Pathophysiologial Role and Diuretic Efficacy of Atrial Natriuretic Peptide in Renal Patients

LUCA DE NICOLA,* VINCENZO BELLIZZI,* BRUNO CIANCIARUSO,* ROBERTO MINUOTOLO,* GIUSEPPE COLUCCI,* MARIO BALLETTA,* GIORGIO FUIANO,† and GIUSEPPE CONTE‡
*Division of Nephrology, School of Medicine, First University of Naples, Naples, Italy; †University of Reggio Calabria, Catanzaro, Italy; and ‡Second University of Naples, Naples, Italy.

Abstract. It has been suggested that renal disease is characterized by the presence of resistance to the natriuretic effects of atrial peptide (ANP). In this study, plasma ANP (pANP) and renal function were evaluated during stepwise infusion of low ANP doses (2, 4, 8, and 16 ng/kg per min) in glomerulonephritic patients with (CRF) or without (GN) moderate renal failure, and in normal subjects (NOR), kept at low-sodium diet (LSD; 35 mEq NaCl/day). To assess the physiological ANP levels, pANP was also measured in the three groups after normal-sodium diet (NSD; 235 mEq NaCl/day). ANP did not affect systemic and renal perfusion at any of the doses tested; a significant increment of GFR was observed only in NOR and GN. The 2-, 4-, and 8-ng/kg doses increased pANP to values overlapping the physiological concentrations measured at NSD; this was associated with a dose-dependent increase of urinary excretion of sodium (UNaV) that reached analogous levels in the three groups. ANP accounted for approximately 40% of the UNaV increment evoked by NSD in patients and in normal subjects. The 16-ng/kg dose led to supraphysiological levels that induced a similar marked enhancement of UNaV (from the basal value of 0.12 ± 0.02 to 0.42 ± 0.08 mEq/min in CRF, from 0.13 ± 0.02 to 0.73 ± 0.08 in GN, and from 0.09 ± 0.02 to 0.49 ± 0.11 in NOR). In CRF, the normal natriuretic response to the highest dose was caused by a larger increase of fractional UNaV that was strictly dependent on the greater pANP increment, as demonstrated by similar changes in the fractional excretion of cGMP, and, in part, on the greater aldosterone decrease. In all groups, ANP also induced a dose-dependent urinary loss of phosphate, potassium, and urea, resulting in a significant 15 to 25% decrease in the plasma levels. Thus, in GN and CRF patients, ANP plays a significant role in the renal handling of sodium; moreover, the achievement of low supraphysiological pANP levels leads to a conspicuous natriuresis associated with unique extranatriuretic effects. (J Am Soc Nephrol 8: 445–455, 1997)

In moderate to advanced chronic renal failure (CRF), the natriuretic response to loop diuretics is blunted by insufficient intratubular secretion of these drugs and the activation of sodium-retaining mechanisms, mainly represented by the stimulation of the renin-angiotensin system and the increase of proximal tubule reabsorption (1,2). Large doses are therefore given as intravenous bolus or continuous infusion (3,4) but are, however, associated with relevant adverse effects, such as hyponatremia as a result of further impairment of free-water generation (5), hyperazotemia (6,7), and ototoxicity (4). Thus, a great effort has been made in recent years to find new diuretics effective in CRF (2,8), but the results have been inconclusive.

More recently, interest in the atrial natriuretic peptide (ANP) has been raised (9). In healthy subjects, endogenous ANP plays a critical role in the physiological renal handling of sodium (10,11); moreover, the administration of low doses of ANP strikingly increases the urinary excretion not only of sodium but also of the primary solutes retained in CRF, such as urea, potassium, and phosphate, with no systemic effect (10–13). The enhancement of renal excretory function is secondary to the reduction of tubular reabsorption in the distal nephron and the associated inhibitory effect on the proximal reabsorption with the consequent increase of free-water clearance (10–14). Importantly, the diuretic effectiveness of low-dose ANP in normal subjects appears to be dependent on the attainment of plasma ANP levels (pANP) immediately above the physiological range (10–12).

In contrast, scarce and discouraging data have been provided on the renal effects of ANP in renal disease. Although recent data from our group have demonstrated that endogenous ANP release is as highly sensitive to the variation of salt intake in CRF patients as in normal subjects (15), the few studies in which ANP was administered have reported that substantial increments of pANP lead to a modest natriuretic response in these patients (16–19). The resistance to the diuretic effects of ANP may be merely dependent on the renal disease per se; alternatively, it may be related to the development of important antinatriuretic side effects, such as arterial hypotension and hemoconcentration in patients, that have been ascribed to systematic vasodilation and extravascular fluid shift directly caused.
by the high pANP levels (20–23). Moreover, it is important to
note that despite the fact that volume status greatly influences
the renal response to ANP (20), the presence of a steady salt
balance has not been ascertained in the early studies, even in
those including salt-losing nephritis (16–18). Therefore, de-
spite the potential clinical implications, both the pathophysio-
logical role and the pharmacological effects of ANP remain
ill-defined in these patients.

