Renal Adaptation to Dietary Sodium Restriction in Moderate Renal Failure Resulting from Chronic Glomerular Disease

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ABSTRACT

The renal response to sodium restriction was evaluated, and the concurrent changes of the plasma levels of aldosterone (ALDO) and atrial natriuretic peptide (ANP), in healthy patients (NOR), in normotensive patients with non-nephrotic chronic glomerulonephritis and normal renal function (GN), and in patients with glomerulonephritis and moderate renal failure (GFR, 41 ± 4 mL/min; CRF). The three groups were studied for 1 wk after changing from a normal-sodium diet (NSD, 235 mEq NaCl/day) to a low-sodium diet (LSD, 35 mEq NaCl/day). All patients reached a steady sodium balance within the 4th and 5th day of LSD with an analogous cumulative loss of sodium. After salt restriction, the fractional urinary sodium excretion diminished by the same extent in the three groups, whereas the fractional free-water reabsorption did not vary in NOR and markedly decreased in GN and CRF. Plasma levels of ALDO were similar in all groups at NSD and similarly increased during LSD. In GN and CRF, as compared to NOR, ANP levels were higher at NSD and decreased by a minor extent during LSD. Notably, in GN and CRF, but not in NOR, the attainment of the new sodium balance after sodium restriction was preceded by a significant parallel reduction of blood pressure and GFR; the GFR decline was secondary to a major decrement of RPF so that filtration fraction (FF) increased. It was concluded that in NOR, distal tubular effects of ANP and ALDO account for the attainment of sodium balance during LSD. As a difference, both GN and CRF patients achieve the new sodium balance primarily through hemodynamic changes: the renal hypoperfusion secondary to a decrease in blood pressure that diminishes the filtered load of sodium, and the increase of FF that enhances the proximal tubular sodium reabsorption. This abnormal response seems related to both the minor suppression of ANP and the increased salt-sensitivity of blood pressure that are likely the result of the presence of volume expansion.

Key Words: Humans, renal hemodynamics, sodium balance, ANP, aldosterone

Salt-intake restriction represents the cornerstone in the management of hypertension secondary to chronic renal failure (CRF). Such a dietary modification is prescribed early in the course of the renal disease because CRF-related hypertension often occurs after only mild impairment of renal function, with a greater prevalence in chronic glomerulonephritis compared with other nephropathies (1). Surprisingly, the renal adaptation to a low-sodium diet (LSD) in the initial stages of CRF still remains ill-defined.

This critical issue has been investigated only in patients with advanced CRF who undergo a salt-free diet (2–7). Under these conditions, the renal adaptation to sodium deprivation is inefficient, with a significant delay in the achievement of a new sodium balance. Moreover, in these patients, a further GFR decline could be observed when sodium balance became markedly negative. In addition, the interpretation of these studies was complicated by the presence of patients with salt-wasting diseases that result from a primary tubulointerstitial lesion.

In moderate renal insufficiency, that is, the stage at which a clinical diagnosis of renal failure is more often made, the day-by-day renal response to an LSD that contains 2 to 5 g NaCl/day has never been object of investigation. It is important to note that a potential influence of salt intake on the GFR outcome may be present in these patients, as in ESRD: a GFR decrement, in fact, has been reported in the initial period of protein restriction in a large number of patients with moderate CRF (8). This phenomenon, which is associated with a parallel reduction of blood pressure, has been attributed to hemodynamic changes subsequent to the lower protein intake. However, because a low-protein diet in CRF patients is generally associated with a parallel reduction of salt intake, it is possible...
that the decrease of dietary sodium may have contributed to the GFR decline observed in that study. Indeed, we have recently demonstrated that in moderate CRF, a low-protein diet does not decrease GFR if the normal salt intake is maintained (9).

