrane-bound specific peptidase (21) and is secreted into circulating blood. In CRF patients, inactive hANP fragments are probably retained in circulating blood. RIA's with antibodies that recognize inactive sites of hANP have a high probability of measuring inactive hANP fragments and thus give results that indicate high concentrations of hANP-LI in plasma for patients with end-stage renal disease. This would falsely suggest that bioactive hANP increases with decreased renal function. Therefore, some investigators suppose that plasma hANP-LI concentration is inversely correlated with renal function.

hANP is secreted mainly from the atrium owing to an increase in atrial wall tension (22, 23) or atrial volume load (24). Thus, hANP concentration in plasma increases from volume overload in the circulation in patients with congestive heart failure (7, 8) and in those undergoing hemodialysis (12, 25). To avoid volume-overload status in kidney disease patients in this study, salt intake was carefully restricted in patients with CRF, hypertension, edema, or heart disease and in patients taking drugs such as digitalis or diuretics. All patients examined had normal plasma renin activity and plasma aldosterone concentration; therefore, volume status in the patients in this study should be normal. It is reasonable that plasma hANP-LI does not increase from renal dysfunction itself, because a half-life of hANP in the plasma is only 1.5 to 2.0 min (26, 27). Because as the present study indicates, plasma hANP-LI concentrations are not affected by decrease of renal function, an RI with an antibody that recognizes the carboxyl-end of hANP should be used in estimating the plasma hANP-LI concentration in CRF patients. The hANP concentration in plasma in renal disease patients seems to be higher than that of controls. In this study, the renal disease patient was on average older than the controls. As Ohashi et a (28) reported, older people have higher hANP concentrations in plasma than do younger subjects; therefore, the mean hANP concentration in renal disease patients may be higher than that in the controls. Thus, in our study, this fact should not affect the correlation between Ccr and hANP or Na in the plasma of renal disease patients.

The increase in $\text{FE}_{\text{hANP}}$ in patients with CRF may result from an increase in single-nephron glomerular filtration rate similar to the $\text{FE}_{\text{Na}}$ increase in these patients, as reported previously (29).

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