In this study, patients with CRF resulting from biopsy-
proven chronic glomerulonephritis underwent stepwise infu-
sion of different low ANP doses, under controlled conditions
of the volume status. The dose-response relationship of the
renal effects of ANP was evaluated in the lowest range of doses
ever tested in renal patients, by using an experimental design
that offers two major advantages: first, the capability to esti-
mate the contribution of physiological pANP levels to the renal
handling of sodium, and second, the possibility of investigating
the natriuretic and extranatriuretic effects of pANP levels that
are immediately above the physiological range (10–12). A
control group of patients with chronic glomerulonephritis and
normal GFR was also enrolled to evaluate whether the poten-
tial abnormalities in the renal effects of ANP in CRF are the
results of alterations related to the loss of functioning nephrons
or to the glomerular disease.

Materials and Methods

Subjects and Criteria for Selection

The study population (Table 1) included 16 male patients with
chronic glomerulonephritis, eight of whom had chronic renal failure
(CRF group) and eight of whom had normal renal function (GN
group). A normal control group was composed of eight healthy male
volunteers from our medical staff (NOR group). A written informed
consent was obtained from each patient before the study. All patients
had histological diagnosis of primary glomerular disease made when
GFR was normal or slightly reduced. They showed a stable renal
function in the last 6 months before the study. Exclusion criteria for
patients consisted of mean arterial pressure > 105 mm Hg despite
antihypertensive therapy, neoplastic disease, protein urinary excre-
tion > 2 g/day, urinary tract infections, clinically recognizable extra-
cellular volume depletion, heart failure, cirrhosis and/or edema of any
cause, diabetes mellitus, and primary tubulointerstitial lesions. The
absence of secondary glomerulopathies was assessed by standard
diagnostic procedure and, whenever indicated, by biopsy of extrarenal
tissue. Pyelonephritis, nephrolithiasis, cystic disease, urinary obstruc-
tion, vesicoureteral reflux, and solitary kidney were excluded by
echography and urography.

Systemic hypertension, defined as blood pressure > 140/90 mm Hg
in three different measurements from the same day throughout the
6-month period of observation, was present in six patients with CRF,
whereas it was absent in GN and NOR. In these CRF patients, blood
pressure was controlled by oral administration of clonidine (0.075 mg
two times daily or three times daily); each patient maintained the same
therapy at constant dosage throughout the study. Any other medica-
tion was withdrawn at least 1 month before the study. The healthy
status in NOR subjects was established by history, physical examina-
tion, and clinical and laboratory analyses. An ultrasound evaluation of
kidneys and bladder excluded a post-voiding urinary volume in all
subjects.

Alcohol, caffeine, cigarettes, and physical exercise were prohibited
within 48 h before and throughout the study. Patients were admitted
to our unit, where accurate daily assessment of body weight (in the
morning before breakfast), and the timing and completion of all 24-h
urine collections were controlled by research personnel. An accurate
daily record of fluid, sodium, potassium, protein, and calorie intake
was kept. All meals were prepared by the institution’s kitchen.

ANP Infusion on Low-Sodium Diet

One week before the experiments, all subjects started a diet that had
a constant daily intake of sodium (35 mEq), potassium (70 mEq),
proteins (1.0 g/kg body wt) and calories (35 kcal/kg body wt). ANP
infusion was performed after equilibration at this diet (low-sodium
diet, LSD); in this regard, it is important to stress that, in each single
subject, the study was performed only if the daily urinary output of
sodium, potassium, and urea nitrogen corresponded to the prescribed
intake ±10% in the last 3 days before the study.

Renal function was assessed according to a standard protocol used
in our unit (15,24,25). On the morning (8:00 a.m.) of the study, after
7 days of LSD, fasting subjects drank 10 mL/kg body wt of tap water
and were placed in a seated position, which was maintained through-
out the study except when patients stood to void. To perform intra-
venous infusion and blood sampling, small Teflon cannulae (Abbott
Labs, Chicago, IL) were inserted into an antecubital vein in each arm.
To measure GFR and RPF, a bolus injection of a priming dose of
insulin (50 mg/kg body wt; provided by Jacopo Monico, Venezia/
Mestre, Italy) and p-aminohippuric acid (PAH, 10 mg/kg body wt;
Jacopo Monico) in 50 mL of saline infusion was performed; thereaf-
there, a continuous infusion (1 mL/min) of insulin (125 mg/creatinine
clearance per 500 mL 5% D-solution) and PAH (12.5 mg/estimated
RPF per 500 mL 5% D-solution) was started and continued through-
out the experiment to maintain a constant plasma concentration of the
two markers. After 60 min of stabilization, clearance periods of 30
min each were obtained. Urinary samples were collected at the end of
the clearance periods after the urinary volume was measured, and
blood was withdrawn at the beginning and at the end of each clearance

| Table 1. Clinical characteristics of normal subjects (NOR), and patients with chronic glomerulonephritis without (GN) and with (CRF) renal failure* |
|-----------------|-----|-----|-----|
| **Characteristic** | **NOR** | **GN** | **CRF** |
| **Patients (N)**  | 8   | 8   | 8   |
| **Age**          | 39.2 ± 2.8 | 41.2 ± 2.1 | 43.7 ± 2.4 |
| **U_pret (g/24h)** | 0.85 ± 0.31 | 0.76 ± 0.24 |
| **Glomerular disease** | 2 MP, 2 M, 3 IgA, 1 FS | 3 MP, 2 MC, 2 IgA, 1 FS |

* Values are mean ± SE. U_pret urinary protein excretion; MP, diffuse mesangial proliferative; M, membranous; IgA, Berger’s disease; FS, focal sclerosis; MC, mesangial capillary.
period through a catheter kept open by a flushing of heparinized solution. Blood pressure (BP) was measured every 10 min with a standard mercury sphygmomanometer, with the fifth Korotkoff sound used as the diastolic value.