To analyze the mechanisms of the renal adaptation to a pure dietary sodium restriction, patients with moderate CRF secondary to biopsy-verified glomerular disease were studied daily while they were maintained on a normal-sodium diet (NSD; 235 mEq NaCl/day) and after starting an LSD (35 mEq NaCl/day) while the protein intake was kept constant (1.0 g/kg body wt per day). We also compared the daily changes of urinary sodium excretion with the adjustments of the plasma levels of atrial natriuretic peptide (ANP) and aldosterone (ALDO) that are the most important "volume sensing" hormones in health (10). No author has previously evaluated the daily variation of these hormones after sodium restriction in CRF patients; on the contrary, for the best assessment of the sensitivity of ANP and ALDO response to LSD, it is crucial to examine the first day's results, that is, before the achievement of a neutral sodium balance (11). Two control groups were included in the analysis: a group of healthy patients and a group of patients with biopsy-proven chronic glomerulonephritis and normal renal function. This latter group was the determinant to better evaluate whether the potential abnormalities in the renal response to LSD in CRF were the result of the glomerular disease per se, or, alternatively, of the functional and structural alterations that characterize the loss of functioning nephrons.

METHODS

Patients

Seven healthy male volunteers of our medical staff and 14 male patients were studied for 15 days after their informed consent was obtained. The clinical characteristics of the patients studied are depicted in Table 1. Healthy patients (NOR group) had a negative history for renal disease, and normal urinalysis and creatinine clearance (Ccr) values. All patients had a primary glomerular disease; the histological diagnosis are reported in Table 1. Seven patients showed a moderate reduction of renal function (CRF group), and seven patients did not have any reduction in renal function (GN group).

Exclusion criteria for patients consisted of mean arterial pressure higher than 105 mm Hg during antihypertensive treatment, neoplastic disease, protein urinary excretion ≥ 1.5 g/day, heart failure, cirrhosis, edema of any cause, diabetes mellitus, or presence of significant tubulointerstitial lesions.

Patients were periodically seen by the same physician in a day-hospital setting for a period of at least 6 months before the study. Systemic hypertension, defined as blood pressure ≥ 140/90 mm Hg in three different measurements from the same day throughout the period of observation, was present in six patients with CRF, and was absent in GN. Antihypertensive therapy consisted only of clonidine (0.075 mg twice daily or three times daily); each patient maintained the same constant dosage throughout the study.

Study Protocol

For 7 days, healthy volunteers and patients received a diet containing 35 mEq of sodium, 70 mEq of potassium, 1.0 g/kg body wt per day of proteins, 35 kcal/kg body wt per day, and a supplement of 200 mEq of sodium as oral capsules. On the 4th day of this diet (normal-sodium diet, NSD), healthy volunteers and patients were admitted to our unit. The constancy of 24-h urinary urea nitrogen and sodium excretion values, showing compliance to the prescribed diet, was proved by steady levels for at least 3 days. Patients underwent renal clearance studies of inulin and p-aminohippuric acid (PAH) on the 3rd day at 8.00 a.m. Thereafter, the sodium supplement of 200 mEq/day was withdrawn while the calorie and protein content of the diet (low-sodium diet, LSD) was kept constant. The renal clearance studies were repeated when a new steady sodium balance could be documented in the last 3 days of LSD.

In each subject, at both dietary regimens, the external sodium balance was considered achieved if the daily urinary output of sodium corresponded to the prescribed intake ± 10%.

Every morning at 8.00 a.m., during the entire period of observation in our unit, blood pressure and pulse rate were registered and blood samples were drawn from all of the patients in the study. All patients had fasted from the previous evening while still laying in bed. The patients were then allowed to stand and void; body weight (with underwear clothing) was then recorded. Twenty-four-hour urinary collection was obtained daily.