Three basal clearances were obtained (basal-LSD). Stepwise infusion of progressively higher doses of human α-(99–126)-ANP (hANP; Novabiochem, Geneva, Switzerland), diluted in NaCl 0.9% solution, was then administered at infusion rates of 2, 4, 8, and 16 ng/kg body wt per min; the infusion volume did not change (30 mL/h). Notably, the hANP doses administered in this study are the lowest ever tested in renal patients. Each infusion step lasted 60 min and was divided into two 30-min renal clearance periods; blood and urinary samples were collected at the beginning and end of each period. During each 30-min period, as in the basal studies, the urinary salt and fluid losses (minus the infusion volumes) were step-by-step intravenously replaced by hypotonic saline (80 mEq/L NaCl) in the contralateral arm.

ANP, aldosterone (ALDO), total protein, and hematocrit values were measured in blood samples. Sodium, potassium, urea nitrogen, phosphorous, osmolality, inulin, and PAH were measured in blood and urinary samples. The urinary levels of cGMP, which is the biological marker for the ANP activity in vivo (26), were also assessed.

A time-control study was also performed in four subjects from each group at LSD. These subjects underwent the identical experimental infusion study, except for the absence of hANP; as in the experimental study, the urinary losses were replaced by intravenous hypotonic saline every 30 min. Such a control study did not show any difference in the urinary excretion of sodium, potassium, urea nitrogen, and phosphorus among the different study periods (data not shown).

**ANP Release on Normal-Sodium Diet**

A further study was performed in the same subjects to evaluate the physiological endogenous release of ANP. All subjects were kept on a normal sodium diet (235 mEq NaCl/day, NSD) for 7 to 10 days, and took in the same amount of calories, protein, and potassium as in the LSD (15). Indeed, the extracellular volume expansion induced by the increase in salt intake to the normal level of 13 g/day represents the proper physiological stimulus to ANP release (10–12). The physiological plasma ANP level (PHY) during ANP infusion was defined in each subject as the value of pANP that, in a single 30-min collection period, was the closest to the mean pANP value detected in the same subject at NSD. Because these PHY levels were reproduced by exogenous infusion during LSD intake, their renal effects were investigated in this setting, without consideration of other natriuretic mechanisms that would be active in a state of volume expansion secondary to the increase of sodium intake. This method therefore allows the assessment of the isolated contribution of ANP to the physiological increment of natriuresis evoked by higher salt intake (10–12).

The NSD study performed was similar to that described above, but because hANP was not infused, it included only the first three 30-min clearance periods after equilibration. Once the new external sodium balance was achieved, blood and urinary samples were attained to measure the plasma levels of ANP, ALDO, and natriuresis.

**Histological Study**

Two cores, which had been obtained by percutaneous renal biopsy from each patient in presence of normal or slightly reduced renal function, were analyzed by light and immunofluorescence microscopy by two renal pathologists (M.B. and G.F.), as previously indicated in other studies from our laboratory (24). In brief, the core selected for light microscopy was embedded in plastic and processed by using conventional techniques. The other core was oriented in standard frozen-section embedding compound and snap-frozen by immersion in liquid nitrogen. The specimens were serially cut in 2-μm-thick sections stained with hematoxylin and eosin, periodic acid Schiff, methenamine-silver, Masson’s trichrome, and van Gieson’s stains. Immunofluorescence microscopy was performed in all cases by using a direct immunofluorescence technique with specific antisera for immunoglobulin (Ig) G, IgA, IgM, IgE, C1q, C3, C4, and fibrinogen. The histological diagnosis was obtained in all 16 patients, and is reported in Table 1. Notably, only a mild and focal interstitial infiltration was detected in five patients (two GN and three CRF).

**Analytical Determinations**

Standard techniques, described in other articles by our group (6,15,24,25), were used to determine the urinary and plasma levels of insulin and PAH. Protein concentration was measured by the biuret method; plasma and urinary levels of urea nitrogen, sodium, potassium, and phosphate were analyzed by a Beckman autoanalyzer (Beckman Instruments, Fullerton, CA). Plasma and urinary osmolality was determined by an osmometer (Model 3MO; Advanced Instruments, Needham Heights, MA). Plasma ANP levels were assessed as described in our previous studies (10–12,15). In brief, blood samples (7 mL) for ANP RIA were collected in chilled polystyrene tubes containing 0.3 mL of 10% EDTA and then immediately centrifuged at 4°C. Plasma was separated and stored at −20°C. To extract α-ANP from plasma, we applied the extraction protocol recommended by Amersham, and used Amersham’s Amprep 100 mg C8 columns (Amersham International plc, Amersham, UK). The assay was performed according to the RIA procedure indicated in the commercial kit from Amersham (human α-ANP RIA system, code RPA 512). The sensitivity of the assay was 1.8 pg/tube. The intra-assay and interassay variation coefficients were lower than 3.5%. Recovery averaged 70% (SE 0.01); in individual samples, recoveries were determined by addition of 125I-ANP (1200 cpm) to the plasma before extraction. All plasma levels of ANP were calculated after correction for single recovery. Aldosterone was measured by using commercial RIA kits (Sorin, Saluggia, Italy), and cGMP was measured by using a commercial kit (cGMP [125I]) assay system by Amersham, UK, code RPA 525).

**Calculations and Statistical Analyses**

The percentage of the physiological contribution of ANP to the upregulation of urinary sodium excretion (UNaV at NSD) was calculated according to our previous studies (10–12), as follows:

\[([\text{PHY-UNaV}] - \text{BASAL-UNaV}) \times (\text{NSD-UNaV} - \text{BASAL-UNaV})] \times 100\]

where PHY-UNaV is the natriuresis corresponding to PHY pANP levels during infusion at LSD, BASAL-UNaV is the natriuresis in basal conditions at LSD, and NSD-UNaV represents sodium excretion at NSD.

GFR and RPF were corrected for body surface area. Effective RPF was calculated by dividing the corresponding PAH clearance by an estimate of the renal extraction ratio of PAH, which was assumed to be 0.85 in subjects with normal renal function and 0.70 in CRF patients (15,25). Protein intake was calculated using a standard formula. All values are reported as mean ± SE. We used one-way analysis of variance for intergroup comparisons, and one-way analysis of variance for repeated measurements to analyze differences in the same group. We also performed a linear correlation analysis. The level of statistical significance was defined as \(P < 0.05\).
Results

Adherence to the Prescribed Diet

In each subject, the daily assessment of urinary excretion of sodium, potassium, and urea nitrogen demonstrated the adherence to the prescribed diet before the clearance studies at both salt intake levels. On the last day of LSD (35 mEq NaCl/day), when ANP was infused, the daily urinary sodium excretion averaged 43 ± 9 mEq in CRF, 40 ± 6 mEq in GN, and 44 ± 9 mEq in NOR. Similarly, the evaluation of the 24-h sodium excretion before the study at NSD (235 mEq NaCl/day) testified the achievement of a steady external sodium balance that matched the prescribed intake. In agreement with our previous findings [15], a neutral external sodium balance was attained by the fifth day of both LSD and NSD in patients and normal subjects, therefore excluding a derangement of sodium handling in the both groups. The protein intake, calculated per kg of body weight, ranged from 0.95 to 1.10 g in the three groups for both diets.

Plasma Levels of ANP and Aldosterone

As expected, NSD was associated with pANP levels significantly higher than the values detected at basal-LSD; the value at NSD was, on average, lower in NOR (P < 0.05 versus GN and CRF) (Figure 1). The extracellular volume expansion induced by the increased salt intake also determined a significant reduction in the ALDO levels. At basal-LSD, CRF patients showed higher pANP and ALDO levels with respect to NOR and GN. In the three groups, the infusion of progressively higher doses of hANP led to a proportional increment in pANP levels that was coupled with a parallel reduction of ALDO.

The PHY-pANP level was considered as the level reached during infusion at LSD that matched the value detected at NSD (10–12). Accordingly, this level was obtained at the hANP dose of 2 ng/kg in two CRF, three GN, and two NOR; at the 4-ng dose in three CRF, three GN, and three NOR; and at the 8-ng dose in three CRF, two GN, and three NOR. During hANP infusion, PHY-pANP (pg/mL) averaged 53.5 ± 8 in CRF, 40.5 ± 4.4 in GN, and 28.8 ± 1.8 in NOR; similar to NSD conditions, the NOR value was significantly lower versus CRF and GN. Notably, ALDO levels corresponding to PHY-pANP were 202 ± 37 pg/mL, 140 ± 28 pg/mL, and 125 ± 11 pg/mL in CRF, GN, and NOR, respectively. These values were similar to those detected at NSD and, moreover, no significant intergroup difference was noted.

The levels attained during the infusion of the highest dose, that is, 16 ng/kg per min can be therefore considered as low supraphysiological levels in the subjects examined. Interestingly, when the changes versus baseline of both ANP and ALDO levels induced by the 16-ng dose were examined, major effects were observed in CRF versus GN and NOR: pANP increased by 88 ± 9.5 pg/mL in NOR, 68 ± 10 in GN, and 134 ± 12 in CRF (P < 0.05 versus other groups); ALDO decreased by 103 ± 14 pg/mL in NOR, 90 ± 17 in GN, and 210 ± 37 in CRF (P < 0.05 versus other groups).

Figure 1. Plasma levels of ANP (top) and aldosterone (bottom) in normal subjects (solid bar) and in patients with chronic glomerulonephritis without (hatched bar) and with CRF (dotted bar) with normal-sodium (NSD) and low-sodium (LSD) diets, in basal conditions and during hANP infusion. * P < 0.05 versus basal; ** P < 0.05 versus other groups; *** P < 0.05 versus NSD.