### TABLE 1. Clinical characteristics of healthy patients (NOR), and patients with chronic glomerulonephritis with (CRF) and without (GN) renal failure

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NOR</th>
<th>GN</th>
<th>CRF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (N)</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>35.4 ± 1.6</td>
<td>44.3 ± 2.7</td>
<td>38.3 ± 5.9</td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>80.1 ± 1.9</td>
<td>73.6 ± 3.3</td>
<td>66.4 ± 2.1</td>
</tr>
<tr>
<td>Ccr (mL/min)</td>
<td>116.5 ± 6.2</td>
<td>115.6 ± 3.0</td>
<td>41.4 ± 4.4</td>
</tr>
<tr>
<td>Urinary Sodium Bicarbonate (mEq/L)</td>
<td>24.9 ± 0.3</td>
<td>23.4 ± 0.9</td>
<td>21.3 ± 0.9</td>
</tr>
<tr>
<td>Urop (%) (g/24 h)</td>
<td>0.8 ± 0.35</td>
<td>0.7 ± 0.28</td>
<td></td>
</tr>
<tr>
<td>Glomerular Disease</td>
<td>2 MSP, 2 M, 2 IgA, 1 FS</td>
<td>2 MSP, 2 MC, 2 IgA, 1 FS</td>
<td></td>
</tr>
</tbody>
</table>

a The values are mean ± SE. Ccr, creatinine clearance; Urop, urinary protein excretion; MSP, diffuse mesangial proliferative; M, membranous; IgA, Berger disease; FS, focal sclerosis; MC, mesangial capillary.
b P < 0.05 versus NOR.
c P < 0.05 versus other groups.

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Blood pressure was measured with a mercury sphygmomanometer as a mean of three consecutive measurements on the same arm. The levels of ANP, ALDO, and total protein were measured from plasma samples. Sodium, potassium, urea, and creatinine values were assessed from blood and 24-h urinary samples.

**GFR and RPF Measurements**

Renal function determination was performed at the same time of the day, (8.00 a.m). According to our previous studies (9,12), renal clearance values were measured during a steady state of maximal water diuresis to gain insights into the tubular function of the proximal nephron. On the morning of the test, fasting patients drank 10 mL/kg body wt of tap water; thereafter, to maintain water balance, patients drank an amount of water equal to the urinary volume collected minus the amount administered with the infusion solution that was given orally. To perform iv infusions and blood sampling, small Teflon cannulae (Abbott Labs, Illinois) were inserted into an antecubital vein of each arm: a bolus injection of a priming dose of inulin (50 mg/kg body wt; Jacopo Monico, Venezia/Mestre, Italy) and PAH (10 mg/kg body wt; Jacopo Monico) in 50 mL of saline solution was performed; thereafter, a continuous infusion (1 mL/min) of inulin (125 mg/CCR per 500 mL 5% D-solution) and PAH (12.5 mg/estimated RPF per 500 mL 5% D-solution) was started and continued throughout the experiment to maintain a constant plasma concentration of the two markers. After 60 min of stabilization, three clearance periods of 30 min each were obtained. Blood was withdrawn at the beginning and at the end of each period through a catheter kept open by a flushing of heparinized solution. In all of the patients studied, urine collection was obtained by spontaneous voiding. A post-voiding residual urinary volume in bladder was previously excluded by ultrasound evaluation. Blood pressure was measured every 10 min during each clearance period.

**Analytical Determinations**

Plasma and urinary concentrations of proteins, nitrogen, sodium, potassium, osmoles, inulin, and PAH, as well as plasma ANP and ALDO levels, were analyzed by using standard techniques described in our previous papers (9,11--14). Blood samples (7 mL) for ANP radioimmunoassay were collected in chilled polystyrene tubes containing 0.3 mL of 10% EDTA, and immediately centrifuged at 4°C. Plasma was separated and stored at −20°C. ANP from plasma was extracted by following the extraction protocol suggested by Amersham, using Amersham’s Amprep 100 mg C8 columns (Amersham International plc. United Kingdom). The assay was performed by following the radioimmunoassay procedure as indicated in the commercial kit from Amersham (Human rANP[125]I radiolmmunoassay system, code RPA 512, Amersham). Plasma ALDO was measured by radioimmunoassay with commercial kits from Sorin (Sorin, Saluggia, Italy).