Systemic and Renal Hemodynamics

No change was observed in the mean arterial pressure (MAP) at any of the hANP doses tested (Table 2). Furthermore, exogenous administration of hANP did not cause a change in volume status in any of the subjects studied. In fact, the hematocrit value did not change during infusion of the different doses of ANP; similar results were obtained in the plasma concentration of total proteins.

The progressive increase of the pANP levels was associated with a proportional increment of GFR in normal subjects and only after the highest dose in GN patients; on the contrary, no GFR change was observed in patients with renal failure. The enhancement of GFR in NOR and GN was not related to an improved renal perfusion, as demonstrated by the constancy of RPF.
Table 2. Blood pressure and renal hemodynamics in basal conditions and during human-α-(99–126)-ANP (hANP) infusion in normal subjects (NOR), and in patients with chronic glomerulonephritis without (GN) and with (CRF) renal failure\(^a\)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Basal</th>
<th>hANP Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 ng/kg per min</td>
<td>4 ng/kg per min</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOR</td>
<td>90.3 ± 2.6</td>
<td>90.1 ± 2.9</td>
</tr>
<tr>
<td>GN</td>
<td>91.7 ± 3.4</td>
<td>92.9 ± 4.0</td>
</tr>
<tr>
<td>CRF</td>
<td>102.0 ± 2.5(^b)</td>
<td>102.9 ± 3.0</td>
</tr>
<tr>
<td>Hct (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOR</td>
<td>46.3 ± 1.1</td>
<td>46.0 ± 1.3</td>
</tr>
<tr>
<td>GN</td>
<td>46.0 ± 2.6</td>
<td>46.1 ± 2.4</td>
</tr>
<tr>
<td>CRF</td>
<td>45.4 ± 2.0</td>
<td>45.3 ± 1.8</td>
</tr>
<tr>
<td>GFR (mL/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOR</td>
<td>108.9 ± 2.9</td>
<td>110.7 ± 3.2</td>
</tr>
<tr>
<td>GN</td>
<td>107.3 ± 5.9</td>
<td>108.8 ± 5.8</td>
</tr>
<tr>
<td>CRF</td>
<td>29.0 ± 2.1(^b)</td>
<td>29.4 ± 2.0(^b)</td>
</tr>
<tr>
<td>RPF (mL/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOR</td>
<td>422 ± 15</td>
<td>397 ± 15</td>
</tr>
<tr>
<td>GN</td>
<td>395 ± 20</td>
<td>361 ± 20</td>
</tr>
<tr>
<td>CRF</td>
<td>154 ± 24(^b)</td>
<td>141 ± 20(^b)</td>
</tr>
</tbody>
</table>

\(^a\) Values are mean ± SE. MAP, mean arterial pressure; Hct, hematocrit; RPF, effective renal plasma flow.
\(^b\) \(P < 0.05\) versus other groups.
\(^c\) \(P < 0.05\) versus Basal.
\(^d\) \(P < 0.05\) versus NOR.

Urinary Excretion of Sodium

During LSD intake, the basal urinary excretion of sodium (\(U_{NaV}\)) was similar in the three groups: 0.119 ± 0.02, 0.127 ± 0.02, and 0.090 ± 0.02 mEq/min in CRF, GN, and NOR, respectively (Figure 2). The infusion of the progressively higher hANP doses induced a dose-dependent increase of \(U_{NaV}\). At each infusion step, the hANP-induced natriuresis was analogous in the three groups. The \(U_{NaV}\) value corresponding to PHY-pANP levels was significantly greater with respect to the basal values: 0.202 ± 0.04, 0.208 ± 0.04, and 0.154 ± 0.02 mEq/min in CRF, GN, and NOR, respectively. A decrease of tubular sodium reabsorption was demonstrated in all subjects by the parallel increment of the fractional \(U_{NaV}\); in CRF patients, such a mechanism accounted for the entire hANP-induced natriuresis, as GFR remained constant. When the absolute changes versus baseline of fractional \(U_{NaV}\) (\(\Delta U_{NaV}/100\) mL GFR) after the 16-ng dose were investigated, a significant major increase was observed in CRF (+0.904 ± 0.18 mEq/min) versus both GN (+0.493 ± 0.06) and NOR (+0.281 ± 0.07).

To gain insights into the renal sensitivity to ANP in patients, the plasma ANP levels measured in the infusive study were plotted against the corresponding absolute and fractional \(U_{NaV}\) values (Figure 3). All linear correlations were highly significant. Notably, when the absolute \(U_{NaV}\) versus pANP was plotted, the resulting slope was less steep in CRF as compared with that attained in NOR; on the contrary, the slope was steeper in patients than in normal subjects if fractional \(U_{NaV}\) was used.

During NSD intake, \(U_{NaV}\) was greater in all groups, compared with basal-LSD, as well as with the values corresponding to PHY-pANP; the mean values were 0.305 ± 0.05, 0.297 ± 0.04, and 0.256 ± 0.03 mEq/min in CRF, GN, and NOR, respectively. As in our previous studies (10–12), we were able to calculate the physiological contribution of ANP because the PHY-pANP during infusion at LSD reproduced the pANP levels measured at NSD, that is, in a setting of physiological stimulus to ANP release. In CRF patients, the \(U_{NaV}\) increase detected at PHY-pANP represented the 38 ± 5% of the \(U_{NaV}\) increment evoked by NSD versus basal-LSD. This value was not statistically different in NOR (46 ± 6%) and GN (46 ± 5%) groups.

Urinary Excretion of cGMP

During hANP infusion, the urinary excretion rate of cGMP progressively increased in parallel with the increment of both pANP and natriuresis (Table 3). The \(U_{cGMP}\) value corresponding to PHY-pANP was similarly increased in the three groups: 402 ± 112, 704 ± 105, and 661 ± 110 pmol/min in CRF, GN, and NOR, respectively (\(P < 0.05\) versus basal-LSD). The fractional urinary excretion of cGMP strikingly matched the changes in fractional sodium excretion, its value being higher in CRF than in NOR at baseline, as well as during infusion.

As depicted in Figure 4, highly significant linear correlations were found between the urinary excretion of cGMP and the respective pANP levels in the three groups. When the absolute urinary excretion of cGMP was considered, the slope that
resulted was less steep in renal patients as compared with NOR, whereas no difference was noted among groups if the fractional cGMP excretion versus pANP was plotted.

**Urinary Excretion of Other Solutes**

The achievement of progressively higher pANP levels was associated with a dose-dependent increase in the urinary excretion of potassium (U_K), phosphate (U_P), and urea nitrogen (U_UN) in all groups (Table 4). Similarly, the respective fractional excretions significantly increased in all groups, indicating an ANP-dependent decrease in the tubular reabsorption of the three solutes.

It is important to note that the urinary losses were effective in producing a proportional decrease in the plasma values of potassium, urea nitrogen, and phosphorus (Figure 5).

As reported in Table 4, hANP significantly increased osmolar clearance, and free-water generation remained unchanged in NOR and GN, although it rose significantly in CRF. Consequently, the plasma concentration of sodium, and plasma osmolality, did not vary in the three groups.

**Discussion**

The low hANP doses administered in this study did not modify either blood pressure or the extracellular volume; similarly, renal perfusion was not affected by any dose tested. Interestingly, a significant increase of GFR, in the absence of RPF increments, was observed in NOR and GN, confirming the positive effect of ANP on the glomerular ultrafiltration
pressure or coefficient, as suggested by micropuncture studies in normal rats (9). The absence of GFR changes in CRF is consistent with our previous data in similar patients, showing a blunted capacity to increase GFR in response to a vasodilating stimulus (25). These findings represent a major difference with respect to the previous infusive studies in renal failure showing a modest increment of natriuresis, in which hypotension and extravascular fluid shift probably developed because of the high values of pANP reached (16,17,19,21). In the study presented here, the preservation of systemic and renal hemodynamics, possibly related to the low pANP levels, allowed the full expression of the natriuretic effects of hANP.

In CRF patients, the resulting pANP levels were higher than in normal subjects at LSD, in baseline as well as during infusion; the same difference was noted after equilibrium at the higher salt intake (NSD). Previous studies reported the increase of pANP levels in ESRD; these data have been ascribed to either enhanced release by volume expansion, or reduced renal clearance of the hormone (22,27). An augmented extracellular volume partially accounted for the high basal levels because after salt intake was increased in the NSD, the pANP level was also above the normal range in glomerulonephritic patients with normal GFR. This finding has been previously ascribed to the high sensitivity of ANP release to even minor changes in the volume status (15,28). Nevertheless, the major increment of pANP levels observed in CRF during hANP infusion was certainly secondary to the lower catabolism because the extracellular volume remained constant.

An elevated pANP level is usually associated with diminished tubular responsiveness to ANP in various edematous disorders, such as nephrotic syndrome, heart failure, and liver cirrhosis (9,29,30). No author has previously evaluated whether tubular resistance to exogenous ANP is present in patients with chronic glomerular disease without nephrotic syndrome. Critical information on this issue is provided in this study by the regression analysis between the urinary excretion of cGMP, the marker for the biological activity of ANP (26), and the pANP levels (Figure 4). When the absolute UcGMPV is considered, the resulting slope was less steep in the two groups of patients as compared with NOR; however, such a difference disappeared when the cGMP excretion per GFR unit was used. Therefore, the ANP/cGMP system appears to be intact at the single nephron level in glomerulonephritic patients, even in those with reduced renal function, whereas the diminished sensitivity of the system in GN and CRF patients—detected in the presence of absolute values of UcGMPV—is only apparent, being merely dependent on the lower cGMP excretion because of the smaller or absent GFR increase observed during hANP infusion in GN and CRF, respectively. In both groups of patients, the integrity of the ANP/cGMP system was associated with a normal natriuretic response to hANP, as demonstrated by the increment of absolute UnaV after each dose to values similar to those measured in NOR subjects.