**Calculation**

GFR and RPF were corrected for body surface area. RPF was calculated by division of the corresponding PAH clearance by an estimate of the renal extraction ratio of PAH. According to other authors that have examined patients with GFR values similar to those recorded in our study, the renal PAH extraction ratio was assumed to be 0.85 in healthy patients, and 0.70 in patients with CRF (15).

**Statistical Analysis**

All values are reported as mean ± SE. We used one-way analysis of variance for comparisons among different groups, and for repeated measurements to analyze differences in the same group. Linear regression analysis was also performed. The level of statistical significance was defined as $P < 0.05$.

**RESULTS**

**Daily Evaluation of the Effects of Low-Sodium Diet**

**Sodium Balance.** No difference was noted among groups in the daily sodium balance after sodium restriction (Figure 1). On the first day of LSD, patients developed a negative sodium balance of 70 to 100 mEq; the difference between salt intake and output progressively decreased in the following days. We considered the external sodium balance to have been achieved if the 24-h urinary output of sodium matched the prescribed intake with a maximal varia-
tion of 10%; accordingly, a steady balance was reached at the 4th day in CRF patients and at the 5th day in GN and NOR.

At the end of the LSD period, the mean cumulative sodium balance result was also similar in the three groups (−134 ± 20 mEq in control patients, −157 ± 66 mEq in GN, and −160 ± 28 mEq in CRF, Figure 1), indicating a net decrease of about 1 L of extracellular volume in all of the patients studied. This finding is in agreement with the significant mean decrement of body weight observed at the end of LSD versus NSD (NOR, from 80.1 ± 1.9 to 78.9 ± 1.0 kg; GN, from 73.6 ± 3.3 to 72.2 ± 3.3 kg; CRF, 66.4 ± 2.1 to 65.3 ± 2.1 kg).

Plasma protein concentration (TP) was slightly but significantly higher at NSD in NOR than in GN and CRF (Table 2); at the end of LSD, this value had increased in all groups: from 7.3 ± 0.1 to 7.6 ± 0.1 g/dL, from 6.5 ± 0.3 to 6.8 ± 0.1, and from 6.4 ± 0.2 to 6.8 ± 0.2 in NOR, GN, and CRF, respectively (P < 0.05 versus NSD). The same difference was maintained at LSD.

Notably, the reduction of dietary salt was associated with a constant protein intake. Indeed, on the basis of the daily measurements of urinary urea nitrogen excretion, the protein intake calculated per kg of body weight ranged from 0.95 to 1.10 g in the three groups. These values remained constant throughout the study.

Blood Pressure and Renal Parameters. After salt restriction, a progressive reduction of blood pressure was recorded in the two groups of patients but not in healthy individuals (Figure 2). In GN and CRF, MAP was significantly lower at the 4th and the 3rd day of LSD, respectively; this phenomenon therefore preceded the attainment of sodium balance in both groups of patients.

The day-by-day study evidenced a significant decrease of GFR that was simultaneous with the reduction of blood pressure in patients. Indeed, Ccr values (Figure 2) diminished at the 4th day in the GN group (107.1 ± 4.7 versus 115.6 ± 3.0 mL/min, P < 0.05) and at the 3rd day of LSD in CRF (36.9 ± 4.1 versus 41.4 ± 4.4 mL/min, P < 0.05). The renal function remained constantly depressed in the subsequent days (Ccr on the last day of LSD: 108.4 ± 6.0 mL/min in GN and 34.2 ± 4.8 mL/min in CRF). On the contrary, Ccr did not vary throughout the period of observation in healthy patients (Figure 2).

The basal fractional urinary excretion of sodium (FENa) was greater in CRF patients with respect to the two groups with normal GFR maintained on both dietary regimens (Figure 3). After salt restriction, FENa progressively decreased by the same extent in the three groups, reaching a steady level once sodium balance was achieved: by the last day of LSD, FENa was reduced by 82 ± 4%, 79 ± 7%, and 74 ± 5% in NOR, GN, and CRF, respectively.