On the other hand, the resistance to the natriuretic effects of hANP in edematous disorders is associated with a reduced fractional excretion of cGMP (26,29,30), and it has therefore been explained as a consequence of diminished sodium delivery to the distal nephron, the site of ANP activity (26). Accordingly, the absence of resistance to the natriuretic activity of hANP in GN and CRF can be related to the presence of adequate sodium delivery to the distal nephron, as evidenced by the respective normal and supranormal basal values of fractional UnaV.

The comparison between pANP and UnaV at NSD and during hANP infusion at LSD allowed the analysis of the pathophysiological role of ANP in the renal handling of sodium. In CRF and GN patients, ANP accounted for approximately 40% of the increase in natriuresis observed with the 235-mEq NaCl diet, as compared with the low-salt intake of 35 mEq/day. A similar value was detected in normal subjects in

**Table 3. Absolute and fractional urinary excretion of cGMP in basal conditions and during hANP infusion in normal subjects (NOR) and in patients with chronic glomerulonephritis without (GN) and with (CRF) renal failure**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Basal</th>
<th>hANP Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>UcGMPV (pmol/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOR</td>
<td>226 ± 20</td>
<td>355 ± 29</td>
</tr>
<tr>
<td>GN</td>
<td>337 ± 67c</td>
<td>517 ± 96c</td>
</tr>
<tr>
<td>CRF</td>
<td>127 ± 21</td>
<td>252 ± 47</td>
</tr>
<tr>
<td>UcGMPV/GFR (pmol/min per 100 mL GFR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOR</td>
<td>203 ± 13c</td>
<td>330 ± 33c</td>
</tr>
<tr>
<td>GN</td>
<td>313 ± 55</td>
<td>471 ± 78</td>
</tr>
<tr>
<td>CRF</td>
<td>458 ± 79</td>
<td>980 ± 232</td>
</tr>
</tbody>
</table>

*a Values are mean ± SE.

b P < 0.05 versus basal.

c P < 0.05 versus CRF.

d P < 0.05 versus other groups.
Figure 4. Correlation of plasma ANP level and either absolute (top) or fractional (bottom) urinary excretion of cGMP in baseline and during hANP infusion in normal subjects (solid line, ■) and in patients with chronic glomerulonephritis without (dashed line, +) and with CRF (dotted line, *) who were maintained on a low-sodium diet. The P value was <0.001 in all groups. In the correlation of pANP and absolute cGMP excretion, r value was 0.89 in NOR, 0.71 in GN, and 0.51 in CRF; when the fractional UcGMP was considered, r value was 0.82 in NOR, 0.68 in GN, and 0.46 in CRF.

The study presented here, as in the previous studies by our group (10–12). Notably, the physiological contribution of ANP to the adaptation of sodium excretion, which was assessed with the low-salt diet, was not influenced by the other natriuretic mechanisms that operate when extracellular volume is expanded by an increased salt intake. A potential effect of aldosterone could be also excluded because the ALDO levels corresponding to PHY-pANP values did not differ from those detected with the NSD.

The key finding of this study is that in renal failure, pANP levels which are higher than that in normal subjects are not only biologically active, but also functional to the achievement of natriuretic effects of normal magnitude. In the regression

Figure 5. Plasma levels of potassium (top), phosphorus (middle), and urea (bottom) at baseline and during hANP infusion in normal subjects (solid line) and in patients with chronic glomerulonephritis without (dashed line) and with CRF (dotted line) who were maintained on a low-sodium diet. * P < 0.05 versus basal; o P < 0.05 versus other groups.
Table 4. Urinary excretion of water and solutes in basal conditions and during hANP infusion in normal subjects (NOR), and in patients with chronic glomerulonephritis without (GN) and with (CRF) renal failure kept on low-sodium diet.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Basal</th>
<th>hANP Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 ng/kg per min</td>
</tr>
<tr>
<td>V (mL/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOR</td>
<td>11.0 ± 0.6</td>
<td>11.0 ± 0.5</td>
</tr>
<tr>
<td>GN</td>
<td>10.0 ± 1.4</td>
<td>8.3 ± 1.4</td>
</tr>
<tr>
<td>CRF</td>
<td>4.9 ± 0.7b</td>
<td>5.0 ± 0.4</td>
</tr>
<tr>
<td>U_K V (µEq/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOR</td>
<td>55 ± 4</td>
<td>65 ± 6</td>
</tr>
<tr>
<td>GN</td>
<td>63 ± 11</td>
<td>81 ± 11</td>
</tr>
<tr>
<td>CRF</td>
<td>56 ± 5</td>
<td>60 ± 6</td>
</tr>
<tr>
<td>U_P V (mg/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOR</td>
<td>0.32 ± 0.06</td>
<td>0.57 ± 0.03c</td>
</tr>
<tr>
<td>GN</td>
<td>0.19 ± 0.03</td>
<td>0.24 ± 0.04</td>
</tr>
<tr>
<td>CRF</td>
<td>0.19 ± 0.05</td>
<td>0.21 ± 0.05</td>
</tr>
<tr>
<td>U_UN V (mg/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOR</td>
<td>9.1 ± 0.8</td>
<td>10.7 ± 1.1c</td>
</tr>
<tr>
<td>GN</td>
<td>10.0 ± 0.5</td>
<td>10.8 ± 1.1</td>
</tr>
<tr>
<td>CRF</td>
<td>8.2 ± 0.7</td>
<td>8.8 ± 0.8</td>
</tr>
<tr>
<td>C_OSM (mL/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOR</td>
<td>3.45 ± 0.25</td>
<td>3.41 ± 0.11</td>
</tr>
<tr>
<td>GN</td>
<td>2.86 ± 0.12</td>
<td>3.08 ± 0.26</td>
</tr>
<tr>
<td>CRF</td>
<td>2.32 ± 0.21</td>
<td>2.13 ± 0.15</td>
</tr>
<tr>
<td>C_H2O (mL/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOR</td>
<td>6.63 ± 0.40</td>
<td>7.43 ± 0.83</td>
</tr>
<tr>
<td>GN</td>
<td>7.34 ± 1.25</td>
<td>5.10 ± 1.39</td>
</tr>
<tr>
<td>CRF</td>
<td>2.45 ± 0.66</td>
<td>2.90 ± 0.43</td>
</tr>
</tbody>
</table>