Plasma Levels of ANP and ALDO. With both NSD and LSD, plasma ANP levels were significantly higher in GN and CRF patients than in healthy patients (Figure 4). In contrast, no difference was detected among groups with regard to the ALDO levels. In all of the patients under study, LSD was associated with a decrease of ANP and a concurrent increase of ALDO. A significant change in the ANP levels was noted as early as the first day after dietary sodium restriction was begun, whereas ALDO changed by the 2nd day of LSD. When the final variation of ANP levels from NSD to the end of LSD was examined, a greater decrease was observed in NOR (−83.4 ± 4%) than in GN and CRF (−43.0 ± 9% and −47.1 ± 7%, respectively, P < 0.05 versus NOR). ALDO increased by the same extent in the two groups.

The linear regression analysis, calculated by plotting the daily ANP concentration with the corresponding value of FENa from each subject, showed a significant direct correlation only in the NOR group (N = 56, significant).
Figure 2. Daily changes of Ccr (column) and mean arterial pressure (dashed line) during the 7 days of low-sodium diet in glomerulonephritic patients with normal (top) or moderately reduced GFR (middle), and in healthy patients (bottom). Day 0 is the last day of the normal-sodium diet. *P < 0.05 versus Day 0.

Figure 3. Daily changes of the fractional urinary excretion of sodium during the seven days of low-sodium diet in glomerulonephritic patients with normal (dashed line) or moderately reduced GFR (dotted line), and in healthy patients (solid line). Day 0 is the last day of the normal-sodium diet. *P < 0.05 versus other groups; **P < 0.05 versus other days; ***P < 0.05 versus healthy patients.

r = 0.6930, P < 0.0001). No correlation was found between plasma ALDO levels and the respective FE_{Na} values in any of the groups studied.

Renal Function Before and After Low-Sodium Diet

The renal clearance data from the last day of NSD and LSD (Table 2) confirmed the results of the day-by-day evaluation. At NSD, GFR, and RPF, measured by inulin and PAH renal clearance, was comparable in NOR and GN groups. At the end of the LSD period, a significant renal hypoperfusion was detected only in GN and CRF. In the GN group, LSD was associated with a 12% decrease of GFR that was secondary to a parallel 24% reduction of RPF; this resulted in a significant increase in the filtration fraction (FF). Similarly, in the CRF group, sodium restriction determined a GFR decline of about 15% that was dependent on a 33% decrease of RPF with a consequent elevation of FF. The observed renal hypoperfusion in GN and CRF at LSD was coupled with a significant increase of the renal vascular resistances.

After salt restriction, the fractional free-water generation strikingly decreased in both GN and CRF groups, and did not change in NOR. These data, attained in a condition of maximal water diuresis, are compatible with a increment of tubular sodium reabsorption at the level of the proximal nephron (9,12).

DISCUSSION

The adherence to the prescribed LSD allowed the correct analysis of the renal response to the pure salt restriction, that is, independently of influences deter-
mEq of sodium developed in the three groups 24 h after sodium restriction; the corresponding extracellular volume depletion of approximately 0.5 L (about -5%) was associated with a simultaneous marked decrease of ANP levels in the absence of significant changes of plasma ALDO. Therefore, the release of ANP was suppressed by very small reductions of the extracellular volume in all groups.

It is probable that the high sensitivity of ANP release was critical to the final change of ANP levels in healthy patients and renal patients after salt restriction. A significant minor ANP suppression was evident in GN (-43%) and CRF (-47%) with respect to NOR (-83%). Such a diverse response may have been dependent on the presence of a mild volume expansion in both GN and CRF groups. Indeed, ANP levels were higher in CRF than in healthy patients at both levels of sodium intake. The same finding has been reported by other authors and interpreted as a consequence of enhanced ANP release secondary to volume expansion and/or determined by a decreased renal clearance of this hormone (16-18). In the study presented here, the significantly lower values of plasma protein concentration, as well as the presence of high ANP levels in the GN group with normal GFR, are consistent with the former hypothesis. We can therefore hypothesize that in both groups of patients, the inhibitory effects of LSD on ANP release may have been smaller because of a volemia that was constantly augmented. Notably, the presence of increased extracellular volume in GN and CRF patients cannot be excluded on the basis of ALDO levels similar to the control value because the ALDO concentrations have been found to be normal or even elevated in moderate to advanced CRF (19-21).

In the NOR group, the fractional urinary sodium excretion significantly correlated with ANP levels but not with ALDO. This finding, as well as the maximal suppression of ANP release in the same group, confirm that ANP is the main determinant of the tubular response to sodium restriction in health (11). In healthy patients, sodium reabsorption likely increased at the distal tubule level as indicated by the decrement of FeNa associated with a constant fractional free-water generation.

On the contrary, no relationship was detected between FeNa and either ANP or ALDO in GN and CRF patients, suggesting that in glomerular disease, the two hormones do not play a major role in downregulating natriuresis after salt restriction. Indeed, in GN and CRF but not in NOR, the daily measurements of blood pressure and Ccr showed a simultaneous decrease of both parameters that took place before the attainment of the new sodium balance. These results show that a reduction of GFR secondary to the decrement of blood pressure represents a primary mechanism adopted by these patients to conserve sodium at LSD.

The inulin and PAH clearances performed on the last day of each dietary regimen not only confirmed the significant GFR decline in GN and CRF after
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sodium restriction, but also demonstrated that this was secondary to a greater reduction of renal plasma flow coupled with an elevation of the FF. In addition, the acute study showed a marked decrease of the fractional free-water generation in the same two groups, measured during maximal water diuretics. It is therefore reasonable to hypothesize that in GN and CRF patients, proximal tubular reabsorption of sodium increased in response to LSD and, moreover, that this phenomenon was primarily attributable to the renal hypoperfusion and the consequent increment of the oncotic pressure at the level of the proximal peritubular capillary (22). We cannot exclude, however, that factors other than the hemodynamic changes may have been involved in the tubular adaptation to LSD in the renal patients.

The functional impairment of renal perfusion after sodium restriction in CRF was dependent on the simultaneous decrease of the systemic arterial pressure. At the end of the LSD period, the depletion of the extracellular volume was comparable in the three groups; however, blood pressure diminished in renal patients but not in healthy patients. These data are therefore compatible with an increased salt-sensitivity of blood pressure that has been previously described in patients with mild to advanced CRF (21,23–26). Importantly, the findings in the GN group indicate that in patients with primary glomerular disease, this peculiar response to salt restriction develops even before hypertension and/or GFR decline become manifest. The augmented volemia may have played a central role in the pathophysiology of this phenomenon. This alteration, in fact, is crucial to the enhancement of the salt-sensitivity of blood pressure in renal patients (21, 23–26).

In conclusion, in glomerulonephritic patients with or without moderate CRF, the renal adaptation to LSD is achieved as promptly as it is in healthy patients, however, the underlying mechanisms appear to be different. In healthy patients, sodium excretion is efficaciously readjusted by hormonal changes with ANP playing a predominant role. On the other hand, renal patients activate the new sodium balance primarily through hemodynamic changes: the renal hypoperfusion secondary to a reduction of blood pressure that diminishes GFR and the filtered load of sodium, and the increase of filtration fraction that enhances the proximal tubular sodium reabsorption. In these patients, the abnormal mechanism of adaptation is likely to be attributable to the presence of volume expansion that is associated with a minor suppression of ANP release and increased salt-sensitivity of blood pressure. These phenomena precede the development of systemic hypertension and GFR decline.

Interestingly, these findings imply that a careful analysis of the possible changes in salt intake is mandatory when examining the impact of a low-protein diet, as with any other therapeutic intervention, on the progressive GFR decline in established renal disease. Indeed, we have recently demonstrated that protein restriction does not decrease GFR in patients with moderate CRF if the usual sodium intake is kept constant (9). Therefore, the results attained in both the previous study and this study strongly suggest that the reduced salt intake, usually associated with protein restriction, is the primary determinant of the GFR decline observed after starting a low-protein diet in patients with renal insufficiency (8).

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