* Values are mean ± SE. V, urinary volume; U_K V, urinary potassium excretion; U_P V, urinary phosphorous excretion; U_UN V, urinary urea nitrogen excretion; C_OSM, osmolar clearance; C_H2O, free-water clearance.

b P < 0.05 versus other groups.

c P < 0.05 versus basal.

Analysis between absolute U_Na V levels and respective pANP values, the resulting slope was less steep in CRF as compared with that in NOR (Figure 3), a finding that is apparently consistent with the early hypothesis that renal failure is characterized by resistance to the natriuretic effects of ANP. Nevertheless, the opposite held true when the fractional U_Na V was considered for the analysis (Figure 3): in CRF, the calculated slope was actually steeper with respect to that attained in the NOR group, suggesting a higher tubular sensitivity to circulating ANP in patients than in normal subjects. Indeed, the infusion of hANP led to a greater sodium excretion per single nephron in CRF as compared with NOR (Figure 2), confirming an exaggerated tubular response to exogenous hANP in these patients. However, because the ANP/cGMP system was functioning normally in the surviving nephrons of CRF patients (Figure 4), the observed greater natriuretic response per single nephron in these patients was at least partially determined by the hANP-induced inhibition of aldosterone release; a major decrease of plasma ALDO was in fact observed during hANP infusion in this group (Figure 1). This is the first time that a greater natriuretic response to ANP at the single-nephron level has been shown in patients with renal failure; in fact, although a similar finding has been reported in animal models of renal failure (31), no author has previously compared the effects of the same dose of hANP in renal patients and normal subjects.

The suppression of the renin-aldosterone system is an essential feature of ANP activity (9–14); it represents a major advantage with respect to the commonly used loop diuretics, because the stimulation of this system is the primary mechanism of resistance to the natriuretic efficacy of these agents (2). An additional difference in comparison with loop diuretics (4) is the fact that the marked hANP-induced natriuresis was not associated with a decrease in free-water clearance and the consequent development of hyponatremia.

Besides the major natriuretic effects, the infusion of progressively higher doses of ANP promoted in patients, as well as in normal subjects, a proportional marked increase of the urinary excretion of the primary solutes retained in renal disease (such as urea, phosphate, and potassium), which, in patients, was mainly dependent on the reduction of the tubular reabsorption.
Importantly, a 15 to 25% decrease of the plasma levels of these three solutes corresponded to the enhanced urinary losses (Figure 3). These data are largely confirmatory of the findings recently attained in normal subjects by our group (12). The increase in urea excretion was possibly a result of ANP-induced medullary washout, as shown by the increment in osmolar clearance. Interestingly, the increase in the fractional phosphate excretion is consistent with the hypothesis that ANP presents an additional inhibitory effect on the reabsorptive rate in the proximal tubule (14), because phosphate is primarily reabsorbed at this level. This phenomenon may also explain the augmented urinary potassium loss that occurred despite decreasing ALDO levels because of the enhanced luminal electronegativity; the increment in the Na⁺/K⁺ exchange in the cortical distal nephron because of the increased distal Na⁺ delivery may also have played a role.

In summary, this controlled study demonstrates that in GN and CRF patients, (1) pANP levels are biologically active; (2) ANP is a primary mediator of the natriuresis that is essential to the attainment of a new sodium balance when salt intake is augmented; (3) low supraphysiological levels of ANP, which do not reduce systemic and renal perfusion, markedly enhance urinary sodium excretion to the same levels observed in normal subjects; (4) the major natriuretic effect is associated with suppression of aldosterone release, as well as with an increased urinary excretion of potassium, urea, and phosphate with consequent decrease of the corresponding plasma levels by 15 to 25%. Therefore, in chronic glomerular disease with or without moderate renal failure, ANP plays a significant pathophysiological role in the renal handling of sodium; moreover, the infusion of low, nonhypotensive doses of ANP leads to a conspicuous natriuresis associated with unique extranatriuretic effects.

Acknowledgments

We thank Mr. Francesco Uccello for his technical assistance and helpful critique. This study was partially supported by Consiglio Nazionale delle Ricerche, Roma, Italy (Fund No. 94/02523.CT 04).

References


